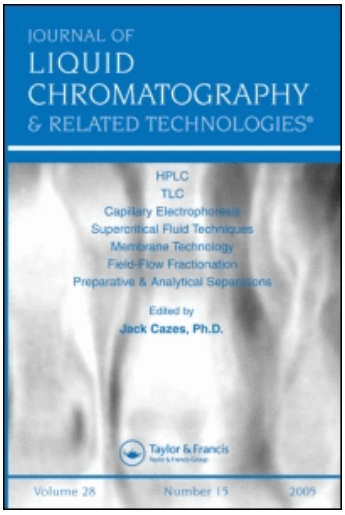

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QUANTITATIVE TLC FOR DETERMINATION OF THE TRIACYLGLYCEROL COMPOSITION OF SESAME SEEDS

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ABSTRACT

Quantitative silver ion TLC and reversed phase TLC were applied in complementary ways to determine the triacylglycerol composition of a commercial sample of sesame seeds. Twenty-three triacylglycerol species were identified and quantified with good reproducibility and accuracy, the main ones being OLL (19.2%) and OOL (18.0%), followed by LLL (9.6%), OOO (8.9%) and POL (8.6%) (P-Palmitic; O-Oleic; L-Linoleic). The number of determined triacylglycerols in sesame seeds exceeds those reported previously in the literature.

Key Words: Silver ion TLC; Reverse-phase TLC; Triacylglycerol composition; Sesame seed oil

INTRODUCTION

Sesame is a food and oilseed crop of great antiquity and is probably one of the oldest of the cultivated oilseed crops.[1] The oil is unusually stable, the

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stability being ascribed to the presence of fat-soluble lignans of the series of 2,6-diaryl-3,7-dioxabicyclooctanes.[2] Important characteristics of the oil are the high and almost equal content of oleic (32.7–53.9%) and linoleic (39.3–59%) acids.[3] Because of these properties, the high quality product is a valuable edible oil while the lower grade products are used to produce margarines, confectionary fats, or as carrier fats in pharmaceuticals. Sesame oil is also a well-known and widely used additive to other edible oils as it increases their antioxidation stability, showing synergistic effect as an antioxidant with α-tocopherol.[3] The seeds are widely used in confectionery and bakery.

Plant, seeds and oil have been used in the folk medicine of India, China, Japan, and many other countries in the South and Southeast and, later, specific chemical constituents were found to be physiologically active, helping in some cases of lung disease[4,5] and some forms of breast and lung cancer.[6–8] The chemical constituents of sesame are, therefore, subject of constant intensive investigations and the literature on the matter, especially by Japanese authors, is very large. With this background, it is interesting to note the lack of detailed information on the triacylglycerol (TAG) composition of the oil. In the latest edition of the Lipid Handbook,[3] the data on sesame TAGs are based on a work of Jurriens, published in 1968,[9] in which nine TAG unsaturation groups were determined. A recent paper dealing with matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry[10] reported only the presence of eight major TAGs in sesame oil.

As a part of a program for examination of the quality of local and imported edible oilseeds and oils offered in the Bulgarian market, we report here on the TAG composition of sesame seeds as determined by successive application of silver ion (Ag-) and reversed phase (RP-) TLC/scanning densitometry.

EXPERIMENTAL

All reagents and solvents were analytical grade. Petroleum ether was of the b.p. 40–60°C fraction, diethyl ether was peroxide-free, and chloroform was washed extensively to remove the stabilizing alcohol. Dimethyldichlorosilane (DMDS) was purchased from Fluka (Buchs, Switzerland); Kieselguhr G, Silica gel G, bromine and sulfuryl chloride were obtained from Merck (Darmstadt, Germany).

Sesame seeds were from a bakery product supplied by a local market.

A reference TAG mixture was prepared by mixing equal quantities of TAGs from lard and sunflower oils. Added to this mixture was 100 mg of tristearin in order to increase the proportion of the trisaturated TAGs (SSS; S, saturated acyl residue) to a reasonable value. This mixture was used to identify the TAGs from SSS to DDD (D, dienoic acyl residues). Pure TAG fractions with known

composition from tangerine oil[11] and from linseed oil[12] were used to better identify TAGs, which contained linoleic and linolenic acid.

Extraction of Lipids

The procedure described by Christie[13] was used to extract the lipids. In brief, 200 mL of isopropanol was added to the ground seeds (2 g) and the mixture was stirred for 30 min with a magnetic stirrer. It was then filtered and the residue was extracted with fresh 100 mL of the solvent for another 30 min.

After filtration, the residue was shaken for 10 h with 10 mL of chloroform–isopropanol, 200 : 1 (by vol.). The filtrates were combined; most of the solvent was removed in a rotary evaporator and then taken to near dryness under nitrogen. The residue was then taken up in 30 mL chloroform–methanol, 2 : 1 (by vol.). The solution was washed with 20 mL of potassium chloride (0.9%). The upper layer was removed by aspiration and the rest was washed twice with 10 mL of methanol–saline, 1 : 1 (by vol.). Finally, the bottom layer was filtered in a round bottom flask; most of the solvent was evaporated in a rotary evaporator, and the rest was transferred quantitatively to a previously weighed glass container. The rest of the solvent was evaporated under nitrogen to a constant weight of the lipid residue.

The residue was dissolved in dichloromethane to give a 10% stock solution of total lipids.

Isolation of TAGs by Preparative Silica Gel TLC

A sample of 200 mg from the stock was applied onto 20 cm × 20 cm plates (*ca.* 1 mm thick layer) and developed with petroleum ether-acetone 100 : 8 (by vol.). The separated zones were detected by spraying the edge of each plate with 2,7-dichlorofluorescein. The TAG zone was scraped, transferred to small glass column and eluted with ethyl ether. The solvent was evaporated under stream of nitrogen and the residue was weighed in small glass containers (of known weight) to a constant weight.

Analysis of TAGs

Quantitative Ag-TLC

The procedure was described in detail elsewhere.[14] Briefly, TAG classes differing in unsaturation were separated on 19 × 4 cm glass plates, coated with an approximate 0.2 mm silica gel G layer and impregnated by dipping into a 0.5%

methanolic solution of silver nitrate. The sample size and the mobile phase composition, depending on the separation needed, are given in Table 1. Continuous ascending development with the respective volume of the mobile phase in open cylindrical tanks (24 cm × 5 cm i.d) was performed. The plate was then dried (1 h at 110°C), and treated consecutively with bromine and sulphuryl chloride vapors (30 min each, in closed tanks and in a fume-cupboard) to ensure the correct quantitative charring (at 180–200°C) of the separated TAG classes.

Preparative Ag-TLC

Preparative Ag-TLC was carried out as described by Nikolova-Damyanova and Amidzhin.[15] TAG classes were separated on 20 × 20 cm home-made glass plates (*ca.* 1 mm thick silica gel G layer which contained 5% silver nitrate). Plates were sprayed with 2,7-dichlorofluorescein and TAG zones were visualized under UV light, scraped off, and eluted with diethyl ether. The purity and identity of each zone was checked by analytical Ag-TLC after co-chromatography with the reference TAG mixture and the source oil, applied alongside. The solvent was removed under nitrogen and samples were redissolved in hexane to give a 0.1% solution.

Quantitative RP-TLC

The procedure described by Chobanov et al.[16] was used. In brief, 19 × 4 cm glass plates, covered with *ca.* 0.2 mm thick Kieselguhr G layer, were first treated for 6 hr with vapors of DMDS and then washed by a single elution with methanol. A 5–10 μL aliquot of the 0.1% TAG chloroform solution was applied on the plate and developed twice in a closed cylindrical tank (dimensions above), each time with fresh 3 mL of the mobile phase, to a solvent front movement of 17 cm. A three component mobile phase, acetone–acetonitrile–water, 7 : 3 : X (by volume) was used with acetone–acetonitrile ratio kept constant and varying the water proportions. The chromatographic conditions are presented in Table 2. Plates were dried (at 110°C for 1 hr) and the separated species were visualized by spraying with 50% ethanolic sulphuric acid and heating at 200–220°C for about 5 min over a temperature-controlled metal plate.

Quantification by Scanning Densitometry

The densities of the charred spots were measured by a CS-930 densitometer, (Shimadzu Corporation, Kyoto, Japan) equipped with DR-2 Shimadzu integrator, in the zigzag reflection mode at 450 nm. Beam-slit was

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Table 1. Chromatographic Conditions for the Separation of TAG Classes by Silver Ion TLC

Separation of S_3, SM_2 and S_2M[a]			Separation of TAGs from S_2M to M_2D			Separation of the Sum of D_2S, D_2M, D_3, SDT		
Sample (μg)	Mobile Phase[b]	Volume (mL)	Sample (μg)	Mobile Phase	Volume (mL)	Sample (μg)	Mobile Phase	Volume (mL)
50	PE : A, 100 : 4	6	30–35	PE : A, 100 : 4	6	25–30	Hx : A : EtOH, 100 : 4 : 2	6

[a]S, saturated, M, monoenoic, D, dienoic and T, trienoic fatty acid residues.
[b]EtOH, ethanol; PE, petroleum ether (b.p. 40–60°C); Hx, hexane; A, acetone; the proportion is in volume parts.

Table 2. Water Proportion in the Mobile Phase Acetone–Acetonitrile–Water, 70 : 30 : X, for the Separation of Triacylglycerol Groups into Molecular Species by RP-TLC

TG Class[a]	TG Species[b]	PN[c]	Water Proportion, by Volume
S_2M	PPO, PStO, StStO	48, 50, 52	12
SM_2	POO, StOO, AOO	48, 50	14
S_2D	PPL, PStL, StStL	46, 48, 50	12
SMD	POL, StOL, AOL	46, 48	18
SD_2	PLL, StLL, ALL	44, 46	20

[a]For the abbreviations see the footnote to Table 1.
[b]The order of designation does not indicate positional isomers, P, palmitic; St, stearic; A, arachidic; O, oleic; L, linoleic; fatty acid residues.
[c]Partition number $PN = CN - 2n$; CN – number of carbon atoms, n – number of double bonds.

varied from 0.4×0.4 mm to 1.2×1.2 mm and the stage step varied depending on the separation achieved. The quantity of each spot was presented as relative area percent, as derived from the integrator. Two sets of densitometric results were obtained: Ag-TLC provided the quantitative data for the TAG classes differing in unsaturation and RP-TLC for the TAG species differing in chain-length within a given class. It is clear that the Ag-TLC results were of vital importance as they were used as the bases to recalculate the RP-TLC results and to produce the final data for the TAG composition of the sample.

Gas Chromatography (GC) of Fatty Acid Methyl Esters

Fatty acid composition of TAGs was determined on the corresponding methyl esters, prepared according to Hartman and Lago.[17] A Hewlett-Packard model 5890 (Hewlett Packard, GmbH, Austria) gas chromatograph was used with a $30\,\text{m} \times 0.25\,\text{mm}$ (I.D.) capillary glass INNOWax column (cross-linked PEG, Hewlett Packard, GmbH, Austria). The column temperature was programmed from 165°C (held for 3 min) to 200°C at 4°C min^{-1} and held at this temperature for 20 min; injector and detector temperatures were 260°C. Nitrogen was used as the carrier gas.

RESULTS AND DISCUSSION

TAGs comprised 90.5% of the lipid fraction. When subjected to qualitative Ag-TLC, the TAG mixture was separated into eleven unsaturation

Table 3. Triacylglycerol Composition of Sesame Seeds

TAG Species[a]	Rel.%
Trisaturated	Traces
PPO	0.5
PstO	1.6
PAO + StStO	1.4
StAO	0.6
POO	6.4
StOO	3.3
AOO	0.3
OOO	8.9
PPL	2.0
PStL	1.8
PAL + StStL	0.8
StAL	0.2
POL	8.6
StOL	4.8
AOL	0.7
OOL	18.0
PLL	6.1
StLL	4.1
ALL	0.8
OLL	19.2
LLL	9.6
SLLn	traces

[a]For the abbreviations, see the footnotes to Table 2; the order of designation does not present the position of the acyl moiety in the molecule.

groups, unambiguously identified as S_3 (traces), S_2M, SM_2, S_2D, M_3, SMD, M_2D, D_2S, D_2M, D_3, SDT (traces) migrating in this order (S, saturated-, M, monoenoic-, D, dienoic- and T, trienoic acyl residues). These groups were quantified by Ag-TLC/densitometry in two stages. All components but D_2S, D_2M, D_3 and SDT (which were determined as a sum) were well resolved and quantified by a single development with 6 mL of petroleum ether-acetone, 100 : 4 (by volume). The whole sample was applied to another plate and the four unresolved TAGs were well separated and determined by using a mobile

phase of hexane–acetone–ethanol, 100 : 4 : 2 (by volume); M_2D was the internal standard. Because of the necessity of two-stage determination of the TAG composition in Ag-TLC, and because this procedure provided the basic quantitative data, each quantitative result was a mean of six independent measurements, the relative standard deviation not exceeding 12%.

The TAG groups that contained saturated acyl residue were isolated from the total mixture by preparative Ag-TLC and each class was then subjected to RP-TLC. This intermediate step allowed for the determination of components with content less than 1% in the total mixture. The identification of TAG species in RP-TLC was based on the fatty acid composition and the respective partition numbers (PN = CN-2n, where CN is the total number of carbon atoms in the three acyl moieties and n is the number of double bonds) and was performed.[18] using triolein (PN = 48) as a reference TAG. Each quantitative result was a mean of four independent measurements, with a standard deviation not exceeding 10% rel.

The results of Ag- and RP-TLC were combined to give the final TAG composition of the sesame seeds (Table 3). Twenty-three TAG species were determined, the main components being OLL (19.2%) and OOL (18.0%), followed by LLL (9.6%) OOO (8.9%) and POL (8.6%) (P, palmitic acid, O, oleic acid, L, linoleic acid residues, the order of designation does not present the position of the acyl moiety in the molecule). Because of certain limitation in the resolution power of RP-TLC, we were not able to resolve species that had the same PN, such as PAO and StStO (PN = 52), and PAL and StStL (PN = 50).

The respective values in the Table are presented as sums, but there is no clear evidence that all four components are present in the sample. It is evident from Table 3 that the unsaturated TAGs contained only oleic and linoleic acyl residues which comprised 55.7% of the total content, i.e., the TAG composition is

Table 4. Comparison Between the Fatty Acid Composition of Sesame TAGs (a) Determined Directly by GLC and (b) Calculated from TAG Data

Fatty Acid	a	b
16 : 0	9.2	10.0
18 : 0	5.4	6.1
20 : 0	0.6	0.8
18 : 1	41.6	40.1
18 : 2	41.5	42.0
18 : 3	0.3	traces

of oleic/linoleic type much like that of corn and cottonseed oils.[3,12] This composition of the sesame seed oil and its high stability determine the high nutrition value of the product.

Based on the TAG composition of the sesame seeds, their fatty acid composition was calculated. The values obtained were compared with those determined directly by GC in Table 4. The good fit between the two sets of results confirms the accuracy of the present determination.

To the best of our knowledge, this is the most detailed determination of sesame seed TAGs at present.

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