QUANTITATIVE ANALYSIS OF CAROTENES IN THE OIL FROM DIFFERENT PALM SPECIES

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

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Carotenes 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

The 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 *pisifera* (P)] and their hybrids (M × D, M × P) and the backcross (MD × P).

EXPERIMENTAL

Materials

Oil palm fruits from *E. guineensis* (*dura* (D), *pisifera* (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 spikelets 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 50 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

removed by washing the mixture with 2% aqueous $Na_2S_2O_3$ followed by distilled water. The solution was then dried over anhydrous Na_2SO_4, the solvent was evaporated, and the isomerization products were redissolved in HPLC 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[a]

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

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

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

[b]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

[a]Total Carotenoids estimated at 446 nm.

RESULTS AND DISCUSSION

Crossing *E. oleifera* and *E. guineensis* has been shown to yield hybrid palms which retain the characteristics of the *E. oleifera* palm in terms of the height increment, fruit shape and fruit colour (Hartley, 1977). However, the fatty acid composition of the oil is intermediate between those found in the two parent species. Similar results were also observed 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

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

The major characteristic of carotenes is the presence of highly conjugated polyene chains, which normally results in absorption of light in the visible region. This is advantageous for carotenoid detection, and the interference from non-carotene compounds can be eliminated when the appropriate wavelengths are selected. This is particularly important since apart from carotenes the non-saponifiable fraction of palm oil contains other minor constituents which absorb in the UV region.

Identification of carotenes was carried out by co-chromatography with the few available authentic carotenes purchased from Sigma; in many instances identification of the peaks was based mainly on their characteristic UV-VIS absorption spectra obtained from the pure carotenes collected from the HPLC. The number of conjugated double bonds as well as differences in end groups determines the nature of the UV-VIS spectra and absorption maxima (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

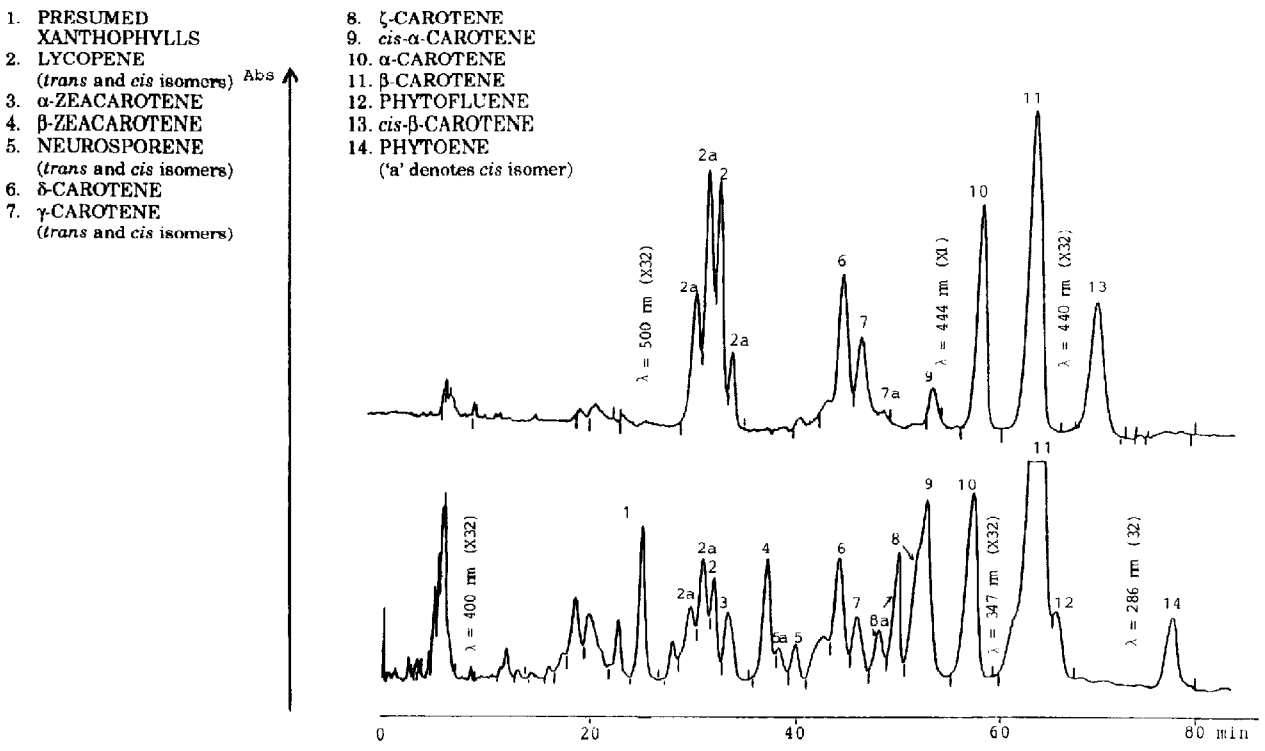

Figure 1. HPLC of carotenoids of palm oil

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

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

of polarity or absorption among the carotenes and lead to the characteristic elution profile observed for them. The appearance of an absorption maximum (the '*cis* peak') in the UV region of the spectrum of most of the *cis* isomers, and chromatographic analysis of the iodine-isomerized products of selected carotenes separated by HPLC, assisted in the identification of some carotenes, particularly those with *cis*-isomers.

As reported earlier (Ng and Tan, 1988), the most 'polar' carotene, lycopene, was the first one to be eluted from reverse phase column. Oxygenated carotenes (xanthophylls) were eluted much faster and were well separated from the hydrocarbon carotenes. The highly conjugated lycopene, which was not found in palm oil samples by Ng and Tan (1988), was detected in the present study in small amounts in palm oil from the commercial *tenera* variety. The 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)
Lycopene	472	3450
α-Zeacarotene	421	2450
β-Zeacarotene	427	2520
Neurosporene	440	2918
δ-Carotene	456	3290
γ-Carotene	462	3100
ζ-Carotene	400	2555
α-Carotene	444	2800
β-Carotene	453	2592
Phytofluene	347	1577
Phytoene	286	915

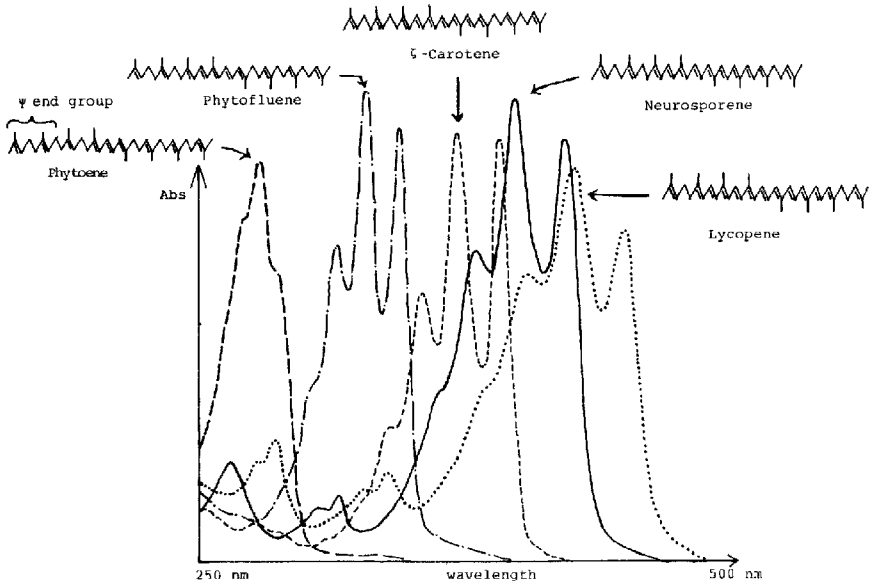

Figure 2. UV-Visible spectra of acyclic carotenes

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

The two major carotenes in palm oil, α- and β-carotenes (peaks 10 and 11 respectively), were identified by co-chromatography with standards 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-

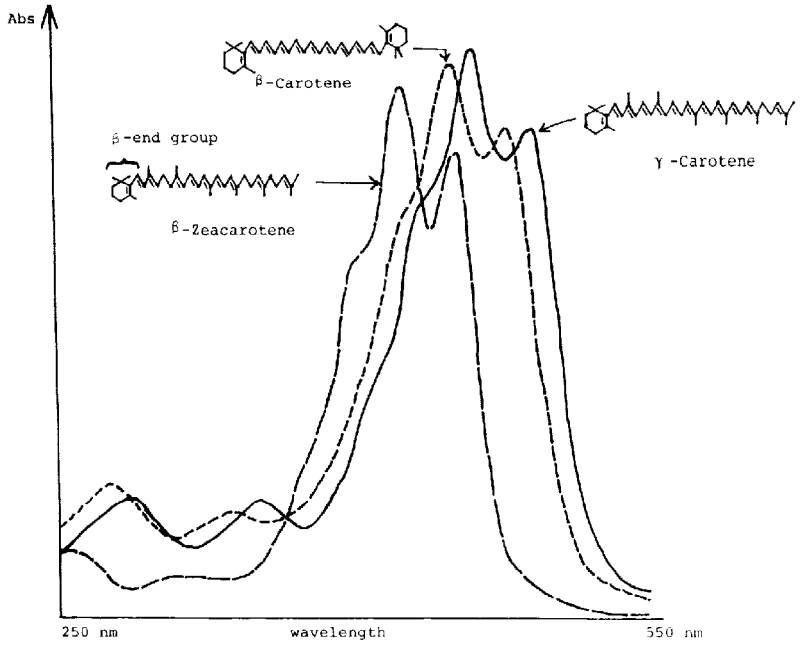

Figure 3. UV-Visible spectra of carotenes with β-cyclic end groups.

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
Phytoene	1.12	1.68	2.49	1.83	2.45	1.30	1.27
cis-β-Carotene	0.48	0.10	0.15	0.38	0.55	0.42	0.68
Phytofluene	Tr[b]	0.90	1.24	Tr	0.15	Tr	0.06
β-Carotene	54.08	54.39	56.02	60.53	56.42	51.64	56.02
α-Carotene	40.38	33.11	54.35	32.70	36.40	36.50	35.16
cis-α-Carotene	2.30	1.64	0.86	1.37	1.38	2.29	2.49
ζ-Carotene	0.36	1.12	2.31	1.13	0.70	0.36	0.69
γ-Carotene	0.09	0.48	1.10	0.23	0.26	0.19	0.33
δ-Carotene	0.09	0.27	2.00	0.24	0.22	0.14	0.83
Neurosporene	0.04	0.63	0.77	0.23	0.08	0.08	0.29
β-Zeacarotene	0.57	0.97	0.56	1.03	0.96	1.53	0.74
α-Zeacarotene	0.43	0.21	0.30	0.35	0.40	0.52	0.23
Lycopene	0.07	4.50	7.81	0.05	0.04	0.02	1.30
Total Carotene (p.p.m.)	4592	428	997	1430	2324	896	673

[a]M = *Elaeis oleifera* (*Melanococca*), P = *E. guineensis* (*pisifera*); D = *E. guineensis* (*dura*).
[a]T = *E. guineensis* (*tenera*) = D × P

[b]Tr = trace.

carotenes (8a) which showed a shift of the spectral bands to shorter wavelengths and the extra 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.

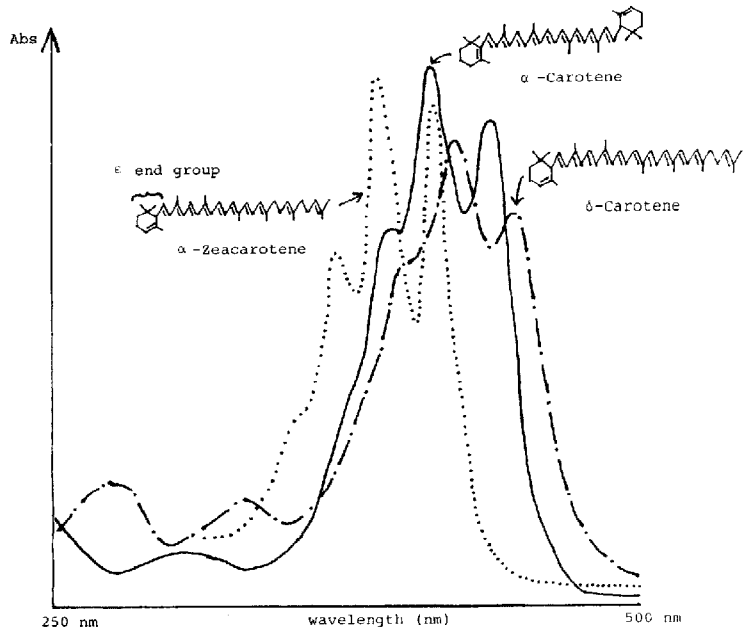

Figure 4. UV-Visible spectra of carotenes with ε-cyclic end group.

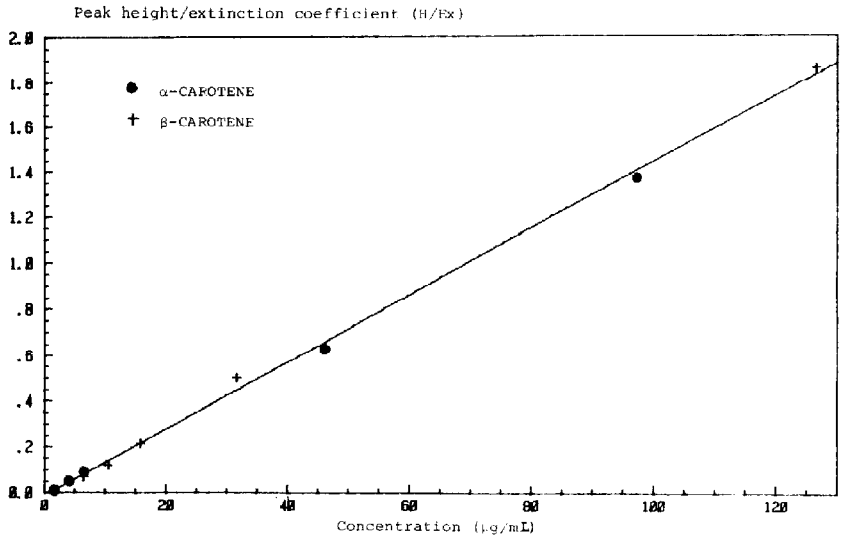

Figure 5. Standard curves for α- and β-carotenes: Peak height / extinction coefficient (H / Ex) versus concentration (μg / mL).

Peaks 5, 6 and 7 were identified as neurosporene, δ- and γ-carotenes respectively, on the basis of the UV-VIS spectral data shown in *Table 3*. The elution order was in accord with that published by Ng and Tan (1988). Peaks 5a and 7a were tentatively assigned as the *cis*-isomers of neurosporene and γ-carotene respectively. These two carotenes show lower absorption maxima than the corresponding *trans* carotenes and the *cis* peaks for the two *cis*-isomers were observed in the near UV region 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

analysis of the carotenes was possible by measuring the heights of the individual carotene peaks in the chromatograms. Individual peaks were recorded at different UV-VIS wavelengths: the wavelength chosen (normally the absorption maximum – λ max – of each carotene) and the published extinction coefficients (Ex) used in this study are shown in *Table 4*. It has been reported that *cis*-carotenoids exhibit lower extinction coefficients than the corresponding *trans* isomers (Davies, 1976). However, because of the uncertainty of the position of the *cis* double bonds in the present study and the limited data on the extinction coefficients for the different types of *cis*-carotenes, extinction coefficients for the *trans* isomers were assumed in the quantification of *cis* carotene isomers; it is expected that the actual values will be slightly lower: for example, if the extinction coefficient for *trans*-β-carotene is used for the quantification of 9-*cis*-β-carotene, about 5% less of the 9-*cis*-β-carotene will be reported (Sweeney and Marsh, 1970).

For calibration, α- and β-carotenes were used as external standards in the present study. The peak heights (H) of these carotenes recorded at the chosen wavelengths showed a linear correlation with their concentrations. By plotting the H/Ex of both α- and β-carotenes against their respective concentrations, a linear relationship was obtained over the range of concentrations of HPLC analysis (*Figure 5*).

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

Table 5 shows the detailed carotene profiles of oils from the various palm species studied. The major constituents found in all these oils were β-carotene and α-carotene, ranging from 54% to 60% and 24% to 40% of the total carotenes, respectively.

No significant variation in the nature of the carotenes was found between *E. oleifera*, *E. guineensis*, their hybrids and the backcross. However, 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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REFERENCES

BOCKENNOOGEN, H A (1974). *Analysis and Characterization of Oils, Fats, and Fat Products*. Interscience, New York. Vol. 2, p. 648.

BUSHWAY, R J (1986). Determination of α- and β-carotenes in some raw fruits and vegetables by High-Performance Liquid Chromatography. *J. Agric. Food Chem., 34*, 409-412.

DAVIES, B H (1976). Carotenoids, In: *Chemistry and Biochemistry of Plant Pigments*, T.W. Goodwin (ed). Academic Press, London. Vol. 2.

GOH, S H; CHOO, Y M and ONG, S H (1985). Minor constituents of palm oil. *J. Am. Chem. Soc., 62*, 237-240.

HARTLEY, C W S (1977). *The Oil Palm*, 2nd edition, Longman, London and New York. pp. 299-305.

JACOBSBERG, B (1974). Palm oil characteristics and quality. In: *Proc. of 1st MARDI Workshop on Oil Palm Technology*. Kuala Lumpur, June 1974, pp. 48-68.

JOSE, A T Q; DELIA, B R-A; ESTEVES, W E-S and GERHARD, F P (1990). Carotenoid composition and Vitamin A values of oils from four Brazalian palm fruit. *Fat Sci. Technol.*, *92*, 222-226.

MATHEWS-ROTH; M M and KRINSKY, N I (1987). Carotenoids affect development of UV-B induced skin cancer. *Photochem. Photobiol.*, *46*, 507-509.

METTLIN, C (1984). Epidemiologic studies on vitamin A and Cancer. *Adv. Nutr. Res.*, *6*, 47-65.

MURAKOSHI, M; TAKAYASU, J; KIMURA, O; KOHMURA, E; NISHINO, H; IWASHIMA, A; OKUZUMI, J; SAKAI, T; SUGIMOTO, T; IMANISHI, J and IWASAKI, R (1989). Inhibitory effect of α-carotene on proliferation of the human neuroblastoma cell line GOTO. *J. Natl. Cancer Inst.*, *81*, 1649-1652.

NELIS, H J C F and DE LEENHEER, A P (1983). Isocratic Nonaqueous Reversed-Phase Liquid Chromatography of carotenoids. *Anal. Chem.*, 55, 270-275.

NG, J H and TAN, B (1988). Analysis of palm oil carotenoids by HPLC with diode-array detection. *J. Chromatogr.*, *26*, 463-469.

NORMAN, J T and TAPAN, K B (1988). Does beta-carotene prevent cancer? A critical appraisal. *Nutr. Res.*, 8, 685-701.

PETO, R; DOLL, R; BUCKLEY, J D and SPORN, M B (1981). Can dietary beta-carotene materially reduce human cancer rates? *Nature, 290*, 201-208.

SIEW, W L and TAN, Y A (1988). Identity characteristics of palm oil. *Palm Oil Dev. 9*, 25-26.

SUDA, D; SCHWARTZ, J and SHKLAR, G (1986). Inhibition of experimental oral carcinogenesis by topical beta-carotene. *Carcinogenesis*, 7, 711-715.

SUNDRAM, K; KHOR H T; ONG, A S H and PATHMANATHAN, R (1989). Effect of dietary palm oil on mammary carcinogenesis in female rats induced by 7, 12-dimethylbenz(a) anthracene. *Cancer Res.*, *49*, 1447-1451.

SWEENEY, J P and MARSH, A C (1970). Vitamins and other nutrients: separation of carotene stereoisomers in vegetables. *J. Ass. Off. Analyt. Chem.*, 53, 937-940.

TAM, T K; LIM, C S; YEOH, G H and OOI, S C (1976). The oil and other characteristics of interspecific (*Elaeis guineensis* × *Elaeis oleifera*) hybrids planted in Malaysia and their significance for future breeding programmes. In: *International Development In Oil Palm*. D.A. Earp and W. Newall (eds). *The Proceedings of the Malaysian International Agricultural Oil Palm Conf.* Kuala Lumpur, June 1976. pp. 27-38.

TAN, B; GRADY, C M and GAWIENOWSKI, A M (1986). Hydrocarbon carotenoid profiles of palm oil processed fractions. *J. Am. Oil Chem. Soc. 63*, 1175-1179.

ZIEGLER, R G (1989). A review of epidemiological evidence that carotenoids reduce the risk of cancer. *J. Nutr.*, *119*, 116-122.

ZECHMEISTER, L (1976). *Cis-Trans Isomeric Carotenoids, Vitamins A and Arylpolyenes*. Academic Press, New York.