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CAROTENOID PROFILES AND PRELIMINARY INVESTIGATION ON CAROTENOID BIOSYNTHESIS IN THE OIL PALM (*Elaeis guineensis*) MESOCARP

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ABSTRACT

*The changes in carotenoid content and profile were studied in oil palm (*E. guineensis*) mesocarp at various stages of development. Spectrophotometric analysis showed that chlorophyll synthesis predominates in the young fruits but it then shifts strongly to carotenoids as the fruit ripens. The major carotenoids in the oil from ripe fruits are α- and β-carotenes. A very wide variation in total carotenoid content was observed in various* E. guineensis *genotypes. High performance liquid chromatography (HPLC) analysis using both C18 and C30 stationary phases showed the C30 phase to be superior in separating the* cis- *and* trans- *isomers of both α- and β-carotenes and resolving other components. Lycopene was not detected in at all.*

Incorporation studies were carried out with various ^{14}C-labelled substrates - acetate, glyceraldehyde-3-phosphate (G3P), isopentenyl, pyrophosphate (IPP), mevalonic acid (MVA) and pyruvate. IPP was the most incorporated showing it to be a major intermediate in carotenoid synthesis in the oil palm. There was also considerable incorporation of acetate, IPP and MVA into α- and β-carotenes. G3P and pyruvate were not incorporated into α- and β-carotenes suggesting that carotenoid synthesis in the oil palm follows the acetate/mevalonate pathway.

Keywords: carotenoids, carotenoid biosynthesis, *E. guineensis*, acetate/mevalonate pathway, oil palm.

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INTRODUCTION

Carotenoids are responsible for most of the yellow, orange and red colours in fruits, vegetables and flowers. Green vegetables also contain carotenoids such as α-carotene, β-carotene and β-cryptoxanthin, but are masked by the green chlorophylls. Carotenoid levels vary with genotype and maturity. Different biological and environmental conditions/stresses affect carotenoid metabolism. Plants exposed to sub-optimal light, water or temperature conditions can produce higher or lower carotenoid levels (Barry *et al*., 1992; Sarry *et al*., 1994). In humans and animals, dietary carotenoids are essential precursors of vitamin A and retinoid compounds required for morphogenesis (Bendich and Olson, 1989). There is considerable evidence that carotenoids act as potent antioxidants and have chemoprotective properties for human health (Haliwell, 1997; Ziegler, 1989). They play beneficial roles in the prevention of chronic diseases (Charleux, 1996; Mayne, 1996) and enhance the immune system (Van Breeman, 1996). Carotenoids function in plants as protectors against damage to the photosynthetic apparatus from excessive light (Demming-Adams *et al*., 1989; Shultz *et al*., 1991). The diverse locations of carotenoids in the cell clearly imply that efficient regulatory mechanisms control the rate of biosynthesis, breakdown and deposition of the pigments at their functional locations. These mechanisms need to be understood in order to manipulate the carotenoid content and composition in oil palm. There is currently considerable interest in the manipulation of carotenoid content and composition in plants to

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improve their nutritional composition for human consumption. Carotenoids have many industrial applications as food and feed additives, and are used in cosmetics and as nutraceuticals. Palm oil is one of the richest sources of plant carotenoids. However, at present, there is little or no information on the biosynthesis of carotenoids in oil palm fruits. The aim of this study was to provide some basic information on the carotenoid profile and synthesis in the oil palm mesocarp with a view to future manipulation of the carotenoid biosynthesis pathway in the palm.

EXPERIMENTAL

Plant Material

Fruits from the commercial oil palm (*Elaeis guineensis* Jacq. var *tenera*) at various stages of development, *i.e.*, 15- to 20-weeks after anthesis (WAA), were obtained fresh from the Malaysian Palm Oil Board (MPOB) Research Station at Universiti Kebangsaan Malaysia, Bangi. The female inflorescences were tagged at anthesis and the fruits harvested at various stages of ripeness.

For the experiments on screening for total carotene content in the oil from different genotypes, fresh fruit bunches at 20 WAA (*E. guineensis* Jacq. var *tenera* or *dura*) were obtained from the MPOB germplasm collection at its Kluang Research Station.

Reagents

Carotene standards, namely, β-carotene, mixed isomers and lycopene, were obtained from Sigma Chemicals Co., USA.

Extraction of Carotenoids

The procedure described by Davies (1976) was modified. Mesocarp slices (5 g) were transferred into a 50 ml polypropylene tube containing 20 ml 0.1 M MES buffer (pH 6.0). An equal volume of acetone was added and the extraction mixture gently shaken for approximately 10 min. The mixture was decanted and the tissue slices re-extracted with fresh acetone twice. Diethyl ether (10 ml) was added to the combined extraction solution and then 1 ml 3.3% sodium chloride solution to effect partitioning. The mixture was gently shaken to allow the carotenoids to preferentially dissolve in the upper diethyl ether layer which was collected while the lower acetone-buffer layer was re-extracted twice with 10 ml fresh diethyl ether. The combined ethereal solution was then concentrated to dryness with nitrogen (N_2) gas to yield the crude carotenoid extract.

Saponification

Cold saponification was carried out to remove unwanted lipid materials. Ethanol (10 ml) and 1 ml 60% KOH were added to the dried extract and left overnight in the dark at 4°C with gentle shaking. Diethyl ether (10 ml) were added to the flask and gently shaken. The upper diethyl ether layer contained the carotenoids (unsaponified material) while the lower ethanolic aqueous phase the unwanted saponified material, especially lipids. Two to three volumes of distilled water were added drop wise to the diethyl layer to gently wash off any residual KOH. The diethyl ether layer was collected and concentrated to dryness with N_2 gas.

HPLC Analysis

A Waters HPLC System with 600E controller and pump and WISP 715 autosampler were used to separate samples by reverse phase chromatography. Two stationary phases were used: Bischoff C30 and Genesis C18 (5 μm, 4.6 x 250 mm) columns fitted with guard columns. A Waters 996 photodiode array detector with wavelength detection range set at 200-600 nm was used to ensure that both the UV and visible (Vis) absorbing carotenoids in the extracts were detected. Peaks were identified by spectrophotometric profiles and retention times. The mobile phase for the C30 column comprised:

Solvent A; TBME methanol water H_2O (15/81/4, v/v/v).
Solvent B: TBME/methanol/H_2O (90/6/4, v/v/v)

The elution programme followed a linear gradient from 100% A to 100% B in 90 min. The injection volume was 20 ml and the flow rate set at 1 ml min^{-1}. The mobile phase for the C18 stationary comprised: acetonitrile/methanol/dichloromethane (70/10/20, v/v/v) run in isocratic mode at a flow rate of 1.5 ml min^{-1}. The Waters chromatography software, Millennium 32, was used to analyse the data.

Spectrophotometric Profiles

Carotenoid extracts/standards dried under nitrogen gas were re-dissolved in iso-octane and one ml transferred into a quartz cuvette. The absorption spectra of the samples were recorded at room temperature and 200 – 600 nm wavelength with a Kontron Uvikon 923 UV-VIS double beam spectrophotometer.

Analysis of total carotene content. Lipids were extracted from oil palm fruits using a manual press following their softening by autocalving at 15 psi for 10 min. The oil (0.1 g) was accurately weighed to the nearest 0.0001 g into a 25 cm^3 volumetric flask

and made up to volume with iso-octane. The absorbance of the solution at 446 nm was determined. The carotene content expressed as carotene (ug g^{-1}) is given by:

$$\frac{25 \times 383 (a_s - a_b)}{100 W}$$

where a_s is the absorbance of the sample, a_b the cuvette error (blank) and W the weight of the sample in g (PORIM, 1988).

Incorporation of ^{14}C-substrates

Various radio-labelled substrates - ^{14}C-acetate, ^{14}C-isopentenyl pyrophosphate (IPP), ^{14}C-glyce raldehyde-3-phosphate (G3P), ^{14}C-mevalonic acid (MVA) and ^{14}C-pyruvic acid (Py) - were ascertained for their incorporation into the carotenoid pathway.

One gram thinly sliced mesocarp (20 WAA) was incubated with 0.25 μCi ^{14}C-labelled substrate in a 25 ml conical flask containing 4 ml 0.1 M MES buffer (pH 6.0) for 4 hr at 25°C. The conical flask was fitted with a stopper fixed with a well containing filter paper saturated with 200 μl 2 M KOH for trapping $^{14}CO_2$. A 10 μl sample from the mixture before and after incubation were transferred to a scintillation vial containing 10 μl scintillation liquid for counting. A blank not containing any radio-labelled substrate was also included for the background subtraction.

Acetone (4 ml) was added to stop the reaction. A stream of N_2 gas was passed through the reaction mixture and the reaction flask capped to reduce oxidation of the carotenoids. The reaction mixture was then left overnight in the dark at 4°C with gentle shaking (120 rpm). The carotenoids in the sample were extracted using acetone and diethyl ether as described earlier. Unwanted lipid materials were removed by cold saponification. The saponified ^{14}C-carotenoid extract was kept at -20°C for further analysis.

To determine the percentage incorporation and efficiency of extraction, a 100 μl sample was removed for ^{14}C-counting at each extraction step. The experiment was carried out in triplicate and a Liquid Scintillation Analyser (PACKARD) used to count the radio-labelled samples.

Thin Layer Chromatography

Thin layer chromatography (TLC) was carried out to separate the carotenoids labelled following incubation with the different ^{14}C-substrates. Commercial TLC plates were used. Prior to application of the radio-labelled carotenoid extracts, the TLC plates were activated in an oven at 110°C for 2 hr. Carotene (25 ml) standard were applied in individual lanes on the TLC plate. Radio-labelled samples (100 μl) were applied with carrier standards on each allocated lane on the TLC plate. The plates were developed using a double solvent system. They were first developed in petroleum ether/diethyl ether at 80:20 (v/v) ratio to separate the polar compounds. The plates were left to dry in the fume cupboard for approximately 20 min before developing a second time in the same dimension in petroleum ether/diethyl ether in the ratio 98:2 (v/v) to separate the non-polar compounds.

Once the solvent vapours had evaporated, the plates were stained in a glass tank containing saturated iodine vapour for approximately 15-20 min in the dark. The individual bands were scraped into scintillation vials containing 10 ml scintillation cocktail. A Liquid Scintillation Analyser (PACKARD) was used to count the radio-labelled samples. All experiments were carried out in triplicate.

Statistical Analysis

All the data presented in this study were analysed by one-way analysis of variance (ANOVA) with the SAS software package (Bender, 1982). Unless otherwise stated, all significant differences were accepted at the 5% level of probability ($p<0.05$).

RESULTS AND DISCUSSION

Spectrophometric Profiles

The spectral profiles of the oil palm carotenoid extracts in 300-750 nm wavelength from fruits of 15 to 20 WAA are shown in *Figure 1*. The profiles reflect the different colour stages of the oil palm fruit characterizing its progressive development. At stage I (12 WAA), with the inflorescence still enclosed within its spate, the fruits would hardly have developed much carotenoids or chlorophyll from the little light received. At stage II (12-14 WAA), increases in chlorophyll and carotenoids were observed with the spectral profiles characterized by peaks of chlorophyll (663 nm) and carotenoids. The carotenoids absorbed at both the UV and visible range. Maximum absorbance in the UV region was observed at 278 nm which probably corresponds to phytoene, the first carotenoid in the (carotenoid) pathway. At Stage III (15 - 18 WAA), there was a steady increase in carotene and a decline in chlorophyll. There was only negligible absorbance in the UV region at 278 nm, indicating little phytoene, possibly with most of it converted to α- and β-carotenes. Stage IV (19 - 20 WAA) saw striking increases in the carotenoids, especially β-carotene, while the chlorophyll pigments fell below detection. The overall maximum absorbance in the visible range is attributed to α- and β-carotenes. Three

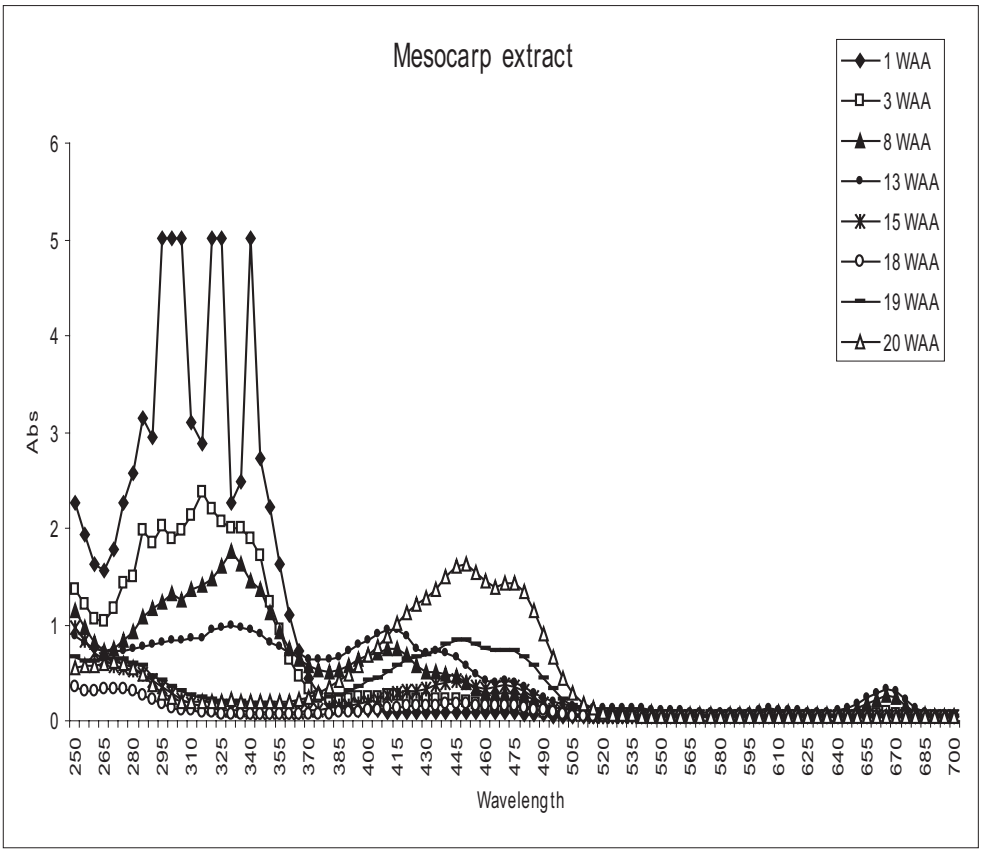

Figure 1. Spectral profile of oil palm carotenoid extracts from mesocarp at various stages of development.

absorbance peaks were observed in the visible region - at 423 nm, 446 nm and 472 nm.

Carotene Content at Different Stages of Development

Spectrophotometry provides a quick, easy and highly accurate method for determination of total carotene content. The carotene content in palm oil is usually expressed per unit weight of oil. The lipids were thus extracted from mesocarp tissue and the carotene content determined using PORIM Test Methods. *Table 1* represents the total carotenoid content in commercial *tenera* oil from fruits of 15 - 20 WAA. Carotene gradually increased from 15 to 18 WAA, then abruptly doubled after 18 WAA. This is similar to oil synthesis in the mesocarp which starts at 15 WAA and reaches the maximum at 20 WAA.

Carotene Content in Different Genotypes

MPOB has the largest oil palm germplasm collection in the world. The oil from several hundred of these palms were screened for total carotene content in their fully ripe fruits, *i.e.*, 20 WAA. *Table 2* shows some sample results. There was a very large variation in the carotene levels - from 242 ppm to almost 3300 ppm – suggesting sufficient variation for breeding improvement. In refining palm oil, the carotenoids are destroyed/bleached to produce a yellow oil to satisfy consumer preference for a light coloured cooking oil. Thus, producing a low carotene oil would reduce the refining cost. On the other hand, α- and β-carotenes have considerable value as nutraceuticals or health supplements, for which high carotene palms can be bred without recourse to genetic manipulation and its attendant controversies. The carotene content in the current unrefined commercial palm oil is 500-600 ppm.

HPLC Analysis

Separation of the carotenoids extracted from mesocarp at 20 WAA was optimized on both the C18 and C30 stationary phases. The mobile phases for the optimized separations were different for the two

TABLE 1. CAROTENE CONTENTS IN PALM OIL FROM MESOCARP OF VARIOUS AGES FROM COMMERCIAL OIL PALM

Age of fruits (WAA)	15 WAA	16 WAA	17 WAA	18 WAA	19 WAA	20 WAA
Carotene content	101.00 ^{E}	115.00 ^{E}	236.00 ^{D}	305.00 ^{C}	457.00 ^{B}	575.00 ^{A}

Note: Each value is the means of triplicate determination. Means with different superscripts are significantly ($p< 0.05$) different.

TABLE 2. CAROTENE CONTENTS IN PALM OIL FROM SOME SAMPLE GENOTYPES IN THE MPOB OIL PALM GERMPLASM COLLECTION

Serial No.	Trial/palm No.	Origin/ cross	Parts per million (ppm)
10803	0.293/614	T	591.9
10877	0.365/168	T	584.6
10884	0.365/229	T	241.8
10887	0.365/235	T	806.0
10899	0.365/332	T	758.1
10902	0.365/376	T	994.3
10916	0.365/495	T	1190.2
10920	0.365/661	T	1044.8
10928	0.365/719	T	1127.8
10945	0.360/2011	D	1736.9
10962	0.360/1895	D	2736.8
10999	0.359/386	D	1569.4
11002	0.359/381	D	2376.3
11004	0.359/437	D	2314.0
11006	0.359/441	D	3293.8
11007	0.359/434	D	2486.9
11025	0.359/425	T	2639.2
11027	0.359/414	D	2471.8
11149	0.290/315	D	2009.5
11150	0.290/261	D	2238.3
11153	0.290/251	D	2548.9

Note: T – *tenera*.
D – *dura*.

stationary phases and are as described in the Experimental Procedure. C18 phases have been routinely used for carotenoid separation but while they are able to separate the major components based on structural isomers they do not efficiently separate the geometrical isomers which have different physiological properties. Zechmeister (1962) and Sweeney (1973) provided strong evidence that the pro-vitamin A activities of the *trans*-isomers of α- and β-carotenes are two-fold more than those of the corresponding *cis*-isomers. Therefore, proper separation of the *cis*- and *trans*-isomers is necessary to properly determine the pro-vitamin activity of a biological sample including palm oil extracts.

Figures 2 and *3* show that the C-30 stationary phase effected superior separations of oil palm carotenoids than the C18 phase. The C30 column was engineered primarily to separate carotenoid isomers (Sander *et al*., 1994). Because of its shorter chain length, the C18 column does not have enough partitioning power to resolve the structurally similar isomers of long-chain molecules. The geometrical isomers (*cis* or *trans*) of α-carotene and β-carotene were successfully separated on the C30 stationary phase. This column (and optimized mobile phase) was thus used for further HPLC separations of extracts from oil palm mesocarp at various stages of development and the results shown in *Figures 4* to *8*.

Figure 9 shows a three-dimensional chromatogram of a carotenoid extract at 20 WAA. Identification of carotenoids in each extract was based on the chromatographic behaviour, absorption spectra and retention time. The ability of the photo diode array detector to simultaneously provide complete spectra of peaks separated provided information for tentative peak identification. The carotenoids from developing oil palm mesocarp (15 - 18 WAA) absorbed strongly in the UV range, but they decreased with fruit ripening (19 WAA onwards), replaced by carotenoids that absorb strongly in the visible range.

The major carotenoids identified in all the extracts were α- and β-carotenes. The limiting factor to this work was the non-availability of commercial

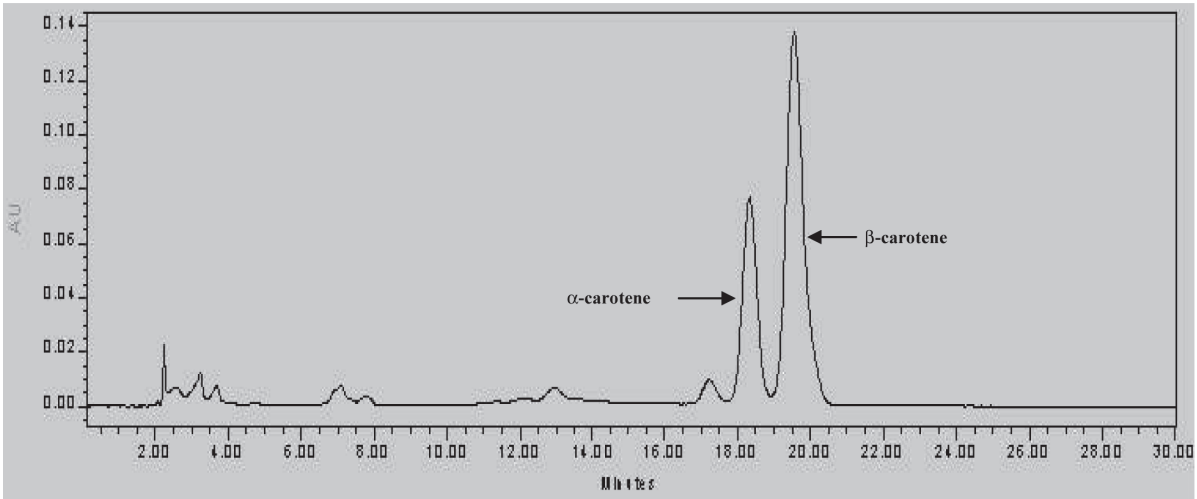

Figure 2. Separation of saponified 20 WAA extract on the C18 genesis column with mobile phase ACN:MeOH:DCM (70:10:20, v/v/v).

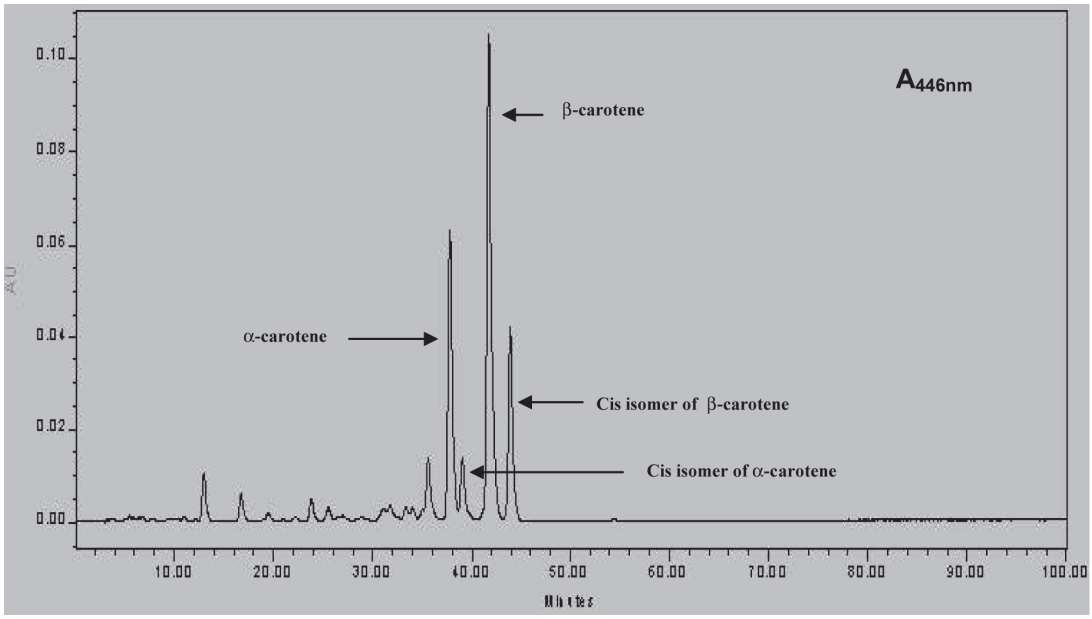

Figure 3. Separation of saponified 20 WAA extract on the C30 Bischoff column with mobile phase TBME:MeOH:H_2O in ratio 15:81:4 to 90:6:4 v/v/v (gradient).

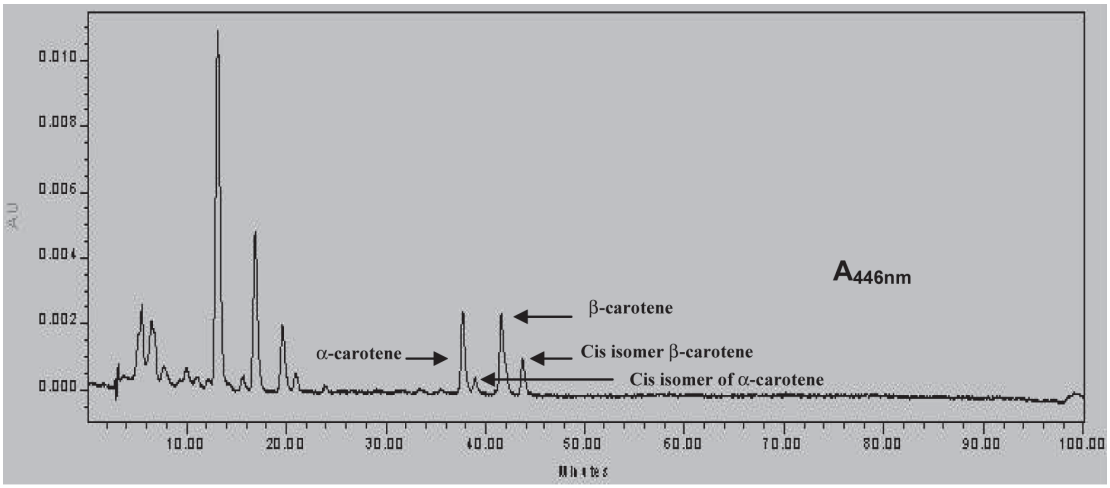

Figure 4. Separation of saponified 15 WAA extract on the C30 Bischoff column with mobile phase TBME:MeOH:H_2O in ratio 15:81:4 to 90:6:4 v/v/v (gradient).

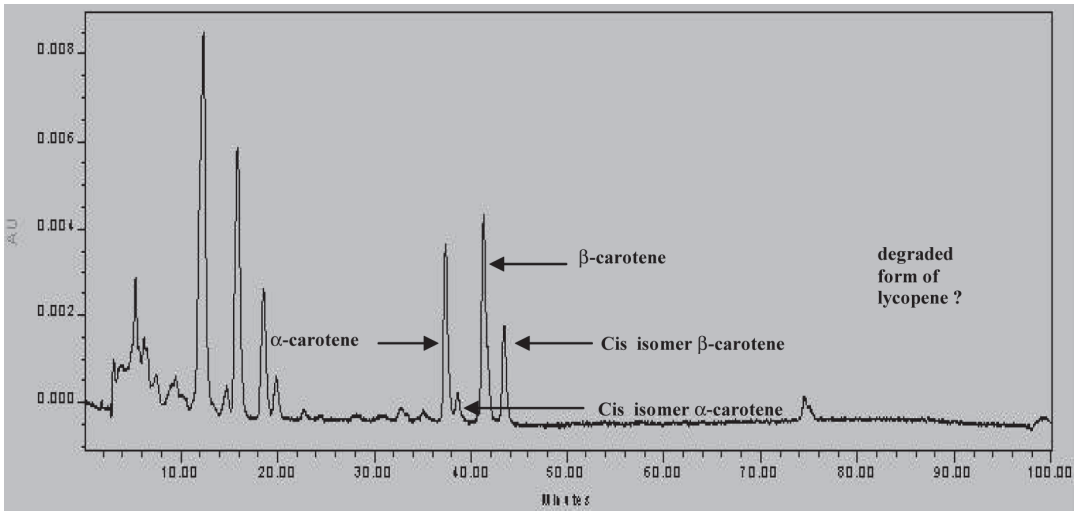

Figure 5. Separation of saponified 16 WAA extract on the C30 Bischoff column with mobile phase TBME:MeOH:H_2O in ratio 15:81:4 to 90:6:4 v/v/v (gradient).

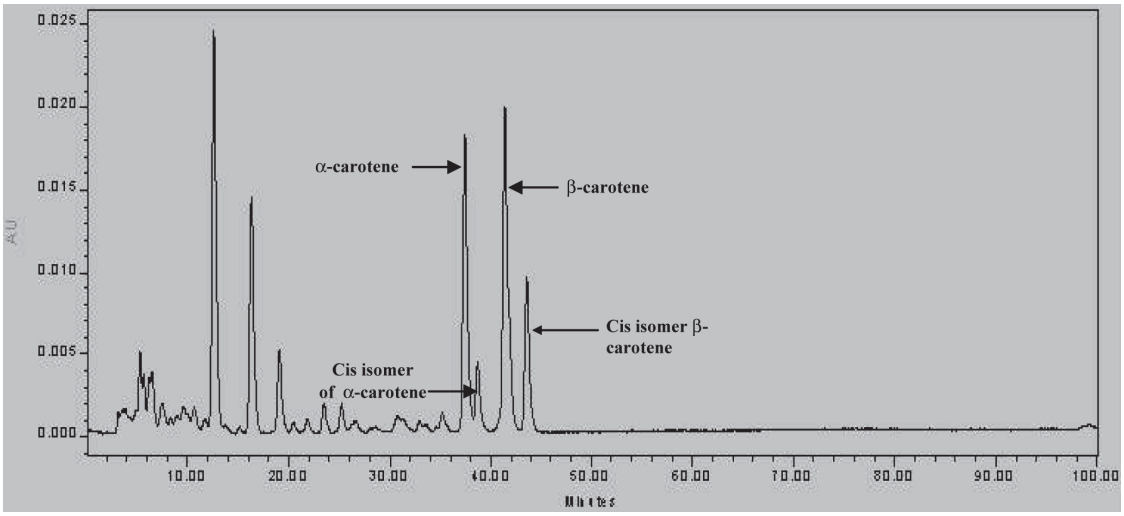

Figure 6. Separation of saponified 17 WAA extract on the C30 Bischoff column with mobile phase TBME:MeOH:H_2O in ratio 15:81:4 to 90:6:4 v/v/v (gradient).

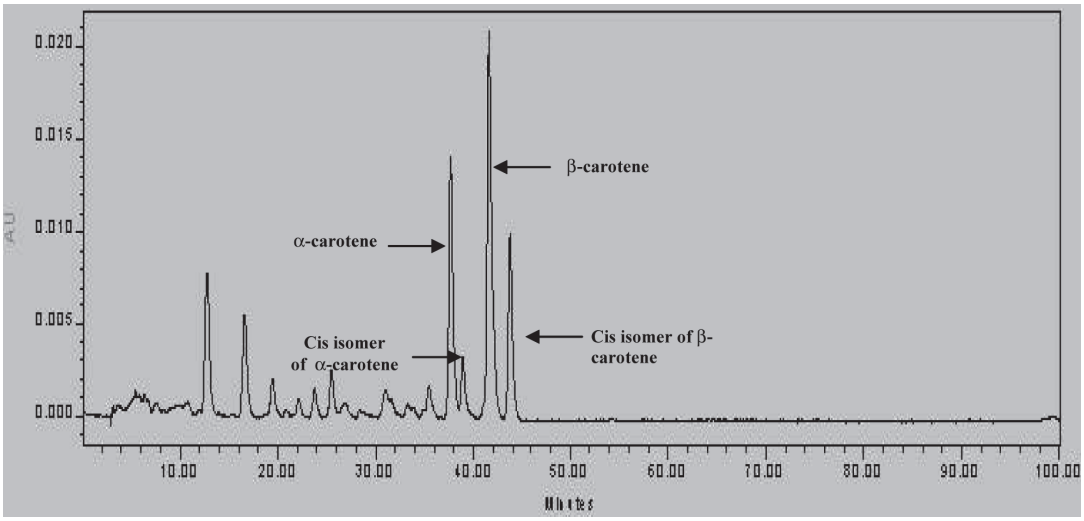

Figure 7. Separation of saponified 18 WAA extract on the C30 Bischoff column with mobile phase TBME:MeOH:H_2O in ratio 15:81:4 to 90:6:4 v/v/v (gradient).

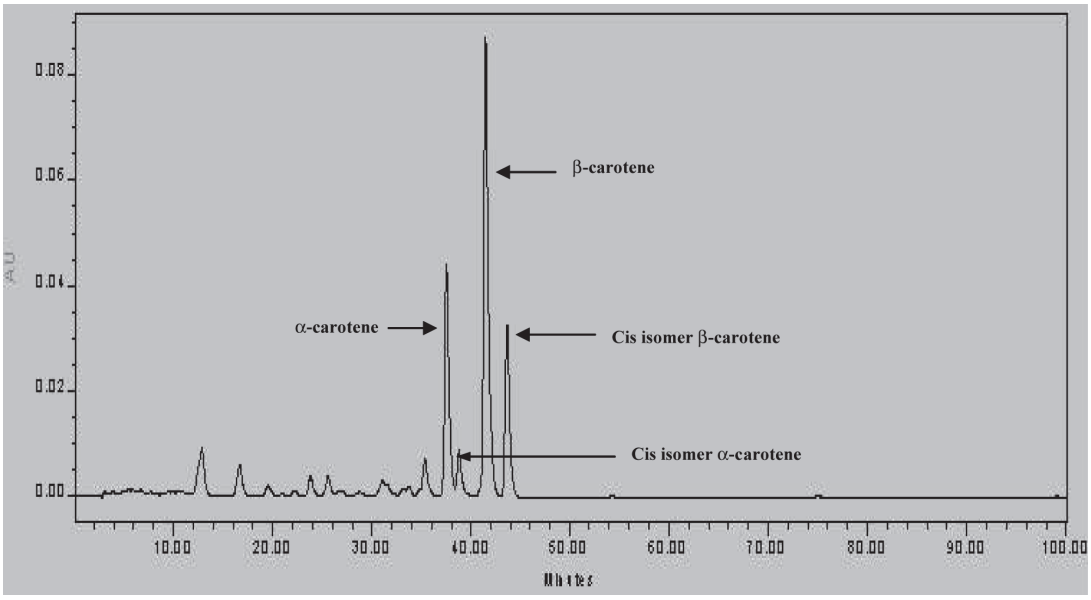

Figure 8. Separation of saponified 19 WAA extract on the C30 Bischoff column with mobile phase TBME:MeOH:H_2O in ratio 15:81:4 to 90:6:4 v/v/v (gradient).

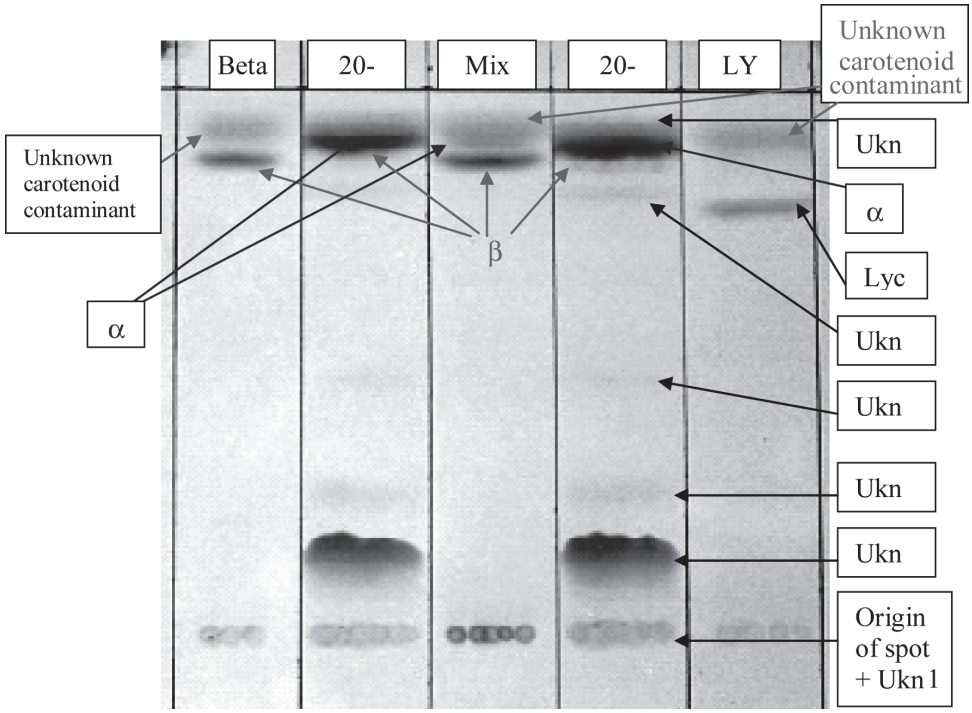

Figure 9. Thin layer chromatography (TLC) separation of saponified 20 WAA extract on a commercial plate developed with PE:DE (80:20 v/v) first, before being developed a second time in the same orientation in PE:DE (98:2 v/v).

Notes:
Beta : β-carotene standard.
20-WAA : carotenoid sample extracted from oil palm mesocarp 20 weeks after anthesis (WAA).
Mix : standard mixture of α- and β-carotenes (1:2).
LYC: lycopene standard.
α : α-carotene.
β: β-carotene.
Lyc: lycopene.
Ukn: unknown (e.g. Ukn 1 stands for Unknown No.1).
Sample volume: 100 µl.
Standard volume: 25 µl.

carotene standards except those for α- and β-carotenes and lycopene. This prevented identification of several of the earlier eluting peaks, the heights of which decreased gradually with maturity of the mesocarp while the *cis*- and *trans*-isomers of α- and β-carotenes increased greatly. Lycopene was not found in any of the carotenoid extracts, suggesting that the oil palm has very active β- and ε-lycopene cyclases that very effectively convert lycopene to α- and β-carotenes. Since lycopene is a valuable nutraceutical, a strategy could be to silence the β- and ε-cyclases to increase lycopene in palm oil.

In the 16 WAA extract (*Figure 5*), a minor peak was detected at 74 min, which correlated with the retention time of the lycopene standard. However, the absorbance profile of the minor peak did not match that of the lycopene standard. The minor peak may thus have been a degraded form of an unknown component. In any case, the minor peak was detected only in minute amounts (<0.0005 AU) and can be ignored. It was not found in all the other extracts at other stages of development.

Incorporation Studies

Until the late nineties, it was assumed that carotenoid biosynthesis occurred mainly through the acetate/mevalonate pathway (Goldstein and Brown, 1990; Bach *et al*., 1999). However, current evidence suggests that there is an alternative pathway - the pyruvate/G3P pathway, also known as the non-mevalonate pathway or deoxy-xylulose 5-phosphate (DxP) pathway (Lichtenhaler *et al*., 1997).

Incorporation of ^{14}C-labelled substrates was carried out to determine the flow of carbon in the carotenoid biosynthesis pathway and to adduce evidence on which pathway is used in the oil palm. *Table 3* gives the mean incorporation of each of the substrates into the saponified carotenoid extract from 20 WAA mesocarp. The average recovery of the ^{14}C-substrates was only about 80% due to unavoidable losses from the experimental procedure. Isopentenyl pyrophosphate (IPP) was the most incorporated of the five substrates. Duncan's multiple range test showed a significant difference ($p<0.05$) between IPP and the other substrates, but no significant difference

TABLE 3. RATES OF INCORPORATION OF ^{14}C-SUBSTRATES INTO THE SAPONIFIED CAROTENOID EXTRACT FROM 20 WEEKS AFTER ANTHESIS OIL PALM MESOCARP

^{14}C-substrate	Incorporation into non-saponifiables (%)*
Acetate	1.1^{B}
Glyceraldehyde-3-phosphate (G3P)	0.7^{B}
Isopentenyl pyrophospate (IPP)	8.9^{A}
Mevalonic acid/mevalonate (MVA)	1.4^{B}
Pyruvic acid/pyruvate (Py)	0.9^{B}

Notes: *Each figure is the mean of two measurements. Means with different superscripts are significantly ($p< 0.05$) different.

($p>0.05$) between the other four. IPP is the building block in the biosynthesis of isoprenoid, and it is not surprising that it was the most incorporated.

The incorporation products were subjected to TLC and the bands corresponding to α- and β-carotenes and lycopene based on the carrier standards (*Figure 9*) were scraped and their radioactive counts determined (*Table 4*). There was considerable incorporation of ^{14}C-acetate, ^{14}C-IPP and ^{14}C-MVA into the α- and β-carotene fractions. However, ^{14}C-G3P and ^{14}C-Py were not incorporated, suggesting that the oil palm uses the acetate/mevalonate pathway.

The results have to be treated with caution because radioactive incorporation studies may give rise to errors. It has to be borne in mind that sterols are also non-saponifiables and their presence even in small amounts can introduce errors in radioactive incorporation data.

Although the evidence remains tentative, it is suggested that in the oil palm, pyruvate is converted to acetyl-CoA which then feeds into the acetate/mevalonate pathway. Since the pyuvate used in this experiment was labelled at the C-1 position, its conversion to acetyl-CoA would result in its loss as $^{14}CO_2$ with unlabelled carotene formed (*i.e.*, α- and β-carotenes). When ^{14}C-G3P was used, the label was not found in the α- and β-carotene fractions even though it was universally labelled. This is further evidence that α- and β-carotene syntheses in oil palm follow the acetate/mevalonate pathway. There was no incorporation of any of the substrates into lycopene confirming that lycopene is not present or present only in negligible amounts in the oil palm.

TABLE 4. INCORPORATION OF VARIOUS ^{14}C-SUBSTRATES INTO DIFFERENT CAROTENOID FRACTIONS OF 20 WEEKS AFTER ANTHESIS EXTRACTS

Carotenoid fraction	^{14}C-Acetate (%)	^{14}C-G3P (%)	^{14}C-IPP (%)	^{14}C-MVA (%)	^{14}C-Py (%)
Ukn 1	22.0^{E}	24.7^{C}	28.3^{B}	63.1^{A}	24.6^{D}
Ukn 2	49.3^{D}	71.6^{A}	65.3^{B}	28.4^{E}	59.0^{C}
Ukn 3	10.2^{A}	2.2^{D}	3.8^{B}	3.3^{C}	1.6^{E}
Ukn 4	1.2^{B}	0.6^{C}	0.4^{E}	0.5^{D}	1.6^{A}
Ukn 5	0.3^{C}	0.9^{B}	0.3^{C}	0	9.9^{A}
β-carotene	2.9^{A}	0	1.6^{B}	0.6^{C}	0
α-carotene	0.3^{A}	0	0.2^{B}	0.1^{C}	0
Ukn 6	13.8^{A}	0	0.1^{D}	4.0^{B}	3.3^{C}

Notes: Each value is the mean of two measurements. Means in the same row with different superscripts are significantly ($p< 0.05$) different.

CONCLUSION

Chlorophylls and carotenoids are synthesized in a co-ordinated manner in the oil palm mesocarp with mainly carotenoids in the ripe fruit and mainly chlorophyll in the young fruit. The mesocarp has a very variable carotene content in its various stages of development as well as in different genotypes. Genotypic variation of carotene content in palm oil from ripe fruits ranged from about 242 ppm to 3300 ppm.

The major carotenoids in the oil palm mesocarp are α- and β-carotenes. Lycopene was below detectable levels based on high performance liquid chromatography (HPLC), TLC and radio-incorporation studies. Lycopene has been touted as the nutraceutical of the millennium and the very high α- and β-carotene contents in oil palm makes the oil palm a good candidate for producing lycopene by metabolic engineering.

Preliminary information based on ^{14}C radio-incorporation studies strongly suggests that the acetate mevalonate pathway occurs in the oil palm mesocarp. Further work needs to be carried out to ascertain whether this is the only pathway used, or that both the acetate mevalonate and pyruvate/G3P pathways operate. Inhibition studies with mevinolin may help clarify the issue.

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