Separation of Crude Palm Oil Components by Semipreparative Supercritical Fluid Chromatography

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ABSTRACT: Successful separation of triglycerides, diglycerides, free fatty acids, carotenes, tocopherol, and tocotrienols from crude palm oil has been achieved by supercritical fluid chromatography (SFC) with a combination of a C18 and a silica gel column. The separation was carried out by the programmed extraction elution method. Free fatty acids were separated into five components by gas–liquid chromatography; tocopherol and tocotrienols were also separated into four components by SFC analysis, and the pure fractionated carotenes were obtained by preparative SFC. Thus, by using supercritical fluid chromatography, crude palm oil components can be separated and fractionated, based on differences in their functional groups.
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KEY WORDS: Carotenes, diglycerides, free fatty acids, palm oil, supercritical fluid chromatography, tocopherol, tocotrienols, triglycerides.

Oil palm trees are grown within approximately 10°N and 10°S of the equator. It is a prolific crop that produces two types of oils: palm oil from the mesocarp and palm kernel oil from the kernel nut.

Palm oil is now one of the four major oils (together with soybean, rapeseed and sunflower oils) traded worldwide. Consumption of palm oil has been increasing each year because of its nutritional value, as evidenced by recent findings (1), as well as its versatility and suitability for various food and nonfood applications. Crude palm oil (CPO) contains mainly glycerides (more than 90% triglycerides, 2–7% diglycerides, and less than 1% monoglycerides), with 3–5% free fatty acids and about 1% minor components. Among the minor components are carotenoids (500–700 ppm), vitamin E (tocopherol and tocotrienols, 600–1000 ppm), and sterols (250–620 ppm) (2). These minor components are valuable compounds because they possess significant physiological and medicinal properties.

In this paper, we report a new method for the separation of triglycerides, diglycerides, free fatty acids, carotenoids, tocopherol, and tocotrienols from CPO by supercritical fluid chromatography (SFC) by using the programmed extraction elution method (PEEM) (3,4). PEEM was also employed for the semi-preparative separation of the above components with supercritical carbon dioxide. Each fraction obtained was analyzed by SFC and gas–liquid chromatography (GLC).

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EXPERIMENTAL PROCEDURES

Apparatus. A JASCO Model SUPER-200 SFE/SFC system with a UV-970 variable-wavelength ultraviolet (UV) detector or an MD-910 photodiode-array multiwavelength UV/VIS detector was used in this experiment. These detectors were equipped with high-pressure flow cells, operating at a pressure of 30 MPa.

Materials. CPO was obtained from a Malaysian palm oil mill. The solvents used were of analytical grade, purchased from Wako Pure Chemicals (Osaka, Japan). Liquefied carbon dioxide was purchased from Shoutan Shoji (Tokyo, Japan).

Columns. Super-Crestpak C18 and Superpak SIL columns were used in separation of CPO. Columns of 10.0 mm i.d. × 100 mm were used for semipreparative separations. Identical columns with dimensions of 4.6 mm i.d. × 150 mm (C18) and 4.6 mm i.d. × 250 mm (SIL) were used for analytical separations.

RESULTS AND DISCUSSION

Analytical separation of CPO with a C18 column. CPO was separated into 11 peaks as shown in Figure 1. By utilizing UV spectra and elution order, the peaks that eluted in a time period between 1 and 3 min were identified as free fatty acids, those in the 3- to 4-min time period as tocopherols and tocotrienols (UV absorption maximum at 292 nm), at 4–6 min as diglycerides, at 8–15 min as triglycerides, and those from 12 to 15 min as carotenes (UV-VIS absorption maximum at 270, 324 and 440 nm, respectively). The carotenes overlapped with the triglycerides upon eluting from the column.

Analytical separation of CPO with only a silica gel column. A typical chromatogram obtained with a silica gel column is shown in Figure 2. The elution order of solutes is similar to that shown in Figure 1 obtained with the C18 column.

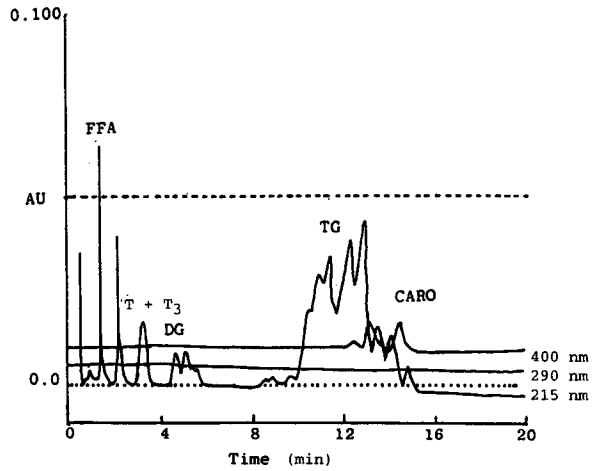

FIG. 1. Supercritical fluid chromatography chromatogram of crude palm oil with a C18 column. Conditions: column, Super Crestpak C18 5 μm, 4.6 mm i.d. × 150 mm; eluent CO_2/EtoH = 3.0/0.2 mL/min; back pressure 18 MPa; FFA, free fatty acids (215 nm); T + T_3, tocopherol and tocotrienol (215 nm); DG, diglycerides (215 nm); TG, triglycerides (215 nm); CARO, carotenes (400 nm); CPO, crude palm oil.

However, the C18 column does not permit complete separation of the carotenes from the triglycerides as does the silica gel column.

Fractionation of CPO by the PEEM. A 500-mg portion of CPO was injected onto the C18 column (10 mm i.d. × 100 mm), and PEEM was performed. Four fractions were obtained as shown in Figure 3.

Fraction 1 (F1) (10 mg) included substances that eluted from 0 to 66 min while using pure carbon dioxide at 18 MPa pressure as the eluent.

Fraction 2 (F2) (8.5 mg) included substances that eluted during the next 20 min with a mixture of carbon dioxide and ethanol (5.0/0.2 mL/min).

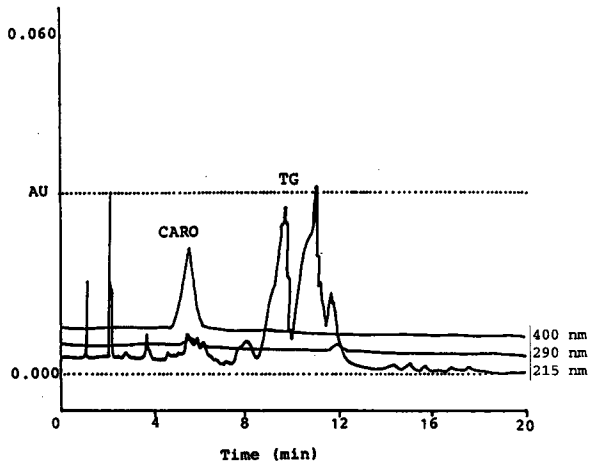

FIG. 2. Supercritical fluid chromatography chromatogram of crude palm oil with a silica gel column. Conditions: column, Superpak SIL 5 μm, 4.6 mm i.d. × 250 mm; other conditions and abbreviations as in Figure 1.

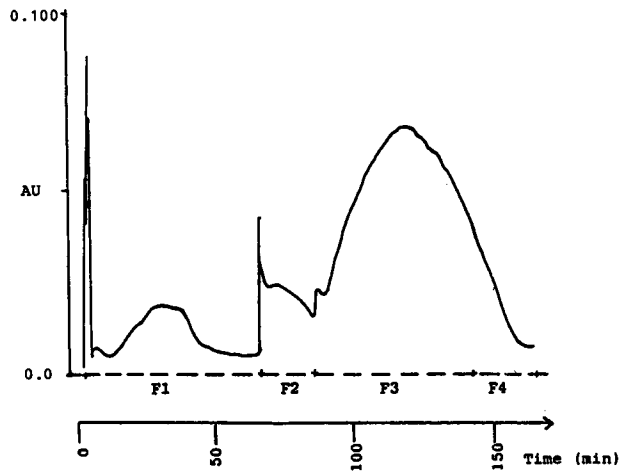

FIG. 3. Semipreparative supercritical fluid chromatography chromatogram of crude palm oil. Refer to text for conditions.

Fraction 3 (F3) (100 mg) included solutes that eluted in the next 54 min by increasing ethanol flow rate to 0.5 mL/min. Finally, fraction 4 (F4) (8 mg) included substances that eluted in the next 10 min under the same elution conditions as were used for F3. This fraction also exhibited a reddish yellow color.

F1. The main constituents of this fraction were free fatty acids and a small amount of tocopherols and tocotrienols. Free fatty acids were analyzed by capillary GLC, and the resultant chromatogram is shown in Figure 4. The free fatty acid constituents that were found in F1 of CPO are myristic acid (0.9%), palmitic acid (47.9%), stearic acid (3.5%), oleic acid (37.1%), and linoleic acid (10.7%). These results are in good agreement with previously reported work (5).

Small amounts of isomers of oleic acid and linoleic acid and a trace amount of linolenic acid were also detected.

F2. Tocopherols, tocotrienols, and diglycerides were in this fraction isolated by PEEM. This fraction was subjected to further purification on the analytical C18 column to isolate the tocopherols and tocotrienols from the diglycerides. The tocopherols and tocotrienols fraction obtained was then separated into four main components by using the SIL column, according to their polarities, as shown in Figure 5.

Peaks were identified as: α-tocopherol (peak 1), α-tocotrienol (peak 2), γ-tocotrienol (peak 3), and δ-tocotrienol (peak 4) by comparing each solute's retention time with that of the standard compound.

F3. F3 consisted of isomeric triglycerides. The major isomeric triglycerides have been identified as 1,3-dipalmitoyl-2-oleyl glycerol, tripalmitin, 1,3-dipalmitoyl-2-linoleyl glycerol, 1-palmitoyl-2,3-dioleyl glycerol, and 1-palmitoyl-2-linoleyl-3-oleyl glycerol, consistent with earlier published data (6).

F4. This fraction contained predominantly carotenes and triglycerides. Carotenes could be separated completely from triglycerides by SFC with the SIL column.

The peak eluting at 14.5 min was identified as β-carotene

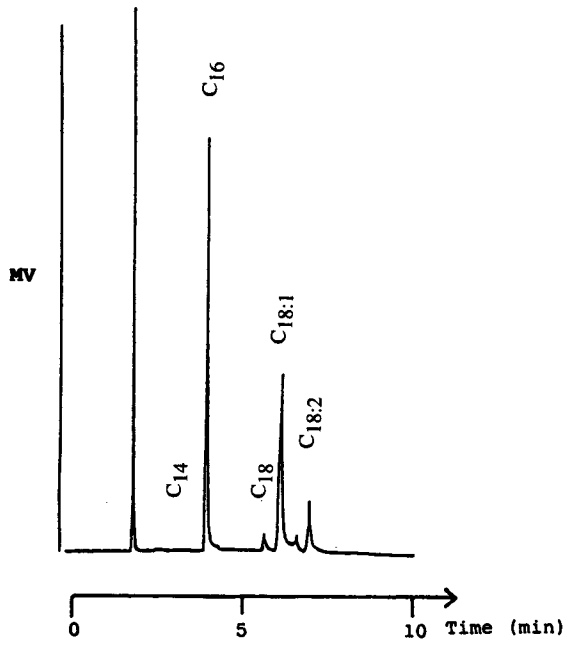

FIG. 4. Gas–liquid chromatography chromatogram of fraction 1 (free fatty acid fraction). Conditions: column, DBWAX 20M, film thickness 25 μm, 0.25 mm × 30 m; carrier gas, He, 1.2 mL/min, split ratio 1/100; injector temperature, 250°C; detector, flame-ionization detector; detector temperature, 280°C; oven temperature program, 180°C for 2 min, 5°C/min to 250°C and maintained for 9 min.

by comparing its retention time with that of the standard. In this case, the resolution obtained was similar to the chromatogram monitored at 400 nm (Fig. 1), but the purity of each peak was considerably improved over that shown in the previous chromatogram.

In our previous work on the separation of carotenes by reversed-phase high-performance liquid chromatography (HPLC) (7), carotenes present in CPO were separated into 11 components, with α- and β-carotenes as the major components. In this study, the separation on SFC-C18 separation gave three main peaks; SFC–HPLC separation will have to be used to obtain better resolution.

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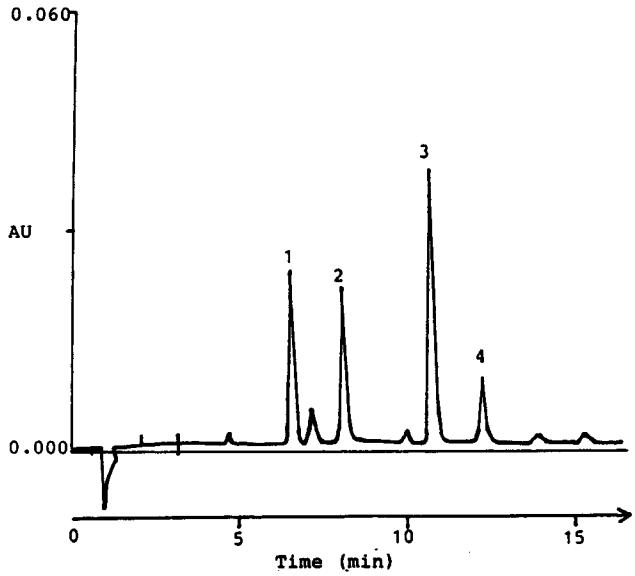

FIG. 5. Supercritical fluid chromatography chromatogram of the separation of tocopherols and tocotrienols in crude palm oil. Conditions: column, Superpak SIL 5 μm, 4.6 mm i.d. × 250 mm; detection, Ultraviolet at 290 nm; other conditions as in Figure 1. Peak 1: α-tocopherol; Peak 2: α-tocotrienol; Peak 3: γ-tocotrienol; Peak 4: δ-tocotrienol.

REFERENCES

1. Cottrell, R.C., Introduction: Nutritional Aspects of Palm Oil, *Am. J. Clin. Nutr. Suppl. 53*:989S–1009S (1991).
2. Goh, S.H., Y.M. Choo, and A.S.H. Ong, Minor Constituents of Palm Oil, *J. Am. Oil Chem. Soc. 62*:237–241 (1985).
3. Yamauchi, Y., and M. Saito, Fractionation of Lemon-Peel Oil by Semi-Preparative Supercritical Fluid Chromatography, *J. Chromatogr. 505*:237–246 (1990).
4. Higashidate, S., Y. Yamauchi, and M. Saito, Enrichment of Eicosapentaenoic Acid and Docosahexaenoic Acid Esters from Esterified Fish Oil by Programmed Extraction-Elution with Supercritical Carbon Dioxide, *Ibid. 515*:295–303 (1990).
5. Tan, B.K., and F.C.H. Oh, Malaysian Palm Oil Chemical and Physical Characteristics, *PORIM Technology No. 3*:1–5 (1982).
6. Tan, Y.A., Applications of GLC in the Analysis of Oils and Fats, *PORIM Bulletin, No. 21*:4–18 (1990).
7. Yap, S.C., Y.M. Choo, C.K. Ooi, A.S.H. Ong, and S.H. Goh, Quantitative Analysis of Carotenes in the Oil from Different Palm Species, *Elaeis 3*:369–378 (1991).

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