A Quantitative Determination of Tocotrienols and Tocopherols in Palm Oil by TLC-GLC [1]

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ABSTRACT

A reliable analytical method is required for determining tocopherols and tocotrienols in palm oil and palm oil fractions. This paper describes a TLC/GLC method which distinguishes between these compounds and is based on their separations from palm oils by saponification and TLC, followed by direct GLC analysis.

INTRODUCTION

Palm oil and palm oil fractions are being used increasingly for the production of fatty foods and for frying fat. In addition to tocopherols, palm oil also contains tocotrienols; both have antioxidative properties, those of tocotrienols being even better than those of tocopherols (1). Since these antioxidative compounds may be removed by the physical and chemical treatments of the palm oil refining process, a reliable analytical method of determining tocopherols and tocotrienols is required. Analysis on the basis of procedures reported in the literature (2-6) is time-consuming and, in our opinion, not reliable. We have developed a TLC-GLC method which distinguishes between tocopherols and tocotrienols and which is based on the separation of these compounds from the oil by saponification and TLC, followed by direct GLC.

The reliability of this TLC-GLC method has been studied by recovery experiments in which known amounts of tocopherols and tocotrienols were added to crude palm oil freed from these compounds by column chromatography.

[1]Dedicated to Professor E. Havina on the occasion of his retirement from the State University of Leyden, The Netherlands.

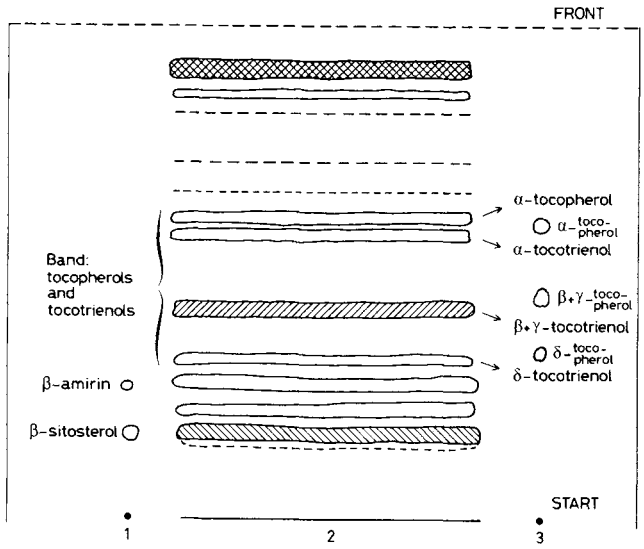

FIG. 1. Separation of the unsaponifiable part of crude palm oil by TLC: 1. β-sitosterol and β-amirin, 2. Unsaponifiable part of crude palm oil, 3. α, β+γ and δ-tocopherol. Adsorbent: silicagel-60 chromatoplates, layer thickness 0.25 mm, ex Merck. Mobile phase: benzene/ethyl acetate (96/4, v/v). Amount of sample: 0.8 mg/cm for unsaponifiable material of crude palm oil and 20 μg for model substances. Spray: 0.2% 2,7-dichlorofluorescein in ethanol.

EXPERIMENTAL

TLC-GLC Method

500 mg Ascorbic acid, 10 ml ethanol and 5 ml of a 60% (m/v) aqueous solution of KOH is added to 5 g crude palm oil. After this mixture has been refluxed for 30 min under nitrogen, it is transferred to a separating funnel with 50 ml distilled water and 50 ml diethyl ether. The unsaponifiable part is extracted by shaking for ca. 1 min. The ether phase is separated and the ethanol/water is extracted with three times 30 ml ether. The ether extract is washed with distilled water until alkali-free, after which the ether is evaporated by a Büchi-Rotavapor. Finally, 30 ml ethanol is added to the residue to remove traces of water by repeated evaporation of the ethanol in vacuum.

The total unsaponifiable part is dissolved in chloroform and brought as a streak of 15 cm length on a Silica Gel G chromatoplate (Silica Gel-60, ex Merck, layer thickness 0.25 mm). β-Amirin, β-sitosterol and α-, (β+γ)- and δ-tocopherol in 20 μg amounts are spotted on the lefthand and righthand side of the chromatoplate respectively (Fig. 1). After ascending TLC with benzene/ethyl acetate (96:4; v/v) as eluant, the tocopherols and tocotrienols separated from other unsaponifiable materials – such as the accompanying 4-demethyl, 4-α-methyl and 4,4-dimethyl sterols – are visualized with 2,7-dichlorofluorescein in ethanol and scraped off the chromatoplate as one band (Fig. 1). An adjusted amount of, for instance, 1.20 mg hexadecyl stearate is pipetted onto the scraped material as internal standard. After extraction with 40 ml chloroform/methanol (70:30; v/v), the collected tocopherols and tocotrienols are

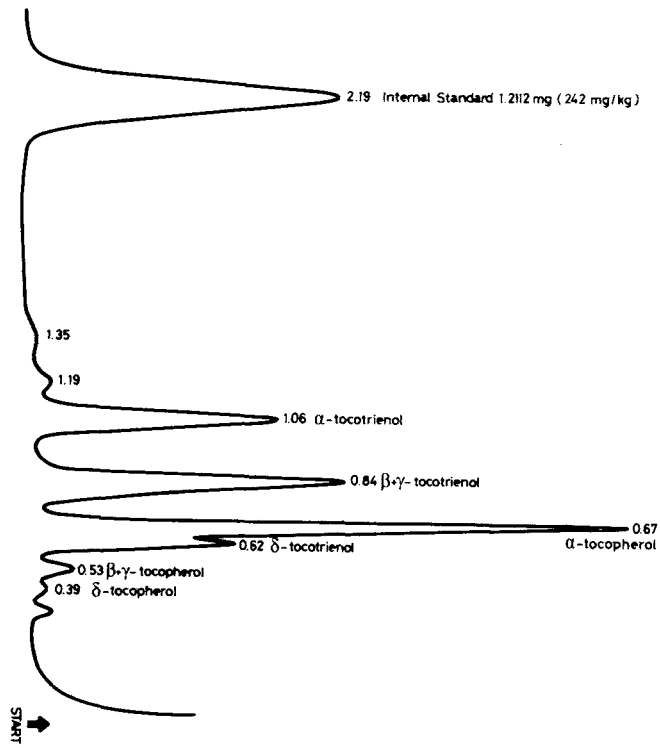

FIG. 2. GLC of the tocopherol and tocotrienol fractions obtained by TLC of the unsaponifiable part of palm oil.

TABLE I

Reproducibility of the Quantitative Determination of Tocopherols and Tocotrienols in Crude Palm Oil by the TLC-GLC Method (Expts. 1a-8a). Recovery Experiments with Isolated Tocopherol and Tocotrienol Fractions of Expts. 1a-8a, Added to Alumina-treated Palm Oil[a] (Expts. 1b-8b)

Expt.		Tocopherol (mg/kg)			Tocotrienol (mg/kg)		
		α	β+γ	δ	α	β+γ	δ
1a		138.7	3.7	0.8	181.4	279.6	33.2
b		137.0	0.8	0.0	167.5	271.2	30.3
2a		132.1	4.7	5.7	167.6	272.4	31.4
b		132.2	1.4	0.0	163.1	261.1	31.5
3a		137.9	4.2	2.2	178.8	276.7	32.4
b		128.9	1.5	0.0	160.5	260.2	31.8
4a		124.4	6.9	15.9	167.3	270.1	27.7
b		134.1	1.0	0.4	165.4	264.3	30.3
5a		141.5	3.3	0.8	181.9	282.3	33.7
b		135.7	1.2	0.0	168.0	267.6	31.4
6a		140.0	4.1	0.8	181.0	280.7	32.6
b		131.6	2.3	0.0	171.1	261.5	29.5
7a		131.3	4.0	0.4	174.1	279.8	33.3
b		127.5	2.8	1.6	174.7	270.4	33.2
8a		137.8	3.9	1.0	184.7	278.2	32.9
b		132.9	2.0	0.0	171.6	265.1	32.0
Mean value	a	135.5	4.4	3.4	177.1	277.5	32.1
	b	132.5	1.6	0.2	167.7	265.2	31.2
Standard deviation (%)	a	4.2	25.4		3.8	1.5	5.9
	b	2.4	42		2.8	1.6	3.8

[a]All values are corrected for the presence of 1-25 mg/kg tocopherol and tocotrienol esters, found by GLC in the alumina-treated palm oil after saponifications.

TABLE II

Evaluation of the Response Factor of Synthetic [a]α-Tocopherol with Respect to Hexadecylstearate by GLC[b]

Sample α-Toc. (mg/ml)	α-Toc. found by GLC (mg)	Calculated response factor for α-tocopherol
1.00	0.89	1.12
2.00	1.85	1.08
3.00	2.77	1.08
4.00	2.67	1.09
5.00	4.57	1.09
6.01	5.56	1.08
7.01	6.43	1.09

[a]Purity 96.29% according to GLC.
[b]Mean value = 1.09. S.D. = 0.014.

dissolved in 1 ml diethyl ether, and then they are quantified by direct GLC by injecting 1 μl of the above mentioned solution on the GLC column (Fig. 2). Column: 2% Silicon oil MS 550/HP chromosorb W 80-100 mesh, AW-DMCS; length 100 cm, diameter 0.4 cm, glass. Column temperature 235 C, injection port temperature 260 C, carrier gas N_2 45 ml/min, FID.

Separation of tocopherols, tocotrienols from the sterols (4-demethyl, 4-α-methyl and 4,4-dimethyl) in the unsaponifiable part of the palm oil by TLC is necessary since on the TLC column the sterols have the same retention times as α-tocotrienol and (β+γ)-tocotrienol. The relative retention times of α-, (β+γ- and δ-tocopherol and α-, (β+γ)-, and δ-tocotrienol measured with respect to β-sitosterol are respectively 0.67, 0.53 and 0.39 for the tocopherols and 1.06, 0.84 and 0.62 for the tocotrienols (Fig. 2).

Analysis of Palm Oils

Eight samples from one batch of crude palm oil were analysed by the TLC-GLC procedure (Expt. 1a-8a; Table I). Additionally, eight recovery experiments were carried out in which the tocopherol and tocotrienol fractions obtained in the first series were added to eight 5 g samples of the same palm oil previously freed from tocopherols and tocotrienols by percolation in hexane on alumina (7) (Expts. 1b-8b). Another three recovery experiments with synthetic α-tocopherol also added to 5 g samples of the same palm oil freed from tocopherols and tocotrienols were carried out at a 25 mg/kg level. The alumina-treated palm oil analysed several times after saponification by the TLC-GLC procedure showed the presence of tocopherol and tocotrienol esters in consistent amounts.

Determination of the Response Factor of Synthetic αTocopherol (αT)

For the evaluation of the response factor of synthetic αT with respect to the chosen internal standard hexadecylstearate, seven GLC analyses have been carried out at different levels of tocopherol concentration (Table II). According to the formula

$$W_{\alpha T} = \frac{\text{Factor} \times O_{\alpha T} \times W_{\text{int.st.}}}{O_{\text{int.st.}}}, \text{ where}$$

$W_{\alpha T}$ = weight of synthetic αT (mg/100 ml; purity 96.29% by GLC)

$O_{\alpha T}$ = peak area of α T measured by triangulation

$W_{int.st.}$ = weight of internal standard hexadecylstearate (mg/100 ml)

$O_{int.st.}$ = peak area of internal standard (triangulation).

The response or correction factor for synthetic αT with respect to hexadecylstearate was calculated to be 1.09 (coefficient of variation 1.3%).

RESULTS AND DISCUSSION

The separation of α-tocopherol, α-tocotrienol, (β+γ)-tocopherol (β+γ)-tocotrienol, δ-tocopherol and δ-tocotrienol from the sterols (4-demethyl, 4-α-methyl and 4,4-dimethyl) in the unsaponifiable part of the palm oil was achieved by TLC, using benzene/ethyl acetate (96:4; v/v) as eluant. The mixture of β- and γ-tocopherol and β- and γ-tocotrienol, however, could be separated by GLC only into fractions of (β+γ)-tocopherol and (β+γ)-tocotrienol; no literature data on the complete separation of the latter compounds by GLC are available yet. The peak areas of the tocopherols and tocotrienols were calculated by computer. So the δ-tocotrienol/α-tocopherol overlapped peaks could be calculated accurately by tracing the minimum between those peaks. The results of TLC-GLC determination of tocotrienols and tocopherols (Expts. 1a-8a) as well as those of the recovery experiments (Expts. 1b-8b) are collected in Table I. In these experiments the losses of tocopherols and tocotrienols occurring during all the different analytical steps (saponifications, spotting onto chromatoplates, exposure to relatively high temperatures during GLC analyses) and air oxidation during the whole procedure, could be checked by comparing the data in Table I.

The losses of α-tocopherol, α-tocotrienol, (β+γ)-tocotrienol and δ-tocotrienol are very low: 2.2, 5.3, 4.4 and 2.8% (mean values) respectively. The recovery of synthetic α-tocopherol was 91.3, 96.3 and 92.1%, the coefficient of variation being 2.9%.

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