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Application of Supercritical Fluid Chromatography in the Quantitative Analysis of Minor Components (carotenes, vitamin E, sterols, and squalene) from Palm Oil

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ABSTRACT: The application of supercritical fluid chromatography (SFC) coupled with a UV variable-wavelength detector to isolate the minor components (carotenes, vitamin E, sterols, and squalene) in crude palm oil (CPO) and the residual oil from palm-pressed fiber is reported. SFC is a good technique for the isolation and analysis of these compounds from the sources mentioned. The carotenes, vitamin E, sterols, and squalene were isolated in less than 20 min. The individual vitamin E isomers present in palm oil were also isolated into their respective components, α-tocopherol, α-tocotrienol, γ-tocopherol, γ-tocotrienol, and δ-tocotrienol. Calibration of all the minor components of palm as well as the individual components of palm vitamin E was carried out and was found to be comparable to those analyzed by other established analytical methods.

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Crude palm oil (CPO) consists of mainly glycerides (TG, >90%; DG, 2–7%; and MG, <1%), FFA (3–5%), and about 1% minor components such as carotenoids (500–700 ppm), vitamin E (600–1000 ppm), sterols (250–620 ppm), and squalene (200–600 ppm) (1). These minor components are also present in palm fiber oil (PFO) but in a much higher concentration: carotenoids (4000–6000 ppm), vitamin E (2000–4000 ppm), sterols (4000–6500 ppm) (2). All these components have important physiological properties, such as being antioxidative, anticareinogenic, and hypocholesterolemic (3–12).

Various chromatographic and analytical techniques have been reported for the isolation and analysis of these minor components: vitamin E by HPLC using fluorescence detection, squalene and sterols by GC-FID after saponification, and total carotenes by UV-vis spectrophotometry (5,13–16). Chromatographic isolations, e.g., by HPLC and GC, of similar compounds from sources other than palm oil also have been reported (5,13–16).

Although supercritical fluid chromatography (SFC) was reported more than 20 years ago, its advantages have only recently been realized. The unique properties of supercritical fluid as a mobile phase in SFC overcome the difficulties of solute thermal instability and volatility encountered in GC and also shorten the relatively longer analysis times of HPLC separations (17). The comparatively lower operating temperature of SFC over GC gives the advantage of preserving the heat-labile compounds in palm oil. Thus, the deterioration of minor components in palm oil caused by thermal degradation can be minimized or avoided. The growth in popularity of SFC as an analytical tool has been slow because some technical deficiencies, such as injection methods, flow restrictors, and interfaces between the supercritical fluid chromatograph and detectors, needed be addressed before it could be more widely used.

Although the SFC of carotenes, vitamin E, and sterols has been reported in the past, it should be noted that most of these separations were carried out using model mixtures, thereby eliminating interference from other compounds that would be present should a real sample matrix be used (18,19). Moreover, successful SFC separations of real samples also differed from one type of oilseed to another, as the types of minor components present were not similar. In addition, the choice of a capillary or packed column influences the choice for SFC as a separation tool. Packed-column SFC has only recently gained popularity because of the rapid development of packed-column technologies in recent years.

The detection of solutes from SFC separations has been reported using detectors such as flame ionization, NMR, evaporative light-scattering, and IR (17–19). However, a UV variable-wavelength detector was chosen in this study, as the λ_{max} of the solutes indicates the identity of the minor components eluted. This is because the minor components in palm absorb UV at different wavelengths: carotenes at 446 nm, vitamin E at 291 nm, sterols at 220 nm, and squalene at 220 nm.

Previously, Ng and fellow researchers (20) reported the SFC separation of tocols from palm oil using both silica and diol columns. To date, a successful single SFC separation of carotenes, vitamin E, sterols, and squalene from any real sample matrix has not been reported. This paper now reports the SFC separation and quantification of these four minor oilseed components from both CPO and PFO.

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Abbreviations: CPO, crude palm oil; PFO, palm fiber oil; SFC, supercritical fluid chromatography;

EXPERIMENTAL PROCEDURES

Apparatus. A JASCO Model SUPER-200 SFC system with a UV-970 variable-wavelength UV/vis detector equipped with high-pressure flow cells was used.

Materials. CPO was obtained from Keck Seng (Malaysia) Berhad (Johor, Malaysia). Palm pressed fiber was obtained from the MPOB Experimental Mill (Negri Sembilan, Malaysia). The fiber was extracted for its oil by soaking it in hexane for 16 h.

All solvents used were of either chromatography or analytical grade and were purchased from Merck (Darmstadt, Germany), J.T.Baker (Phillipsburg, NJ), or HmBG (Hamburg, Germany). Solvents used for chromatography were degassed before use. CO_2 (99.995%) was purchased from Malaysian Oxygen Berhad (Kuala Lumpur, Malaysia).

Squalene, β-carotene, β-sitosterol, and α-tocopherol standards were purchased from Sigma-Aldrich (St. Louis, MO). Tocotrienol standards were obtained from Calbiochem (San Diego, CA).

Preparation of the unsaponifiable sample. Approximately 5 g of CPO was saponified with 5 mL of 50% ethanolic KOH and 1 g of pyrogallol (which acts as an antioxidant) by heating it in the dark under nitrogen flushing using a steam bath for 1 h. The saponified sample was then cooled to room temperature and extracted using a 50-mL portion of a single-phase system of 10% distilled water in *n*-hexane until the supernatant turned colorless. The pooled hexane extracts were washed with 50-mL portions of 10% ethanol in distilled water until the washed water turned neutral. The extract was then taken to dryness using a rotary evaporator and was further dried under vacuum.

The same saponification procedure was repeated using 3 g of PFO.

The yield of unsaponifiable sample from 5 g of CPO was 0.1 g, or 2%, whereas the yield from 3 g of PFO was 0.08 g, or 2.7%. Each unsaponifiable sample from CPO and PFO was dissolved in dichloromethane and injected into a supercritical fluid chromatograph, a high-performance liquid chromatograph, or a gas chromatograph after silylation for further analysis of sterols. Wavelengths for the detection of minor components were: carotenes, 446 nm; vitamin E, 291 nm; sterols, 220 nm; and squalene, 220 nm.

SFC. SFC was carried out using a 5-μm, 4.6 × 250 mm LiChrosorb® 60A Silica analytical column (Merck), with a flow rate of 3.12 mL/min CO_2 with 4% ethanol as entrainer under 180 kg/cm^2 pressure at 50°C. The loop at the injector port was 20 μL.

HPLC. Analysis of vitamin E was also carried out using HPLC coupled with a fluorescence detector (λ_{ex}: 295 nm; λ_{em}: 325 nm) to compare the results obtained by SFC. A LiChrosorb® 60A Silica analytical column (Merck), 4.6 mm i.d. × 250 mm length, was used. The mobile phase was hexane/THF/isopropanol (1000:60:4) at 1 mL/min.

GC. Results obtained from the analysis of squalene and sterols using SFC were compared with those obtained using a known GC method. Samples for GC analyses were prepared by adding 0.3 mL of 30% *N,O*-bis(trimethylsilyl) trifluoroacetamide in dichloromethane to a weighed sample (*ca.* 0.02 g). The solution was brought up to 1.5 mL using dichloromethane. Thereafter, the sample mixture was heated at 60°C for 2 h before injection into the gas chromatograph-FID.

A Hewlett-Packard 5890 Series II Plus gas–liquid chromatograph was used. The column used was an SGE 15 m × 0.32 mm i.d. BPX5 0.25 μm capillary column (SGE, Melbourne, Australia); the initial oven temperature was set at 100°C for 1 min and increased to 400°C at the rate of 10°C/min. The injector and detector temperatures were set at 370°C. The oven equilibrium time was 3 min under a pressure of 6.60 psi. The carrier gas (helium) was set at flow velocity ranges of 1.99–2 mL/min/cm/s. The range of split ratio between the compressed air and H_2 gas was 0.0–1.

Quantification of carotene, vitamin E, sterols, and squalene. Standard curves of the vitamin E standards (α-tocopherol, α-tocotrienol, γ-tocopherol, γ-tocotrienol, and δ-tocotrienol) were obtained by injecting different concentrations of the standard into the supercritical fluid chromatograph. Peak areas were obtained, and calibration graphs were then plotted.

Similar steps were applied for the squalene and sterols, which were calibrated as β-sitosterol. Statistical data from the analysis were obtained based on the results of 15 repetitive analyses.

The total carotene content was confirmed using the established method of UV-vis spectrophotometry, by which its spectra and absorbance were obtained at 446 nm.

RESULTS AND DISCUSSION

The isolation of minor components of palm using SFC is a more straightforward method than other procedures. Conventional analyses of palm minor components are tedious, as different techniques such as UV-vis spectrophotometry, HPLC, and GC are necessary to quantify all four minor components of palm in just one sample. Because different analyses require different sample preparations, such as silylation, to convert the nonvolatile compounds to their more volatile derivatives for GC analyses, the whole procedure is time-consuming and labor-intensive. Using SFC, sample preparation, which takes less than 5 min, is carried out only once, thus saving time and labor. Moreover, isolation of the minor components of palm oil by SFC can be accomplished in less than 20 min (Fig. 1). This is much shorter than HPLC and GC, which takes 45 min to complete.

Figure 1 shows the SFC chromatograms of carotenes, vitamin E, sterols, and squalene in CPO and PFO, respectively. Squalene was eluted first, as observed at a wavelength of 220 nm. The amounts of squalene in CPO and PFO were 600 ± 10 and 1730 ± 30 ppm, respectively. The results obtained by the SFC analysis were comparable to those obtained by GC.

Carotenes were eluted as total carotenes, as observed at 446 nm. This was chosen as the wavelength of palm carotenes since it corresponds to the wavelength with maximal absorption in the UV. The amount of carotenes in palm oil was calibrated as

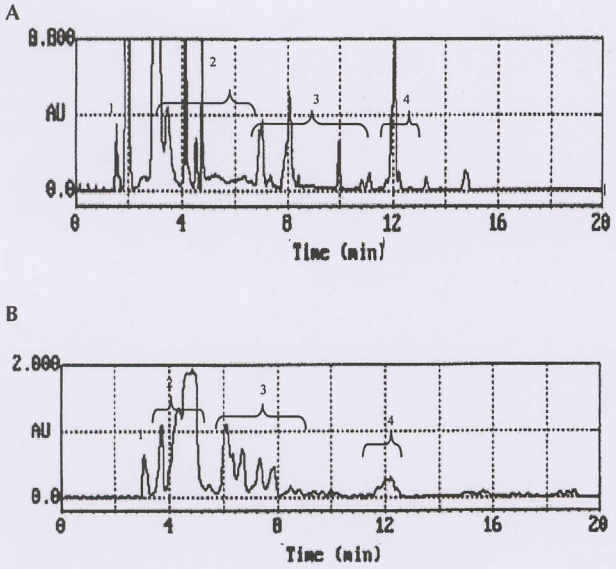

FIG. 1. Supercritical fluid chromatogram of crude palm oil (CPO) and palm fiber oil (PFO) unsaponifiable matter at 210 nm. CPO (A) and PFO (B) unsaponifiable matter was separated by supercritical fluid chromatography (SFC) with a LiChrosorb® 60A Silica column (4.6 mm i.d. × 250 mm length; Merck, Darmstadt, Germany) at 180 kg/cm^2 and 50°C. The compounds separated were: 1, squalene; 2, carotenes; 3, vitamin E; and 4, sterols.

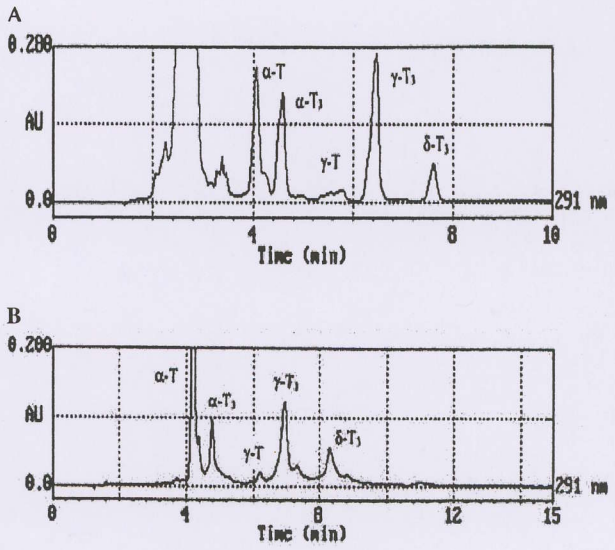

FIG. 2. Supercritical fluid chromatogram of vitamin E in CPO and PFO at 291 nm. Vitamin E in CPO (A) and PFO (B) was separated by SFC with a LiChrosorb 60A Silica column (4.6 mm i.d. × 250 mm length; Merck) at 180 kg/cm^2 and 50°C. α-T, α-tocopherol; α-T_3, α-tocotrienol; γ-T, γ-tocopherol; γ-T_3, γ-tocotrienol, and δ-T_3, δ-tocotrienol. For other abbreviations see Figure 1.

total β-carotene since it is the major carotene present in palm oil. The total carotenes in CPO were 550 ± 10 ppm, whereas they were 2400 ± 30 ppm in PFO. Using UV-vis spectrometry, the total carotenes in CPO and PFO were 600 ± 20 and 2290 ± 190 ppm, respectively. These results are in agreement with the results obtained by SFC.

Since vitamin E isomers have their maximal absorbance (fluorescence) at 291 nm, this wavelength was chosen for quantification of the vitamin E isomers. At 291 nm, the vitamin E isomers in palm oil were separated into five components, with α-tocopherol as the first vitamin E isomer to be eluted, followed by α-tocotrienol, γ-tocopherol, and γ-tocotrienol, with δ-tocotrienol as the last. Figure 2 shows the SFC chromatograms of vitamin E isomers in the CPO and PFO unsaponifiable samples at 290 nm. The concentrations of the five types of vitamin E isomers detected in CPO and PFO are presented in Table 1 and compared with HPLC analyses. There was a distinct difference in the concentrations of the vitamin E isomers analyzed by SFC and HPLC. Analyses carried out using SFC gave higher concentrations than those by HPLC. This can be attributed to the fact that in HPLC, vitamin E is destroyed due to prolonged exposure to organic solvents. Because SFC uses supercritical carbon dioxide (SC-CO_2) as the mobile phase, the oxidation of minor components of palm (especially vitamin E isomers) is avoided because SC-CO_2 is nondestructive. Other organic solvents used with HPLC or other chromatographic methods have been shown to degrade the tocols.

Sterols were the last to be eluted by SFC and were quantified as total β-sitosterol, as this is the major sterol present in palm oil. The concentrations of total sterols in CPO and PFO

TABLE 1
Concentration of Vitamin E in Unsaponifiable Samples of Crude Palm Oil (CPO) and Palm Fiber Oil (PFO)[a]

	Concentration (ppm) recovered from			
	SFC		HPLC	
	PFO	CPO	PFO	CPO
α-Tocopherol	1260 ± 50	300 ± 40	1250 ± 70	310 ± 70
α-Tocotrienol	260 ± 10	230 ± 20	230 ± 40	240 ± 5
γ-Tocopherol	80 ± 5	20 ± 5	100 ± 20	10 ± 10
γ-Tocotrienol	480 ± 30	540 ± 30	350 ± 30	460 ± 30
δ-Tocotrienol	200 ± 50	90 ± 10	160 ± 10	100 ± 70
Total	2280 ± 145	1180 ± 105	2090 ± 170	1120 ± 185

[a]Results obtained by calibration with authentic standards using both supercritical fluid chromatography (SFC) and HPLC.

TABLE 2
Concentration of Individual Sterols Analyzed by GC[a]

	Concentration (ppm)	
	CPO	PFO
Cholesterol	60	620
Stigmasterol	30	340
β-Sitosterol	60	300
Campesterol	80	990
Total	230	2250

[a]Breakdown of the sterol composition of CPO and PFO as analyzed by GC. In contrast, the composition of sterols for SFC is reported in this paper as total sterols. For abbreviations see Table 1.

were 180 ± 10 and 2410 ± 50 ppm, respectively. GC-FID is an established method for the quantification of sterols by which the individual sterols can be calibrated (2). Table 2 shows GC results for the quantification of individual sterols. The results obtained by SFC for the determination of total sterols were comparable to those obtained by GC.

In conclusion, SFC can be applied for the isolation of minor components of palm oil, as SFC results for the isolation of these compounds are comparable to those obtained by known methods.

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REFERENCES

1. Goh, S.H., Choo, Y.M., and Ong, A.S.H. (1985) Minor Constituents of Palm Oil, *J. Am. Oil Chem. Soc. 62*, 237–240.
2. Choo, Y.M., Yap, S.C., Ooi, C.K., Ma, A.N., Goh, S.H., and Ong, A.S.H. (1996) Recovered Oil from Palm-Pressed Fiber: A Good Source of Natural Carotenoids, Vitamin E, and Sterols, *J. Am. Oil Chem. Soc. 73*, 599–602.
3. Yu, W., Zhao, Y., Xue, Z., Jin, H., and Wang, D. (2001) The Antioxidant Properties of Lycopene Concentrate Extracted from Tomato Paste, *J. Am. Oil Chem. Soc. 78*, 697–701.
4. Martinez-Valverde, I., Periago, M.J., Provan, G., and Chesson, A. (2002) Phenolic Compounds, Lycopene and Antioxidant Activity in Commercial Varieties of Tomato (*Lycopersicum esculentum*), *J. Sci. Food Agric. 82*, 323–330.
5. Qureshi, A.A., Mo, H.B., Packer, L., and Peterson, D.M. (2000) Isolation and Identification of Novel Tocotrienols from Rice Bran with Hypocholesterolemic, Antioxidant and Antitumor Properties, *J. Agric. Food Chem. 48*, 3130–3140.
6. Canfield, L.M. (1995) β-Carotenoid Metabolites: Potential Importance to Human Health, *Malaysian Oil Sci. Technol. 4 (1)*, 43–46.
7. Ashfaq, M.K., Zuberi, H.S., and Waqar, M.A. (2001) Vitamin E and β-Carotene Affect Anticancer Immunity: *In vitro* and *in vivo* Studies, in *2001 PORIM International Conference*, Malaysian Palm Oil Board, Kuala Lumpur, Malaysia.
8. Goh, S.H., Hew, N.F., Norhanom, A.E., and Yadar, M.C. (1994) Inhibition of Tumour Growth Promotion by Various Palm Oil Tocotrienols, *Int. J. Cancer 57*, 529–531.
9. Guthrie, N., Nesaretnam, K., Chambers, A.F., and Carroll, K.K. (1993) Inhibition of Breast Cancer Cell Growth by Tocotrienols, *FASEB J. 7*, A70.
10. Farquhar, J.W. (1996) Plant Sterols: Their Biological Effects in Humans, in *Handbook of Lipids in Human Nutrition*, CRC Press, Boca Raton, FL, pp. 101–105.
11. Nesaretnam, K., Kor, H.T., Ganesan, J., Chong, Y.H., Sundram, K., and Gapor, A. (1992) The Effect of Vitamin E Tocotrienols from Palm Oil on Chemically-Induced Mammary Carcinogenesis in Female Rats, *Nutr. Res. 12*, 63–75.
12. Tay, B.Y.P., and Choo, Y.M. (2000) Valuable Minor Constituents of Commercial Red Palm Olein: Carotenoids, Vitamin E, Ubiquinones and Sterols, *J. Oil Palm Res. 12 (1)*, 14–24.
13. Villén, J., Blanch, G.P., del Castillo, M.L.R., and Herraiz, M. (1998) Rapid and Simultaneous Analysis of Free Sterols, Tocopherols, and Squalene in Edible Oils by Coupled Reversed-Phase Liquid Chromatography–Gas Chromatography, *J. Agric. Food Chem. 46*, 1419–1422.
14. Boven, M.V., Daenens, P., Maes, K., and Cokelaere, M. (1997) Content and Composition of Free Sterols and Free Fatty Alcohols in Jojoba Oil, *J. Agric. Food Chem. 45*, 1180–1184.
15. Senorans, F.J., Villen, J., Tabera, J., and Herraiz, M. (1998) Simplex Optimization of the Direct Analysis of Free Sterols in Sunflower Oil by On-line Coupled Reversed Phase Liquid Chromatography–Gas Chromatography, *J. Agric. Food Chem. 46*, 1022–1026.
16. Senorans, F.J., Tabera, J., and Herraiz, M. (1996) Rapid Separation of Free Sterols in Edible Oils by On-line Coupled Reversed Phase Liquid Chromatography–Gas Chromatography, *J. Agric. Food Chem. 44*, 3189–3192.
17. Smith, R.M. (ed.) (1993) *Supercritical Fluid Chromatography*, RSC Chromatography Monographs, Royal Society of Chemistry, London.
18. King, J.W., and List, G.R. (1996) *Supercritical Fluid Technology in Oil and Lipid Chemistry*, AOCS Press, Champaign.
19. Laakso, P. (1992) Supercritical Fluid Chromatography of Lipids, in *Advances in Lipid Methodology–One* (Christie, W.W., ed.), Oily Press, Dundee, Scotland, pp. 81–120.
20. Ng, M.H., Choo, Y.M., Ma, A.N., Chuah, C.H., and Hashim, M.A. (2004) Isolation of Palm Tocols Using Supercritical Fluid Chromatography, *J. Chromatogr. Sci. 42*, 536–539.

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