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Article

Chromatographic Analyses of Tocopherols and Tocotrienols in Palm Oil

Ng Mei Han* and Choo Yuen May

Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

*Author to whom correspondence should be addressed. Email: meihan@mpob.gov.my

Analyses of tocols (tocopherols and tocotrienols) in palm oil have been extensively reported in the past. However, due to the scarcity of individual tocotrienol standards, calibrations have mostly been carried out using only α-tocopherol as standard. Moreover, even if the individual tocotrienols are being used, their reliability is often questioned, because tocotrienols are highly susceptible to oxidation and deterioration. This paper reports on the study of the deterioration rate of individual tocotrienol standards upon storage as well as different calibration methods for the tocols in palm oil.

Introduction

Palm oil is known to contain both tocopherols and tocotrienols (1–4). Their amount in crude palm oil (CPO), the oil obtained upon pressing palm fruits, range from 700–1000 ppm (1–3). Earlier studies documented that palm tocols consist of α-tocopherol (α-T), α-tocotrienol (α-T_3), γ- tocopherol (γ-T), γ-tocotrienol (γ-T_3) and δ-tocotrienol (δ-T_3) (1, 3–4). Studies in later years reported α-tocomonoenol (α-T_1) to also be present in palm oil (2). Many studies have indicated that tocotrienols exhibit superior antioxidative and anticancer properties (5–16). Palm tocotrienols have been products of interest in recent years for applications in various industries such as nutraceuticals, pharmaceuticalsand cosmeceuticals.

The analyses of both tocopherols and tocotrienols are carried out by high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) (3, 17–27). The American Oil Chemists' Society (AOCS) documented an official method for the analyses of tocopherols and tocotrienols in vegetable oils and fats (28). Ng et al reported a method for the analyses of palm tocopherols and tocotrienols using SFC (3). Ong et al reported a comprehensive analysis of tocopherols and tocotrienols in blood serum (4). Although chromatographic analyses methods have been documented, the analysis results of tocopherols and tocotrienols have often raised trade disputes, the most debated being the use of individual standards for the analyses of the α-, β-, γ- and δ-tocotrienols. The question on the concentration of the standards has often been raised because the tocotrienols, especially δ-tocotrienol, are easily susceptible to oxidation. The scarcity of tocotrienols and tocomononeol standards also contributed to the difficulty in analyses and calibrations. The official AOCS method recommended the use of α-T as reference in the absence of the tocotrienol standards, which is a common practice (28). This is not often accepted by the industry players, primarily because of the different responses of the individual tocopherols and tocotrienols in ultraviolet (UV) spectroscopic analyses. In addition, the concentration of α-tocomonoenol is often not reported although its presence is quite significant in palm oil.

This paper reports on the analyses of tocopherols, tocomonenols and tocotrienols in palm oil, taking into consideration the effect of mobile phase and calibration by individual standards versus the use of only α-T as standard.

Materials and Methods

Materials

All solvents used were of chromatographic grade, purchased from Merck (Darmstadt, Germany). Tocotrienol standards were purchased from Davos Life Sciences (Singapore). The tocol calibration kit was a gift from the same company. CPO was obtained from the Malaysian Palm Oil Board Milling Technology Centre (POMTEC) in Negri Sembilan, Malaysia.

Preparation of tocol standards stock solution

Ten milligrams α-T standard were weighed and transferred to a 100 mL volumetric flask, making up the volume with hexane. The same procedure was repeated for all other tocol standards. Another set of standards was prepared in a similar manner by substituting hexane with heptane as the dissolving solvent.

Determination of concentration of tocol standards

Determination of the concentration of tocol standards was performed in the manner outlined in the AOCS method (28). Ten milliliters of the α-T standard stock solution was transferred to a 50 mL round bottom flask and the hexane was removed by rotary evaporator at temperature less than 40°C. The content of the flask was then transferred to a 10 mL volumetric flask, making up the volume with methanol. The UV absorbance of the α-T methanolic solution was measured at 292 nm. The concentration of α-T was determined by dividing the absorbance value obtained by its E value (1% / 1 cm). A similar procedure was repeated for all other tocol standards. The E values for each corresponding tocol are shown in Table I.

The tocols were sealed and stored at −5°C. The absorbance was taken again one, two and three weeks after the initial analyses. The whole procedure was repeated five times.

Chromatography analyses of palm tocopherols and tocotrienols

A Waters HPLC coupled with photodiode array detector was used for HPLC analyses of palm tocols. The column used was silica 4.6 mm i.d. x 250 mm length. Chromatography was carried out via two methods using a similar column, as depicted in Table II. The flowrate for both methods was 1 mL/min.

CPO was dissolved in hexane for Method A and in heptanes for Method B. Different concentrations of CPO ranging from

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Table I
E Values of Corresponding Tocopherols and Tocotrienols

Tocols	Absorbance measured at (nm)	E Values (1% / 1cm)
α-tocopherol / tocotrienol	292	0.0076
β-tocopherol / tocotrienol	296	0.0089
γ-tocopherol / tocotrienol	298	0.0091
δ-tocopherol / tocotrienol	298	0.0087

Table II
Mobile Phase for HPLC Analyses

Method	Mobile phase
A	Hexane–THF–IPA (95:4:1)
B	Heptane–ethyl acetate (95:5)

Table III
λ_{max} of Individual Tocotrienols

Tocols	λ_{max} (nm)[a]	λ_{max} (nm)[b]
α-tocotrienol	292	291
β-tocotrienol	296	297
γ-tocotrienol	298	298
δ-ttocotrienol	298	298

[a] AOCS method
[b] This study

4–12 mg/mL were injected to study the effect of oil sample concentrations on the HPLC separations.

Results and Discussion

Determination of concentration of tocol standards

AOCS method Ce 8–89 outlined that the individual tocotrienols should follow their respective tocopherols where the wavelengths at which they absorb maximum UV (λ_{max}) is the concern (28). Table III depicts the λ_{max} of each tocopherol and tocotrienol obtained in this study.

A slight deviation was found ($\pm$ 1 nm) for the λ_{max} of α-T_3 as opposed to the literature. As such, the λ_{max} and E values of individual tocopherols can also be used for individual tocotrienols.

The concentrations of the tocopherol and tocotrienol standards are shown in Figure 1. All tocols showed a deterioration of approximately 3% after one week, approximately 6% after two weeks and less than 10% after three weeks when stored at −5°C over three weeks (Table IV). Concerns have been raised about the reliability of the tocotrienol standards used for analyses because they are more susceptible to oxidation than tocopherols. Deterioration of the tocol standards will result in a report of lower tocol content in samples. It would be best to prepare the standards only when they are needed. However, due to the scarcity of the tocotrienol standards, it is recommended that standards older than two weeks should be discarded or recalibrated.

The elution of palm tocols in normal stationary phase follows the order of α-T, α-T_1, α-T_3, γ-T, and γ-T_3, and δ-T_3 was the last to be eluted. Method A (Figure 2) completed the analyses at less

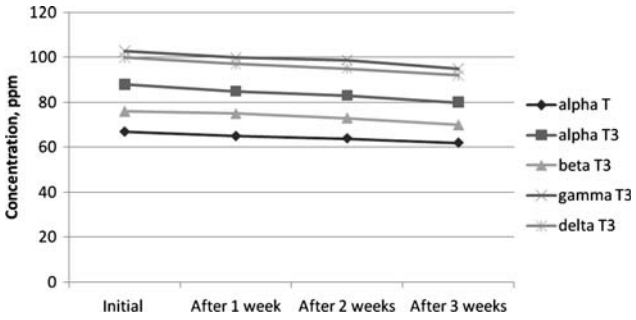

Figure 1. Concentrations of tocopherol and tocotrienols stored over three weeks Chromatography analyses of tocopherols and tocotrienols.

Table IV
Deterioration (%) of Tocols Compared to Initial Concentration

Tocol	Deterioration (%)		
	After 1 week	After 2 weeks	After 3 weeks
α-tocopherol	3.0	4.5	7.5
α-tocotrienol	3.4	5.7	9.1
β-tocotrienol	1.3	3.9	7.9
γ-tocotrienol	2.9	3.9	7.8
δ-tocotrienol	3.0	5.0	8.0

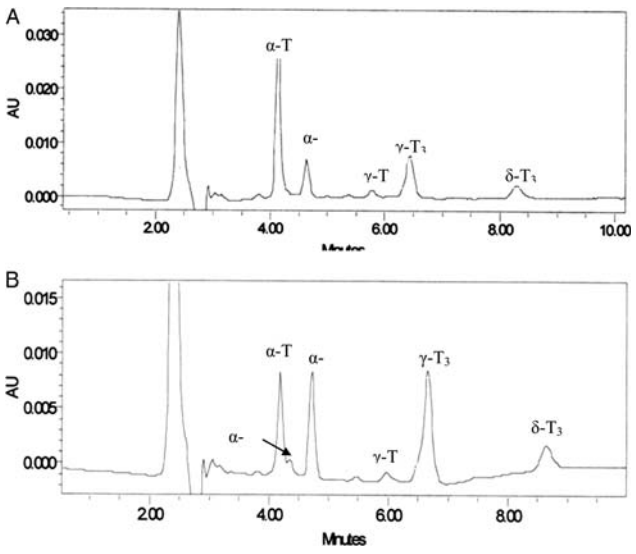

Figure 2. HPLC of CPO by Method A: CPO concentration 4 mg/mL, (B) CPO concentration 12 mg/mL.

than 10 minutes, whereas Method B (Figure 3) took approximately 13 minutes to be completed. Detection was carried out using Photodiode Array Detector at 280 nm. Some studies have reported detection via fluorescence detector. Although both detection methods are comparable, it would nevertheless be advisable to state the method of detection in analysis reports.

A loss of detection was experienced for α-T_1 in Method A when a CPO concentration of less than 5 mg/mL was injected (Figure 2). Analyses carried out by Method B however, did not encounter such a problem.

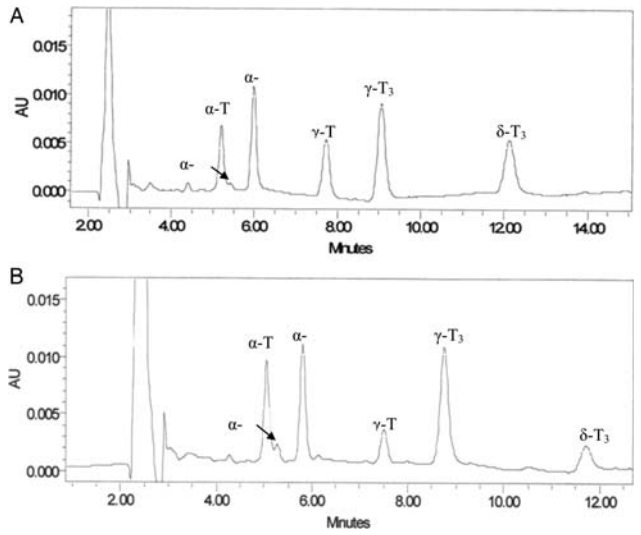

Figure 3. HPLC of CPO by Method B: (A) CPO concentration 4 mg/mL, (B) CPO concentration 12 mg/mL.

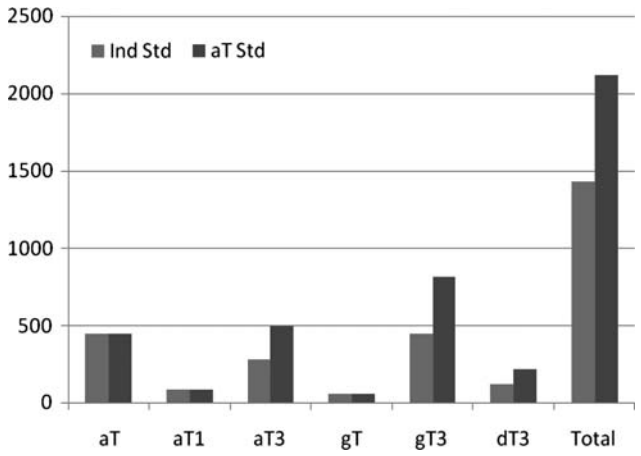

Figure 4. CPO tocol composition analyzed by Method A.

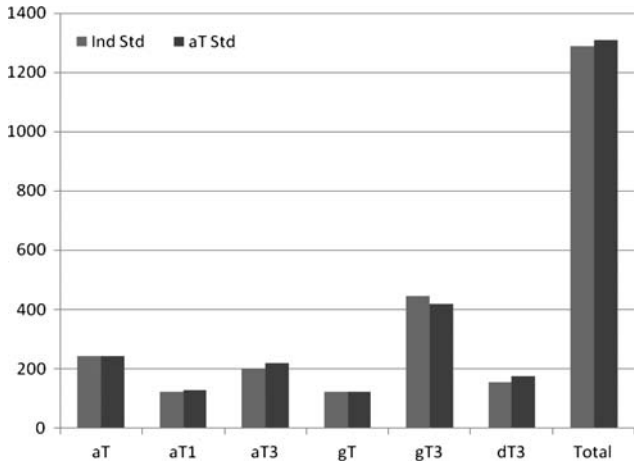

Figure 5. CPO tocol composition analyzed by Method B.

AOCS method Ce 8–89 recommended the use of α-T in the event of unavailability of tocotrienol standards (28). In Method A, it was found that the use of only α-T as standard resulted in a concentration of almost 40% higher than when each of the tocols was calibrated using individual standards (Figure 4). On the other hand, only a 1.7% difference in concentration was reported when HPLC analysis was carried out by Method B (Figure 5).

Analyses carried out by Method B reported the total tocol concentration to be approximately 1300 ppm, regardless of the type of standards used. This is also true for analyses by Method A using individual standards for calibration. Analyses by Method A using α-T as standard, however, reported total tocols of more than 2100 ppm, a deviation of almost 40% compared to when calibration was carried out using individual standards. As such, it is not recommended to use only α-T as standard when analysis is carried out using Method A.

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