Palm Oil Carotenoids: Chemistry and Technology

Y M Choo*, S C Yap*, A S H Ong*, C K Ooi* and S H Goh†

2

ABSTRACT

nsiderable interest has been generated on the anti-cancer operties of carotenoids normally available from dietary, urces e.g. carrots. However, the richest and most abundant urce of carotenoids (in terms of retinol equivalents) comes m palm oil of the Elaeis guineensis *palm. Our studies on chemistry and technology of palm oil carotenoids show t the carotenoids can be extracted from palm oil and its ctions, palm pressed fibres, oil from oleifera palms and hybrids (*Elaeis guineensis × Elaeis oleifera*). Various thods of extraction have been explored but emphasis would given to extraction from esterified palm oil.*

An HPLC method has been applied to the analyses of otenes from the various sources mentioned above and the ults show differences and similarities in their compositions. orts have been made to present the carotene concentrates he form of capsules, powder and emulsion. Preliminary ults show that palm oil carotenoids in capsular and powder ms have good storage stability.

INTRODUCTION

rotenes, in particular β-carotene, are known for their vitamin A activities as they can be transformed into amin A *in vivo*. The vitamin A equivalents of α, β-and γ-otenes and β-zeacarotenes are 0.9, 1.67, 0.75 and 0.42 pectively (Isler, 1971 and Morton, 1970). β-Carotene, sides being a precursor of vitamin A, has been shown to be efficient quencher of singlet oxygen and as such is an ective antioxidant (Krinsky *et al.*, 1982; Santamaria *et al.*, 88; Wefers *et al.*, 1988 and Machlin *et al.*, 1987). Recent earch findings have indicated that three micro-nutrients nely β-carotene, vitamin E (both of these are present in m oil) and vitamin C have protective properties against e radical damage which are believed to be responsible for nerous degenerative diseases such as atherosclerosis, ritis, carcinogenesis, *etc*. In fact epidemiological studies he 1980s strongly associate β-carotene with the prevention certain types of cancers such as oral, pharyngeal, lung and mach cancer (Bianchi *et al.*, 1982; Tan *et al.*, 1986; ndram *et al.* 1989; Suda *et al.* 1986; Schwartz *et al.* 1986; ehelin *et al.*, 1986; Staehelin *et al.*, 1984.; Stich *et al.*, 84; Winn *et al.*, 1984; Shekelle *et al.*, 1981; Menkes *et al.*, 86; Matthews-Roth *et al.*, 1987; Nomura *et al.*, 1985; ey *et al.*, 1987; Alam *et al.*, 1984; Mathews-Roth *et al.*, 82; Peto *et al.*, 1981; Hirayama 1979; Mettlin 1984; Bollag 74; Ilixan 1987; Saffioti *et al.*, 1967; Chu *et al.* 1965; klav *et al.*, 1980 Swartz *et al.*, 1986). In this connection, National Institute of Health has identified β-carotene as e of the first top ten cancer preventive agents. What is re interesting is the recent report on α-carotene which has been shown to be ten fold more potent as an anti-cancer agent than β-carotene (Lion Corporation 1989). Both α- and β-carotenes constitute 90% of the total carotenes present in palm oil, the world's largest natural plant source of carotenes in terms of retinol (pro-vitamin A) equivalent (Tan, 1987). Carrots, green leafy vegetables and tomatoes which are considered to have significant quantities of pro-vitamin A activities contain about 15 to 300 times less retinol equivalents than palm oil. The growing importance of carotenes has prompted re-investigation of carotenes in palm oil in terms of their chemistry and physiological activities. This paper, however, will cover only the chemistry and technology of the palm-based carotenoids.

DISCUSSION

Carotenes, a class of C40 polyunsaturated hydrocarbons, impart orangy-red colour in palm oil. The concentration of the carotenoids has been analysed to be 500–700 ppm (Jacobsberg, 1974 and Goh *et al.*, 1985). The present study shows that other than the crude palm oil of the Tenera palm (*Elaeis guineensis* family), carotenoids can also be recovered from other oil palm species (Choo *et al.*, Submitted for publication), palm pressed fibres (Choo *et al.*, 1987) and esterified palm oil (Choo *et al.*, 1987 and Choo *et al.*,1988).

The present commercially planted oil palm in Malaysia is Tenera (T), a cross between Dura (D) and Pisifera (P), all belonging to the *Elaeis guineensis* family originating from West Africa. *Elaeis oleifera* or Melanococca (M), the South American species, exhibits numerous drawbacks to enable them to be planted in commercial planting but it produces oils with higher concentration of carotenes. Interest in oil palm breeding for oils of greater unsaturation, higher yields, shorter trees and disease resistant palms has led to the breeding of *Elaeis oleifera* × *Elaeis guineensis* hybrids (Task Force 1985). The present results show that the concentration of carotenoids in Dura, Pisifera, Melanococca, M×D and M×P hybrids are 948, 380,4347, 1846 and 1289 ppm respectively based on UV analysis. Melanococca oil has a higher carotenoid content while *Elaeis guineensis* oil has the lowest with the hybrids having carotenoid content of intermediate concentration. Carotenoids can also be recovered from pressed fibres (Choo *et al.*, submitted for publication), which are normally burnt as fuel in the oil palm mills. About 5%–6% residual oil can be recovered from pressed fibres with carotenoids ranging from 4000–6000 ppm. In addition, the preparation of palm oil alkyl esters for oleochemicals also present a unique opportunity for the recovery of the carotenoids.

The known methods of recovery of carotenoids directly from palm oil include extraction by saponification (Eckey,

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TABLE 1. COMPOSITION (%) OF CAROTENOIDS IN PALM OIL

	Elaeis guineensis (E.g.)		*Elaeis oleifera* (E.o.) or	E.g.× E.o.		Palm Pressed	Reported Known Carotenoids	
	Tenera	Pisifera (P)	Dura (D)	Melanococca (M)	M × P	M × D	Fibre	(d) A	(e), (f) B
Phytoene	1.27	1.68	2.49	1.12	1.83	2.45	11.87	1.2[a]	
Cis β-Carotene	0.68	0.10	0.15	0.48	0.38	0.55	0.49		
Phytofluene	0.06	0.90	1.24	Tr	Tr	0.15	0.40	0.04[a]	
β-Carotene	56.02	54.39	56.02	54.08	60.53	56.42	30.95		55
α-Carotene	35.06	33.11	24.35	40.38	32.78	36.40	19.45	89.3[b]	36
Cis α-Carotene	2.49	1.64	0.86	2.30	1.37	1.38	1.77		
ζ-Carotene	0.69	1.12	2.31	0.36	1.13	0.70	7.56		
γ-Carotene	0.33	0.48	1.16	0.08	0.23	0.26	2.70	1.8	3
δ-Carotene	0.83	0.27	2.00	0.09	0.24	0.22	6.94	1.9	
Neurosporene	0.29	0.63	0.77	0.04	0.23	0.08	3.38	0.3	
β-Zeacarotene	0.74	0.97	0.56	0.57	1.03	0.96	0.37		
α-Zeacarotene	0.23	0.21	0.30	0.43	0.35	0.40	Tr		
Lycopene	1.30	4.50	7.81	0.07	0.05	0.40	14.13	1.03	4
Xanthophylls[c]								4.3	2
Total Carotenes (ppm)	673	428	997	4292	1430	2324	5162		

[a] Found in crude palm olein.
[b] Mixture of α- and β-carotenes.
[c] Xanthophylls are not analysed.
[d] Tan *et al.*, 1986
[e] Malaysian Palm Oil Producers Association, 1975
[f] Meara *et al.*, 1976

1945; Eckey, 1949; Tabor *et al.*, 1948; Gebhart ,1951; Blaizot 1953) adsorption (Ong *et al.*, 1980; Unilever Ltd., 1953; Lange *et al.*, 1949; Mamuro *et al.*, 1986; Hama *et al.*, 1987), selective solvent extraction (Passino 1952 and Larner 1947), molecular distillation (Ooi *et al.*, 1986) and transesterification followed by distillation of the esters (Lion Fat and Oil Company 1976; Hama *et al.*, 1986; Hara *et al.*, 1988). In the present study, various methods of isolation have been developed to recover carotenoids from palm oil, palm pressed fibres and esterified palm oil. In this context, two simple and practical methods for the concentration and recovery of carotenoids via alkyl esters of palm oil route had been achieved (Choo *et al.*, 1987 and Choo *et al.*, 1988). The first method involves selective absorption of carotenoids from alkyl esters in an open column (Choo *et al.*, 1987). High recovery of carotenoids (> 90%) could be obtained with the column being reused over 30 times without any loss of activity. The second method involving distillation has been successful in producing a carotenoid concentrate of 40 000 ppm from both laboratory and pilot plant scale (Choo *et al.*, 1988). Currently the toxicology and cancer study of these carotenoid concentrate produced from the second method are being conducted.

In view of the complex composition of the palm oil carotenoids, analytical methods have to be developed. While total carotenoids in palm oil is determined by UV-visible spectrophotometer at 446 nm as ppm β-carotene, HPLC could be used to determine qualitatively and quantitatively the various components (Choo *et al.*, submitted for publication and Ng *et al.*, 1988). In the present study, a binary solvent system using acetonitrile and methylene chloride was used on a C18 reverse phase column with variable wavelength detector. 11 Types of carotenes have been identified using this technique. These include lycopenes, α-zeacarotene, β-zeacarotene, neurosporene, δ-carotene, γ-carotene, ζ-carotene, α-carotene, β-carotene, phytofluene and phytoene in terms of their elution order in reversed-phase system. The structures of these carotenes (trans) are shown in *Figure 1*. This method has been applied to the analysis of the unsaponifiable fraction of palm oil from Dura, Pisifera, Tenera Melanoccoca, the hybrids (M×D and M×P), and pressed fibres (Choo *et al.*, 1987). The results are tabulated as shown in *Table 1* and typical HPLC chromatogram of the carotenes are shown in *Figure 2*. From *Table 1*, it can be seen that the carotene profile of Dura, Pisifera, Melanococca and the hybrids (M × D, M × P) is similar to that of the Tenera palm. α-and β-Carotenes are the two major carotenes present, with the former present at 24%–40% and the latter at 54%–60%. The only difference is the amount of lycopene which appears to be higher in the *Elaeis guineensis* palms but lower in other palms. In the case of palm pressed fibres, the carotene profile is quite different from that of the Tenera palm. α- and β-Carotenes constitute only about 50% of the total carotenes present, others such as phytoene (11.87%) ζ-carotene (7.56%), δ-carotene (6.94%) and lycopene (14.13%) are comparatively much higher.

Preparation of carotenoid concentrate from palm oil in three different forms namely capsule, powder and emulsion has been effected successfully for pharmaceutical applications (Choo *et al.*, application for Patent). Preliminary storage tests of the capsules and powder at 28°C–30°C and 4°C for five months show that there is no noticeable change in the carotenoid content. Work in this area is still in progress.

CONCLUSION

Extraction of carotenoids from different oil palm sources has been successful. The composition of these extracts have been analysed quantitatively by HPLC. A practical way of making pharmaceutical applications from these extracts has also been found.

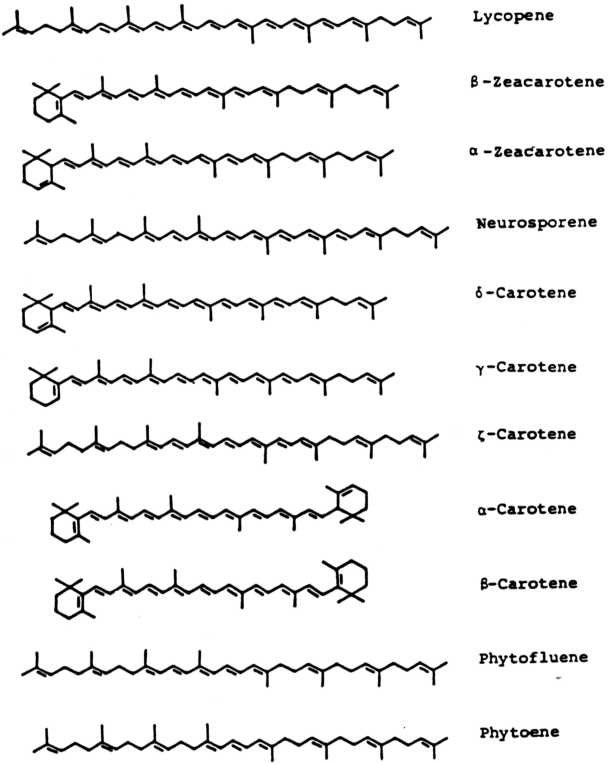

Figure 1. Carotenes in Palm Oil

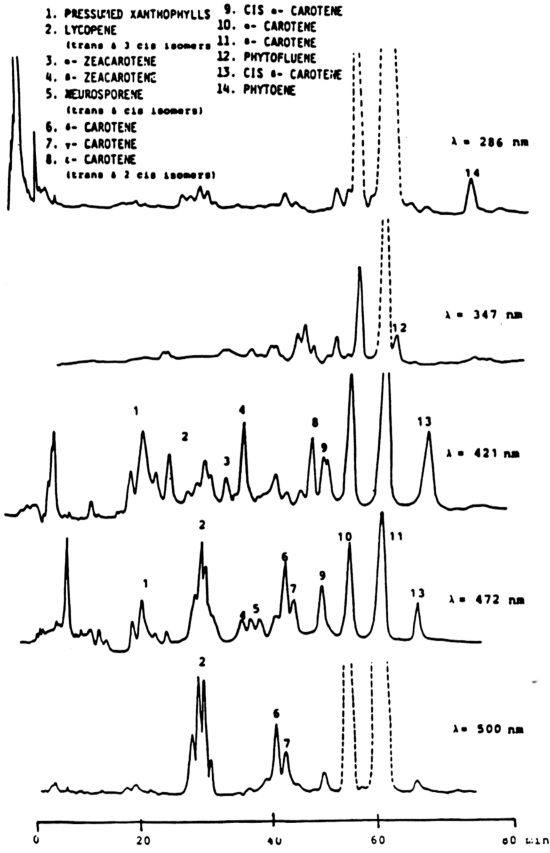

Figure 2. HPLC of Carotenes of Palm Oil

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xtraction of Carotenoids from Palm Oil

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has been well documented that crude palm oil tains the highest concentration of agro-derived otenoids with a total concentration in the range -700 ppm (1-3). Some studies have already been ried out to analyze the detail composition of the otene profiles in crude palm oils: it was found that nd β-carotenes in a ratio ranging from 2:3 to 1:2 stituted more than 80% of the total carotenoid tent (3,4).

arotenes, in particular β-carotene and to a lesser ent α-carotene, are known for their provitamin A vities as they can be transformed into vitamin A *vivo*. Because of this, crude palm oil can be sidered the world's largest natural plant source of otenes in terms of retinol (provitamin A) equival-e. Both vitamin A and carotenes have been gested to possess properties that reduce the risk ancer, and can effectively prevent or delay tumor elopment in animals treated with chemical car-gens (5,6). Epidemiological studies inthe 1980's e also strongly associated β-carotene with the ention of certain types of cancers such as oral, ryngeal, lung and stomach cancers (7-12). The β-otene has also been identified as one of the first ten cancer preventive agents by the U.S. National itutes of Health.

nce carotenoids are likely to grow in importance value, the recovery of carotenoids from palm oil palm oil by-products is important, and numer- extraction methods have been developed to ver the carotenoids from the crude palm oil: e include the saponification method (13,14), process (15), adsorption (16-19), selective sol- extraction (19,20), molecular distillation (21), transesterification followed by distillation of rs (22-25).

this paper, various methods of extraction and entration of carotenoids from palm oil and palm -products will be described; work on recovery of igh carotenoid oil from the hybrids of *Elaeis ra* × *Elaeis guineensis (E.o. × E.g.)* as well as parent species is also included. A nonaqeous se phase high performance liquid chromato-hy (NARP HPLC) technique was used to deter- qualitatively and quantitatively the detailed ene profiles of all the extracts. Palm carotenoid ntrates are now made available in capsule, der and emulsion forms, and preliminary results heir storage stability in capsules and powder s have been obtained.

om correspondence should be addressed.

Experimental Procedures

Extraction of Oil from E. oleifera, E. quineensis *(Dura & Pisifera) and hybrids.* Crude palm oils from various oil palm species were Soxhlet extracted with hexane (AR grade) from the dried mesocarp. The mesocarp was peeled from the oil palm fruits after sterilization at 1.032 bar steam pressure at 120°C for 15 min and drying at 60°C for 1 hr.

Extraction of Residual Oil from Pressed Fibers. Fresh pressed palm fruit fibers, collected from oil palm mills, were dried at 50-60°C for 1 hr and the residual oil was extracted with hexane using a Soxhlet apparatus. High pressure Soxhlet extraction was also used to extract the residual oil from fibers using liquid CO_2 as an extraction medium at a pressure of 700-750 psi for 2 hr.

Fractionation of Crude Palm Oil. Crude palm olein was obtained from the palm oil fractionation industry, where the crude palm oil was mixed with 10% of detergent (sodium lauryl sulphate) and cooled to about 20-21°C in batch crystallizers using external cooling. The stearin and olein, together with the detergent were then separated by high-speed centrifugation. The olein and stearin were washed with warm water and separated by centrifugation.

Double Pressing Oil Extraction. The double pressing process was carried out in the palm oil mill. In the conventional process, fruitlets from fresh fruit bunches having been sterilized, threshed and separated from the stalk, were conveyed into the digester, and then oil was extracted (pressed out) by a single pressing using screw presses. In the double pressing process, the oil from the fruitlets was first extracted at a low pressure (16 rpm 0 bar), the fiber was then separated from the nuts in the pressure cake and a second extraction was carried out at higher pressure (12 rmp 90 bars) on the fiber alone. The second-stage pressed oil was washed and dried separately from the first-stage pressed oil which had a normal cartenoid level.

Preparation of Methyl Esters through Transesterification. Crude palm oil was transesterified with methanol (AR grade) at a molar ratio of oil to methanol 2:1, catalyzed by 0.5% (w/w) excess of sodium hydroxide (AR grade) after the free fatty acid had been neutralized. The reaction mixture was stirred, heated to reflux and monitored by thin layer chromatography (TLC) (silica gel, solvent chloroform/hexane 1:1) until all the triglycerides were converted to methyl esters. The ester layer was then separated from the glycerol layer, and was washed with distilled water until the washings became neu-

l. The final ester product was obtained after the er was dried with Na_2SO_4, and the solvent removed der reduced pressure.

ncentration Via Removal of Methyl Esters. The rotenoids in the volatile methyl esters obtained m transesterification were recovered through two ferent methods: (i) Methyl esters were distilled der reduced pressure (under nitrogen), b.p. range 7–158°C at 0.23 torr. The carotenoid residue tained was stored at -20°C under nitrogen, or atile methyl esters were distilled under high vac- m using a Sibata falling film molecular apparatus a pressue of $<10 \times 10^{-3}$ torr with a temperature nging from 100–150°C; the carotenoid concentrate s collected as a residue. (ii) Methyl esters obtained re dissolved in methanol (1:2 v/v), and the mixture s introduced onto the glass column packed with reverse phase Silica gel. The colorless esters were ted first, and excess methanol was introduced til the carotenoids' band (red color) was about to eluted out; hexane and methanol (98:2 v/v) or oroform was then used to elute out this high otenoid fraction adsorbed in the reverse phase.

rotene Profiles of Various Palm Oil Extracts. tailed analyses of the carotene profile for various tracts were carried out using a Varian 5000 HPLC uipped with a variable wavelength UV-100 detec- . Isocratic separation was performed on a 5 µl rbax ODS column (4.6 mm ID × 25 cm) with a vent system of acetonitrile (89%) and dichlorome- ne (11%) at a flow rate of 1 ml/min. Carotenes re used as external standards for quantitative dies.

bility of Carotene in Various Forms. Carotenoid bility of the prepared oil capsule and powder ms has been determined for a period of one year at bient and freezer (-15°C) temperature.

esults and Discussion

brid palms, a cross between the African oil palm *eis guineensis* (*E.g*) and the South American oil m *Elaeis oleifera* (*E.o*), have been shown to vide several advantages including a more unsatu- ed oil, a lower height increment in trunk growth d resistance to certain diseases (26, 27). It was also orted that the carotenoid content of this hybrid s intermediate between the *E. oleifera* and *E. neensis* (27). In this study, the hybrid fruits tained from an oil palm plantation have provided mparable results, as shown in Table 1. The *E. ifera* species has a 7-fold greater carotenoid con- ntration than the present commercially planted ecies, Tenera, followed by the hybrids of *E.o* × *E.g* ura) and *E.o.* × *E.g.* (Pisifera). These hybrid oils are refore good natural sources of carotenoids, and provide dietary vitamin A and carotenoids if the is consumed as it is or as red palm oil after a mild ining process (28), i.e., refined and deodorized der low pressure and low temperature without the struction of carotenoids.

A carotenoid-rich oil can also be obtained from the m pressed fiber (29) — a palm oil by-product which is presently being burned as a fuel in palm oil mills. The pressed fiber was found to contain about 5–6% (w/w) of residual oil with a carotenoid concentration of 4000–6000 ppm (Table 2). It was also found that the carotenoid content of the residual oil in the pressed fiber of hybrid oil palm fruits was even higher, at 6000–7000 ppm. The carotenoid content was not much different from two different extraction techniques used, i.e. organic solvent extraction and liquid CO_2 extraction methods, except that the oil extracted by liquid CO_2 was cleaner: extraction of less polar impurities could be due to the lower temperature and low polarity of liquid CO_2. In fact, it was found that the total phosphorus content was much lower (<10 ppm) as compared with the organic solvent extraction (>600 ppm).

Recently, a new system of palm oil extraction based on a double pressing technique has been implemented by several mills in Malaysia. The expected advantages of double pressing over the conventional single-stage pressing are: lower oil loss in fiber, higher kernel extraction rate, less wear on screw worm and cages, reduction of contamination of kernel oil in crude palm oil and, more interestingly, a higher concentration of carotenoids in the extracted oil from second pressing. This could be due to the fact that the first extraction (first pressing) in a double-pressing process was carried out at lower pressure (to avoid cracking of the nuts), and relatively higher oil was extracted as compared to carotenoids. After removal of nuts, the fiber is then subjected to higher

TABLE 1

Total Carotenoids from Various Oil Palm Species

Oil palm species	Total carotenoids[a] (ppm)
E.o	4347
E.o × *E.g* (D)	1846
E.o × *E.g* (P)	1289
E.o × *E.g* (D) × *E.g* (P)	864
E.g (P)	380
E.g (D)	948
E.g (T)	610

[a]Total carotenoids estimated at 446 nm; D = Dura; P = Psisifera; T = Tenera; *E.o.*, *Elaesis oleifera; E.g.*, *Elaesis guineensis.*

TABLE 2

Carotenoid Contents of Various Palm Oil Extracts

Palm oil extract	Carotenoid content[a] (ppm)
Crude palm oil	630 - 700
Crude palm olein	680 - 760
Crude palm stearin	380 - 540
Second pressed oil	1800 - 2400
Residual oil from fiber	4000 - 7000

[a]Total carotenoids estimated at 446 nm.

essure. More carotenoids were extracted out of the esocarp together with some residual oil from first essing. On analysis, the second-pressed oil shows a gher concentration of carotenoids as shown in ble 2 and the detailed carotene profile is shown in ble 3.

Carotenoids are also being concentrated in an dustrial process called fractionation. Fractionation carried out to extend the uses of palm oil; the oducts obtained are the liquid oil (olein 70–80% w) and the solid fat (stearin 20–30% w/w). The ain processes used in the palm oil industries are mple winterization, detergent fractionation process d solvent fractionation; the advantages and disad-ntages of these processes have been reported (30,). However, regardless of which process was used, was observed that the carotenoid content in the lm olein (lower-melting) fraction is being enriched m 10 to 20% (w/w) as shown in Table 2; the opherol and tocotrienols are also found to be 20% her than crude palm oil.

Further to the work on carotenoid-rich palm oils scribed above, some studies are also being carried t to recover and concentrate carotenoids from ious palm oil sources to obtain an oil with high ncentrations of palm-based carotenoids for phar-ceutical uses. Most of the reported methods of overy of carotenoids directly from palm oil are too fcult, inefficient or too costly. Recently, prepara-n of volatile palm oil methyl esters on a large scale oleochemical or diesel substitute has been carried t (32–34). This mild reaction processes palm oil to atile methyl esters leaving the valuable minor components unchanged (33). This allows for a unique opportunity for the recovery of carotenoids in palm oil. The first method involves selective adsorption of the carotenoids by reverse phase adsorption; esters with higher polarity were first eluted out from the column and the carotenoids were recovered after this. The carotenoid concentration recovered ranged from 8000 to 9000 ppm (Table 4). A recovery of greater than 90% (w/w) can be obtained through this method, and the column can be regenerated and reused for more than 30 times without any loss of activity. The carotenoid concentrate obtained through the carbon adsorption of the crude palm methyl ester is also shown in Table 3; the recovery as well as the carotenoids concentration was low as compared to the C_{18} reverse phase method. Data on the carotenoid content through the activated carbon adsorption as well as the molecular distillation of crude palm oil are also included in Table 4.

The second method involved the distillation of the volatile alkyl ester using normal vacuum distillation (33) or molecular distillation techniques (35). Residual concentrates of >2.0% (w/w) carotenoids (Table 4) content can be achieved by normal vacuum distillation with a recovery of about 46% (w/w); this residual carotenoid can be further concentrated to 8.4% (w/w) by normal phase column chromatography and at the same time other separated minor components can also be concentrated; total tocopherol and tocotrienol content was increased to 37% (w/w), and the sterols were concentrated to 32% (w/w) with a recovery of >65% (w/w) and >82% (w/w) respectively based on the crude methyl ester. An oil

BLE 3

nposition (%) of Carotenes in Palm Oil

	Elaeis guineensis (E.g)		*Elaeis oleifera* or	Hybrids (*E.g.* × *E.o*)		Palm	Second-
	Tenera	Pisifera (P)	Dura (D)	Melanococca (*E.o.*)	*E.o.* × *E.g* (P)	*E.o.* × *E.g* (D)	pressed fiber oil	press oil
toene	1.27	1.68	2.49	1.12	1.83	2.45	11.87	6.50
tofluene	0.06	0.90	1.24	tr*	tr	0.15	0.40	1.63
-β-carotene	0.68	0.10	0.15	0.48	0.38	0.55	0.49	0.28
arotene	56.02	54.39	56.02	54.08	60.53	56.42	30.95	31.10
arotene	35.06	33.11	24.35	40.38	32.78	36.40	19.45	20.68
-α-carotene	2.49	1.64	0.86	2.30	1.37	1.38	1.77	1.70
rotene[a]	0.69	1.12	2.31	0.36	1.13	0.70	7.56	4.62
rotene	0.83	0.27	2.00	0.09	0.24	0.22	6.94	2.13
arotene	0.33	0.48	1.16	0.08	0.23	0.26	2.70	2.48
rosporene[b]	0.29	0.63	0.77	0.04	0.23	0.08	3.38	1.88
acarotene	0.74	0.97	0.56	0.57	1.03	0.96	0.37	0.58
acarotene	0.23	0.21	0.30	0.43	0.35	0.40	tr	0.15
opene[c]	1.30	4.50	7.81	0.07	0.05	0.04	14.13	26.45
l (ppm)	673	428	997	4592	1430	2324	5162	2510

ether with 2 *cis* isomers.
ether with a *cis* isomer.
ether with 3 *cis* isomers.

ABLE 4
rotenoid Concentrates from Various Methods

ethod	Carotenoids content[a] (% of recovery)	(ppm)
cuum distillation[b]	>20800	(<46%)
olecular distillation[b]	>40000	(>80%)
isorption[b]: (a) C_{18}	8000-9000	(>90%0
(b) Carbon	5000-7000	(<50%)
olecular distillation of rude palm oil[21]	1290-1990	
isorption from crude alm oil (activated arbon)[24]	3700-5600	(<80%)

otal carotenoids estimated at 446 nm.
hrough methyl ester route total carotenoids are estimated . 446 nm).

th a final carotenoid concentration of >40,000 pm has been achieved through molecular distilla- n. The process was carried out at lower pressure 10×10^{-3} torr) and lower temperature (<170°C), d the ester residence time on the heater is very ort as compared to vacuum distillation. This was tained with recovery at greater than 80% (w/w), d this process has been scaled up to pilot scale ing "Centrifugal Vacuum Thin Film Evaporator CE-0B."

Generally, the total carotenoids in palm oil are termined by UV-visible spectroscopy at 446 nm as pm of β-carotene; however, due to the complex mposition of palm oil carotenoids, a NARP HPLC as used to study the carotene profile both qualita- ely and quantitatively; identification was based on injection with standard samples, order of elution d the UV-visible spectra of the collected individual rotenes from HPLC. Eleven types of carotenes were entified from various palm oil sources. From Table it can be seen that the carotene profile of the *E. ineensis* species (D, P&T), *E. oleifera* and their brids has a comparable carotene profile where α- d β-carotenes are the major components. The oil pm the pressed fibers and the oil obtained from cond pressing are similar and contain a lower level α- and β-carotenes compared to other palm oil urces. However, it has relatively higher concentra- on of phytoene, δ-carotene and lycopene.

Presentation of carotenoid concentrate in three fferent forms for pharmaceuticals has been carried t; this includes capsules (both soft and hard), wder and emulsion. The powder formulation has en successfully formulated and it could be made to carotene tables, or encapsulated in hard cap- les (36). Preliminary results on the storage stability sts show that the carotenoids in powder form ring storage at room temperature were not as able as carotenoid concentrate in the capsules. This uld be due to greater exposure to light and air in e powder form, leading to increased oxidation or gradation of carotenoids. However, only a slight cline (<4%) in carotenoid content was observed for the powder if it was kept in the freezer (-15°C) for a period of one year.

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IN THE OIL FROM DIFFERENT PALM SPECIES

ywords: *Elaeis guineensis; Elaeis oleifera;* Hybrids; Carotene; Lycopene; Phytoene; Phytofluene; Neurosporene; Zeacarotene; Non-Aqueous Reverse-Phase Liquid Chromatography.

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arotenes in palm oils from Elaeis guineensis – dura *(D),* pisifera *(P) and* tenera *(T),* Elaeis oleifera *or melanococca (M), from the hybrids M × D and M × P and from the backcross MP × D were analysed using HPLC and UV-Visible spectrophotometry. Eleven types of carotenes were identified, the major ones being* α- *and* β-*carotenes, which constituted about 90% of the total carotenes. Oil from* E. oleifera *(originally from South America) had the highest carotene content (4000 p.p.m.), while that from* Elaeis guineensis *(from West Africa) had the lowest (380 p.p.m.); their hybrids and the backcross had intermediate carotene contents.*

INTRODUCTION

he major oil palm planted in Malaysia is the *tenera* (T) variety (obtained from the cross between the *dura* and *pisifera* varieties, D×P) of *Elaeis guineensis*, which originated from West Africa. Crude palm oil from the fruits of *tenera* palms has a carotenoid content of about 500 – 700 p.p.m. (Jacobsberg, 1974; Goh *et al.*, 1985). However, the carotenoid content of oils from other oil palm species such as *Elaeis oleifera* [*melanococca* (M)] has been reported to be about 4000 p.p.m. (Tam *et al.*, 1976). The major carotenes present in Malaysian palm oil are α- and β-carotenes which constitute about 90% of the total carotenoids (Goh *et al.*, 1985; Tan *et al.*, 1986).

Some carotenes, particularly β-carotene, have pro-vitamin A activity and recent studies have shown that certain of them, such as α- and β-carotenes and lycopene, also possess protective properties against various types of cancer (Peto *et al.*, 1981; Mettlin, 1984; Suda *et al.*, 1986; Mathews-Roth *et al.*, 1987; Murakoshi *et al.*, 1989; Norman *et al.*, 1988; Sundram *et al.*, 1989; Ziegler, 1989).

In recent years, plant breeders have conducted various studies aimed at producing palms with different characteristics, *e.g.* palms

with more highly unsaturated oil, higher-yielding palms, shorter palms and disease-resistant palms. In the course of such breeding trials, it was found that the carotene content of palm oil from various hybrid palms also varies (Hartley, 1977).

The carotenoids present in palm oil from *E. guineensis* have been identified and reported (Tan *et al.*, 1986; Ng and Tan, 1988; Jose *et al.*, 1990). However, little is known about the detailed carotene profiles of oil from *E. oleifera* and various hybrid palms.

This paper reports a detailed analysis by Non-Aqueous Reverse-Phase High Performance Liquid Chromatography (NARP-HPLC) of the carotene profiles of extracts of the oil from *E. oleifera* (M), *E. guineensis* [*dura* (D) and *oisifera* (P)] and their hybrids (M × D, M × P) and the backcross (MD × P).

EXPERIMENTAL

Materials

Oil palm fruits from *E. guineensis* (*dura* (D), *oisifera* (P) and *tenera* (T)), from *E. oleifera* (M) and from their hybrids M × D, M × P, as well as from the backcross MD × P were collected from Johore Labis Estate, Johor, Malaysia from November 1988 to February 1989. Lycopene and α- and β- carotenes, used as authentic standards in the study, were from Sigma. HPLC grade acetonitrile was from Koch-Light; methylene chloride of analytical grade (AR) from Merck was redistilled before used. Petroleum ether (b.p. 40°C – 60°C) and ethanol used during saponification were of AR grade from Merck.

Procedure

Fresh oil palm bunches were cut into small bikelets and autoclaved at 1.032 bars steam pressure (120°C) for 15 minutes. The mesocarp was then separated from the nuts and dried. The oil was extracted from the dried mesocarp with hexane in a Soxhlet apparatus for 5 hours: it was shown that the total carotene content was not affected significantly during the extraction because of the presence of natural antioxidants (*i.e.* tocopherols and tocotrienols) in the palm oil.

About 5 g of each oil extract was then saponified with 5 ml of 50% ethanolic KOH heated at 50°C in the dark on a water bath under a stream of nitrogen for 45 minutes. The saponified sample was then cooled to room temperature and extracted with 50 ml portions of petroleum ether until the supernatant became colourless. The combined petroleum ether extracts were washed four times with 5 ml portions of distilled water and dried over sodium sulfate. A portion of the extract was brought to dryness in a rotary evaporator at 30°C. The residue was dissolved in a suitable volume of mobile phase, 100 µl of which were injected into the HPLC.

The isocratic separation was performed on a ZORBAX ODS, column (4.6 mm ID × 25 cm stainless steel, 5µm spherical particles protected with a Du Pont guard column (20 microns ZORBAX ODS). A solvent system of acetonitrile (89%) and methylene chloride (11%) was used and the flow rate was 1 ml per minute.

Analysis and detection of carotenes were carried out using a Varian 5000 HPLC instrument equipped with a variable wavelength (190 – 900 nm) UV-100 detector and an SP 4270 integrator. Detection was recorded at different wavelength maxima and attenuated for the display of the various types of carotenes present.

A non-aqueous solvent system with 11% of dichloromethane in acetonitrile was chosen for reverse-phase liquid chromatography to provide separation of the carotenoids as well as to allow for sample solubility. It has been reported that non-aqueous reverse-phase liquid chromatography can enhance chromatographic efficiency, recovery, and sample capacity, as well as column lifetime (Nelis and De Leenheer, 1983).

Individual separated carotenes were collected and the absorbance spectra were recorded using a Hitachi 150-20 spectrophotometer. The total carotene content was determined spectrophotometrically at 446 nm as described by Bockennoogen (1974).

Photoisomerization was carried out by exposing the solutions of the carotenes collected from HPLC (redissolved in hexane – 0.1-1 mg/ml – after the removal of the HPLC mobile phase) to diffuse daylight under nitrogen for 1 hour in the presence of iodine (2% of the weight of carotene) (Davies, 1976). The iodine was

moved by washing the mixture with 2% queous $Na_2S_2O_3$ followed by distilled water. he solution was then dried over anhydrous a_2SO_4, the solvent was evaporated, and the omerization products were redissolved in PLC mobile phase and reanalysed.

fatty acid composition of the hybrids and their parent species (*Table 1*). As shown by the data in *Table 2*, each hybrid and the backcross in the study had total carotene contents intermediate between those found in the parent species. Because of differences in the colour intensity of the skin (exocarp) of fruits from different oil

TABLE 1. FATTY ACID COMPOSITION OF OIL FROM DIFFERENT PALM SPECIES*

	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
	–	0.2	18.5	1.7	1.0	55.9	21.2	1.1	tr
P	–	0.4	32.4	0.3	3.1	52.4	10.3	0.4	0.4
D	–	0.4	35.6	0.1	4.4	44.8	13.5	0.6	0.4
D × P	–	1.5	42.9	0.2	3.8	34.2	16.8	0.5	tr
	–	1.1	42.1	–	4.9	39.6	11.8	0.3	–
	–	2.1	54.0	–	2.7	29.7	10.9	0.3	0.1
	0.3	1.2	44.3	–	4.3	39.3	10.0	0.4	0.3

M = *Elaeis oleifera* (or melanococca); P = *Elaeis guineensis* (*pisifera*); D = *E. guineensis* (*dura*); = *E. guineensis* (*tenera*).

he fatty acid compositions were determined according to ISO 5508: Animal and Vegetable Fat and il Analysis by Gas-Liquid Chromatography of methyl esters of fatty acids.

Siew and Tan (1988).

TABLE 2. TOTAL CAROTENOID CONTENT OF OILS FROM VARIOUS PALM SPECIES

Oil Palm	Total Carotenoids[a] (p.p.m.)
Elaeis oleifera (M)	4347
Elaeis oleifera × *dura* (MD)	1846
Elaeis oleifera × *pisifera* (MP)	1289
MD × *pisifera*	864
Pisifera	380
Dura	948

Total Carotenoids estimated at 446 nm.

RESULTS AND DISCUSSION

rossing *E. oleifera* and *E. guineensis* has been shown to yield hybrid palms which etain the characteristics of the *E. oleifera* alm in terms of the height increment, fruit nape and fruit colour (Hartley, 1977). owever, the fatty acid composition of the oil is ntermediate between those found in the two arent species. Similar results were also bserved in the present study as regards the palm species, and because of the differences in pro-vitamin A activity and anti-cancer properties of various carotenes, it was of interest to obtain the carotene profiles of *E. oleifera*, the hybrids and the backcross by using the NARP-HPLC method.

A typical experimental chromatogram from this study depicting the separation of a complex mixture of carotenes from palm oil is shown in *Figure 1*. The two major components, α- and β-carotenes, and the other nine minor

arotenes present in palm oil were all well solved from one another. In the present study ne nine *cis*-isomers identified included three s lycopenes, two *cis*-ζ-carotenes and one *cis*-omer each for phytofluene, and γ-, α- and carotenes.

The major characteristic of carotenes is the resence of highly conjugated polyene chains, hich normally results in absorption of light in ne visible region. This is advantageous for arotenoid detection, and the interference from on-carotene compounds can be eliminated hen the appropriate wavelengths are selected. his is particularly important since apart from arotenes the non-saponifiable fraction of palm l contains other minor constituents which sorb in the UV region.

Identification of carotenes was carried out by -chromatography with the few available uthentic carotenes purchased from Sigma; in any instances identification of the peaks was ased mainly on their characteristic UV-VIS sorption spectra obtained from the pure arotenes collected from the HPLC. The umber of conjugated double bonds as well as fferences in end groups determines the ature of the UV-VIS spectra and absorption axima (λ max) of carotenes. The spectral maxima (normally three) for the carotenes identified in this study (together with previously published data) are shown in *Table 3*, the compounds being arranged in their order of elution (*cf. Figure 1*).

Figures 2, *3* and *4* show the UV-VIS spectra of the carotenes found in this study. *Figure 2* shows, as expected, that as the number of conjugated double bonds in the acyclic carotenes increases, the absorption maxima also shift to longer wavelengths. The effects of the ring closure of the ψ-end group to form ε- and β-end groups, already described elsewhere (Davies, 1976), are also clearly shown in *Figures 3* and *4*: the displacement of the absorption maxima to shorter wavelengths with a concomitant loss of persistence of spectra is clear in the β-carotene spectrum in *Figure 3*, whereas for α-carotene there is no loss in persistence, merely a shift to a lower wavelength, because there is one conjugated double bond less and a cyclic ε-end group has been formed. These characteristics allow for the detection of some partially resolved carotene components by selecting the UV-VIS wavelength of the detector as described below. Structural differences such as conjugation of double bonds and end groups cause differences

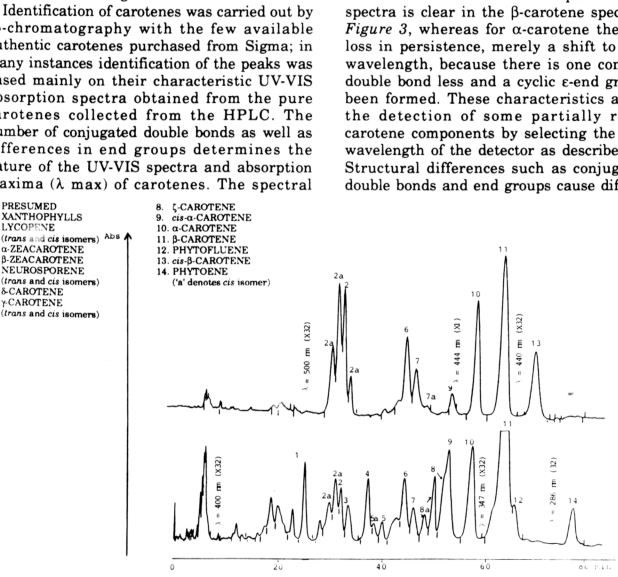

Figure 1. HPLC of carotenoids of palm oil

TABLE 3. MAIN ABSORPTION MAXIMA (nm) OF CAROTENES IN HEXANE

			This Study			Davies, 1976		
	cis peak							
. Xanthophylls		not determined			–		
. Lycopene	*cis*	362	438	464	495			
	cis	362	442	477	497			
	trans		444	470	500	448	473	504
	cis	362	438	464	495			
. α-Zeacarotene			398	420	448	398	421	449
. β-Zeacarotene			404	426	452	407	427	454
. Neurosporene	*cis*	330	414	436	467			
	trans		416	438	468	416	440	470
. δ-Carotene			431	456	484	428	458	490
. γ-Carotene	*trans*		435	462	490	437	462	492
	cis	348	434	459	487			
. ζ-Carotene	*cis*	295	376	397	423			
	cis	295	378	399	424			
	trans		380	401	426	380	400	425
. *cis*-α-Carotene		330	415	438	470			
. α-Carotene			420	440	471	420	442	472
. β-Carotene			426	449	477	425	450	477
. Phytofluene			331	347	366	331	347	366
. *cis*-β-Carotene		334	420	444	472			
. Phytoene			276	286	297	276	286	297

polarity or absorption among the carotenes d lead to the characteristic elution profile served for them. The appearance of an absorp- n maximum (the '*cis* peak') in the UV region the spectrum of most of the *cis* isomers, and romatographic analysis of the iodine-isomerized oducts of selected carotenes separated by PLC, assisted in the identification of some otenes, particularly those with *cis*-isomers. As reported earlier (Ng and Tan, 1988), the st 'polar' carotene, lycopene, was the first e to be eluted from reverse phase column. ygenated carotenes (xanthophylls) were ted much faster and were well separated m the hydrocarbon carotenes. The highly njugated lycopene, which was not found in lm oil samples by Ng and Tan (1988), was ected in the present study in small amounts palm oil from the commercial *tenera* variety. e lycopene content was also found to be comparatively higher in the *dura* and *pisifera* varieties of *E. guineensis*. Besides *trans*-lycopene three *cis*-lycopenes were also detected: two were eluted before and one after the *trans* lycopene peak; all three *cis*-isomers show the '*cis* peak' at 362 nm; their spectra have lower absorption maxima than that of *trans*-lycopene; their identities were confirmed by iodine-catalyzed photoisomerization.

The least polar carotene, phytoene, (peak 14) with seven conjugated double bonds, was the last to elute and it was well separated from the preceeding peak 13 (*cis*-β-carotene) as shown in *Figure 1*. The spectral maxima of phytoene observed in this study were identical with the published data (Davies, 1976). Identification of *cis*-β-carotene (peak 13) and *cis*-α-carotene (peak 9), which eluted after *trans* β-carotene (peak 11) and before *trans*-α-carotene (peak 10) respectively, was based on their characteristic

TABLE 4. EXTINCTION COEFFICIENTS OF VARIOUS CAROTENES AT THE CHOSEN WAVELENGTHS (Davies, 1976)

	Absorption Maxima (nm)	Extinction Coefficient (Ex)
opene	472	3450
eacarotene	421	2450
eacarotene	427	2520
rosporene	440	2918
arotene	456	3290
arotene	462	3100
arotene	400	2555
arotene	444	2800
arotene	453	2592
tofluene	347	1577
toene	286	915

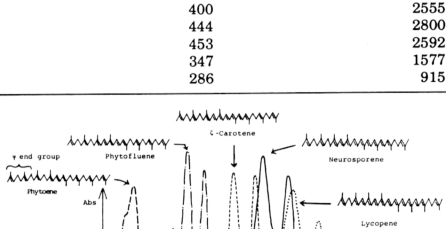

Figure 2. UV-Visible spectra of acyclic carotenes

peaks at 338 nm and 332 nm respectively, d also on the hypsochromic shift of the ectral bands of the *cis*-isomers. Further ifirmation was based on the rechromato- phy of the iodine-isomerized products from respective pure *cis* carotenes collected by LC. Based on the reported UV-VIS spectrum d the elution order (Bushway, 1986), the *cis*-arotene (peak 9) found in this study was st probably 9-*cis*-β-carotene.

The two major carotenes in palm oil, α- and arotenes (peaks 10 and 11 respectively), re identified by co-chromatography with ndards as well as by spectral comparison. Phytofluene (peak 12), which was only observed when the chromatogram was run at λ max 347 nm, was not well resolved because of the comparatively large peak of β-carotene; phytofluene gives a characteristic greenish fluorescence on thin layer chromatograms exposed to long-wavelength (360 nm) UV radiation.

Trans-ζ-carotene (peak 8) was eluted just before (but not well separated from) *cis*-α-carotene. However, when the UV-VIS detector was set at 375 nm, a better resolution from *cis*-α-carotene, which has a low absorptivity at this wavelength, could be obtained. Two *cis*-ζ-

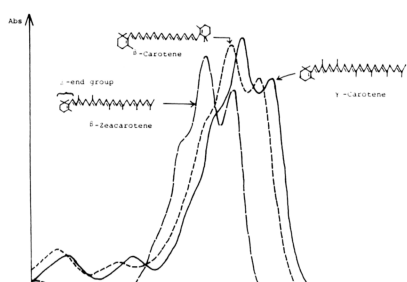

Figure 3. UV-Visible spectra of carotenes with β-cyclic end groups.

TABLE 5. CAROTENE PROFILES OF PALM OIL EXTRACTED FROM *Elaeis guineensis*, *Elaeis oleifera* AND THEIR HYBRIDS

	Carotene Composition (%)						
	M[a]	P	D	MP	MD	MD × P	T
oene	1.12	1.68	2.49	1.83	2.45	1.30	1.27
-Carotene	0.48	0.10	0.15	0.38	0.55	0.42	0.68
ofluene	Tr[b]	0.90	1.24	Tr	0.15	Tr	0.06
rotene	54.08	54.39	56.02	60.53	56.42	51.64	56.02
rotene	40.38	33.11	54.35	32.70	36.40	36.50	35.16
-Carotene	2.30	1.64	0.86	1.37	1.38	2.29	2.49
rotene	0.36	1.12	2.31	1.13	0.70	0.36	0.69
rotene	0.09	0.48	1.10	0.23	0.26	0.19	0.33
rotene	0.09	0.27	2.00	0.24	0.22	0.14	0.83
rosporene	0.04	0.63	0.77	0.23	0.08	0.08	0.29
acarotene	0.57	0.97	0.56	1.03	0.96	1.53	0.74
acarotene	0.43	0.21	0.30	0.35	0.40	0.52	0.23
pene	0.07	4.50	7.81	0.05	0.04	0.02	1.30
Carotene .p.m.)	4592	428	997	1430	2324	896	673

Elaeis oleifera (*Melanococca*), P = *E. guineensis* (*pisifera*); D = *E. guineensis* (*dura*).
E. guineensis (*tenera*) = D × P

trace.

tenes (8a) which showed a shift of the ral bands to shorter wavelengths and the peak at 296 nm (*cis* peak) not present in the *trans*-isomer, were eluted before *trans*-ζ-carotene. However the positions of the *cis* double bonds were not determined.

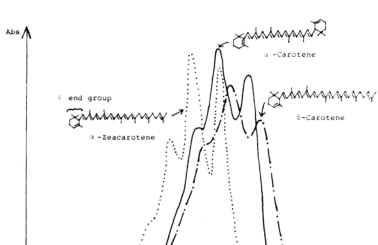

Figure 4. UV-Visible spectra of carotenes with ε-cyclic end group.

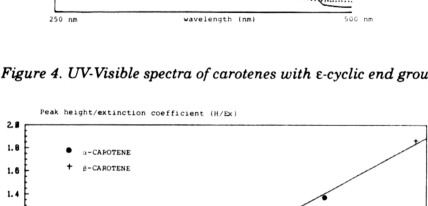

Figure 5. Standard curves for α- and β-carotenes: Peak height/extinction coefficient (H/Ex) versus concentration (µg/mL).

Peaks 5, 6 and 7 were identified as rosporene, δ- and γ-carotenes respectively, the basis of the UV-VIS spectral data shown *Table 3.* The elution order was in accord with t published by Ng and Tan (1988). Peaks 5a 7a were tentatively assigned as the *cis*-mers of neurosporene and γ-carotene pectively. These two carotenes show lower orption maxima than the corresponding s carotenes and the *cis* peaks for the two isomers were observed in the near UV on as has been reported (Zechmeister, 1962; Davies, 1976).

α-Zeacarotene (peak 3) and β-zeacarotene (peak 4), which were not reported by Ng and Tan in the HPLC chromatogram of their palm oil samples, were eluted after lycopene. The elution order of α- and β-zeacarotenes was in agreement with the theory that the end group of α-zeacarotene is more polar than the end group of β-zeacarotene. Hence, α-zeacarotene elutes before β-zeacarotene.

From the well-resolved chromatograms obtained in this study, a detailed quantitative

nalysis of the carotenes was possible by easuring the heights of the individual rotene peaks in the chromatograms. ndividual peaks were recorded at different V-VIS wavelengths: the wavelength chosen ormally the absorption maximum – λ max – each carotene) and the published extinction efficients (Ex) used in this study are shown *Table 4*. It has been reported that *cis*-rotenoids exhibit lower extinction coefficients han the corresponding *trans* isomers Davies, 1976). However, because of the ncertainty of the position of the *cis* double nds in the present study and the limited data the extinction coefficients for the different pes of *cis*-carotenes, extinction coefficients for e *trans* isomers were assumed in the antification of *cis* carotene isomers; it is pected that the actual values will be slightly wer: for example, if the extinction coefficient r *trans*-β-carotene is used for the antification of 9-*cis*-β-carotene, about 5% less the 9-*cis*-β-carotene will be reported weeney and Marsh, 1970).

For calibration, α- and β-carotenes were used external standards in the present study. The ak heights (H) of these carotenes recorded at e chosen wavelengths showed a linear rrelation with their concentrations. By otting the H/Ex of both α- and β-carotenes ainst their respective concentrations, a linear lationship was obtained over the range of ncentrations of HPLC analysis (*Figure 5*).

It was noted in the present study that the tinction (Ex) for α- and β-carotenes in troleum ether could be applied to the mobile ase solvent used (11% methylene chloride in etonitrile), and thus it was assumed that the chosen for other carotenes (in petroleum ner or hexane) could also be applied to the antification of the peak height recorded in e present chromatograms.

Table 5 shows the detailed carotene profiles oils from the various palm species studied. e major constituents found in all these oils re β-carotene and α-carotene, ranging from % to 60% and 24% to 40% of the total otenes, respectively.

No significant variation in the nature of the rotenes was found between *E. oleifera*, *guineensis*, their hybrids and the backcross. wever, in the case of *E. oleifera*, carotenes other than α and β were found in relatively smaller amounts than in the extracts from varieties of *E. guineensis* (*i.e. dura*, *pisifera* or *tenera*). The most significant difference between *E. oleifera* and *E. guineensis* is the amount of lycopene; the oil from *E. guineensis* contains a relatively high level of lycopene, whereas only trace amounts were found in the oil from *E. oleifera* and in that from the hybrids between *E. oleifera* and *E. guineensis*. This may be the cause of differences in the colour of the fruits of the different species. Lycopene imparts a dark red colour to palm oil, and the fruits of *E. guineensis* are dark red when ripe, whereas *E. oleifera* fruits, and those of the hybrids and the backcross, remain orange when they are ripe, in spite of a much higher total carotene content.

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Lipid-Soluble Antioxidants: Biochemistry and Clinical Applications
A.S.H. Ong & L. Packer (eds)

PRODUCTION OF PALM OIL CAROTENOID CONCENTRATE AND ITS POTENTIAL APPLICATION IN NUTRITION

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Summary

Crude palm oil is known to be the richest natural plant source of carotenoids in terms of retinol (provitamin A) equivalents. Currently, however, these carotenoids are destroyed during physical refining of the oil. In view of the physiological importance of these carotenoids, various methods of isolation have been attempted and these include saponification, urea inclusion, selective solvent extraction, adsorption, molecular distillation and transesterification followed by distillation of esters. This paper will describe the processes developed in PORIM which yield carotenoid concentrates of > 80,000 ppm. These are based on (a) adsorption and (b) molecular distillation methods. Another method for the production of red palm oil will also be described. Chemical analysis of the carotenoid concentrates and red palm oil reveals eleven components viz α-carotene, β-carotene, lycopene, phytoene, phytofluene, α-zeacarotene, β-zeacarotene, ζ-carotene, γ-carotene, δ-carotene and neurosporene. The presentation of the carotenoid concentrate in different forms (capsules, powder and emulsion) has been developed and the stability of carotenoids in these forms has been evaluated. Toxicological tests showed that the carotenoid concentrate is safe. A review of the biological activities of these carotenoids e.g. their ability to quench singlet oxygen, anti-tumour activity and anti-atherosclerotic activity, is presented.

Introduction

Carotenoids, a class of C40 polyunsaturated hydrocarbons, impart an orangy-red colour to palm oil. Crude palm oil contains the highest concentration of agro-derived carotenoids with a total concentration in the range of 500 - 700 ppm (Goh et al., 1985). It is in fact the world's richest natural plant source of carotenoids in terms of retinol (provitamin A) equivalents, having 15 times more retinol equivalents than carrots and 300 times more than tomatoes (Tan et al., 1987). Carotenes, in particular β-carotene, are known for their provitamin A activities as they can be transformed into vitamin A in vivo. The vitamin A equivalents of α-, β-and γ-carotenes and β-zeacarotene which are present in crude palm oil are 0.9, 1.67, 0.75 and 0.42 respectively (Isler, 1971; Morton, 1970). β-Carotene, besides being a precursor of vitamin A, has been shown to be an efficient quencher of singlet oxygen and as such is an effective antioxidant (Krinsky et al., 1982; Santamaria et al., 1988; Wefers et al., 1988; Machlin et al., 1987). α-Carotene and lycopene have also been reported to be effective singlet oxygen quenchers (Mascio et al., 1989). In fact, epidemiological studies in the 1980s strongly associate β-carotene with the prevention of certain types of cancers such as oral, pharyngeal, lung and stomach cancers (Sundram et al., 1989; Norman et al., 1988; Suda et al., 1986; 1986; Mathews-Roth et al., 1987; Peto et al., 1989; Mettlin, 1984). In this connection, the National Institute of Health has identified β-carotene as one of the first top ten cancer preventive agents. What is more interesting is the recent report on α-carotene which has been shown to be tenfold more potent as an anti-cancer agent than β-carotene (Murakosh et al., 1989). Of late, research has also indicated that β-carotene has a positive effect in the reduction of atherosclerosis (Gaziano et al., 1990).

Since carotenoids have grown in importance and value, their recovery from crude palm oil and palm oil by-products is of interest. The commercially planted oil palm in Malaysia is the Tenera palm, a cross between Dura and Pisifera, all belonging to Elaeis guineensis family, originating from West Africa. A higher concentration of carotenoids can be obtained from oils of Melanococca and their crosses with Elaeis guineensis palm

(Yap et al., 1992; Tam et al., 1976). Carotenes can also be obtained from a palm oil by-product such as palm pressed fibre (Choo et al. , 1991) and oil from a second pressing in the mills (Choo et al., 1989).

In the current technology of physical refining, the carotenoids in crude palm oil undergo thermal decomposition during deodorisation/deacidification processes (240°-270°C). As a result, the processed products, normally known as refined, bleached and deodorised (rbd) palm oil contain no carotenoids at all. This represents a tremendous loss of pro-vitamin A. It has been estimated that the loss of carotenoids through thermal destruction in 1991 is about 3,660 tonnes. This is certainly a paradoxical situation in view of the fact that carotenoids have been found to have important nutritional and pharmacological properties as mentioned earlier.

In view of the importance of carotenoids for public health applications, three methods have been developed to extract and concentrate them from palm oil and palm oil products.

Material and Methods

Preparation of Alkyl Esters Through Transesterification

Malaysian crude palm oil from the commercially planted Tenera oil palm species was transesterified with methanol or ethanol (AR grade) at a molar ratio of oil to alcohol 2:1, catalyzed by 0.5% (w/w) sodium hydroxide (AR grade) after the free fatty acid had been neutralized. The reaction mixture was stirred, heated to reflux and monitored by thin layer chromatography (TLC) (silica gel, solvent chloroform/hexane 1:1 (v/v)) until all the triglycerides were converted to alkyl esters. The ester layer was then separated from the glycerol layer, and was washed with distilled water until the washings became neutral. The final ester product was dried with Na_2SO_4 and the solvent removed under reduced pressure.

Concentration via Removal of Alkyl Esters

The carotenoids in the volatile alkyl esters obtained from transesterification were recovered through two different methods: (i) The alkyl esters were distilled under high

vacuum using a Sibata falling film molecular apparatus at a pressure of $< 10 \times 10^{-3}$ torr with a temperature ranging from 100 - 170°C; the carotenoid concentrate was collected as a residue. (ii) Alkyl esters obtained were dissolved in alcohol (methanol or ethanol, depending on whether methyl or ethyl esters were used) (1:2 v/v), and the mixture introduced onto the glass column packed with C_{18} reverse phase Silica gel. The colorless esters were eluted first, and excess alcohol was introduced until the carotenoids' band (red color) was about to be eluted out; hexane and alcohol (methanol or ethanol 98:2 v/v) or chloroform were then used to elute out this high carotenoid fraction adsorbed in the reverse phase.

Carotene Composition of Carotenoid Concentrate and Red Palm Oil

Qualitative and quantitative carotene profiles of carotenoid concentrates and red palm oil were carried out using a Varian 5000 HPLC equipped with a variable wavelength UV-100 detector. Isocratic separation was performed on a 5 μm Zorbax ODS column (4.6 mm ID X 25 cm) with a solvent system of acetonitrile (89%) and dichloromethane (11%) at a flow rate of 1 ml/min. α- and β-carotenes were used as external standards for quantitative studies.

Stability of Carotenoid

Carotenoid stability of the prepared oil capsule and powder forms has been determined for a period of one year at ambient and freezer (-15°C) temperatures.

Preparation of Deacidified and Deodorised Red Palm Oil

The oil sample was pretreated with 20% phosphoric acid (0.5% wt. of oil) at 90°C for 10 minutes, followed by bleaching earth (0.5% wt. of oil) at 110°C for 30 minutes. The oil was then filtered to remove the bleaching earth. The pretreated oil was then subjected to deacidification and deodorisation through molecular distillation, the oil was deacidified and deodorised and recycled from 130°C to 170°C at a flow rate of about 8 - 12 kg/hr. The various quality parameters of the pretreated deacidified and deodorised red palm oil were determined following either the AOCS or IUPAC methods.

Results and Discussion

Carotenoids have been recognised to be important nutritionally and thus numerous methods of extraction have been developed to recover them from crude palm oil; These include saponification methods (Tabor et al., 1948; Blaizot et al., 1953), urea process (Knafo, 1952), adsorption (Ong et al., 1980, Unilever Ltd., 1953; Mamuro, et al., 1986; Tanaka et al., 1986) selective solvent extraction (Tanaka et al., 1986; Passino, 1952), molecular distillation (Ooi et al., 1986) and transesterification followed by distillation of esters (Lion Fat and Oil Company, 1976; Eckey, 1949; Hara et al., 1988; Hama et al., 1986).

In this paper, three methods of extraction and concentration of carotenoids from palm oil and palm oil products are reported. The first method which involves transesterification of crude palm oil to alkyl esters (mainly methyl and ethyl esters) followed by molecular distillation of the volatile esters has led to the production of carotenoid concentrate of > 80,000 ppm. As the process involves very mild distillation conditions with pressure < 10×10^{-3} torr, temperature < 170°C and short residence time for ester in the heater, the valuable minor components such as carotenoids and vitamin E originally present in crude palm oil are still found unchanged (Ooi et al., 1988). This process has also been demonstrated on a pilot plant scale giving 75% recovery of carotene based on 17 kg of ester per batch. Analytical data of the carotenoid concentrate by HPLC is shown in Table 1 and a typical HPLC chromatogram of the palm oil carotenoid concentrate is shown in Fig. 1. 11 types of carotenes have been detected of which α- and β-carotenes constitute about 90% of the total carotenes present. It can be seen from Table 1 that the carotene profile of the carotenoid concentrate is similar to that of the starting material, indicating that the process has not destroyed the carotenes. The carotenoid concentrate prepared by this process has also been subjected to a toxicological study (Tan et al., 1991). This study which involved 4 groups of Spraque-Dawley rats (n=12 per group) were fed on a semi-purified diet supplement with 0.2% palm oil based carotenoid concentrate (20,000 ppm), methyl ester, ethyl ester and a control diet for 16 weeks. Histopathological examinations of the major organs such as heart, lungs , adrenals, kidneys, liver and spleen were found to be normal in all dietary groups. No extensive or

significant amount of fat was deposited in the heart and the coronary vessels and aorta was found to be normal in all dietary groups. It was concluded that the carotenoid concentrate and other dietary test groups do not have any toxicological effects on the major organs of the male rats.

The carotenoid concentrate prepared by this process has also been presented in three different forms for pharmaceutical application. These include capsules (both soft and hard) , powder and emulsion. The powder formulation has been successfully formulated and it could be made into carotene tablets, or encapsulated in hard capsules. Preliminary results on the storage stability tests show that the carotenoids in powder form during storage at room temperature were not as stable as carotenoid concentrate in the capsules. This could be due to greater exposure to light and air in the powder form, leading to increased oxidation or degradation of carotenoids. However, only a slight decline (< 4%) in carotenoid content was observed for the powder if it was kept in the freezer (-15°C) for a period of one year (see Figures 1 and 2).

The second method which involves preparation of carotene enriched palm oil has been effected and the process involves degumming of the oil with phosphoric acid followed by treatment with bleaching earth. The treated oil is then subjected to deodorisation and deacidification at mild reaction temperature to remove odoriferous materials as well as free fatty acids (Choo et al., 1988). More than 80% of the carotenes originally present in crude palm oil is retained. Analysis by HPLC shows that the profile of the carotenes is similar to that of the starting material, again indicating that carotenes are not destroyed during the process (see Table 1). The quality of this red palm oil (as shown in Table 2) has also been found to be good. It has been determined by sensory panel that it is suitable in food application (Nor Aini Idris, 1991). This process has also been upgraded to pilot scale operation and under these conditions, 75% of carotene in red palm oil is retained. It is observed that more than 80% of vitamin E originally present in crude palm oil is also retained.

It must be noted that in the second process, the triglycerides remain intact unlike the first process where all the triglycerides have been converted to alkyl esters. However, the first process could yield a higher concentration of carotenoids after the alkyl esters are removed.

Table 1. Carotene Composition (%) of Carotenoid Concentrate, Red Palm Oil and Crude Palm Oil.

Carotene	Carotenoid concentrate	Red palm Oil	Crude palm
phytoene	1.5	2.0	1.3
phytofluene	0.3	1.2	0.1
cis-β-carotene	0.9	0.8	0.7
β-carotene	49.9	47.4	56.0
α-carotene	33.3	37.0	35.1
cis-α-carotene	5.5	6.9	2.5
ζ-carotene	1.7	1.3	0.7
γ-carotene	1.3	0.5	0.3
δ-carotene	0.6	0.6	0.8
neurosporene	0.1	trace	0.3
β-zeacarotene	1.3	0.5	0.7
α-zeacarotene	0.4	0.3	0.2
Lycopene	3.4	1.5	1.3
Total (ppm)	80,560	545	673

Table 2 Quality Parameters of Red Palm Oil

Carotenes	> 80% intact
Tocopherol and Tocotrienols	>80% intact
Free fatty acids	< 0.1%
Peroxide value	< 0.2
Phosphorus content	< 2 ppm
Moisture and impurities	< 0.1%

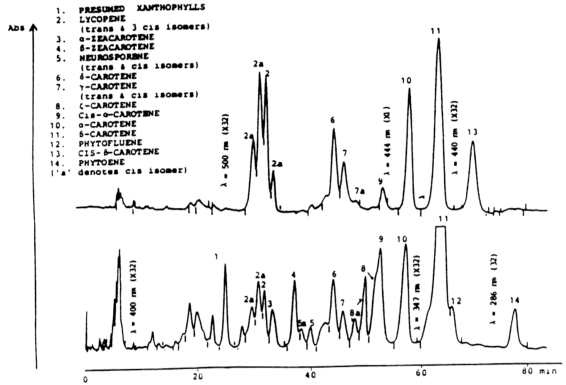


FIGURE 1. HPLC OF CAROTENOIDS OF PALM OIL

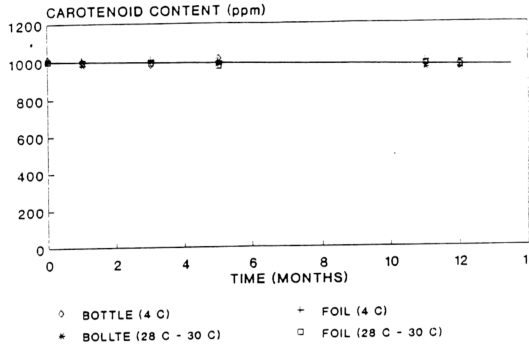
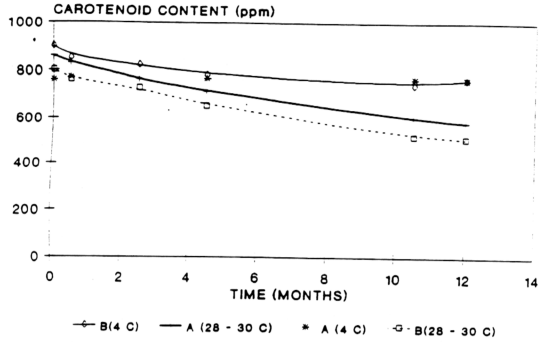


FIGURE 2. STORAGE STABILITY OF CAROTENOIDS IN CAPSULE FORM



A=Samples kept in sample bottle
B=Samples kept in clear bottle

FIGURE 3. STORAGE STABILITY OF CAROTENOID IN POWDER FORM

The third method of carotenoid concentrate has been investigated using C_{18} reverse phase column chromatography (Choo et al., 1991). A recovery of >90% (w/w) can be obtained through this method and the column can be reused for >50 times without any loss of activity. This process, however, requires further work.

Conclusion

Palm oil carotenoids can be successfully obtained for food and pharmaceutical applications. The safety of application is assured by the results of the toxicological study.

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Production and Applications of Deacidified and Deodorized Red Palm Oil

Choo Y M; Ma, A N; Yap,S C; Ooi, C K and Yusof Basiron

INTRODUCTION

Crude palm oil is the richest natural plant source of carotenes in terms of retinol (pro-vitamin A) equivalents. It has 15 times more retinol equivalents than carrots and 300 times more than tomatoes (Tan *et al.*, 1989). Carotenes, in particular α- and β-carotenes, have long been associated with their pro-vitamin A activities as they can be transformed into vitamin A *in vivo*. Recent studies have indicated that they also act as protective anti-cancer agents in relation to certain types of cancers such as lung, oral, pharygeal, colon and stomach (Mathews-Roth *et al.*, 1987, Mettlin 1984, Norman *et al.*, 1988; Peto *et al.*, 1989; Suda *et al.*, 1986). β-Carotene has also been reported to possess anti-atherosclerotic effect (Gaziano *et al.*, 1990). The carotenoid content of Malaysian crude palm oil ranges from 500–700 ppm of which more than 90% are α- and β-carotenes (Goh *et al.*, 1985). These carotenoids impart the characteristic orangey red colour to crude palm oil.

Malaysia is the world's largest producer and exporter of palm oil. The production figure in 1992 was 6.4 million tonnes and more than 90% of this was exported. Palm oil and its products are exported in refined, bleached and deodorized forms. The present refining process causes the destruction of most of the carotenes present in the crude oil. As a result the final product is light golden in colour and devoid of carotenes. It also means that palm oil refining has allowed 3200–4480 tonnes of carotenes to be destroyed in 1992.

This paper describes a newly developed process which is able to yield deodorized and deacidified red palm oil of similar quality to that of the presently available refined, bleached and deodorized palm oil but retaining most of the carotenes as well as vitamin E originally present in the crude palm oil (Ooi *et al.*, 1988).

PROCESS AND PRODUCT

The process to produce deodorized and deacidified red palm oil involves two stages, *i.e.* pretreatment of the crude palm oil followed by deodorization and deacidification by molecular distillation. The pretreatment is carried out in a conventional manner using phosphoric acid followed by bleaching earth. This allows the impurities and oxidative products in the crude palm oil to be removed.

The deodorization and deacidification stage is carried out using molecular distillation unit at temperatures < 165°C and a pressure of 20–35 x 10^{-3} Torr.

The results (*Table 1*) show that more than 80% of the carotenes originally present in crude palm oil and almost 100% of the

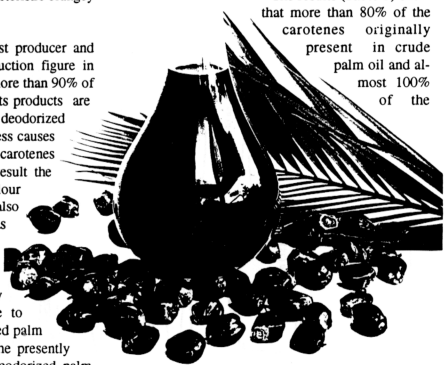

Figure 1. Red palm olein product by PORIM patented technology.

otenes from the feed material *i.e.* the pre-ated palm oil are retained. Analysis by high formance liquid chromatography (HPLC) shows t the profile of the carotenes is similar to that of starting material, again indicating that carotenes not destroyed during the process. A typical PLC chromatogram of carotenes present in red m oil is shown in *Figure 1*. The quality of this l palm oil as shown in *Table 2* has also been nd to be good meeting PORAM's specifica-ns for refined, bleached and deodorized (RBD) m oil. It is also observed that more than 80% vitamin E originally present in crude palm oil is ained. In terms of stability, carotenes, as well as er quality parameters are found to be stable en the red palm oil was kept at 10°C over a iod of one year.

It must be mentioned that degumming and aching stages could be carried out in a typical m oil refinery, and this has been demonstrated treating the sample obtained from the refinery er degumming and bleaching stage with olecular distillation. The results are shown in *ble 3*. A sample of red palm olein produced by aforementioned process is shown in *Figure 2*.

TABLE 1. CAROTENE COMPOSITION (%) OF DEACIDIFIED AND DEODORIZED RED PALM OIL AND CRUDE PALM OIL

Carotene	Red Palm Oil	Crude Palm Oil
phytoene	2.0	1.3
phytofluene	1.2	0.1
cis-β-carotene	0.8	0.7
β-carotene	47.4	56.0
α-carotene	37.0	35.1
cis-α-carotene	6.9	2.5
carotene	1.3	0.7
ζ-carotene	0.5	0.3
γ-carotene	0.6	0.8
neurosporene	trace	0.3
β-zeacarotene	0.5	0.7
α-zeacarotene	0.3	0.2
Lycopene	1.5	1.3
Total (ppm)	**545**	**673**

TABLE 2. QUALITY PARAMETERS OF RED PALM OIL

Carotenes	> 80% intact
Tocopherols and Tocotrienols	> 80% intact
Free fatty acids	< 0.1%
Peroxide value	< 0.2
Phosphorus content	< 2 ppm
Moisture and impurities	< 0.1%

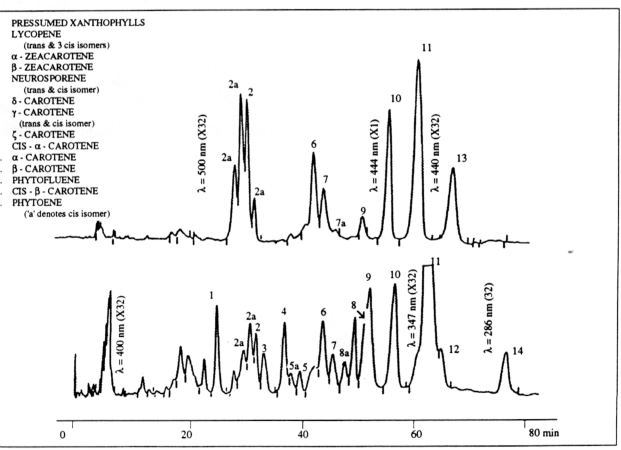

Figure 2. HPLC chromatogram of Red Palm Oil.

APPLICATIONS OF RED PALM OIL

ie red palm oil has been demonstrated in its plications for curry, satay sauce (*Figure 3*) and mbals (*Figure 4*). It can be used in margarine rmulation (*Figure 5*) to give the required colora- n for the final product and the desired level of o-vitamin A. It has also been used for frying nch fries which acquire attractive coloration igure 6). The red palm oil can be expected to be plicable to other dishes which are reddish in our. It is definitely ideal for dishes which are r-fried (*Figure 7*) as most carotenes are not stroyed (Choo *et al.*, 1992). It can also be used salad dressing (*Figure 8*) and cake making igure 9).

Red Palm Olein: most suitable for dishes which are reddish in nature.

Sensory evaluation carried out on the red palm showed that the red palm oil is of very good lity, and is comparable to freshly prepared de palm oil in the laboratory. It is of better lity compared to a normal crude palm oil ob- ed from a refinery. The red palm oil is bland flavour, therefore it receives a low flavour nsity rating (*Table 4*). Fresh crude palm oil pared in the laboratory has a sweet, pleasant amel-like flavour.

As deacidified and deodorized red palm oil tains high level of carotenes in particular α-and rotenes, it can serve as a potential source of vitamin A in developing countries where vita- A deficiency is prevalent.

CONCLUSION

In conclusion, the technology for the production of deacidified and deodorized red palm oil for edible and nutritional uses is available for commercial exploitation. Hence, there is no doubt in the near future that palm oil rich in carotenes can be expected to be available in the market place for use in food applications.

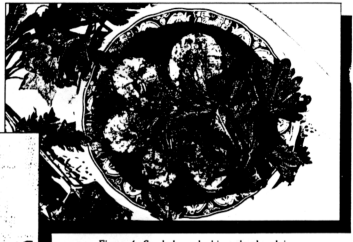

Figure 4. Sambals cooked in red palm olein.

Figure 5. For margarine formulation: gives required coloration for final product and desired level of pro-vitamin A.

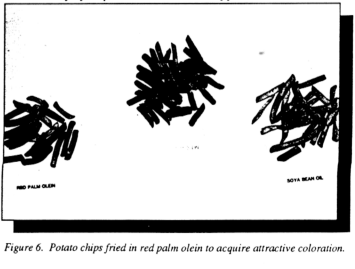

Figure 6. Potato chips fried in red palm olein to acquire attractive coloration.

Figure 7. Ideal for stir-fried dishes.

Figure 8. Used in salad dressing.

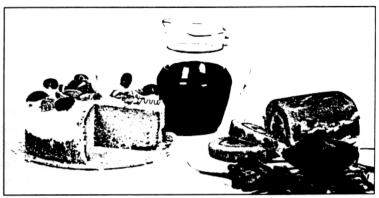

Figure 9. Butter cake baked with red palm oil.

TABLE 3. ANALYSES OF DEACIDIFIED AND DEODORIZED RED PALM OLEIN

ses es	FFA (%)	Carotenes (ppm)	Vitamin E (ppm) α-T	α-T_3	τ-T_3	δ-T_3	PV (meq/kg)	$E^{1\%}_{1cm\,233}$	$E^{1\%}_{1cm\,269}$	Fe (ppm)	M&I %	P ppm
palm olein*	3.53	643	187	207	374	96	2.32	1.47	0.69			
ted lein*	3.53	514	220	214	353	82	0.44	1.34	0.69			
ified and ized red lein*	0.04	513	160	202	275	64	0.10	0.89	0.62	0.2	0.02	1.6
alm olein*	0.04	NIL	139	163	205	54	0.10	0.69	0.60	0.2	0.03	1.6

te :
es obtained from palm oil refinery
ated palm olein samples from refinery, treated with molecular distillation in PORIM

:	Free fatty acids	Fe	:	Iron
:	Peroxide value	P	:	Phosphorus
:	Refined, bleached and deodorized	M&I	:	Moisture and impurities

BLE 4. SENSORY EVALUATION OF DEACIDIFIED AND DEODORIZED RED PALM OIL AND CRUDE PALM OIL (CPO)

ple description	Sensory rating	
	Flavour intensity*	Quality**
idified and deodorized red oil	1.0	5
CPO	4.0	5
age CPO	4.5	3
quality CPO	5.0	1

Flavour intensity rating is from 1 to 5, 1 being bland and 5 being extreme.

Quality rating is from 1 to 5, 1 being very poor and 5 being very good.

ACKNOWLEDGEMENT

e authors wish to thank Keck Seng (M) Bhd. for supply of crude palm olein and bleached palm n samples. Thanks are also due to Dr. Nor i Idris for the sensory evaluation of red palm Yahaya Hawari, Jaafar Ismail, Fatah Yah, nana Makrof and Anita Taib for technical assisce.

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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 (Sibat 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)				SE	LSD
	110	130	150	170		
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)				SE	LSD
	110	130	150	170		
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.

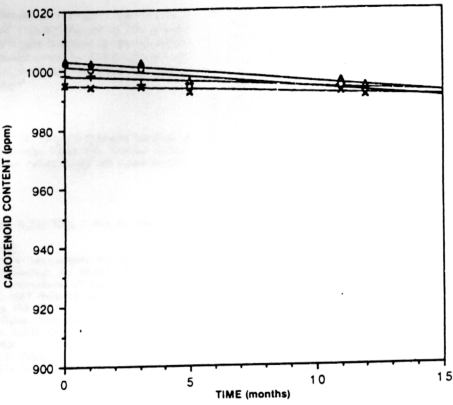

FIG. 1. Storage stabilities of carotenoids in capsule form: ○—bottle (4°C); ×—bottle (28–30°C); △—foil (4°C); +—foil (28–30°C).

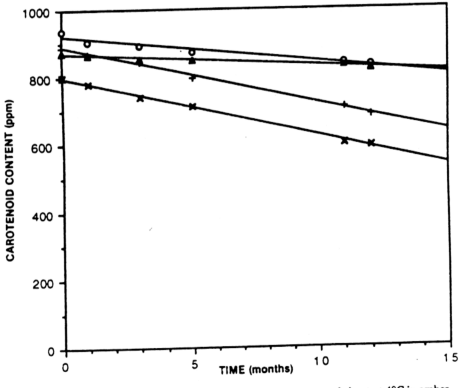

FIG. 2. Storage stabilities of carotenoids in powder form: △—sample kept at 4°C in amber bottle; +—sample kept at 28–30°C in amber bottle; ○—sample kept at 4°C in clear bottle; ×—sample kept at 28–30°C in clear bottle.

stored at 4°C. However, at storage temperatures of °C, the carotenoid concentrations of the powder ed by 20–25%. The bigger decrease in the carotenoid ntration in the powder could be due to greater ex- e to light, leading to increased oxidation and degra- n of the carotenoids.

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