Recovery of Carotenoids from Palm Oil

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The carotenoids from palm oil were recovered through a two-stage process involving transesterification of palm oil followed by molecular distillation of the ester. The carotenoid fraction contained more than 80,000 ppm carotenoids. α- and β-Carotenes were the major components. Vitamin E and sterols were also present.

KEY WORDS: Alkyl esters, carotenes, molecular distillation, palm oil, stability, sterols, transesterification, vitamin E.

It is well known that palm oil contains a high concentration of natural carotenoids of 500–700 ppm (1–3). The major carotenoids of palm oil are α- and β-carotene; together they constitute more than 80% of the total carotenoids in palm oil (3,4). Carotenes, in particular β-carotene and, to a lesser extent, α-carotene, are known for their provitamin A activities, as they are transformed into vitamin A *in vivo*. Compared with other sources of natural carotenoids, palm oil has 15 times more retinol equivalents than carrots and 300 times more than tomatoes (5). Recent studies have also strongly associated β-carotene with the prevention of certain types of cancer, such as oral, pharyngeal, lung and stomach cancers (6–11).

Most of the carotenoids in palm oil are destroyed in the present refining process to produce light-colored oils. This represents a loss of a potential source of natural carotenoids. The importance of carotenoids is well documented, and various methods of extraction and recovery from palm oil have been developed. These include extraction by saponification (12–16), adsorption (17–20), precipitation (21), selective solvent extraction (22,23), molecular distillation (24), transesterification followed by distillation (25–27) and others (28). However, only the transesterification and distillation process has been further developed into a commercial-scale process (29). The present paper describes a potential commercially viable process to recover carotenes from palm oil through transesterification and molecular distillation.

MATERIALS AND METHODS

Preparation of alkyl esters through transesterification. Crude palm oil obtained from Tenera oil palm species was transesterified with methanol/ethanol (AR grade) at a 2:1 molar ratio of oil to alcohol, catalyzed by 0.5% (w/w) sodium hydroxide (AR grade) after the free fatty acids had been neutralized. The reaction mixture was stirred and refluxed until all the triglycerides were converted to alkyl esters. The extent of reaction was monitored through thin-layer chromatography (silica gel, solvent chloroform/hexane, 1:1, vol/vol). The esters were then separated from glycerol and washed with distilled water until neutral. The esters were dried with anhydrous sodium sulfate, and solvent was removed under reduced pressure. The concentrations of carotenoids in crude palm oil and esters were 645 and 650 ppm, respectively.

Carotene concentration via removal of alkyl esters. The carotenes in the transesterified reaction mixture were recovered by distilling off the esters under high vacuum in a falling-film molecular distillation apparatus (Sibata, Scientific Technology Inc., Tokyo, Japan). Various proportions of refined and deodorized (RD) red palm oil; refined (physical), bleached and deodorized (RBD) palm olein; RBD palm oil; and neutralized (chemical), bleached and deodorized (NBD) palm oil were also added to the transesterified mixture. Distillation was carried out at pressures of less than 30×10^{-3} torr with temperatures ranging from 110–170°C, and the carotene concentrate was collected as a residue. The total carotene content was measured with a UV-VS spectrophotometer (Hitachi, Ltd., Tokyo, Japan) at 446 nm.

Carotene profile of the concentrate. Qualitative and quantitative carotene profiles of the concentrates were obtained with a Varian 5000 HPLC (Varian Instrument Group, Palo Alto, CA) equipped with a variable wavelength (190–900 nm) UV-100 detector. Isocratic separation was performed on a 5-μm Zorbax ODS column (Du Pont Biotechnology System, Wilmington, DE).

RESULTS AND DISCUSSION

Complete conversion of the triglycerides into alkyl esters took place without destroying the carotenoids during the transesterification reaction. The carotenoid content of the alkyl esters at this stage was almost the same as that of the starting crude palm oil. The alkyl esters are long-chain fatty acid esters and have boiling points of more than 200°C at atmospheric pressure.

The distillation of the transesterified reaction mixture gave various concentrations of carotenoids. In the single-stage molecular distillation, the concentration of the carotenoid concentrate obtained ranged from 6,600 to 20,000 ppm (Tables 1 and 2). The carotene concentration of the

TABLE 1

Concentrations of Carotenoids from Single-Stage Distillation of Palm Oil Methyl Esters (ppm)[a]

RD red palm oil (%)	Temperature (°C)					
	110	130	150	170	SE	LSD
2.5	18,900	18,900	18,900	19,000	40	130
5	9,750	9,800	9,860	9,980	20	60
10	6,650	6,690	6,750	6,990	20	50

[a]Mean value of three replicates ± SE (standard error) and LSD (least significant difference) at 5% significance level. RD, refined and deodorized.

TABLE 2

Concentrations of Carotenoids from Single-Stage Distillation of Palm Oil Ethyl Esters (ppm)[a]

RD red palm oil (%)	Temperature (°C)					
	110	130	150	170	SE	LSD
2.5	18,500	18,600	18,700	18,700	20	80
5	9,620	9,700	9,780	9,900	10	40
10	6,600	6,640	6,750	6,890	10	30

[a]Mean value of three replicates ± SE (standard error) and LSD (least significant difference) at 5% significance level. See Table 1 for abbreviation.

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residue depended on the percentage of RD red palm oil added to the alkyl esters. Because the distillation process removes most of the esters, the addition of RD red palm oil to the transesterified reaction mixture was necessary to improve the flow of the concentrate during the process. Another reason was to show whether the carotene contents of the various concentrates were proportionate to the percentage of oil added and whether carotenes were destroyed during the process. Results showed that higher concentrations of carotenoids were obtained with addition of 1% RD red palm oil compared with 2.5, 5 and 10% of the oil. The carotene content of 1% oil was 36,000 ppm compared to 18,000 ppm with 2.5% oil. The carotene concentration of the 2.5% oil was about twice the concentration of the 5% oil, and half that of the 1% oil. This showed that the difference in the carotenoid concentration was due mainly to dilution with oil. However, the carotenoid concentration of 1% oil was only two times higher than 2.5% oil, although it should have been 2.5 times higher. The lower carotene content of 1% oil was probably due to some degradation of carotene because of longer retention time in the process. Similar carotenoid concentrations (18,000 ppm and above) were obtained when 2.5% RBD palm olein, RBD palm oil and NBD palm oil were added to the alkyl esters (Table 3). The percentage of carotenes recovered from single-stage distillation ranged from 50–90%, depending on the temperature of the distillation. Higher temperature increased the degradation of the carotenoids and at the same time distilled off the carotenoids. When two-stage distillation was carried out, the carotenoid concentration increased to 75,000 ppm (Table 4). This increase in carotenoid concentration was due to removal of some of the monoglycerides and diglycerides from the residue. The yield of carotenoids recovered from two-stage distillation was about 75%.

Analyses of the carotenoid concentrates showed the presence of various carotenes, vitamin E and sterols. The carotene concentrations of the concentrates were similar to that of crude palm oil (Table 5). The carotenes present in the concentrate were phytoene, phytofluene, *cis*-β-carotene, β-carotene, *cis*-α-carotene, α-carotene, γ-carotene, δ-carotene, ζ-carotene, neurosporene, β-zeacarotene, α-carotene and lycopene. The major carotenes in the concentrate were α- and β-carotene, which made up 83–92% of the total carotenoids. The concentrate was also found to have higher concentrations of vitamin E and sterols (Table 6). The vitamin E concentration was about 10 times higher than that of the alkyl esters, while the sterol concentration was 36 times higher than that of crude palm oil (Table 7).

The storage stabilities of carotenoids in the form of capsules and powder were observed for a period of 12 mon. The carotenoids were more stable in capsule form than in powder form, even at 28–30°C (Figs. 1 and 2). The carotenoid content of the capsule was stable for the 12-mon period, even at 28–30°C. There was a slight decrease (4%) in the carotenoid content of the powder

TABLE 3

Concentrations of Carotenoids in Concentrates with 2.5% of Different Palm Oils[a]

Palm oil	Carotenoid concentration (ppm)
RBD palm olein[b]	18,600 ± 60
RBD palm oil[b]	18,700 ± 50
NBD palm oil[c]	19,000 ± 50

[a]Mean value of three replicates ± SD (standard deviation).
[b]RBD—refined, bleached and deodorized (physical refining).
[c]NBD—neutralized, bleached and deodorized (chemical refining).

TABLE 4

Concentrations of Carotenoids from Two-Stage Distillation of Palm Oil Ethyl Esters with 1% RD Red Palm Oil[a]

Stage	Carotenoid concentration (ppm)
First	36,000 ± 3,250
Second	74,600 ± 6,000

[a]Mean value of three replicates ± SD (standard deviation). See Table 1 for abbreviation.

TABLE 5

Carotenoid Compositions (%) of Carotenoid Concentrates, RD Red Palm Oil and Crude Palm Oil

Carotenoid	Carotenoid concentrate[a]	RD red palm oil[b]	Crude palm oil[a]
Phytoene	1.5 ± 0.4	2.0 ± 0.3	1.3 ± 0.2
Phytofluene	0.3 ± 0.2	1.2 ± 0.4	0.1 ± 0.1
cis-β-Carotene	0.9 ± 0.3	0.8 ± 0.2	0.7 ± 0.2
β-Carotene	49.9 ± 2.9	47.4 ± 4.0	56.0 ± 2.5
α-Carotene	33.3 ± 4.5	37.0 ± 2.5	35.1 ± 2.7
cis-α-Carotene	5.5 ± 0.6	6.9 ± 1.2	2.5 ± 0.2
ζ-Carotene	1.7 ± 0.3	1.3 ± 0.4	0.7 ± 0.2
γ-Carotene	1.3 ± 0.3	0.5 ± 0.1	0.3 ± 0.2
δ-Carotene	0.6 ± 0.2	0.6 ± 0.1	0.8 ± 0.2
Neurosporene	0.1 ± 0.1	trace	0.3 ± 0.1
β-Zeacarotene	1.3 ± 0.3	0.5 ± 0.2	0.7 ± 0.2
α-Zeacarotene	0.4 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
Lycopene	3.4 ± 0.9	1.5 ± 0.3	1.3 ± 0.4
Total (ppm)	80,600 ± 2,500	550 ± 30	670 ± 80

[a]Mean value of four replicates ± SD (standard deviation).
[b]Mean value of three replicates ± SD. See Table 1 for abbreviation.

TABLE 6

Concentrations of Vitamin E and Sterols in Crude Palm Oil and Carotenoid Concentrate[a]

Component	Crude palm oil	Concentrate[b]
Vitamin E (ppm)	350 ± 20	3,840 ± 300
Sterols (ppm)	500 ± 30	18,200 ± 2,000

[a]Mean value of two replicates ± SD (standard deviation).
[b]Mean value of three replicates ± SD.

TABLE 7

Compositions of Sterols in Carotenoid Concentrate and Crude Palm Oil (ppm)[a]

Sterol	Concentrate	Crude palm oil[b]
Cholesterol	1690 ± 190	7–13
Campesterol	3217 ± 310	90–157
Stigmasterol	1877 ± 190	46–66
β-Sitosterol	11,440 ± 1,000	218–370
Total	18,224 ± 2,000	361–600

[a]Mean value of three replicates ± SD (standard deviation).
[b]See Reference 1.

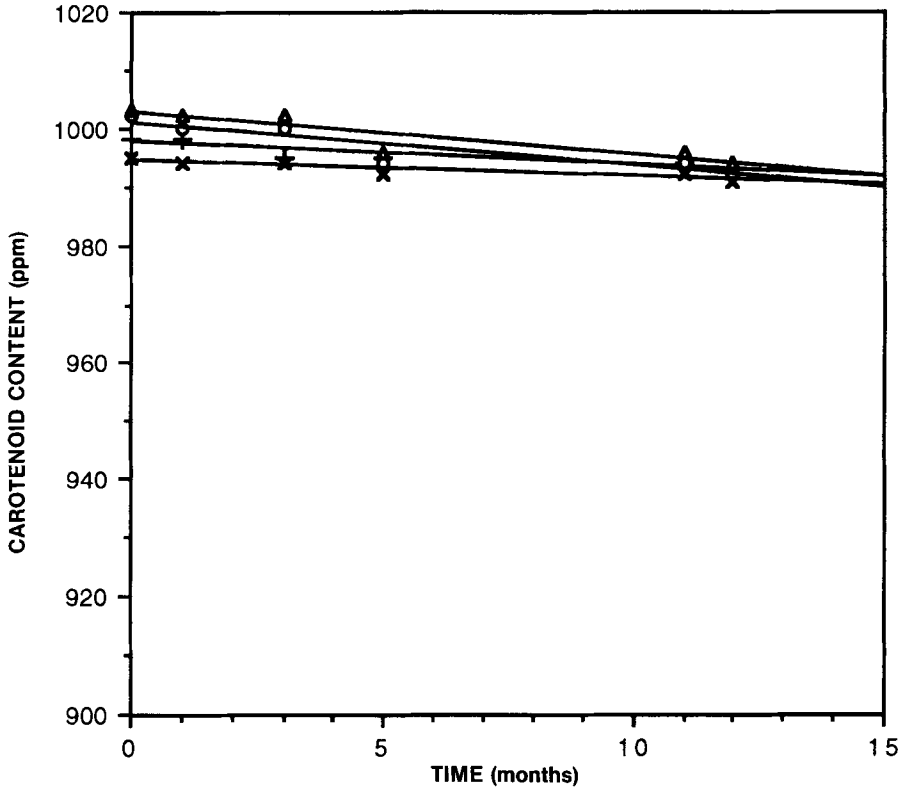

FIG. 1. Storage stabilities of carotenoids in capsule form: O—bottle (4°C); X—bottle (28–30°C); △—foil (4°C); +—foil (28–30°C).

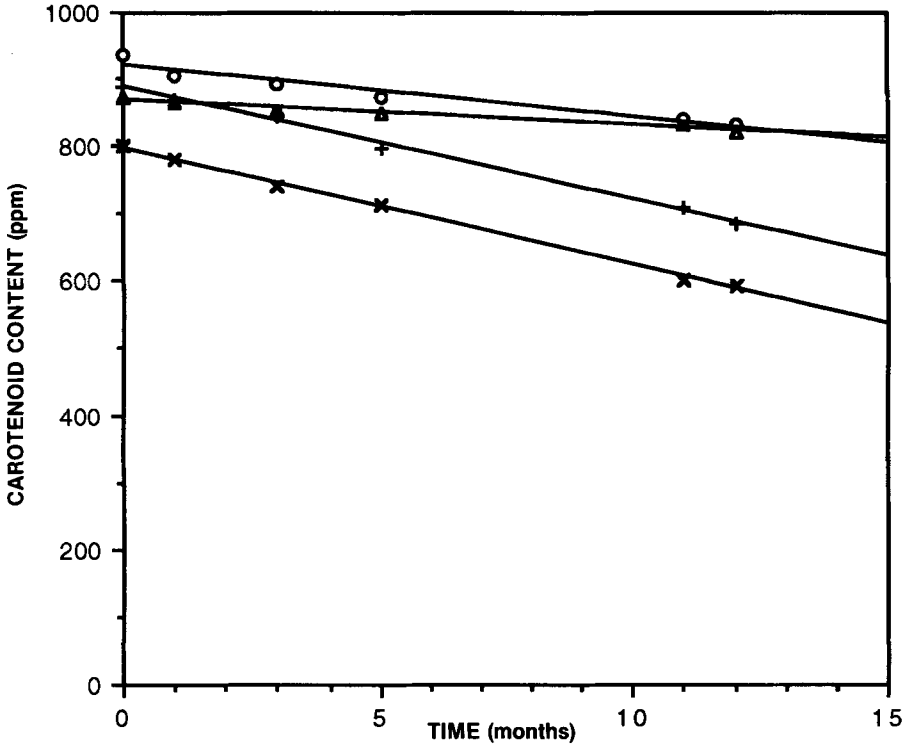

FIG. 2. Storage stabilities of carotenoids in powder form: △—sample kept at 4°C in amber bottle; +—sample kept at 28–30°C in amber bottle; O—sample kept at 4°C in clear bottle; X—sample kept at 28–30°C in clear bottle.

when stored at 4°C. However, at storage temperatures of 28–30°C, the carotenoid concentrations of the powder declined by 20–25%. The bigger decrease in the carotenoid concentration in the powder could be due to greater exposure to light, leading to increased oxidation and degradation of the carotenoids.

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