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GEOMETRICAL ISOMERS OF THE MAJOR PROVITAMIN A PALM CAROTENES, α- and β-CAROTENES IN THE MESOCARP OIL OF FRESH AND STERILIZED PALM FRUITS, CRUDE PALM OIL AND PALM CAROTENE-BASED PRODUCTS: RED PALM OLEIN AND CAROTENE CONCENTRATES

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ABSTRACT

Polymeric C_{30} reversed-phased (RP) liquid chromatography (LC) columns were used to identify the geometrical isomers of the major carotenes from the mesocarp oil of fresh (MOFPF) and sterilized palm fruits (MOSPF), crude palm oil (CPO) and palm carotene-based products (red palm olein [RPOo] and carotene concentrates). In MOFPF, the isomers of α-carotene were all trans, *13-*cis, *13'-*cis *and 9-*cis *and the isomers of β-carotene all* trans *and 13-*cis. *MOSPF had similar geometrical isomers plus two unidentified* cis *α- and β-carotenes. CPO had the geometrical isomers of MOSPF plus 9-*cis*-β-carotene. The isomer profiles of carotenoid extracts from RPOo and carotene concentrates (CC1 and CC2) had similar patterns to that of CPO. The percentage compositions of total* cis*-isomers of α-and β-carotene in MOFPF, MOSPF, CPO, RPOo, CC1 and CC2 were 18.2%, 44.9%, 43.5%, 45.4%, 55.9%, and 38.8%, respectively.*

Keywords: geometrical isomers, mesocarp oil of palm fruits, crude palm oil, red palm olein, carotene concentrates.

NTRODUCTION

Trans α- and β-carotenes are the major carotenes in CPO and other palm carotene-based products (Tay and Choo, 2000; Ooi *et al.*,1994). The analyses of palm carotenoids are based on reverse-phase LC with C18 as the stationary phase (Yap *et al.*, 1991; Tay and Choo, 2001) as the column is able to separate most of the major carotenoids on their structural differences. However, it is not efficient for separating the geometrical isomers of asymmetrical carotenes (which have the *cis* bonds at the same carbon number but at the opposite end of their molecules). The geometrical isomers may well have different physiological properties but this aspect has yet to be explored thoroughly. The provitamin A activities of the *cis*-isomers of α- and β-carotenes and β-cryptoxanthin are only 50% or less than those of their corresponding all *trans* forms (Zechmeister, 1962; Sweeny and Marsh, 1973). Therefore, the proper determination of provitamin A activity for

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any biological sample should take into account the *cis* form. Lessin *et al*. (1997) were among the first to quantify the geometrical isomers of provitamin A carotenoids for fresh, processed fruits and vegetables.

Studies (Ben-Amotz *et al*., 1989; Mokady *et al*., 1990; Levin *et al*., 1994a; Levin and Mokady, 1994b) have shown that 9-*cis* β-carotene was preferentially absorbed and stored when a mixture of all *trans* and the *cis*-isomer was administered to rats and chicks. Recent human trials (Stahl *et al*., 1992; 1993; Tamai *et al.,* 1993) showed that when the diet was supplemented with an oily mixture of all *trans* β-carotene and its 9-*cis*-isomer, only a negligible amount of 9-*cis* was found in the blood but considerable amounts of 9-*cis* and all the *trans* forms (Stahl *et al.,* 1993) were present in the tissues. These studies suggest that the absorption, metabolism and distribution of β-carotene isomers may be different in animals and humans.

The antioxidant activity can be different for the geometrical isomers. The 9-*cis*-β-carotene has a higher potency than the all *trans* forms (Jimenez and Pick, 1993; Levin *et al*., 1994a; Levin and Mokady, 1994b). An *in vivo* animal study showed that the geometrical isomer mixture in the unicellular alga, *Dunaliella bardawil*, protected the central nervous system against oxygen toxicity. But the effect was not observed with the all *trans* or oxidized natural β-carotenes (Bitterman *et al.,* 1994).

Details on the geometrical isomers of the major carotenes in palm oil and its products are still lacking. The C_{30} stationary phase column gives outstanding shape selectivity towards the geometrical asymmetrical isomers in pure systems (Emenhiser *et al.,* 1995; 1996a), and also in biological extracts (Emenhiser *et al.,* 1996b). This study, using the C_{30} column, was therefore undertaken to identify the geometrical isomers of α- and β-carotenes in palm oil of various origins, such as MOFPF, MOSPF, CPO and palm carotene-based products like RPOo and carotene concentrates.

MATERIALS AND METHODS

Materials

Fresh ripe and sterilized palm fruits were collected from the Malaysian Palm Oil Board (MPOB) Experimental Palm Oil Mill in Labu. CPO was obtained from two local mills. RPOo was purchased from the local supermarket. The carotene concentrates were obtained from the experimental plant of MPOB (CC1) and Carotech Sdn. Bhd. (CC2). Hexane and ethanol used for saponification were of analytical grade from Merck, Darmstadt, Germany. The HPLC solvents, methanol and methyl *tert*-butyl ether (MTBE), were of HPLC grade from Merck. Butylated hydrotoluene (BHT) was from Sigma Aldrich Chemical Company, Steinheim, Germany.

Methods

Extraction of oil from fresh and sterilized palm fruit. A day after the fresh palm fruits were received, their mesocarp was cut away from the nuts and ground in a food processor. The ground mesocarp (1 kg) was weighed into a beaker and soaked in 4 litres hexane at room temperature for a day to extract the oil. BHT 0.01 % (w/v) was added to the extracting solvent to minimize degradation of the carotenes. For the sterilized fruit, the softened mesocarp was peeled off and 1 kg weighed into a beaker and soaked in 4 litres hexane at room temperature for one day. The oil samples were obtained after the hexane was removed *in vacuo*.

Extraction of carotenoids from mesocarp oil of fresh sterilized fruits and CPO. The oil samples kept in bottle containers were first homogenized by immersing in a water bath at 35°C - 40°C. About 3 g of the oil were dissolved in 30 ml ethanol and saponified with 5 ml 50% (w/v) potasium hydroxide (KOH) *in vacuo* at 35°C - 40°C for 40 min. The saponified samples were then extracted with 50 ml portions of hexane until the supernatant became colourless. To minimize oxidation of the carotene, the saponification mixture and extracting solvent were added with 0.01% BHT (w/v). The combined hexane extracts were washed five times with 50 ml portions of distilled water and dried over sodium sulphate. The carotene extracts were obtained after removing the hexane *in vacuo*. Since light promotes isomerization, the whole procedure was carried out under dim diffused light. The saponification reaction flask was wrapped in aluminium foil and the entire extraction carried out within 2 hr. The dried carotene extracts were stored at -20°C under a headspace of nitrogen and used within 72 hr. Prior to injection into the HPLC-DAD, the extracts were dissolved in 2-5 ml 89:11 methanol:MTBE mixture.

Instrumentation. Separation was done using two analytical scales (25 cm x 0.46 cm I.D.), DV1 and DV2, of the Develosil ODS-UG 5 µm polymeric C_{30} column protected by precolumns with the same stationary phase. The HPLC system consisted of a HP 1100 series quaternary solvent delivery system with a HP 1100 series on-line degasser and was connected to a HP 1100 series diode array detector (DAD) covering the spectral range 190 - 800 nm. The data were stored and processed by HP Chemstation.

HPLC analysis and peak identification. The HPLC separation was done isocratically using a mobile phase of 89:11 (v/v) methanol:MTBE at a flow rate of 1 ml min^{-1}. The chromatographic peaks were tentatively identified by comparison with a previous separation on a polymeric C_{30} column (Emenhiser *et al.*, 1996b) and by comparing their accumulated absorption visible light spectra (*Figures 1, 2* and *Table 1*) with the absorption maxima from previous studies (Emenhiser *et al.,* 1995; 1996a).

RESULTS AND DISCUSSION

Mesocarp Oil from Fresh Palm Fruit (MOFPF)

Figure 3 shows a typical separation of carotenoid extract from CPO performed on a C18 reverse phase column. The two major peaks between the retention times of 50 min and 65 min were assigned as all

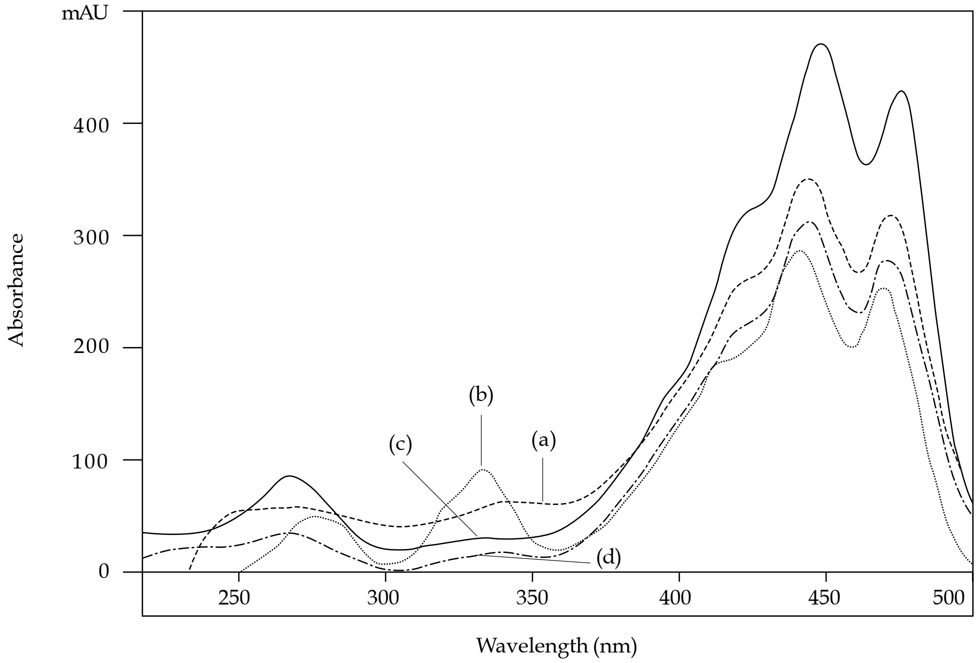

*Figure 1. Electronic absorption spectra of α-carotene isomers in MOFPF, MOSPF, CPO, RPOo and carotene concentrates, CC1 and CC2: a) 13-*cis*-α-carotene, b) an unidentified* cis*-α-carotene, c)* trans *α-carotene, and d) 9-*cis*-α-carotene.*

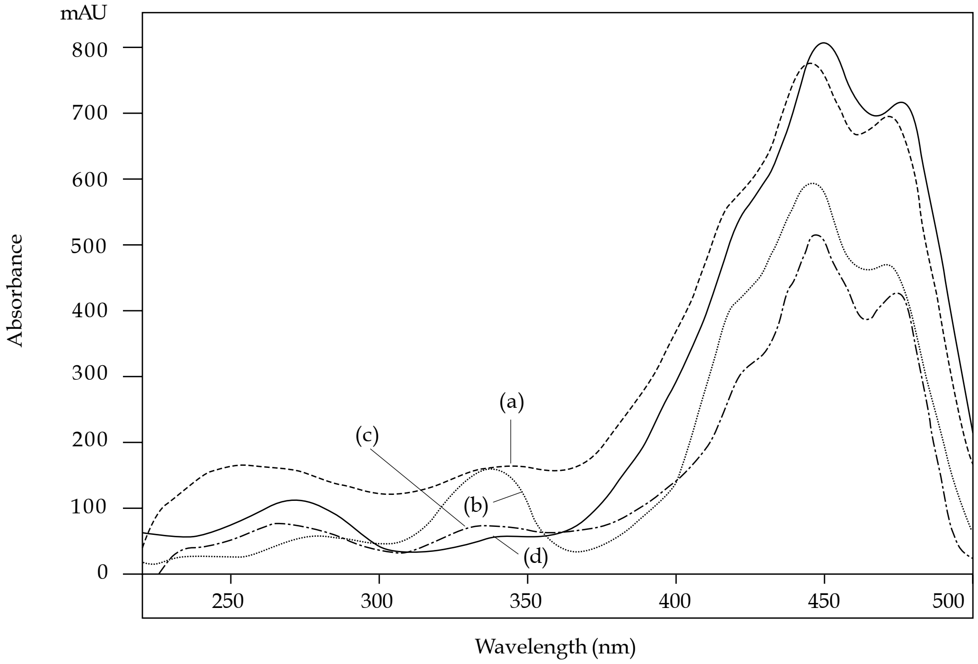

Figure 2. Electronic absorption spectra of β-carotene isomers in MOFPF, MOSPF, CPO, RPOo and carotene concentrates, CC1 and CC2: a) an unidentified cis*-β-carotene, b) 13-*cis*-β-carotene, c) 9-*cis*-β-carotene, and d)* trans *β-carotene.*

TABLE 1. ELECTRONIC ABSORPTION MAXIMA OF THE GEOMETRICAL ISOMERS OF α- AND β-CAROTENES IN MOFPF, MOSPF, CPO, RPOo AND CAROTENE CONCENTRATES

Isomer	**Absorption maxima (nm) [$\varepsilon_2/\varepsilon_2$]**[c]	
	This Study	**Previous Studies**[b]
13-*cis*- α-carotene	*332* 410 (440)[a] 463 [0.368]	*331* nd (438) 466
13'-*cis*- α-carotene	*331* 410 (440) 464 [0.378]	*331* nd (438) 465
13-*cis*- β-carotene	*338* 421 (444) 470 [0.438]	(443)
cis- α-carotene	*331* 420 (440) 468 [0.089]	-
trans α-carotene	nd 423 (445) 476 [0.056]	nd (445) 474
9- *cis*- α-carotene	*330* 412 (440) 469 [0.078]	*329* 420 (442) 470
cis- β-carotene	*338* 421 (445) 468 [0.094]	-
trans β-carotene	nd 422 (452) 478 [0.082]	(450)
9-*cis*- β-carotene	*338* 420 (448) 475 [0.056]	(444)

Notes:

[a] Values in parentheses represent the main absorption maxima.

[b] Absorption maxima for α-carotene isomers in MTBE (Emenhiser *et al.,* 1996a) and main absorption maxima for β-carotene isomers (Emenhiser *et al.,* 1995).

nd - no maxima detected.

[c] Ratio of absorption intensity (ε_2) at the near UV maxima (328-341 nm) to absorption intensity (ε_1) at the main absorption maxima (435 - 456 nm).

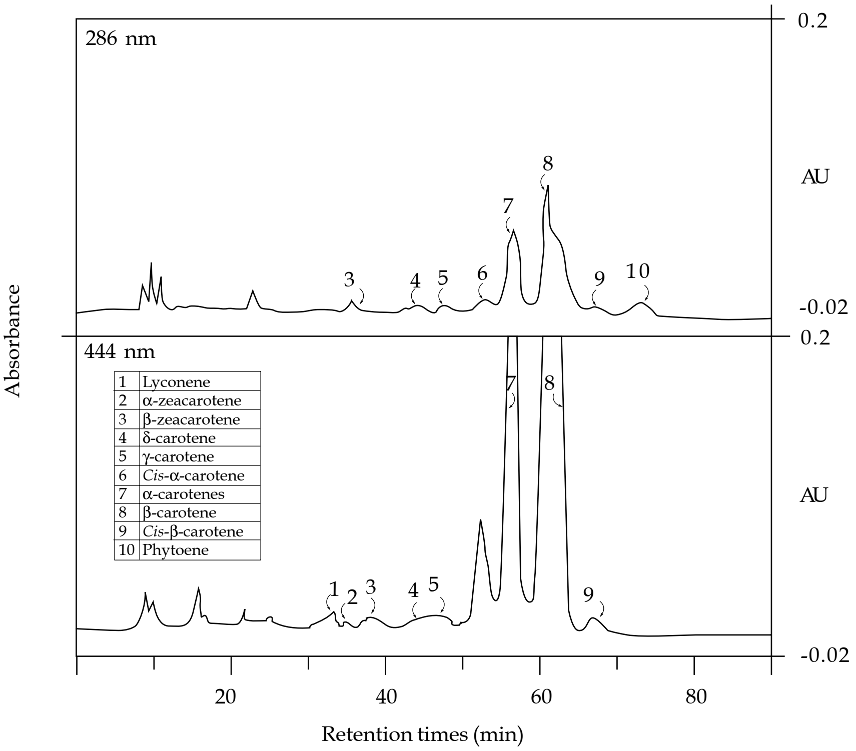

Figure 3. C_{18} chromatographic profiles of carotenoid extract from CPO at: a) 286 nm, and b) 444 nm. The mobile phase was 89:11v/v acetonitrile:methylene chloride at 1 ml min^{-1}.

trans α- and β-carotenes, respectively. But these peaks were asymmetrical and broad, and their visible spectra on the slopes different from on the apices, intimating that some structurally similar compounds, possibly geometrical isomers, were also eluted.

Figures 4a and *b* show the chromatograms of the carotenoid extract from MOFPF using the Develosil columns, DV1 and DV2, respectively. In *Figure 4a,* the isomers of α-carotene (13-*cis* and all *trans*) and β-carotene (13-*cis* and all *trans*), and in *Figure 4b,* two additional α-carotene isomers (13'-*cis* [the

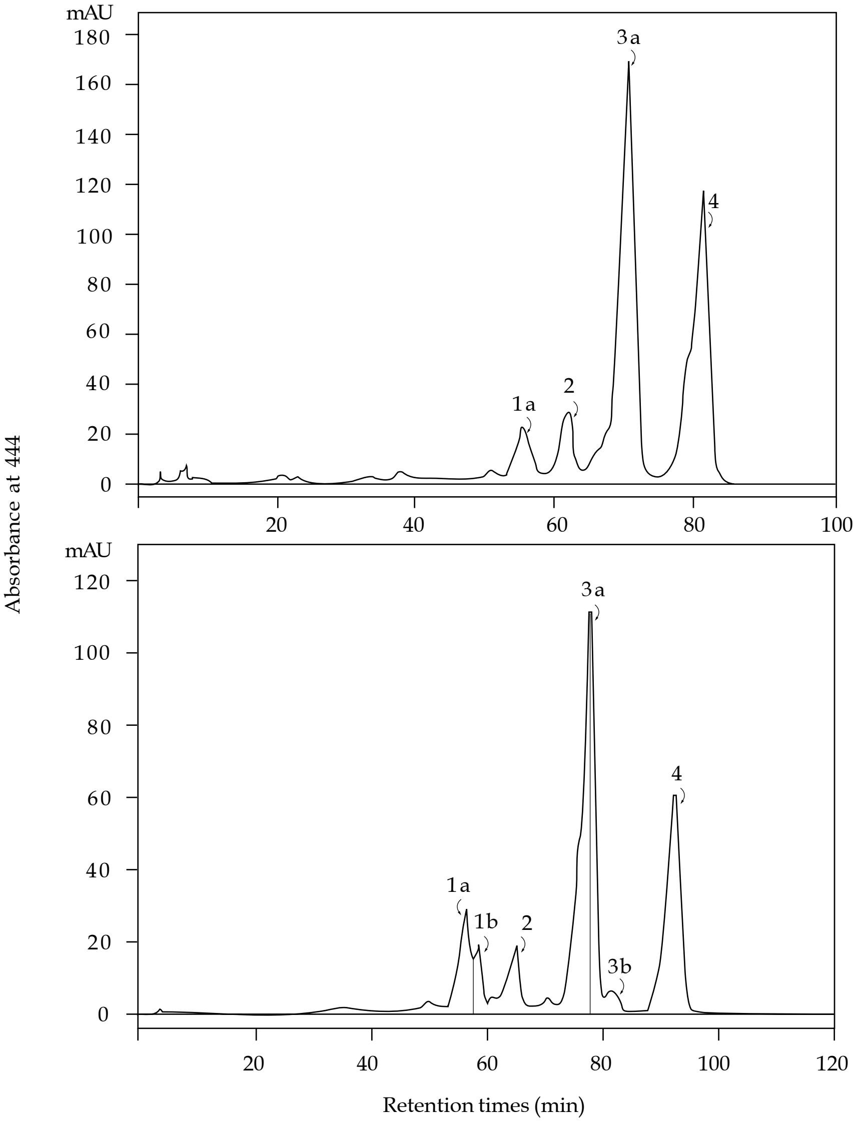

*Figure 4. C_{30} chromatographic profiles of carotenoid extract from MOFPF: a) DV1, and b) DV2. Tentative peak identifications: 1a) 13-*cis*-α-carotene; 1b) 13'-*cis*-α-carotene; 2) 13-*cis*-β-carotene; 3a)* trans *α-carotene; 3b) 9-*cis*-α-carotene, and 4)* trans *β-carotene.*

shoulder on the down slope of the 13-*cis*-α-carotene] and 9-*cis* [beside the all *trans*-α-carotene]), were tentatively identified. This showed that the separation performance for columns with the same stationary phase but from different batches can be different. Therefore, analysis with only one column will be insufficient to detect all the isomers.

Mesocarp Oil from Sterilized Palm Fruit (MOSPF)

In the sterilization of oil palm fruits, a high steam pressure of 3 kg cm^{-2} (the temperature of saturated steam at this pressure is 141.5°C) is applied for 60 - 72 min. High temperatures and pressures are detrimental to carotenes. *Figures 5a* and *b* show the C_{30} chromatographic profiles of carotenoids from MOSPF. The isomers of α-carotene (13-*cis*, 13'-*cis*, 9-*cis* and all *trans*) and β-carotene (13-*cis* and all *trans*) in MOFPF were also present in MOSPF. In *Figure 5a*, two additional unknown *cis* β- and α-carotenes are indicated. These carotenes were only detected when DV1 was used as the analytical column. Thus, the overall qualitative profile of the isomers was unchanged after the fruits were sterilized although additional isomers were formed.

Crude Palm Oil (CPO)

The next step after sterilization is digestion where heat and mechanical abrasion are applied to break the oil cells. In pressing out the oil, the fruits are subject to high heat (100°C) and pressure by the screw press. These are the conditions under which isomerization can occur, *e.g.* in thermal processing of vegetable carotenoids (Sweeny and Marsh, 1971; Chandler and Schwartz, 1988) and during extrusion

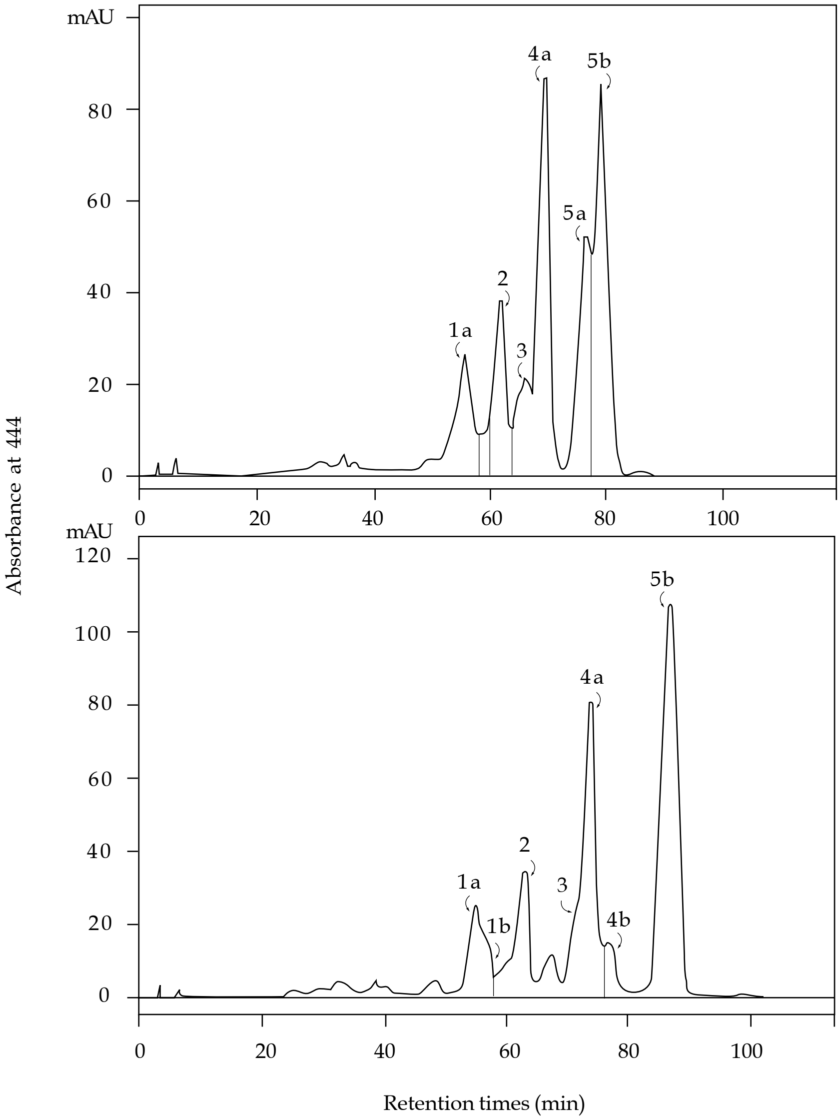

*Figure 5. C_{30} chromatographic profiles of carotenoid extract from MOSPF: a) DV1, and b) DV2. Tentative peak identifications: 1a) 13-*cis*-α-carotene; 1b) 13'-*cis*-α-carotene; 2) 13-*cis*-β-carotene; 3)* cis*-α-carotene; 4a)* trans *α-carotene; 4b) 9-*cis*-α-carotene; 5a)* cis*-β-carotene; and 5b)* trans *β-carotene.*

cooking (Marty and Berset, 1986).

Although the CPO analysed was from two sources, their carotene profiles were similar and thus only one profile is shown of the extracts (*Figures 6a* and *b*). All the isomers in MOSPF were also detected in CPO with the addition of 9-*cis* β-carotene. Thus, even after several stages of mill processing, all the original isomeric forms were still intact.

Carotene Concentrates

Two carotene concentrates, CC1 and CC2, were analysed. CC1 was produced by a process developed in MPOB. CPO was transesterified to alkyl esters, followed by molecular distillation of the volatile esters to produce a concentrate of >80 000 ppm carotene (Choo *et al.*, 1992). This concentrate contained little esters and it was possible to be injected directly into the HPLC system without any chromatographic pretreatment. *Figures 7a* and *b* show the C_{30} chromatographic profile of the isomers in CC1 with the same isomers of α- and β-carotenes as in CPO.

Figures 8a and *b* show the chromatographic profile for CC2 produced by transesterification of CPO followed by phase separation of the carotenoids from the ester-rich layer (Tan and Saleh, 1990). The isomer profile was similar to those of CPO and CC1. Both the processing treatments did not form additional geometrical isomers.

Red Palm Olein (RPOo)

RPOo is a new commercial palm carotene product, produced through a refining process

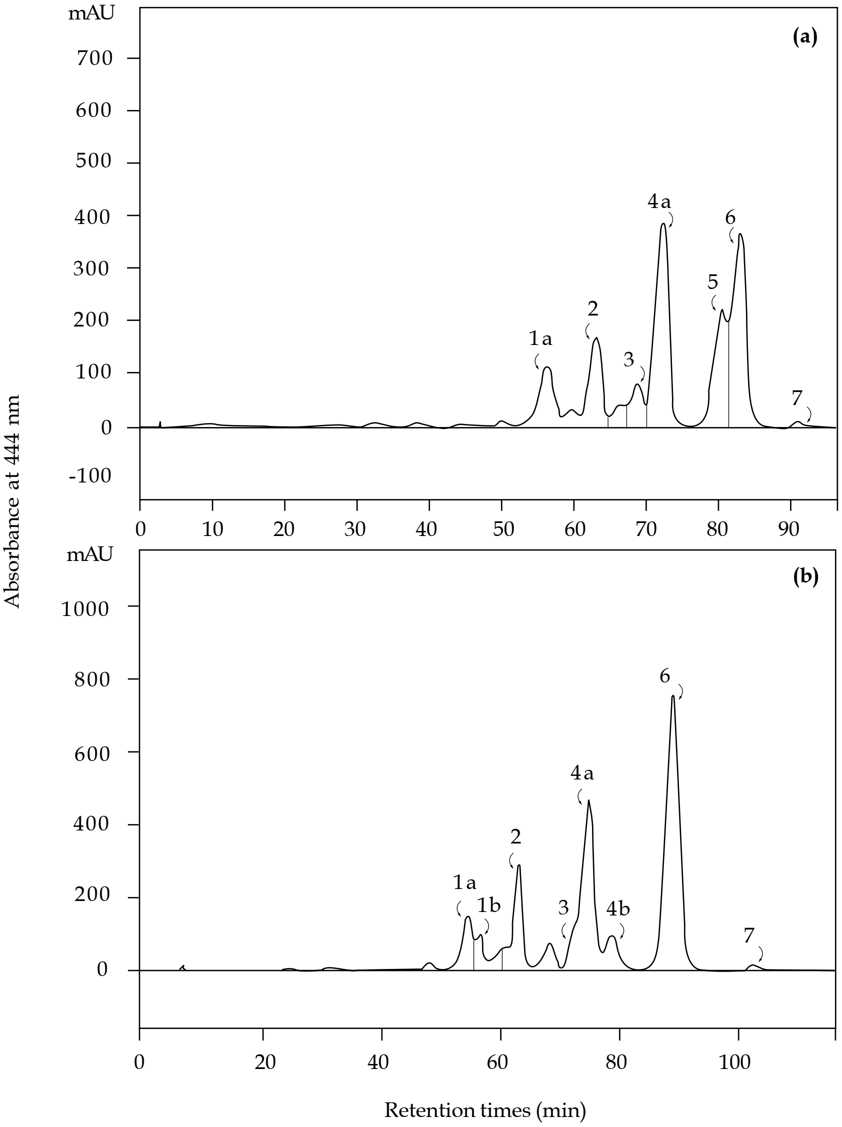

Figure 6. C_{30} chromatographic profiles of carotenoid extract from CPO: a) DV1, and b) DV2.
*Tentative peak identifications: 1a) 13-*cis*-α-carotene; 1b) 13'-*cis*-α-carotene; 2) 13-*cis*-β-carotene; 3)* cis*-α-carotene; 4a)* trans *α-carotene; 4b) 9-*cis*-α-carotene; 5)* cis*-β-carotene; 6)* trans *β-carotene, and 7) 9-*cis*-β-carotene.*

developed by MPOB. There are two stages - pretreatment of CPO followed by deodorization and deacidification by molecular distillation. The milder conditions in the second stage allow most of the carotenes to be retained. *Figures 9a* and *b* show the profile from RPOo in which the isomers of α- and β-carotenes in CPO and carotene concentrates were also observed.

Percentage Composition

Table 2 shows the percentage compositions of the α- and β-carotene isomers for MOFPF, MOSPF, CPO, RPOo and the carotene concentrates, CC1 and CC2. The total *cis* forms of α- and β-carotenes in MOFPF, MOSPF and CPO were 18.2%, 44.9% and 43.5%, respectively. For RPOo, the total *cis*-isomers was almost similar to those in MOSPF and CPO at 45.4%. Although the qualitative profiles of CC1 and CC2 were similar to that of CPO, their *cis*-isomer compositions were different at 55.9% and 38.8%, respectively. Isomerization to the *cis* forms was greater for CC1 than CC2, showing that the different processing treatments had affected the isomers quantitatively.

CONCLUSION

All *trans,* 13-*cis*, 13'-*cis* and 9-*cis*-α-carotenes and 13-*cis* and all *trans*-β-carotenes were ubiquitous among the main carotenoids of MOFPF, MOSPF, CPO, RPOo and palm oil carotene concentrates. Their occurrence in palm oil was not unexpected as various ratios of such geometrical isomers are known to occur in most biological samples. With sterilization, two unknown *cis* α- and β-carotenes

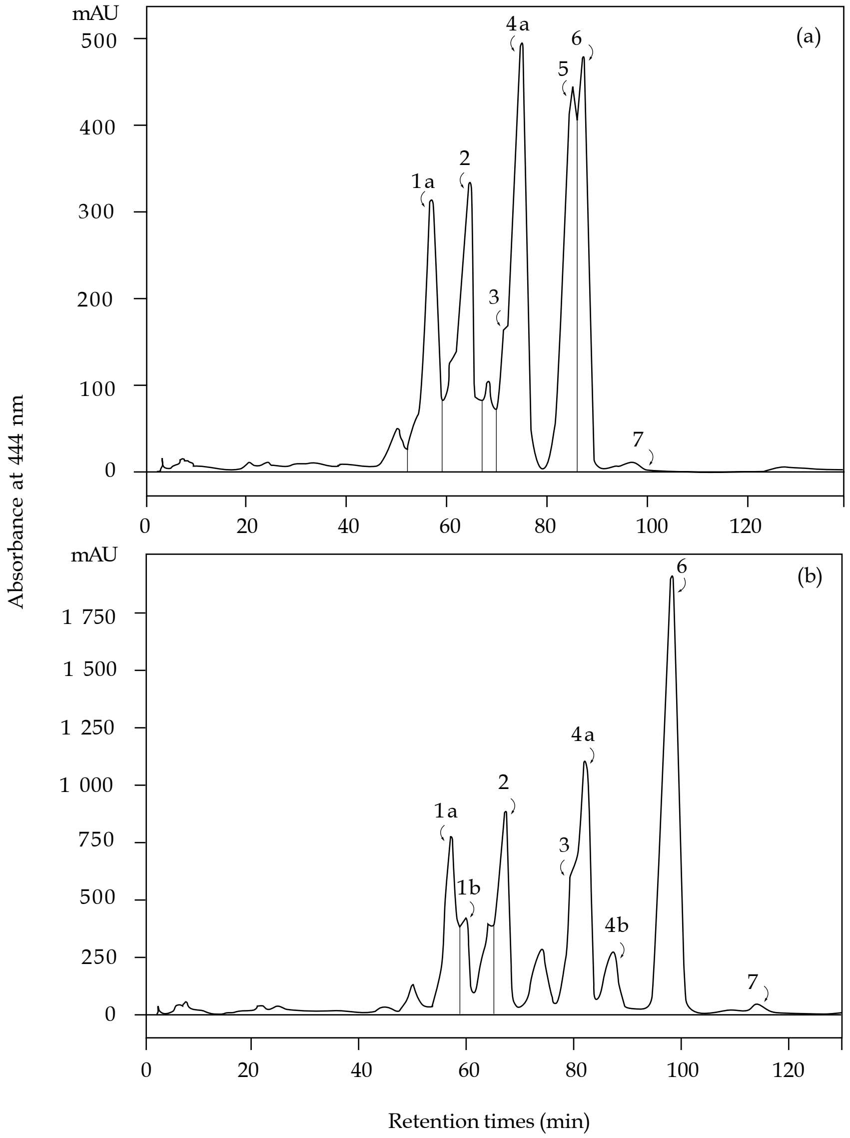

Figure 7. C_{30} *chromatographic profiles of carotene concentrate, CC1: (a) DV1, and (b) DV2.*
*Tentative peak identification: 1a) 13-*cis*-α-carotene; 1b) 13'-*cis*-α-carotene; 2) 13-*cis*-β-carotene; 3)* cis*-α-carotene; 4a)* trans *α-carotene; 4b) 9-*cis*-α-carotene; 5)* cis*-β-carotene; 6)* trans *β-carotene, and 7) 9-*cis*-β-carotene.*

TABLE 2. PERCENTAGE COMPOSITIONS OF α- AND β-CAROTENE ISOMERS FOR MOFPF, MOSPF, CPO, RPOo, CC1 and CC2

Carotene	**MOFPF**[a]	**MOSPF**[a]	**CPO**[a]	**RPOo**[b]	**CC1**[a]	**CC2**[a]
13-*cis*-α-carotene	9.9 ± 3.4	7.3 ± 0.4	9.5 ± 1.3	11.2 ± 2.3	14.5 ±1.0	7.5 ± 0.1
13-*cis*-β-carotene	8.4 ± 0.5	11.2 ± 1.2	13.5 ± 1.0	10.4 ± 1.6	15.8 ± 1.8	6.5 ± 1.2
cis-α-carotene	nd	6.4 ± 0.5	4.3 ± 0.2	8.0 ± 0.9	7.3 ± 0.3	6.7± 0.5
trans-α-carotene	49.6 ± 0.6	26.1 ± 2.1	26.4 ± 1.3	31.6 ± 1.5	23.2 ± 2.2	27.8 ± 0.7
cis-β-carotene	nd	20.1 ± 3.4	14.9 ± 0.6	15.6 ± 0.9	17.9 ± 2.9	16.3 ± 0.3
trans-α-carotene	32.2 ± 4.0	29.0 ± 0.5	30.1 ± 1.1	23.0 ± 3.2	20.9 ± 1.5	33.4 ± 0.8
9-*cis*-β-carotene	nd	nd	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.2

Notes:

[a]Means from four replicates ± standard deviation (SD). [b]Means from five replicates ± SD.

The percentage compositions were calculated from the carotene isomer profiles of DV1; therefore 13'- *cis* and 9-*cis* α- carotene were not included as they were only detected by DV2.

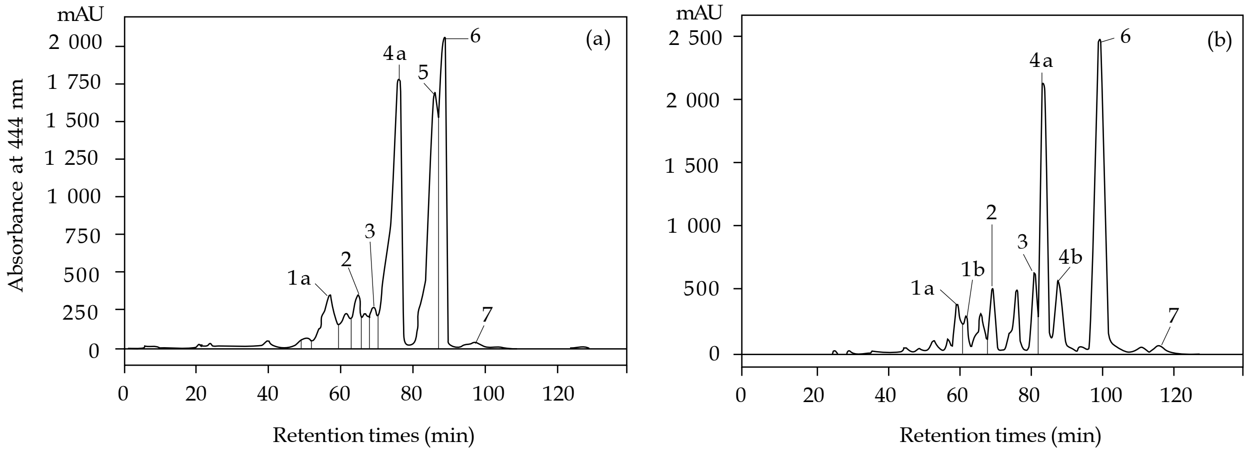

Figure 8. C_{30} chromatographic profiles of carotene concentrate, CC2: (a) DV1, and (b) DV2.
*Tentative peak identification: 1a) 13-*cis*-α-carotene; 1b) 13'-*cis*-α-carotene; 2) 13-*cis*-β-carotene; 3) *cis*-a-carotene; 4a)* trans *α-carotene; 4b) 9-*cis*-α-carotene; 5)* cis*-β-carotene; 6)* trans *β-carotene, and 7) 9-*cis*-β-carotene.*

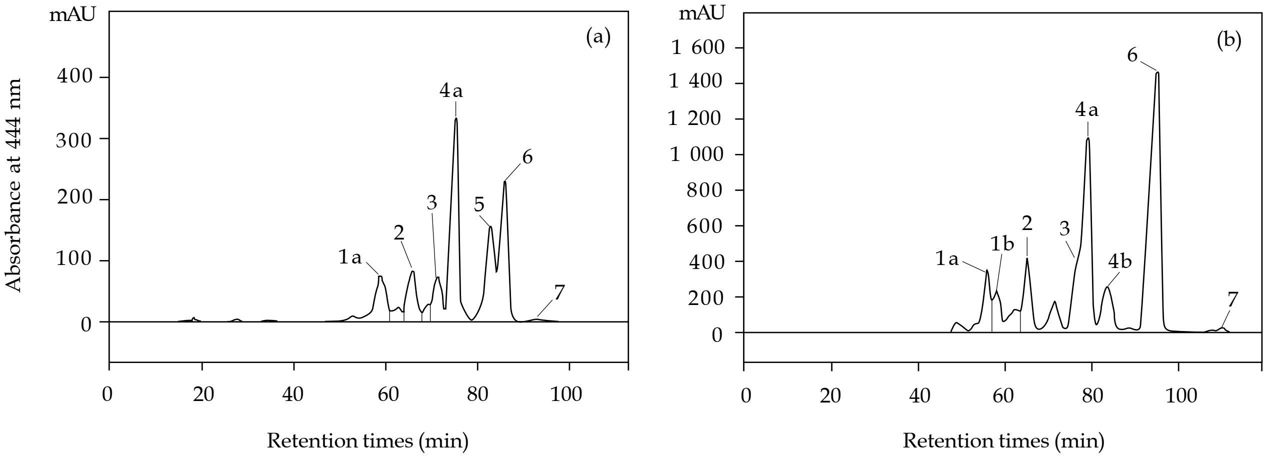

Figure 9. C_{30} chromatographic profiles of carotenoid extract from RPOo: (a) DV1 and (b) DV2.
*Tentative peak identification: 1a) 13-*cis*-α-carotene; 1b) 13'-*cis*-α-carotene; 2) 13-*cis*-β-carotene; 3)* cis*-α-carotene; 4a)* trans *α-carotene; 4b) 9-*cis*-α-carotene; 5)* cis*-β-carotene; 6)* trans *β-carotene, and 7) 9-*cis*-β-carotene.*

were formed. Further downstream in the mill, an additional 9-*cis*-β-carotene was identified in CPO. The overall qualitative profiles of the carotenoid isomers were similar for MOSPF, palm-based products and CPO. However, the quantities of isomers formed varied from product to product depending on the treatment received.

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