Journal of Chromatographic Science, 2016, Vol. 54, No. 4, 633–638
doi: 10.1093/chromsci/bmv241
Article

Article

Improved Method for the Qualitative Analyses of Palm Oil Carotenes Using UPLC

Mei Han Ng* and Yuen May Choo

Engineering and Processing Research Division, Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

*Author to whom correspondence should be addressed. Email: ngmh@yahoo.com

Received 26 September 2014; Revised 11 October 2015

Abstract

Palm oil is the richest source of natural carotenes, comprising 500–700 ppm in crude palm oil (CPO). Its concentration is found to be much higher in oil extracted from palm-pressed fiber, a by-product from the milling of oil palm fruits. There are 11 types of carotenes in palm oil, excluding the *cis*/*trans* isomers of some of the carotenes. Qualitative separation of these individual carotenes is particularly useful for the identification and confirmation of different types of oil as the carotenes profile is unique to each type of vegetable oil. Previous studies on HPLC separation of the individual palm carotenes reported a total analyses time of up to 100 min using C30 stationary phase. In this study, the separation was completed in <5 min. The qualitative separation was successfully carried out using a commonly used stationary phase, C18.

Introduction

Oil palm is the largest source of natural carotenes (1). There are 500–700 ppm of carotenes in crude palm oil (CPO) and 4,000–6,000 ppm in the oil obtained from the palm-pressed fiber, a by-product from the oil palm fruits milling (1–7). The individual carotenes in both CPO and PFO comprise 11 types (2, 3, 8, 9) (Table I). These carotenes, however, comprise different composition in the CPO and PFO (3). Palm oil can be distinguished from other types of oil by reviewing its carotenes content.

Separation of the individual carotenes in palm oil is based on structural differences, such as the conjugation of double bonds and end groups resulting in differences in polarity. Analysis of the individual carotenes in palm oil is a challenge as each of these individual carotenes absorbs UV at different wavelength and thus has different λ_{max} (4, 10–13) (Table II). In addition, not every type of these individual carotene is available in the form of standard reference material. The qualitative analysis of the individual carotenes in palm oil, however, has been reported in the past (4, 6, 11). These analyses were carried out using high performance liquid chromatography (HPLC) (5, 6, 11, 13–16). Different stationary and mobile phases were in used in these reports. The similarity in all these methods, however, is that a photodiode array detector (PDA) is used. The PDA is the most suitable detector for the detection of carotenes as different wavelengths can be monitored simultaneously in a single injection (5, 9, 11–18).

This paper reports on a new method for the qualitative analyses of carotenes in palm oil using ultra performance liquid chromatography (UPLC). This method offers a more efficient and time-saving analyses as opposed to the HPLC methods reported in the past.

Experimental

Instrumentation and reagents

CPO and palm-pressed fiber were obtained from POMTEC in Labu, Negri Sembilan.

Palm-pressed fiber oil (PFO) is obtained by soaking the fresh palm-pressed fiber overnight in hexane, followed by filtration. Excess solvent is removed by way of rotary evaporation.

Mobile phase: all solvents were of chromatographic grade and obtained from Merck (Darmstadt, Germany).

Waters UPLC with Acquity H class Quaternary Solvent Manager, Acquity Sample Manager-FTN and Acquity PDA ελ Detector were used for the qualitative analyses of palm carotenes. Column used was Acquity UPLC BEH C18 1.7 µm 2.1 × 50 mm.

Method

CPO was dissolved in acetonitrile to make into concentration of 2 mg/mL and injected into UPLC. Injection volume was 10 µL. Mobile

Table I. Composition of Carotenes in CPO and PFO

Carotenes	CPO (%)	PFO (%)
Phytoene	1.27	11.87
Phytofluene	0.06	0.40
β-Carotene	56.02	30.95
α-Carotene	35.06	19.45
cis-α-Carotene	2.49	1.17
ξ-Carotene	0.69	7.56
γ-Carotene	0.33	2.70
δ-Carotene	0.83	6.94
Neurosporene	0.29	3.38
β-Zeacarotene	0.74	0.37
α-Zeacarotene	0.23	trace
Lycopene	1.30	14.13

Table II. Maximum Absorption Wavelengths (λ_{max}) of Palm Carotenes in Hexane

Carotene	Yap, 1991 (λ_{max})				Tay, 1999 (λ_{max})			
Phytoene	276	286	297		276	287	299	
Phytofluene	331	347	366		330	343	360	
β-Carotene	426	429	477		430	444	480	
α-Carotene	420	440	471		425	444	475	
cis-α-Carotene	330	415	438	470	330	415	438	470
ζ-Carotene	380	401	426		383	406	420	
γ-Carotene	435	462	490		436	461	488	
Neurosporene	416	438	468		416	448	467	
β-Zeacarotene	404	426	452		404	426	452	
α-Zeacarotene	398	420	448		401	424	449	
Lycopene *trans*-	444	470	500		446	470	503	
cis-	362	438	464	495	349	438	467	488

Source: Yap *et al.* (4); Tay and Choo (5).

phase was acetonitrile and dichloromethane at the ration (98.5:1.5). Flowrate was 0.6 mL/min.

Above procedure was repeated with PFO.

Results

All the individual carotenes in palm oil were completely eluted in <5 min. Figures 1 and 2 depict the carotenes profile of CPO and unsaponifiable fraction of CPO. Figure 3 is an attenuation of Figure 1 between 0 and 2.85 min for the portrayal of the carotenes that are present in minute quantity compared with α- and β-carotene. Similarly, Figure 4 is an attenuation of Figure 2 of the same. Identification of the individual carotenes was carried out based on their λ_{max} from previous study. The λ_{max} value of each of the palm carotenes in this study when compared with previous literature is depicted in Tables III and IV. Specific assignment of the *cis* position was not carried out due to the unavailability of standards. Figures 5 and 6 depict the carotenes profile of PFO and unsaponifable fraction of PFO.

The carotenes profile of palm oil follows the sequence of: *cis* lycopene, *cis* lycopene, lycopene, α-zeacarotene, β-zeacarotene, *cis*-neurosporene, neurosporene, δ-carotene, γ-carotene, *cis* γ-carotene, *cis* ξ-carotene, *cis* ξ-carotene, *cis* ξ-carotene, ξ-carotene, *cis* α-carotene, α-carotene, β-carotene, phytofluene, *cis* β-carotene and phytoene.

Discussion

There are 11 types of carotenes present in both CPO and PFO. The composition of these carotenes, however, differs in both types of oil. Although quantitative analysis of all the individual carotenes in palm oil has yet to be carried out successfully, the qualitative analysis of the carotenes is particularly useful as the wide range of individual

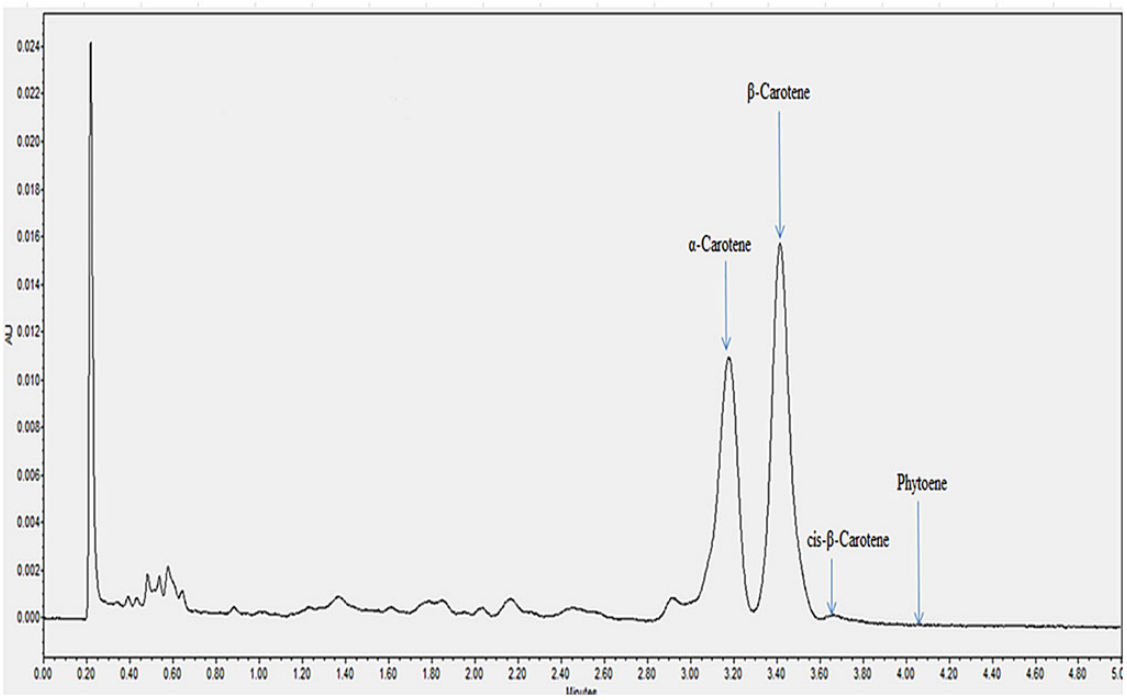

Figure 1. Carotenes profile of CPO. This figure is available in black and white in print and in color at *JCS* online.

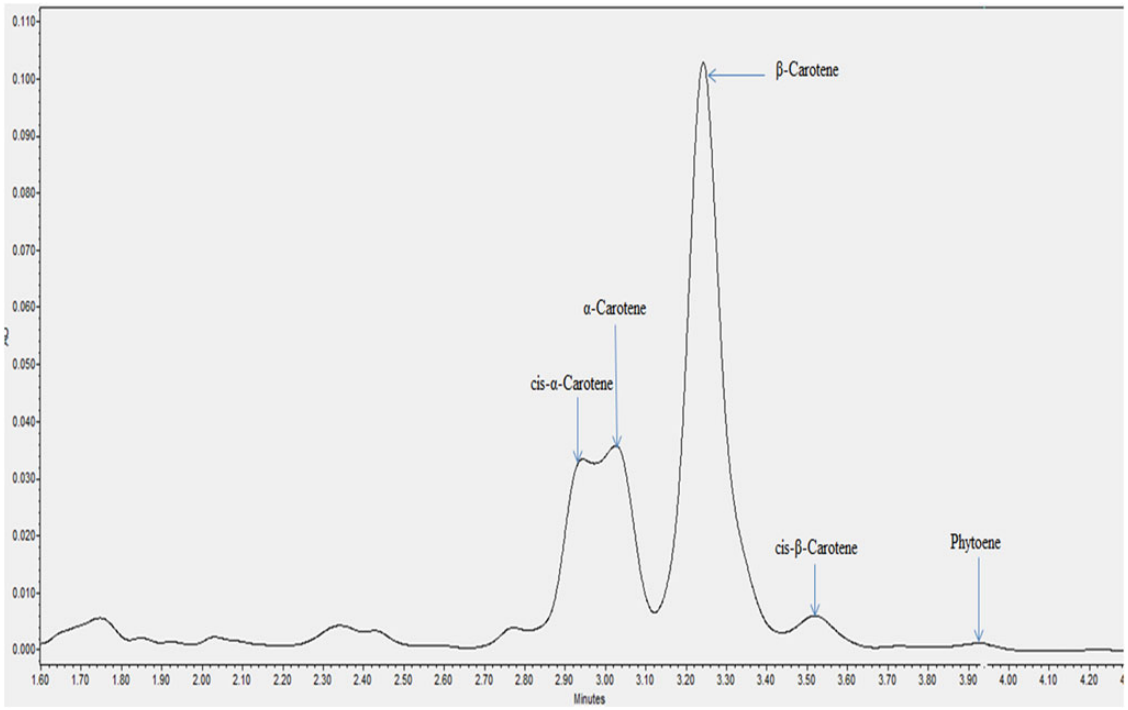

Figure 2. Carotenes profile of unsaponifiable fraction of CPO. This figure is available in black and white in print and in color at *JCS* online.

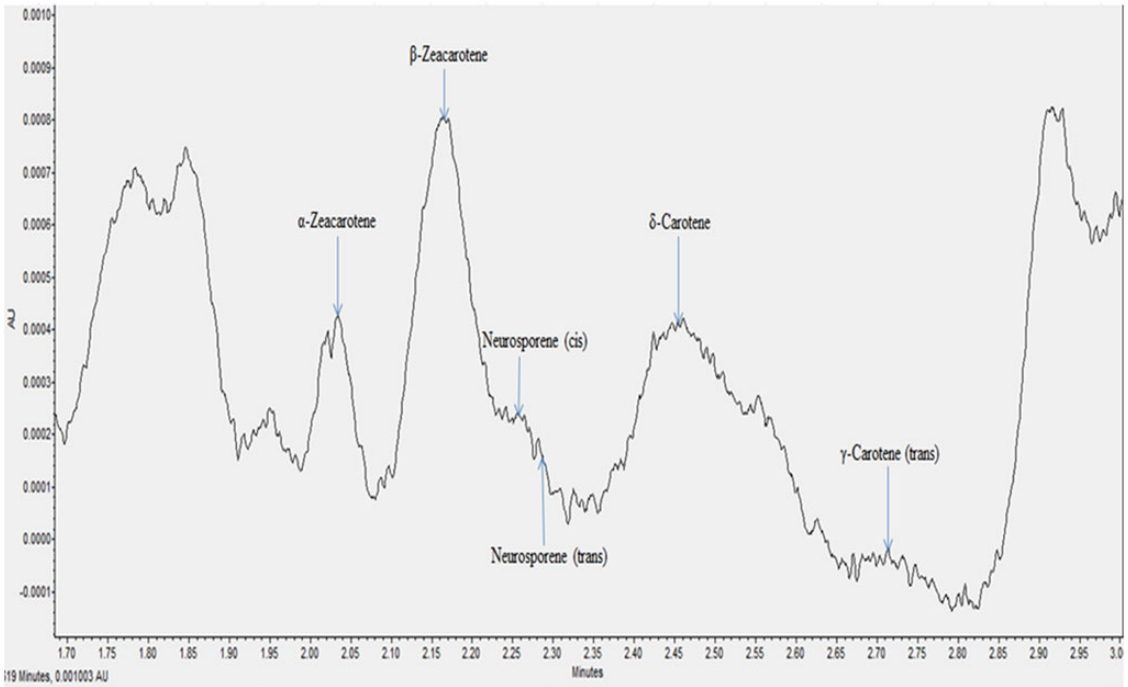

Figure 3. Magnified carotenes profile of CPO. This figure is available in black and white in print and in color at *JCS* online.

carotenes present can be used as indication of the type of oil in the absence of proper labeling.

Due to the low concentration of carotenes in CPO, which is ~500–700 ppm, the carotenes profile is not as prominent as that of PFO. Increasing the concentration of the sample resulted in saturation of the column. By removing the oil components through saponification, the concentration of carotenes in the unsaponifiable fraction of the CPO is greatly enhanced. This led to cleaner and sharper elution of the carotenes from the column. As the concentration of carotenes in PFO is already quite high, 4,000–6,000 ppm, its carotenes profile was more clearly defined.

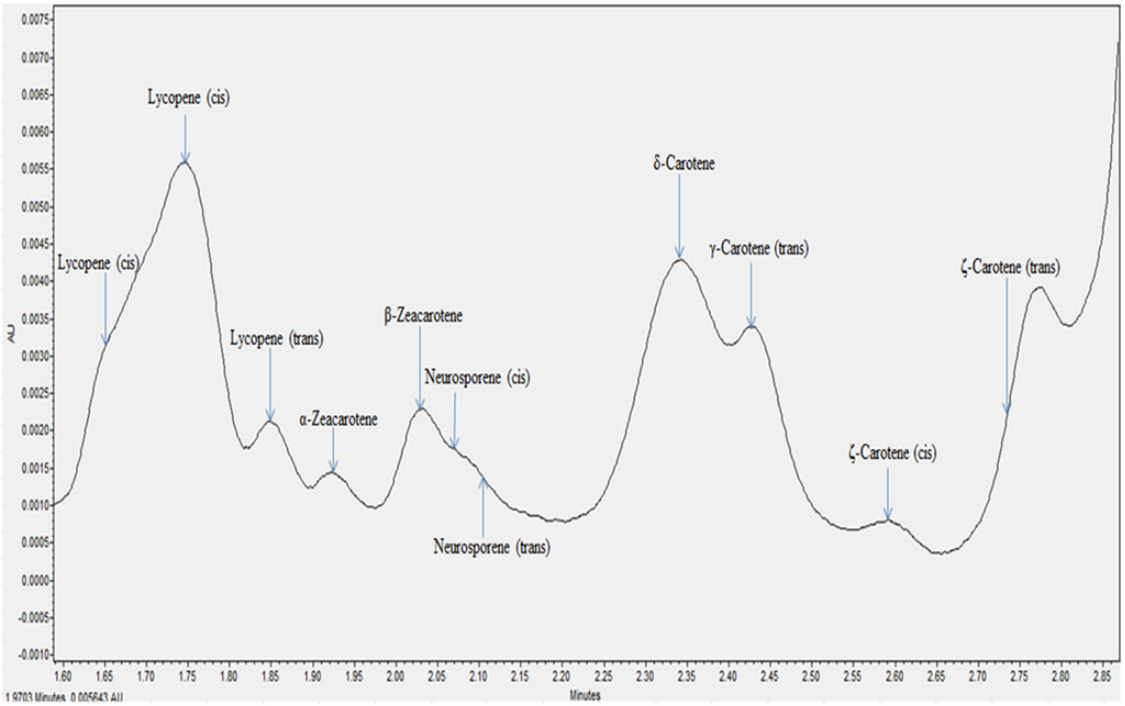

Figure 4. Magnified carotenes profile of unsaponifiable fraction from CPO. This figure is available in black and white in print and in color at *JCS* online.

Table III. Main Absorption Maxima (nm) of Carotenes in CPO and Saponified CPO

Carotenoids	CPO				Saponified CPO			
Lycopene (*cis*)	—				361	442	467	496
Lycopene (*cis*)	—				362	443	470	499
Lycopene (*trans*)	—				444	471	501	
α-Zeacarotene	398	421	449		398	421	449	
β-Zeacarotene	404	430	453		403	427	450	
Neurosporene (*cis*)	331	412	433	464	331	412	433	464
Neurosporene (*trans*)	417	440	466		417	440	466	
δ-Carotene	430	456	485		432	459	486	
γ-Carotene (*trans*)	—				436	462	489	
γ-Carotene (*cis*)	—				—			
ζ-Carotene (*cis*)	—				296	380	401	426
ζ-Carotene (*cis*)	—				—			
ζ-Carotene (*trans*)	382	398	427		379	401	424	
cis-α-Carotene	—				333	416	442	468
α-Carotene	420	444	472		420	445	472	
β-Carotene	426	449	478		426	451	478	
Phytofluene	—				—			
cis-β-Carotene	334	419	444	473	334	419	444	473
Phytoene	276	287	299		276	287	299	

Table IV. Main absorption maxima (nm) of carotenes of unsaponifiable PFO and PFO

Carotenoids	PFO				Unsaponifiable PFO			
Lycopene (*cis*)	362	441	467	496	361	442	467	498
Lycopene (*cis*)	362	442	470	500	362	443	470	500
Lycopene (*trans*)	444	468	500		444	468	499	
α-Zeacarotene	397	421	448		398	422	446	
β-Zeacarotene	404	430	457		403	425	450	
Neurosporene (*cis*)	330	415	439	468	328	414	433	462
Neurosporene (*trans*)	413	437	467		416	439	468	
δ-Carotene	431	457	485		432	457	485	
γ-Carotene (trans)	433	459	489		434	460	489	
γ-Carotene (*cis*)	349	435	459	487	350	433	460	488
ζ-Carotene (*cis*)	296	380	401	426	296	380	400	426
ζ-Carotene (*cis*)	296	379	401	425	297	380	401	425
ζ-Carotene (*trans*)	380	402	426		381	402	426	
cis-α-Carotene	332	417	442	469	332	415	441	468
α-Carotene	420	445	473		419	445	472	
β-Carotene	424	450	477		424	450	477	
Phytofluene	331	349	368		333	349	368	
cis-β-Carotene	333	418	442	471	334	420	445	473
Phytoene	276	286	298		276	287	299	

Each carotene absorbs UV at wavelengths that are different from others. This is called the λ_{max} and it is unique for each carotene. The λ_{max} of each of the carotene depends on the structure of the molecule and the number of double bonds they contain. The λ_{max} of each of the carotene in palm oil has been well documented in the past and it is used to identify the individual carotenes in palm oil in the absence of authentic standards (Table I).

In comparison with past reports using HPLC, UPLC is a fast and efficient method for the qualitative analyses of palm carotenes. The analysis was completed in <5 min, compared with HPLC which took more than 100 min. This is a definite improvement as it saves time and mobile phase consumption. The detection of the individual carotenes is sufficient to prove whether the oil is of palm origin as this carotenes profile is specific to palm oil.

Cis/trans isomer of the individual carotenes was resolved using C30 stationary phase in HPLC separation. In this study, using the UPLC, the C18 stationary phase is able to resolve the *cis/trans* isomers of the carotenes, which otherwise could not be done using HPLC. This shows that UPLC is a more powerful separation tool compared with HPLC.

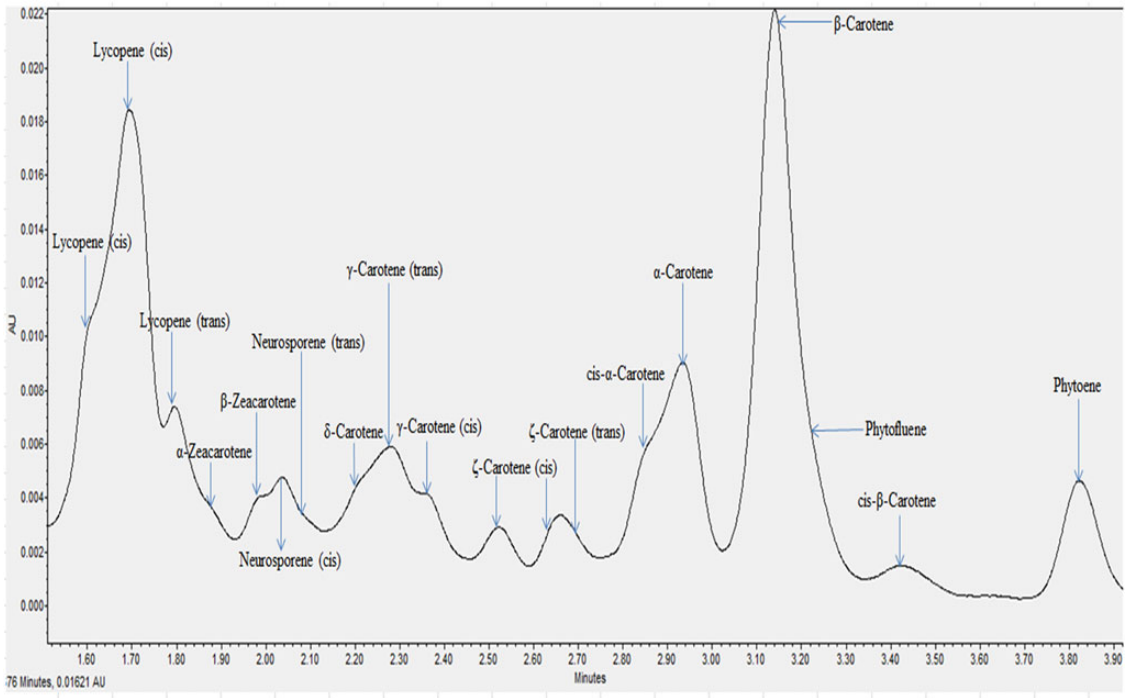

Figure 5. Carotenes profile of PFO. This figure is available in black and white in print and in color at *JCS* online.

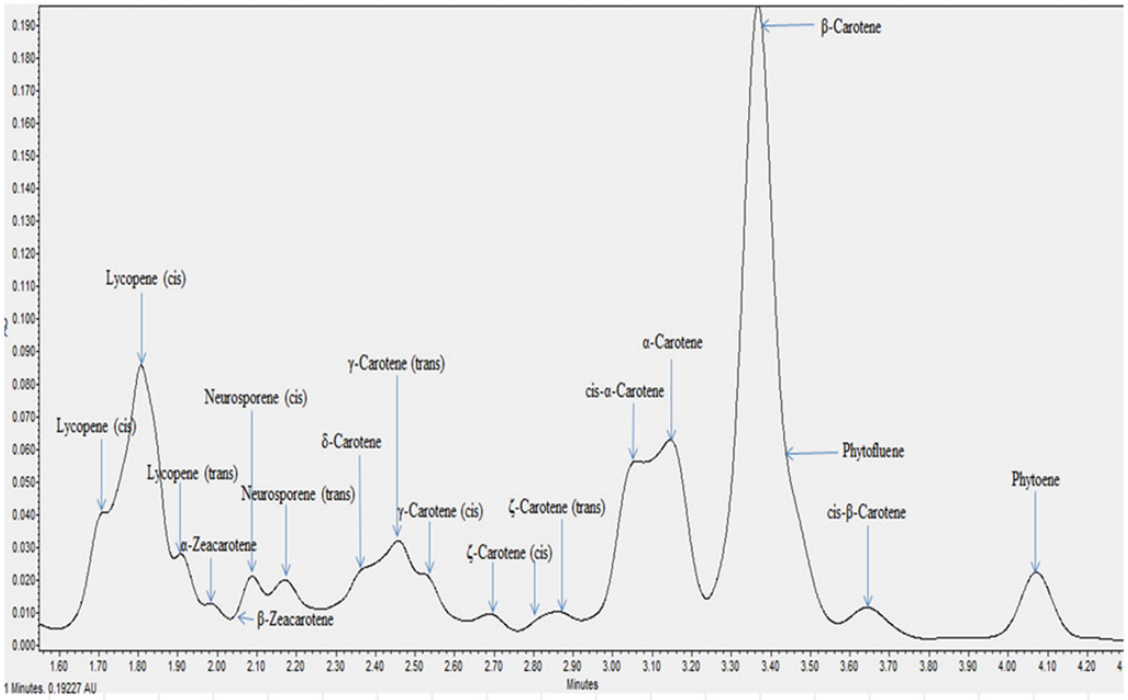

Figure 6. Carotenes profile of unsaponifiable fraction from PFO. This figure is available in black and white in print and in color at *JCS* online.

Acknowledgments

The authors wish to thank the staff of the Clean and Emerging Technologies Group for their technical assistance.

References

1. Goh, S.H., Choo, Y.M., Ong, A.S.H.; Minor constituents of palm oil; *Journal of American Oil Chemists Society*, (1985); 62(2): 237–240.

2. Ooi, C.K., Choo, Y.M., Yap, S.C., Basiron, Y., Ong, A.S.H.; Recovery of carotenoids from palm oil; *Journal of American Oil Chemists Society*, (1994); 71(4): 423–426.
3. Choo, Y.M., Yap, S.C., Ooi, C.K., Ma, A.N., Goh, S.H., Ong, A.S.H.; Recovered oil from palm-pressed fiber: a good source of natural carotenoids, vitamin E and sterols; *Journal of American Oil Chemists Society*, (1996); 73(5): 599–602.
4. Yap, S.C., Choo, Y.M., Ooi, C.K., Ong, A.S.H., Goh, S.H.; Carotenes in the oil from different palm species; *Elaeis*, (1991); 8(2): 369–378.
5. Tay, B.Y.P., Choo, Y.M.; Valuable minor constituents of commercial red palm olein: carotenoids, vitamin E, ubiquinones and sterols; *Journal of Oil Palm Research*, (2000); 12(1): 14–24.
6. Ng, M.H., Choo, Y.M., Ma, A.N., Chuah, C.H., Mohd, A.H.; Isolation and identification of individual palm carotenes using supercritical fluid chromatography; *Malaysian Journal of Science*, (2006); 25(2): 139–145.
7. Ng, M.H., Choo, Y.M.; Isolation and recovery of phytonutrients in palm by isocratic and isobaric flash chromatography; *Journal of Oil Palm Research*, (2012); 25(2): 165–169.
8. Tay, B.Y.P., Choo, Y.M., EE, G.C.L., Goh, S.H.; Geometrical isomers of major provitamin A palm carotenes, α- and β-carotenes in the MEsocarp oil of fresh and sterilised palm fruits, crude palm oil and palm carotene-based products: red palm olein and carotene concentrates; *Journal of Oil Palm Research*, (2002); 13(2): 23–32.
9. Tan, B., Grady, C.M., Gawrenowski, A.M.; Hydrocarbon carotenoid profiles of palm oil processed fractions; *Journal of American Oil Chemists Society*, (1986); 63: 1175–1179.
10. Britton, G.; UV/Visible spectroscopy. In Britton, G., Liaaen-Jensen, S., and Pfander, Birkhauser-Verlag, H. (eds.). *Carotenoids*, Vol. 1B. Birkhauser-Verlag, Basel, Switzerland, (1995), pp. 13–64.
11. Tay, B.Y.P.; Palm carotenoids profile as a quality control tool for palm carotene producers: introducing an improvised method by HPLC-photodiode array and a C30 column; *Journal of Oil Palm Research*, (2006); 18: 253–259.
12. Tay, B.Y.P., Choo, Y.M.; Practical guide to establishing palm carotenoids profiles by HPLC with three dimensional diode array detector; *Palm Oil Developments*, (2000); 22: 13–17.
13. Maoka, T., Fujiwara, Y., Hashimoto, K., Akimoto, N.; Rapid identification of carotenoids in a combination of liquid chromatography/UV–visible absorption spectrometry by photodiode-array detector and atomospheric pressure chemical ionization mass spectrometry (LC/PAD/APCI-MS); *Journal of Oleo Science*, (2002); 51(1): 1–9.
14. Tay, B.Y.P., Gwendoline, E.C.L.; Identification of lutein in crude palm oil and evaluation of carotenoids at various ripening stages of the oil palm fruit; *Journal of Oil Palm Research*, (2003); 18: 189–197.
15. Pettersson, A., Jonsson, L.; Separation of cis–trans isomers of alpha and beta carotene by adsorption HPLC and identification with diode array detection; *Journal of Micronutrient Analysis*, (1990); 8: 23–41.
16. Emenhiser, C., Sander, L.C., Schwartz, S.J.; Capability of a polymeric C30 stationary phase to resolve cis–trans carotenoid isomers in reversed-phase liquid chromatography; *Journal of Chromatography A*, (1995); 707: 205–216.
17. Ng, J.H., Tan, B.; Analysis of palm oil carotenoids by HPLC with diode array detection; *Journal of Chromatographic Science*, (1988); 26: 463–469.
18. Darnoko, D., Cheryan, M., Moros, E., Jerrel, J., Perkins, E.G.; Simultaneous HPLS analyses of palm carotenoids and tocopherols using a C30 Column and Photodiode Array Detector; *Journal of Liquid Chromatography and Related Technologies*, (2000); 23(12): 1873–1885.