Journal of Oil Palm Research Vol. 18 December 2006 p. 253-259

PALM CAROTENOIDS PROFILE AS A QUALITY CONTROL TOOL FOR PALM CAROTENE PRODUCERS: INTRODUCING AN IMPROVISED METHOD BY HPLC-PHOTODIODE ARRAY AND A C_{30} COLUMN

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ABSTRACT

This study establishes a more detailed HPLC profile of palm carotenoids than that possible from reverse phase C_{18} column analysis that detects 11 types of carotenes but can only partially resolve the cis/trans *geometrical isomers. Carotenoid extracts of palm pressed fibre, crude palm oil (CPO) and commercial red palm olein (CRPo) were analysed by HPLC-photodiode array detection using a reverse phase C_{30} column and the palm carotenoids elution sequence was found to be lutein, neurosporene (*trans*), neurosporene (*cis*), α-zeacarotene (*cis*), α-zeacarotene (*trans*), α-zeacarotene (*cis*), phytoene, phytofluene, β-zeacarotene, 13 and 13' cis α-carotene, 13* cis *β-carotene,* trans *α-carotene, 9* cis *α-carotene,* trans *β-carotene, δ-carotene a (*cis*), δ-carotene b (*cis*), δ-carotene (*trans*), γ-carotene a (*cis*), γ-carotene (*trans*), γ-carotene b (*cis*), lycopene (*cis*) and lycopene (*trans*). However, ξ-carotene was not detected using this new method The run time for complete analysis was about 200 min.*

Keywords: palm carotenoid profile, C_{30} column; HPLC-PDA.

Date received: 4 July 2005; **Sent for revision:** 13 July 2005; **Received in final form:** 6 March 2006; **Accepted:** 1 August 2006.

INTRODUCTION

The HPLC carotenoids profile is used by local palm carotene producers as a qualitative monitoring tool on, for example, carotene concentrates or red palm oil. The current method uses a reverse phase C_{18} column (Tay and Choo, 2000), in which up to 11 types of palm carotenes and two *cis* isomers of the major carotenes, α- and β-carotenes can be detected. Separation of these carotenoids is based on structural differences, such as the conjugation of double bonds and end groups resulting in differences in polarity (Yap *et al*., 1991; Ng and Tan, 1988). This method, however, cannot achieve complete separation of the *cis* forms of the major carotenes. The use of a C_{30} column for HPLC-PDA palm carotene analysis was reported previously (Tay *et al*., 2002).

From various epidemiological studies, the *cis* forms of carotenes were found to have different physiological properties in their antioxidant (Jimenez and Pick, 1993; Levin and Mokady, 1994) and pro-vitamin A activities (Deming *et al*., 2002) and in their different bioavailaiblities (Deming *et al*., 2002; During *et al*., 2002). Because of the use of palm carotenes in health supplements, it is important that these *cis* forms are well resolved and displayed in the carotenoid profiles. This paper discusses improvements to the current HPLC palm carotene profiling that allow the *cis* forms of the major and other minor carotenes to be included within a single analysis.

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MATERIALS AND METHODS

Materials

CPO and palm pressed fibre were obtained from the Malaysian Palm Oil Board (MPOB) Experimental Palm Oil Mill in Labu, Malaysia. CRPo was purchased from a local supermarket. Hexane and ethanol used for saponification were of analytical grade from Merck, Darmstadt, Germany. The HPLC solvents, methanol and *tert*-butyl methyl ether (TBME), were of HPLC grade from Merck.

Extraction of oil from palm pressed fibre. The oil in palm pressed fibre was extracted with hexane in a Soxhlet apparatus for 5 hr. About 5 g of the oil were dissolved in 30 ml ethanol and then saponified with 5 ml 50% (w/v) potassium hydroxide in the dark and *in vacuo* at 45°C for 40 min. The saponified samples were then extracted with 50 ml portions of hexane until the supernatant became colourless. Butylated hydroxy toluene (0.01%) was added to minimize oxidation of the carotenes. The combined hexane extract was washed five times with 50 ml portions of distilled water and dried over anhydrous sodium sulphate *in vacuo*. All the procedures above were carried out within 2 hr under dim diffused light to prevent induced isomerization.

Extraction of carotenoids from CPO. The saponification of CPO and CRPo was carried out as described for palm pressed fibre to produce an unsaponifiable extract rich in carotenoids.

Instrumentation and HPLC analysis. The HPLC system consisted of an HP-1100 series on-line degasser connected to a HP 1100 series diode array detector (DAD) equipped with a HP Chemstation (Hewlett Packard, Wilmington, DE) for data processing. The spectral range covered was 190 – 800 nm. Scanning was obtained at 200 – 500 nm for palm carotenoids. The monitoring wavelengths selected were 444 nm, 347 nm (phytofluene) and 286 nm (phytoene). The isocratic separation consisted of mobile phase with 89:11 (v/v) methanol: *tert* methyl butyl ether (TBME) at a flow rate of 1 ml min^{-1} through a Develosil ODS-UG 5 mm polymeric C_{30} column protected by pre-columns with the same stationary phase. The carotenoid extracts from the palm pressed fibre, CPO and CRPo were dissolved in the mobile phase prior to injection into the HPLC system. Identification was based on comparison of the electronic absorption spectra with published data (Emenhiser *et al*., 1995; Davies, 1976). The same carotenoid extracts from palm pressed fibre were reanalysed by HPLC-PDA with an analytical reverse phase 5 μm C_{18} column under isocratic elution at a flow rate of 1 ml min^{-1} with a mobile phase of 89:11 (v/v) acetonitrile: dichloromethane.

RESULTS AND DISCUSSION

Figures 1 to *3* show the chromatographic carotenoid profiles of palm pressed fibre, CPO and CRPo unsaponifiable extracts, respectively. These chromatographic profiles were attenuated for portrayal of phytoene (detected only at 286 nm) and phytofluene (detected only at 347 nm) while the rest were monitored at 444 nm. *Tables 1* to *4* list the retention times and absorption maxima for the various peaks from *Figures 1* to *4*, respectively. Identification was based on the absorption maxima of previous studies (Darnoko *et al*., 2000; Sander *et al*., 1994). *Figure 4* shows the C_{18} carotenoid profile of the same palm pressed fibre sample. From a comparison of *Figures 1* and *4*, it is clearly seen that analysis with a C_{30} column achieved a better *cis*/*trans* separation for neurosporene, α-carotene, β-carotene, δ-carotene, γ-carotene and lycopene. However, ℑ-carotene was not detected. Phytofluene was not detected using the reverse phase C_{18} column, but detected and baseline resolved using the reverse phase C_{30} column. With the C_{18} column, phytofluene was usually found on the slope of the large β-carotene peak or coeluted (Yap *et al*., 1991). In the reverse phase C_{18} chromatographic profiles, the *cis*/*trans* isomers of carotene peaks were not well resolved because the separation was based on the structural features and was limited therefore in geometrical isomer separations. The *cis* carotene peaks in the C_{30} chromatographic profiles were assigned based on the *cis* peaks between 310 – 370 nm, but specific assignment of the *cis* position was not done because standards were not available. The chromatographic profiles of the carotene isomers from the three palm-based samples in *Figures 1 to 3* show that the palm carotenes elution sequence to be lutein, neurosporene (*trans*), neurosporene (*cis*), α-zeacarotene (*cis*), α-zeacarotene (*trans*), α-zeacarotene (*cis*), phytoene; phytofluene; β-zeacarotene; 13 and 13' *cis* α-carotene; 13 *cis* β-carotene, *trans* α-carotene; 9 *cis* α-carotene, *trans* β-carotene, δ-carotene a (*cis*), δ-carotene b (*cis*), δ-carotene (*trans*); γ-carotene a (*cis*); γ-carotene (*trans*); γ-carotene b (*cis*), lycopene (*cis*) and lycopene (*trans*). The complex mixture of peaks before lutein were presumed to be xanthophylls or carotene oxidation products. The types of *cis* geometrical isomers of carotenes were also observed to vary with each sample.

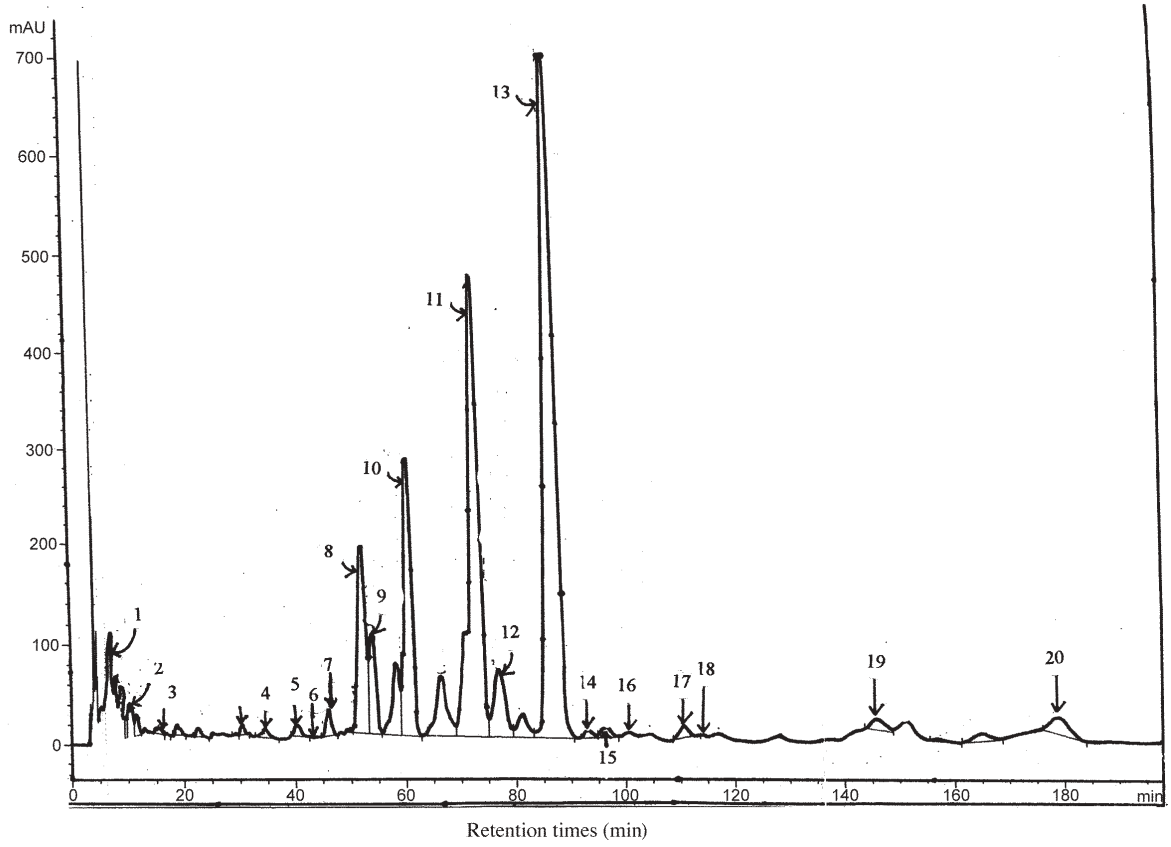

Figure 1. C_{30} chromatographic profile of carotenoid extracts from palm pressed fibres attenuated to display carotenes at various wavelength.
Note: Numberings of peaks corresponded to Table 1.

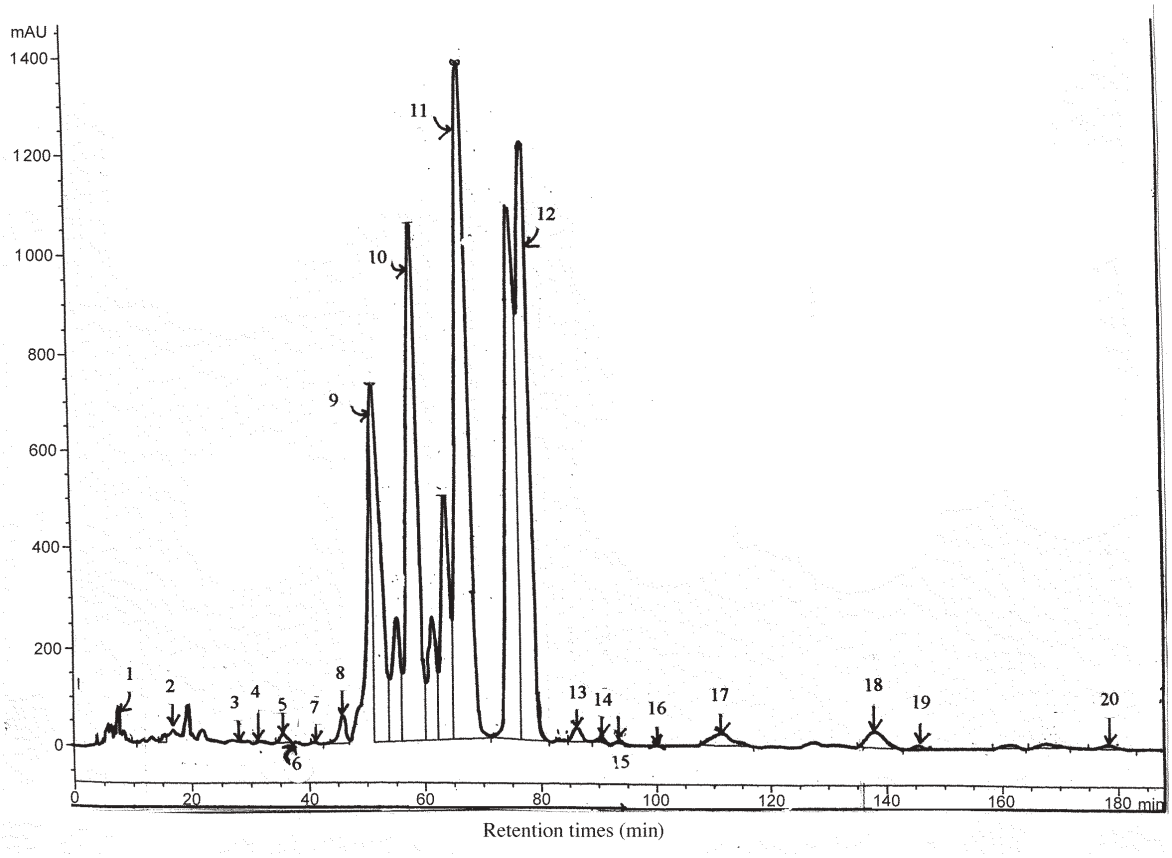

Figure 2. C_{30} chromatographic profile of CPO extract attenuated to display carotenes at various wavelength.
Note: Numberings of peaks corresponded to Table 2.

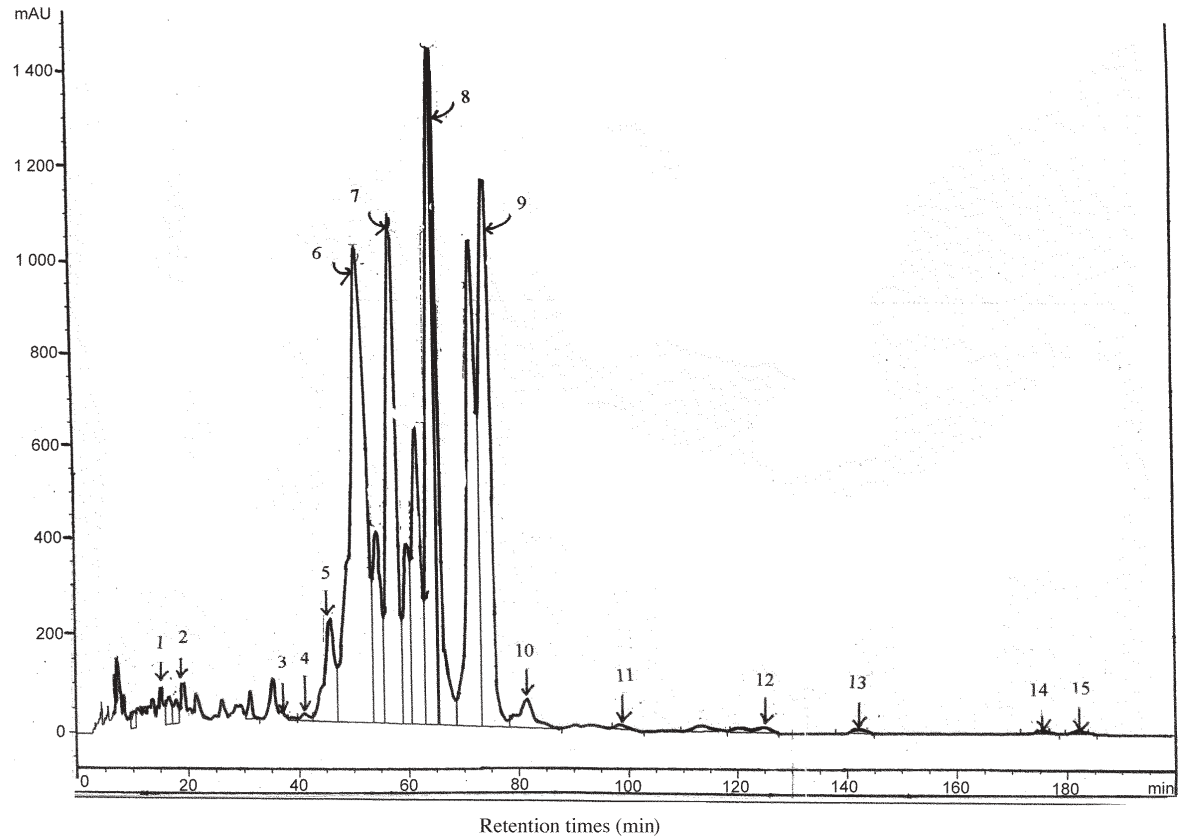

Figure 3. C_{30} chromatographic profile of CRPo extract attenuated to display carotenes at various wavelength. Note: Numberings of peaks corresponded to Table 3.

TABLE 1. ELECTRONIC ABSORPTION MAXIMA OF PEAKS OF CAROTENOID EXTRACTS FROM PALM PRESSED FIBRE (*Figure 1*)

No.	Retention time (min)	Absorption maxima (nm)	Carotenoid
1	6.7	420 444 475	lutein
2	11.4	416 438 468	neurosporene (*trans*)
3	15.7	*330* 414 436 467	neurosporene (*cis*)
4	34.5	398 420 448	α-zeacarotene
5	40.4	276 286 298	phytoene
6	42.5	326 347 368	phytofluene
7	46.0	412 429 453	β-zeacarotene
8	52.1	417 437 465	13 *cis* α-carotene
9	54.1	419 438 467	13′ *cis* α-carotene
10	60.6	*339* 423 444 470	13 *cis* β-carotene
11	73.1	418 440 468	*trans* α-carotene
12	77.1	*330* 412 440 469	9 *cis* α-carotene
13	87.0	423 448 475	*trans* β-carotene
14	93.3	*350* 434 458 481	δ-carotene (*cis*)
15	96.6	*345* 433 455 484	δ-carotene (*cis*)
16	100.4	428 459 489	δ-carotene (*trans*)
17	110.7	*350* 430 454 492	γ-carotene (*cis*)
18	117.1	428 459 489	γ-carotene (*trans*)
19	145.8	*360* 438 465 498	lycopene (*cis*)
20	178.5	435 464 495	lycopene (*trans*)

TABLE 2. ELECTRONIC ABSORPTION MAXIMA OF PEAKS OF CAROTENOID EXTRACTS FROM CRUDE PALM OIL (*Figure 2*)

No.	Retention time (min)	Absorption maxima (nm)	Carotenoid
1	8.4	418 440 472	Lutein
2	16.4	*330* 416 440 465	Neurosporene (*cis)*
3	27.5	*316* 403 423 446	α-zeacarotene (*cis*)
4	29.8	402 421 448	α-zeacarotene (*trans*)
5	35.2	*332* 408 428 455	α-zeacarotene (*cis*)
6	39.7	276 286 298	Phytoene
7	41.9	330 347 368	Phytofluene
8	45.8	408 430 459	β-zeacarotene
9	51.6	417 437 465	13 *cis* α-carotene
10	58.7	*339* 423 444 470	13 *cis* β-carotene
11	67.6	418 440 468	*trans* α-carotene
12	75.7	423 448 475	*trans* β-carotene
13	86.4	*338* 420 448 475	9 *cis* β-carotene
14	93.1	*350* 437 458 481	δ-carotene (*cis*)
15	96.4	*345* 433 455 484	δ-carotene (*cis*)
16	100.6	428 450 480	δ-carotene (*trans*)
17	111.3	*361* 438 460 490	γ-carotene (*cis*)
18	136.8	*360* 436 458 488	γ-carotene (*cis*)
19	145.8	*360* 438 465 498	lycopene (*cis*)
20	178.5	430 460 490	lycopene (*trans*)

TABLE 3. ELECTRONIC ABSORPTION MAXIMA OF PEAKS OF CAROTENOID EXTRACTS FROM COMMERCIAL RED PALM OLEIN (*Figure 3*)

No.	Retention time (min)	Absorption maxima (nm)	Carotenoid
1	16.5	415 440 475	neurosporene (*trans*)
2	17.8	*330* 415 440 467	neurosporene (*cis*)
3	40.9	276 286 298	phytoene
4	42.1	334 347 370	phytofluene
5	46.1	404 428 458	β-zeacarotene
6	51.5	417 437 465	13 *cis* α-carotene
7	54.6	419 438 467	13′ *cis* α-carotene
8	57.7	*339* 423 444 470	13 *cis* β-carotene
9	65.5	418 440 468	*trans* α-carotene
10	75.0	423 448 475	*trans* β-carotene
11	81.6	*332* 423 449 475	9 *cis* β-carotene
12	98.7	434 454 485	δ-carotene (*trans*)
13	120.3	432 464 490	γ-carotene (*trans*)
14	142.3	*310* 443 470 497	lycopene (*cis*)
15	180.9	*310* 444 465 500	lycopene (*trans*)

TABLE 4. ELECTRONIC ABSORPTION MAXIMA OF PEAKS OF CAROTENOID EXTRACTS FROM PALM PRESSED FIBRE (*Figure 4*)

No.	Retention time (min)	Absorption maxima (nm)	Carotenoid
1	27.3	440 468 495	lycopene
2	31.2	405 433 455	β-zeacarotene
3	34.1	416 440 469	neurosporene
4	35.9	429 455 484	δ-carotene
5	38.0	434 458 490	γ-carotene
6	41.6	382 403 428	ℑ-carotene
7	45.5	322 420 442 467	*cis* α-carotene
8	49.1	422 445 473	α-carotene
9	53.0	426 452 478	β-carotene
10	60.2	278 288 300	phytoene

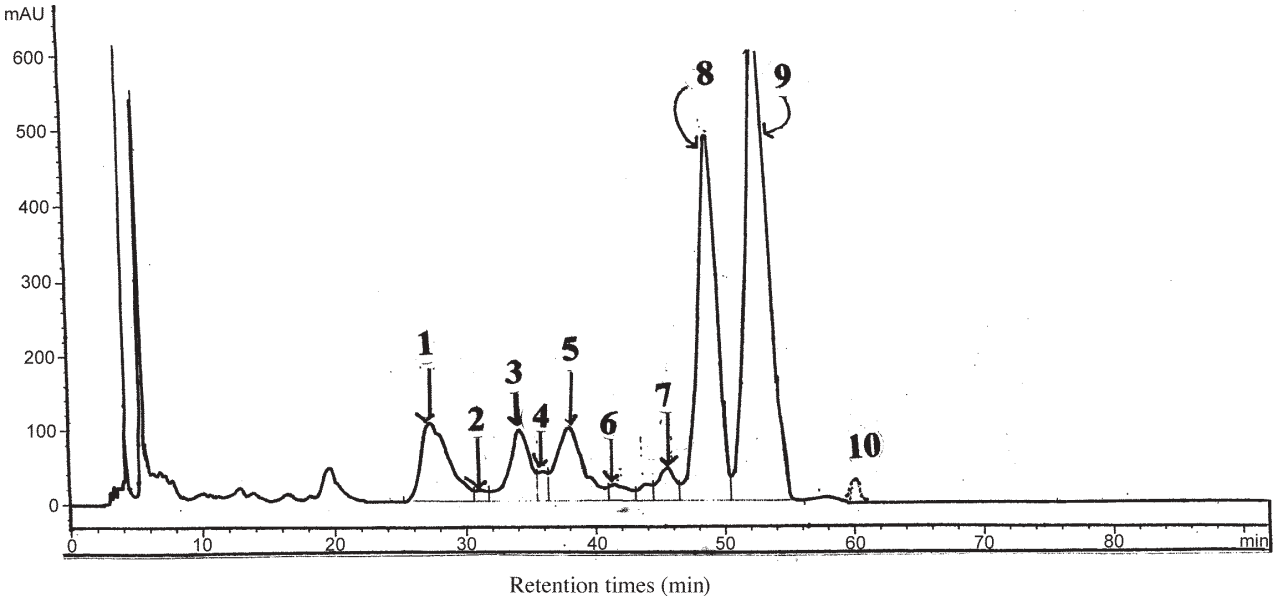

Figure 4. Reserved phase C_{18} chromatographic HPLC-PDA profile of carotene extract from palm pressed fibres. Note: Numberings of peaks corresponded to Table 4.

CONCLUSION

The reverse phase C_{30} column offers a far superior chromatographic separation than reverse phase C_{18} for carotenoid analysis because of its ability to achieve *cis*/*trans* geometrical separation and to separate carotenoids from a wide range of polarity (from polar xanthophylls to non-polar lycopene) and, therefore provides a more detailed qualitative profile of palm carotenoids. Currently, local producers of palm-based carotenes, use HPLC chromatographic profiles, together with the total carotene content as measured by a UV-spectrophotometer, as a quality control tool. With this new analytical procedure, additional information on *cis* forms of carotenoids become available. This information is useful in the light of knowledge that support the superiority of the *cis* form over the *trans* form in some important and favoured physiological activities. This new procedure is a more accurate determination of pro-vitamin A (as a function of the *cis* form activities of palm carotenoids) for use in nutraceuticals.

ACKNOWLEDGEMENT

The author would like to thank the Director-General of MPOB for permission to publish this paper.

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