Residual Amounts of Chlorophylls and Pheophytins in Refined Edible Oils

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ABSTRACT

The quantities of chlorophyll (CHL) A and B, and pheophytin (PHY) A and B in 10 kinds of refined edible oils were estimated by the fluorometric method. The results revealed that CHL and PHY were present in commercial edible oils. PHY A showed the highest content at ca. 67% in total pigments. Compositional ratios of CHL and PHY were similar in different kinds of plant oils. Through the analysis of rapeseed oils at every refining step, we determined that PHY is not formed during oil refining. In the autoxidation of soybean oils to which various amounts of CHL mixtures had been added, the peroxide value of tested oils increased in proportion to the total chlorophyll content. In addition, the compositional changes of the 4 components during autoxidation were investigated.

INTRODUCTION

Exposure to light is largely responsible for oxidation of fats and oils, especially in the presence of chlorophyll (CHL). However, few papers (1-3) have been published on the CHL and pheophytin (PHY) content of fats and oils and these evaluated only crude oils.

In our previous papers (4,5), we reported that the prooxidant activity in PHY was higher than in CHL. From this result, we claimed that not only the CHL content but also the PHY content should be noted in considering oxidative stabilities of edible oils and fats.

The quantity of CHL that remain in refined edible fats and oils is unknown, because the CHL contents are below the limits of detection by spectrophotometric analysis. Recently, we designed a new method for simultaneous fluorometric estimation of CHL and PHY (6), from the modification of the method of White et al. (7), to determine the residual amounts in refined edible fats and oils.

In this paper, the CHL and PHY content were determined in refined edible oils commercially produced at several representative manufacturers in Japan. Compositional changes during the refining process, as well as autoxidation, were investigated.

MATERIALS AND METHODS

The majority of sample oils were deodorized oils, without any additives, obtained from oil manufacturers in Japan.

CHL and PHY Determinations

CHL A and B, and PHY A and B were fluorometrically determined, as in a previous paper (6); 3.64 g of sample oil was dissolved in 16 mL of spectral-grade acetone, and the fluorescence intensities in 4 measurement systems (excitation maximum (Ex) 425-emission maximum (Em) 663 nm, Ex 454-Em 646 nm, Ex 404-Em 670 nm and Ex 434-Em 655 nm) were measured by spectrophotofluorometer (Hitachi 204 type, selector 10, sensitivity 12). Concentration of each component was calculated from the following formula.

$$\text{CHL A}=(10.8F_{425}^{663}+0.307F_{454}^{646}-3.30F_{404}^{670}-4.91F_{434}^{655})\times 10^{-3}\ \text{mg/kg}$$

$$\text{CHL B}=(0.566F_{425}^{663}+5.09F_{454}^{646}-0.199F_{404}^{670}-0.950F_{434}^{655})\times 10^{-3}$$

$$\text{PHY A}=(-8.09F_{425}^{663}-0.214F_{454}^{646}+11.7F_{404}^{670}+2.74F_{434}^{655})\times 10^{-3}$$

$$\text{PHY B}=(-4.81F_{425}^{663}-1.71F_{454}^{646}+1.23F_{404}^{670}+7.92F_{434}^{655})\times 10^{-3}$$

Detection of CHL and Its Derivatives in Crude Rapeseed Oil

Fifty grams of silica gel (Wako-Gel C-100, 40-100 mesh) was packed into a column (24 × 300 mm) in a slurry with hexane and 5 g of crude rapeseed oil was added. This was eluted successively with 10% n-hexane in diethyl ether (DEE), 50% n-hexane in DEE and DEE.

For the removal of triglyceride and carotene, the second fraction was chromatographed again on a silica cartridge (SEP-PAK, Water Associate Inc., Farmingham, MA), using the same solvent system as above. Fifty percent n-hexane eluate was hydrolyzed with lipase (8) to remove small amounts of triglyceride from the chlorophylls, followed by DEE extraction.

Thin layer chromatographic (TLC) analysis was carried out by the method of Sievers and Hynninen (9).

RESULTS AND DISCUSSIONS

Contents of CHL and PHY in Refined Edible Oils

The CHL and PHY content were estimated for 10 kinds of refined edible plant oils commercially produced at several representative oil manufacturers in Japan. The results are shown in Table I. The following facts were revealed. For each edible oil, different total amounts of chlorophyll analogs (tCHL) were observed. The tCHL content increased in the following order; soybean<corn<safflower or sunflower<rapeseed<cottonseed<palm olein. Compositional ratios, defined as the sum of the percentages of CHL and PHY, of A-type and B-type were similar in different plant oils, except seasame oil. Mean content ratio of A and B were 70% and 30% (SD +6%) in all measurements in Table I. PHY was present in a much higher proportion than CHL. PHY A showed the highest content, over 60%, whereas PHY B content was 15-25%. The quantity of CHL A was the smallest, near the limit of detection by fluorometric method. Relative compositional ratio of the 4 components was calculated as CHL A/CHL B/PHY A/PHY B = 4:10:67:19 in 31 measurements, except for seasame oil. Safflower, corn, sunflower and palm oils contained similar quantities of CHL B and PHY B, whereas soybean and rapeseed oils contained a greater quantity of PHY B than CHL B. A relatively constant compositional ratio of the 4 components in the same kind of oil at the same factory was noted, regardless of different production dates.

Although the majority of analyzed oils were obtained after the deodorization process, we assumed that the same results could be expected in the final products available to consumers.

We have already detected higher prooxidant activity in PHY than CHL in previous studies (4,5). The results in Table I also support the conclusion that the PHY, as well as (or more than) the CHL, content must be noted in considering oxidative stabilities of edible oils.

Change of CHL and PHY Composition During the Refining Process

In order to elucidate the cause of the high PHY content in refined edible oils, the fluorometric estimation of rapeseed oils at every refining step was carried out. Before analysis, sample oils, except for bleached and deodorized oils, were diluted with photosensitizer-free oil, prepared by the treatment on an activated carbon-Celite column (10).

The contents of CHL and PHY decreased with the pro-

TABLE I

Chlorophyll and Pheophytin Content of Several Refined Edible Oils in Japan

Oil	Manufacturer[a]	Total	CHL A	CHL B	PHY A	PHY B
		μg/kg oil				
Soybean	A	69.1	0.0	4.9	50.8	13.5
Soybean (S)[b]	B	66.7	3.9	2.7	44.7	15.5
Soybean (S)	B	65.4	3.6	2.8	44.0	15.0
Soybean (S)	B	116.0	2.0	13.3	73.1	27.7
Soybean (F)[c]	B	77.6	6.8	4.1	51.5	15.2
Soybean (F)	B	79.5	4.8	3.8	52.6	18.4
Soybean	C	57.0	0.0	0.3	49.3	7.4
Rapeseed	A	187.6	6.3	27.8	119.2	34.4
Rapeseed	A	180.2	3.6	21.4	107.5	47.7
Rapeseed	A	219.7	15.2	30.6	131.5	42.3
Rapeseed	B	126.0	4.0	11.3	83.5	27.1
Rapeseed	B	126.2	3.2	7.6	85.8	29.6
Rapeseed	B	132.0	3.4	7.4	90.0	31.2
Rapeseed	B	132.4	0.1	7.2	92.5	32.6
Rapeseed	C	154.0	0.2	11.9	92.1	49.8
Cottonseed	A	278.8	9.7	39.8	184.9	44.3
Cottonseed	C	213.9	0.0	10.3	168.7	35.5
Safflower	A	311.9	31.7	60.2	167.2	52.8
Safflower	C	114.8	0.8	16.7	82.4	14.8
Safflower	D	91.6	1.7	9.0	69.9	11.1
Safflower	D	131.9	9.2	11.7	96.0	15.0
Corn	A	161.7	0.0	18.9	114.4	40.5
Corn	B	101.4	2.2	12.0	73.5	13.6
Corn	B	95.4	2.7	10.1	68.5	14.2
Sunflower	B	146.1	8.3	23.1	88.8	25.7
Sunflower	B	160.0	11.0	26.9	95.6	26.5
Olive	E	142.0	7.7	16.9	97.0	20.6
Palm olein	A	582.9	30.3	113.8	341.2	97.6
Palm olein	A	579.4	15.3	106.4	359.3	98.5
Rice bran	F	626.0	29.6	79.5	396.7	120.3
Rice bran	F	533.0	25.8	63.7	368.7	74.8
Seasame	B	1189.5	125.1	318.9	542.8	202.7
Seasame	B	1324.7	162.6	338.9	562.3	310.9
Mean compositional ratio ± SD (%)			4.1 ± 3.0	11.3 ± 6.0	65.7 ± 7.9	19.0 ± 4.7

[a]Same kinds of oil from same manufacturer were produced at different date.
[b]Oils for salad use.
[c]Oils for frying use.

TABLE II

Change of Chrolophyll and Pheophytin Composition During Refining Processing of Rapeseed Oils

	Total	CHL A (%)	(CHL B (%)	PHY A (%)	PHY B (%)
	mg/kg oil				
Normal oil					
Pressed oil	39.9	2.53 (6.3)	4.91 (12.3)	30.3 (76.9)	1.75 (4.4)
Extracted oil	46.1	2.62 (5.7)	2.92 (6.3)	35.6 (77.2)	4.99 (10.8)
Crude oil	35.7	0.88 (2.5)	0.30 (0.9)	27.8 (77.9)	5.35 (18.8)
After degumming	43.1	1.78 (4.1)	1.13 (2.6)	33.3 (77.2)	6.93 (16.1)
After neutralization	39.1	0.89 (2.3)	0.00 (0.0)	31.5 (80.5)	6.84 (17.5)
After bleaching	0.386	0.028 (7.3)	0.059 (13.6)	0.235 (60.9)	0.071 (18.3)
After deodorization	0.171	0.007 (4.1)	0.023 (11.9)	0.108 (62.9)	0.036 (21.1)
Greenseed oil					
Pressed oil	85.3	4.18 (4.9)	6.99 (8.2)	65.6 (76.9)	8.50 (10.0)
Extracted oil	90.3	3.02 (3.3)	4.03 (4.5)	68.9 (76.4)	14.31 (15.8)
Crude oil	81.2	2.95 (3.6)	6.46 (8.0)	64.0 (78.8)	7.83 (9.6)
After neutralization	66.4	1.09 (1.6)	0.00 (0.0)	53.4 (80.4)	11.9 (17.9)
After bleaching	0.436	0.042 (7.8)	0.037 (8.5)	0.299 (65.8)	0.058 (13.3)
After deodorization	0.103	0.0016 (1.6)	0.0047 (4.6)	0.079 (76.9)	0.018 (17.2)

gress of purification (Table II). However, a relatively constant compositional ratio of the 4 components was observed, indicating that PHY is not formed during oil refining. That is, a high proportion of PHY was present, even in the pressed oil.

In the case of green-seed oil, the result the larger quantities of tCHL were observed at all refining steps than those observed in normal oil was inevitable, but the proportional ratios of the 4 components were similar to those of normal oils.

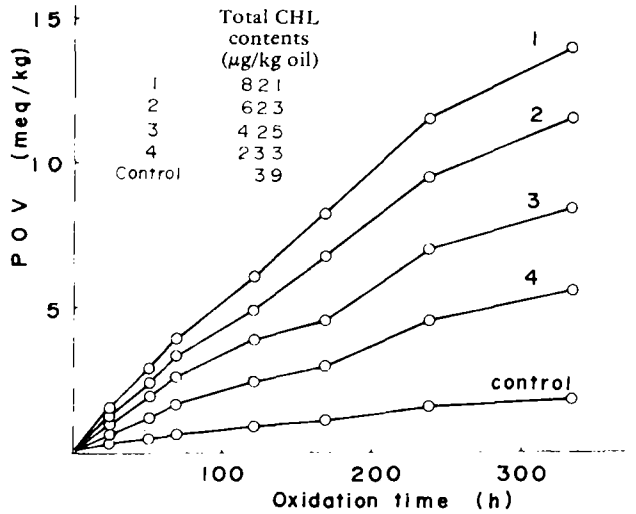

FIG. 1. Autoxidation of soybean oils under room light, to which various amounts of chlorophyll mixture (CHL A/CHL B/PHY A/PHY B = 1:3:10:3) were added.

Change of CHL and PHY During Autoxidation

How CHL and PHY were altered during photooxidation or autoxidation, how the quantities of these substances present would promote autoxidation and so forth, are interesting.

Fifteen mL of chlorophylls-acetone solution (CHL A/CHL B/PHY A/PHY B = 1:3:10:3) were added to 100 g of refined soybean oil (tCHL content 39 μg/kg oil) and autoxidized at room temperature (ca. 15 C) under room light. From the determination of peroxide value (Fig.1), we show that the oxidative stability of tested oils depends on tCHL content. A similar result was also obtained in the experiment on refined rapeseed oil (tCHL content 171 μg/kg oil). These results suggested that in refined edible oils, the amounts of CHL and PHY will affect the oxidative stability.

Figure 2. showed the compositional changes of the 4 components in sample 1 of Figure 1. The CHL content decreased rapidly within a short period, whereas PHY remained almost unchanged. The quantity of tCHL was constant after oxidation for 50 hr, owing to the relative stability of PHY. These results were identical with those in model experiments (4) using methyl linoleate as a substrate. Similar results were also obtained in the experiments on refined rapeseed oil (Fig. 3).

Absence of Pheophorbide in Crude Rapeseed Oil

We suspected that the apparently large quantities of PHY might be caused by contamination by pheophorbide (PHO), since PHY and PHO give the same absorption spectra.

To ascertain the presence of chlorophyll analogs in plant oils, pigment mixtures were separated from crude rapeseed oil, which was rich in tCHL, by silicic acid column chromatography and lipase treatment. In TLC developed in n-heptane-pyridine (7:3) using cellulose plate, CHL analogs were detected as a broad greenish band, including PHY A (Rf 0.84), PHY B (0.76), CHL A (0.68) and CHL B (0.62), which were colored fluorescent red under UV light. However, spots of PHO A (0.18) and B (0.12) could not be detected.

Because the compositional ratio of CHL and PHY were constant at all refining steps, as shown in Table II, we presume that PHO will also be absent in the final refined oil.

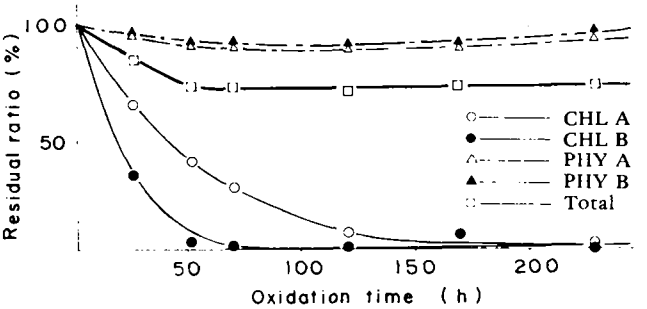

FIG. 2. Compositional changes in the added chlorophylls and pheophytins (total content: 821 μg/kg oil, CHL A/ CHL B/PHY A/PHY B = 1:3:10:3) during photooxidation of soybean oil.

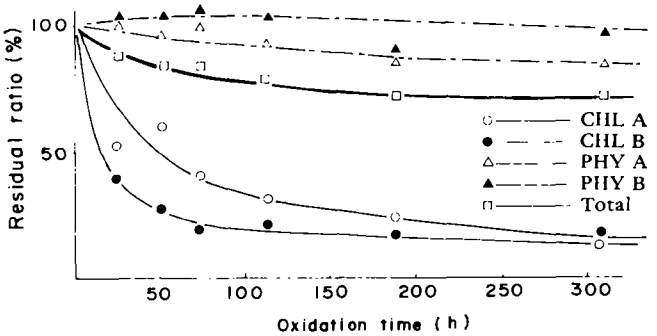

FIG. 3. Compositional changes in the added chlorophylls and pheophytins (total content: 559 μg/kg oil, CHL A/ CHL B/PHY A/PHY B = 1:3:10:3) during photooxidation of rapeseed oil.

Many factors affect autoxidation of edible oils in light: oxygen, temperature, fatty acids and triglycerides composition, tocopherols, carotenoids, chlorophylls, conjugated oxodiene (11), metal. Oxidative stability of edible oils is supposed to be caused by the total balance of these factors, though the prooxidant action of tocopherol (12,13) and of β-carotene (14) and the synergistic effect of tocopherol and β-carotene (15) were reported under the given experimental conditions.

The prooxidant activities of CHL and PHY were confirmed in the other experiments using photosensitizer-free methyl linoleate (4) and refined edible oils (5) (tocopherol content below 10 μg/kg) as substrate. The result is that PHY shows a higher prooxidant activity and higher stability through the photooxidation of triglycerides than CHL. This reconfirmed that the PHY content in oils must be noted as well as CHL. However, the compositional ratio of CHL and PHY was relatively constant in all kinds of plant oils. In addition, the results in Figure 1 indicated that the amount of tCHL will be responsible for the oxidative stability of edible oils, at least, in the range of normal tocopherol content in refined edible oils. From these observations, we recommend that the quantity of tCHL present in refined oils should be minimized and the oils protected from exposure to light in order to ensure oxidative stability.

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Relationship Between Titer and Fatty Acid Composition of Beef Tallow

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ABSTRACT

The effect of titer increase on the composition of 60 samples of Uruguayan beef tallow was studied. The results demonstrate that the titer depends fundamentally on the stearic/oleic ratio.

The effect of the composition of binary and ternary fatty acid mixtures on the titer and the melting point has been reported by several researchers (1-7). From the publications, we may conclude that melting and solidification phenomena are complicated and unpredictable. As beef tallow contains different kinds of fatty acids, no relation would be expected between composition and titer; nevertheless this is not so.

TABLE I

Titers and Fatty Acid Distribution for Uruguayan Beef Tallows

	Maximum value	Minimum value
Titer (C)	47.6	37.3
Myristic (%)	5.5	0.8
Palmitic (%)	30.4	20.2
Palmitoleic (%)	10.1	2.8
Stearic (%)	34.3	9.3
Oleic (%)	51.8	31.6
Linoleic (%)	3.8	0.6

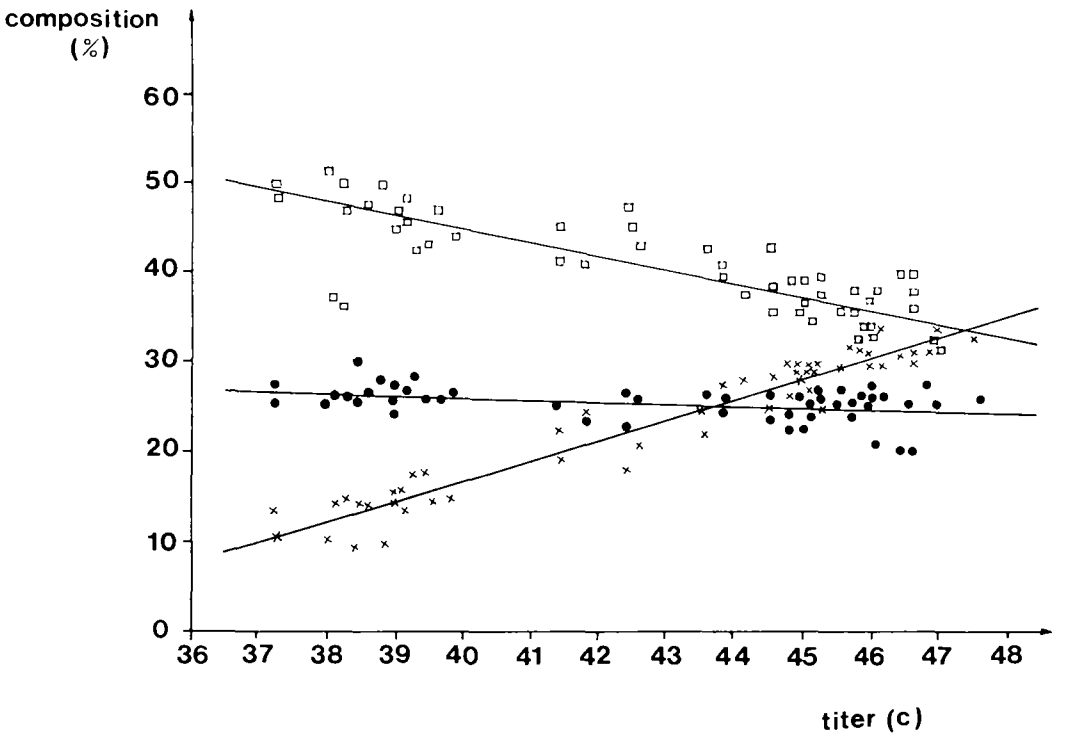

FIG. 1. Effect of titer increase on the composition of beef tallow. × = stearic acid; □ = oleic acid; • =palmitic acid.