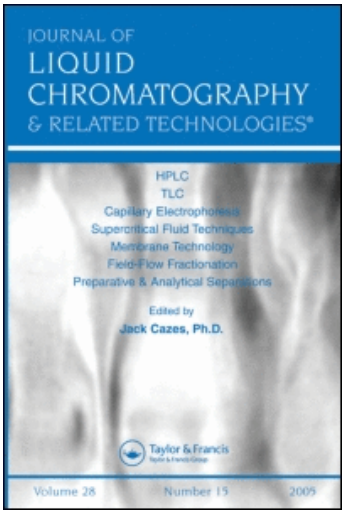

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Quantitative TLC and Gas Chromatography Determination of the Lipid Composition of Raw and Microwaved Roasted Walnuts, Hazelnuts, and Almonds

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Abstract: Analytical and preparative thin-layer chromatography (TLC) on intact silica gel and silica gel layers modified with either silver nitrate (Ag-TLC) or dimethyldichlorosilane (RP-TLC), combined with densitometric quantification and gas chromatography (GC), have been used to elucidate the lipid classes, their fatty acid profiles, the triacylglycerol, and sterol compositions of raw and microwaved roasted walnuts, hazelnuts and almond kernels harvested in Bulgaria. The results on fatty acid and triacylglycerol compositions are in good agreement with those reported for other geographical regions. Microwave roasting on full power for 3 min produced ready-to-consume kernels and no changes in lipids were detected. The combination of TLC techniques used for identification and quantification of triacylglycerols gave results comparable with those obtained using reversed phase liquid chromatography-mass spectrometry (LC-MS).

Keywords: Thin-layer chromatography, Gas chromatography, Lipid composition, Walnut, Hazelnut, Almond

INTRODUCTION

Walnuts, hazelnuts, and almonds are three nuts of major importance in the human diet, and are especially popular in the Mediterranean and nearby regions where they are harvested. Shelled and peeled, these nuts are used

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either as is, usually after appropriate roasting, or as ingredients in many bakery and confectionary products, e.g., ice creams. Their wide use dictates the interest in elucidating the chemical composition of these nuts and the effect of any treatment on them.

Lipids are the major constituents of these nuts, with a content in the range of 52 to 68%, depending on the species[1,2] and are characterized by the high content of oleic (*cis* 9–18:1) and linoleic (*cis* 9,12–18:2) acids.[2–4]

As a part of our program for studies of the lipid composition of different food products harvested and consumed in Bulgaria, we report here on the lipid classes, their fatty acid profiles, and the triacylglycerol composition of raw and microwaved roasted, ready-to-consume, walnut, hazelnut, and almond nuts harvested locally. Analytical and preparative TLC on intact and modified silica gel layers, combined with densitometric quantification and gas chromatography (GC) have been used for these purposes.

EXPERIMENTAL

All reagents and solvents were of analytical grade. Hexane was left for 24 h over potassium hydroxide and then distilled; diethyl ether was peroxide-free. Chloroform was first washed to remove the stabilizing alcohol, then dried over $CaCl_2$ and distilled. Dimethyldichlorosilane (DMDS) was purchased from Fluka (Buchs, Switzerland); Kieselguhr G, Silica gel G, bromine and sulphuryl chloride were obtained from Merck (Darmstadt, Germany). Walnuts, hazelnuts, and almonds were purchased from local markets.

A reference mixture of lipid classes was prepared by mixing equal aliquots of 100 mg/mL solutions of docosane, cholesteryl oleate, methyl oleate, oleyl alcohol, cholesterol, 1,3-diolein, 1-monoolein-rac-glycerol, L-α-dioleylphosphatidyl-choline (all purchased from Sigma-Aldrich Chemie GmbH, Germany) and a purified (preparative silica gel thin-layer chromatography, see below) triacylglycerol (TAG) fraction from sunflower oil in dichloroethane. A reference TAG mixture was prepared by mixing equal quantities of TAGs from lard and sunflower oils; added to this mixture was 10%, by weight, of tristearin in order to increase the proportion of the trisaturated, SSS, (S, saturated acyl residue) TAG to a reasonable value. This mixture was used to identify the TAGs from SSS to DDD (D, dienoic acyl residue). Pure TAG fractions with known compositions from tangerine oil[5,6] and from linseed oil[7] were used to identify TAGs which contained linoleic and linolenic acyl residues.

Preparation of the Kernel Oil Samples

Portions of the walnut, almond, and hazelnut kernels were roasted in a microwave oven (3 min at 100% power). Raw and roasted kernels were

ground in a coffee grinder and 20 g of the material were used for further investigation. The oil content was determined on three separate samples (10 g each) by Soxhlet extraction with hexane for 6 h.

Extraction of Lipids

The procedure described by Christie[8] was used. In brief, each sample (2 g) was homogenized for 30 min with 200 mL of isopropanol using a magnetic stirrer. The mixture was 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 chloroform-isopropanol, 200:1 by volume. The filtrates were combined; most of the solvent was removed in a rotary evaporator and the residue was taken to dryness under nitrogen. The residue was then dissolved in 30 mL of chloroform-methanol, 2:1 by volume. The solution was washed with 20 mL of potassium chloride (0.9%). The upper layer was removed by aspiration and the remainder was washed twice with 10 mL of methanol-saline, 1:1 by volume. Finally, the bottom layer was filtered in a round bottom flask; most of the solvent was then evaporated in a rotary evaporator and the rest was transferred quantitatively in 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 dichloroethane to give a 100 mg/mL stock solution of total lipids. An aliquot was taken to give a 5 mg/mL solution in dichloroethane for identification of the lipid classes.

Identification of the Main Lipid Classes by Analytical Silica Gel TLC

To determine the main lipid class composition, an aliquot of the 5 mg/mL lipid solution (sample size of about 50–100 μg) in dichloroethane was placed on a 19 cm × 4 cm glass plate (ca. 0.2 mm thick silica gel G layer). The lipid classes were identified by comparison with a reference lipid mixture (20 μL of 10 mg/mL solution in dichloroethane) which was placed on the side of the plate. The plate was developed once with ca. 4 mL hexane-acetone, 100:8 (by volume). The lipid zones were detected by spraying with 50% ethanolic sulphuric acid and heating at 200°C on a temperature controlled hot plate.

Isolation and Quantification of Lipid Classes by Preparative Silica Gel TLC

Aliquots of the 100 mg/mL stock solution (sample size of 100 mg, precisely measured) were applied on 20 cm × 20 cm glass plates (ca. 1 mm thick silica

gel G layer) and developed with hexane-acetone, 100:8 (by volume). The separated zones were detected under UV light by spraying the edges of each plate with 2′,7′-dichlorofluorescein, they were then scraped, transferred to small glass columns and eluted with diethyl ether. The solvent was evaporated under a stream of nitrogen and the residue was weighed in a small glass container to a constant weight.

Analysis of Triacylglycerols (TAG)

Quantitative Ag-TLC

The procedure described by Nikolova-Damyanova et al.[9] was used. Briefly, TAG classes were separated according to their unsaturation on 19 cm × 4 cm glass plates, coated with ca. 0.2 mm silica gel G layer and impregnated by dipping into 0.5% or 2.0% methanolic solution of silver nitrate. The silver nitrate concentration, the sample size and the mobile phase composition depended on the required separation and are shown in Table 1. Continuous ascending development with the specified volume of the mobile phase in open cylindrical tanks (24 cm × 5 cm i.d.) was performed. The plates were then dried (1 h at 110°C), and treated consecutively with bromine and

Table 1. Chromatographic conditions for separation of the TAG classes by Ag-TLC

	Walnut	Hazelnut	Almond
TAG classes	from S_2M to M_2D	from S_2M to M_2D	from S_2M to D_3
Mobile phase: composition (v/v/v)	Hx:A:EtOH 100:4:1	Hx:A:EtOH 100:4:1	Hx:A:EtOH 100:4:1
volume (mL)	5.5	6.0	6.0
Sample (μg)	100	10–50	10
% $AgNO_3$	0.5	0.5	0.5
TAG classes	from M_2D to M_2T	from M_2D to D_3	D_2S and D_2M
Mobile phase: composition (v/v/v)	Hx:A:EtOH 100:8:1	Hx:A:EtOH 100:7:1	Chl:MeOH 100:4
volume (mL)	8.0	6.0	10.0
Sample (μg)	20	50	10
% $AgNO_3$	0.5	0.5	2
TAG classes	from M_3 to DT_2		
Mobile phase: composition (v/v/v)	Hx:A:EtOH 100:8:3		
volume (mL)	8.0		
Sample (μg)	20–30		
% $AgNO_3$	0.5		

S - saturated, M - monoenoic, D - dienoic and T - trienoic fatty acyl residues. Hx - hexane, A - acetone, EtOH - ethanol, Chl - chloroform, MeOH - methanol.

sulphuryl chloride vapors (30 min each, in a closed tank and in a fume-cupboard) to ensure the correct quantitative charring (at 180–200°C on a temperature controlled hot plate) of the separated TAG classes.

Preparative Ag-TLC

Preparative Ag-TLC was carried out according to Nikolova-Damyanova et al.[10] TAG classes were separated on 20 cm × 20 cm glass plates (ca. 1 mm thick silica gel G layer impregnated in 2% silver nitrate methanolic solution) using the following mobile phases: chloroform-acetone, 100:1.6 (by volume) for S_2M and SM_2 classes; chloroform-acetone, 100:2 (by volume) for S_2D class; chloroform-methanol, 100:3.5 (by volume) for SMD and SD_2 classes, and chloroform-methanol, 100:4.5 (by volume) for SDT class. 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 along the side. The solvent was removed under nitrogen and the sample was redissolved in dichloroethane to give a 1 mg/mL solution.

Quantitative RP-TLC

Quantitative RP-TLC was carried out according to Chobanov et al.[11] In brief, 19 cm × 4 cm glass plates covered with ca. 0.2 mm thick Kieselguhr G layer were first treated for 6 h with vapors of DMDS and then washed with a single elution of methanol. A 5–10 μL aliquot of the 1 mg/mL TAGs dichloroethane solution was applied on the plate and developed twice in a closed cylindrical tank (dimensions as above), each time with fresh 3 mL of the mobile phase to a solvent front of 17 cm. A three component mobile phase (acetone/acetonitrile/water) was used with a constant acetone/acetonitrile ratio (7:3, by volume). The mobile phase compositions depended on the unsaturation of the TAG class and are presented in Table 2. Plates were dried at 110°C for 1 h 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 hot plate.

Quantification by Scanning Densitometry

The densities of the charred spots were measured with a CS-930 densitometer (Shimadzu Corporation, Kyoto, Japan) equipped with a DR-2 Shimadzu integrator, in the zigzag reflection mode at 450 nm. The beam-slit was 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 the relative area percent, as derived from the integrator. Two sets of densitometric results were obtained: Ag-TLC provided the quantitative data for the TAG

Table 2. Water proportion in the mobile phase acetone/acetonitrile/water, 70:30:W, for separation of TAG classes into molecular species by RP-TLC

TAG class[a]	TAG species[b]	PN[c]	Water proportion, W (by volume)
S_2M	PPO, PStO, StStO	48, 50, 52	12
SM_2	POO, StOO	48, 50	12
S_2D	PPL, PStL, StStL	46, 48, 50	14
SMD	POL, StOL, AOL	46, 48, 50	16
SD_2	PLL, StLL, ALL	44, 46, 48	18
SDT	PLLn, StLLn	42, 44	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; Ln - linolenic fatty acyl residues.

[c]Partition number, PN = CN-2NDB (CN, number of carbon atoms, NDB, number of double bonds).

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 great importance and were used as the base to recalculate the RP-TLC results and to produce the final data for the TAG composition of the sample. The standard deviation of Ag-TLC in the present analysis (three separate TLC runs) did not exceed 10% rel.

Gas Chromatography (GC) of Fatty Acid Methyl Esters (FAME) and Sterols

FAME of the total sample as well as of the fatty acids from sterol esters (SteE), TAG and diacylglycerols (DAG) were prepared according to Hartman and Lago.[12] Free fatty acids were methylated according to Christie[13] with 1% methanolic sulfuric acid for 2 h at 50°C. FAME were then purified by preparative TLC on silica gel as described above (separation of lipid classes). A Hewlett Packard model 5890 (Hewlett Packard GmbH, Austria) gas chromatograph was used with a 30 m × 0.25 mm (I.D.) capillary INNOWax column (cross-linked PEG, Hewlett Packard GmbH, Austria). The column temperature was programed from 165°C to 240°C at 4°C/min and held at this temperature for 10 min; injector and detector temperatures were 260°C. Nitrogen was the carrier gas at a flow rate 0.8 mL/min; split 100:1.

The proportions of free sterols and those derived from the sterol esters were determined on a 30 m × 0.25 mm (I.D.) HP-5MS capillary column (Agilent Technologies, Santa Clara CA, USA) under the following conditions: temperature gradient from 90°C (held for 2 min) to 290°C at 15°C/min then to 310°C at 4°C/min and held at this temperature for 10 min; the injector

temperature was 300°C and the detector temperature was 320°C. Nitrogen was the carrier gas at a flow rate 0.8 mL/min; split 100:1.

The results were presented as the mean area percent (as derived from the integrator) of three separate injections ± standard deviation (S.D.).

RESULTS AND DISCUSSION

Lipid Class Composition

The raw walnut, hazelnut, and almond kernels contained, respectively, 80, 70, and 42% oil (Table 3), which agreed, in general, with data published elsewhere.[1,14–16] The three kernel oils consisted almost entirely of triacylglycerols (>95%). The other lipid classes were sterol esters (except for almond oil), sterols, diacylglycerols and polar lipids not exceeding 2% each. An unidentified lipid zone in negligible quantity, less polar than triacylglycerols, was determined in walnut and hazelnut oils as well. After kernel roasting, the oil content increased but no marked changes in lipid class composition were observed (Table 3).

Fatty Acid Composition

The fatty acid composition of the total lipids from the three analyzed oils is presented in Table 4. The results are in general agreement with the data from other authors.[3,4,14,16–20] Nine fatty acids were identified and quantified and myristic acid (14:0) was in trace amounts in all the three kernel oils. Eicosanoic acid (20:0) in negligible quantity (up to 0.1%) was measured in hazelnut and walnut oils. The other saturated fatty acids, palmitic (16:0) and stearic (18:0), were in almost equal amounts in the latter oils, respectively about 6% and 2%, and in slightly higher proportions in hazelnut oil. In walnut oil linoleic acid (*cis* 9,12–18:2) was the major fatty acid (about 63%) whereas in hazelnut and almond oils oleic acid (*cis* 9–18:1) was in the greatest quantities, respectively about 78% and 66%. Although only the walnut oil contained considerable amount of linolenic acid (*cis* 9,12,15–18:3), its total unsaturation, represented by U/S (U–unsaturated-, S–saturated fatty acids), was very similar to that of almond oil. Hazelnut oil had a little lower unsaturation (Table 4). Other authors have determined negligible amounts of fatty acids such as 15:0, 17:0, 17:1, 20:1, 21:1, 22:0 in hazelnut oil[4,17,20] as well as 20:5 and 22:0 in walnut oil[14] but, as discussed in the literature,[15,21,22] the lipid composition is greatly influenced by genetic and environmental factors.

No significant changes in fatty acid composition of the three kernel oils, after the roasting time chosen, were observed (Table 4). This finding was in agreement with published data claiming that noticeable alteration of other

Table 3. Major lipid classes in walnut, hazelnut and almond kernel oils (wt.%)

	Walnut		Hazelnut		Almond	
Lipid class	Raw	Roasted	Raw	Roasted	Raw	Roasted
Sterol esters	1.3 ± 0.4[a]	1.4 ± 0.2	0.4 ± 0.1	0.6 ± 0.3	n.d.[b]	n.d.
Triacylglycerols	95.5 ± 1.1	96.4 ± 1.4	97.1 ± 1.3	96.0 ± 1.2	98.2 ± 1.5	98.2 ± 1.4
Unidentified	1.0 ± 0.3	0.7 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	n.d.	n.d.
Sterols	0.7 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.4 ± 0.1
Diacylglycerols	1.3 ± 0.3	0.9 ± 0.1	1.0 ± 0.2	1.3 ± 0.3	0.6 ± 0.2	0.9 ± 0.2
Polar lipids	0.3 ± 0.05	0.2 ± 0.05	0.7 ± 0.1	1.0 ± 0.1	0.3 ± 0.1	0.4 ± 0.3
Oil content	80.3 ± 1.4	83.4 ± 1.6	70.3 ± 1.2	80.1 ± 1.4	42.3 ± 0.8	57.8 ± 0.9

[a]Mean ± S.D. (S.D., standard deviation), n = 3.
[b]Not detected.

Table 4. Fatty acid composition[a] of the total lipids in walnut, hazelnut and almond kernel oils (wt.%)

	Walnut		Hazelnut		Almond	
Fatty acid	Raw	Roasted	Raw	Roasted	Raw	Roasted
14:0	tr.[b]	tr.	tr.	tr.	tr.	tr.
16:0	6.0 ± 0.2[c]	6.2 ± 0.2	6.7 ± 0.1	6.4 ± 0.1	6.0 ± 0.1	6.3 ± 0.1
16:1	tr.	tr.	0.2 ± 0.03	0.4 ± 0.03	0.4 ± 0.1	0.5 ± 0.1
18:0	2.0 ± 0.1	2.1 ± 0.1	3.5 ± 0.1	4.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.1
cis 9-18:1	14.8 ± 0.3	15.4 ± 0.5	78.8 ± 1.2	77.9 ± 1.4	65.7 ± 1.3	66.5 ± 1.4
18:1 isom.	0.7 ± 0.1	0.6 ± 0.1	n.d.	n.d.	1.0 ± 0.1	0.9 ± 0.1
cis 9,12-18:2	63.1 ± 1.2	62.7 ± 1.4	10.5 ± 0.8	10.8 ± 0.5	24.8 ± 0.8	24.0 ± 0.7
cis 9,12,15-18:3	13.4 ± 0.3	13.0 ± 0.2	0.2 ± 0.04	0.3 ± 0.05	tr.	tr.
20:0	tr.	tr.	0.1 ± 0.02	0.1 ± 0.03	n.d.	n.d.
U/S[d]	11.5	11.1	8.7	8.4	11.4	11.2

[a]Determined by GC on the fatty acid methyl esters.
[b]Traces (<0.1%).
[c]Mean ± S.D. (S.D., standard deviation), n = 3.
[d]U - unsaturated fatty acids; S - saturated fatty acids.

nuts,[23,24] soybeans,[25] and seed[26–28] lipids occur first after 20 min of microwave roasting. Fatty acids are specifically distributed between the lipid classes as is evident from data presented in Table 5. According to the unsaturation (U/S) values, sterol esters appeared the most saturated ($U/S < 1.5$) and triacylglycerols were the most unsaturated lipid class ($U/S > 9$). Expectedly, in all three oils the fatty acid composition of triacylglycerols (Table 5) resembled that of the total lipids (Table 4).

TAG Composition

A sequence of three chromatographic techniques, namely analytical Ag-TLC, preparative Ag-TLC, and analytical RP-TLC, were applied for determination of TAG composition of raw walnut, hazelnut, and almond oils. As has been shown previously[29] this combination provides results comparable to the detailed TAG analysis achieved so far only by the complementary application of Ag-HPLC and RP-HPLC.[30] Using analytical Ag-TLC, nine TAG classes were unambiguously identified as S_2M, SM_2, M_3, S_2D, SMD, M_2D, D_2S, D_2M and D_3 (S, saturated-, M, monoenoic-, D, dienoic acyl residues; according to the migration order starting from the less retained) in hazelnut and almond oils. Six additional (15 in total), more unsaturated TAG classes: M_2T, SDT, MDT, D_2T, MT_2 and DT_2 (T, trienoic acyl residue, ordered according to the migration from front to start) were detected in walnut oil. TAG groups were quantified by Ag-TLC/densitometry using multiple separate runs for complete resolution and correct quantification. The respective chromatographic conditions and TAG classes resolved in a given run are shown in Table 1 (Experimental). As is evident, a resolution satisfying the requirement of correct densitometric quantification is achieved by varying the mobile phase composition and volume, the silver ion content in the silica gel layer and the sample size. The TAG composition of the three nut oils is presented in Table 6. TAG classes of higher saturation predominated in hazelnut oil in accordance with the fatty acid composition (Table 5). TAG classes containing saturated acyl residue(s) were isolated by preparative Ag-TLC under the conditions given in the Experimental, and subjected to RP-TLC/densitometry for quantification of their TAG molecular species (Table 6, TAG classes and species are ordered according to the migration on the plate starting from the less retained). The RP-TLC differentiates TAG species according to their partition number (PN), where PN = CN-2NDB (CN, number of carbon atoms, NDB, number of double bonds) and clearly resolves species differing in the chain length of the saturated acyl moieties. Nineteen TAG molecular species were determined in walnut oil, the main being LLL, LLLn, PLL and OLL (P, palmitic-, O, oleic-, L, linoleic- and Ln, linolenic acyl residues) comprising about 70% of the total TAG content (Table 6). Fifteen TAG molecular species were determined in hazelnut oil with OOO, OOL and OOP comprising about 80% of the total TAG content.

Table 5. Fatty acid composition[a] of the main lipid classes of walnut, hazelnut and almond kernel oils (wt.%)

Lipid class	Walnut		Hazelnut		Almond	
Fatty acid	Raw	Roasted	Raw	Roasted	Raw	Roasted
Sterol esters						
14:0	n.d.	n.d.	4.6 ± 0.3	4.2 ± 0.2	—	—
16:0	9.5 ± 0.4	9.9 ± 0.5	24.0 ± 0.9	24.8 ± 0.8	—	—
16:1	7.9 ± 0.5	8.2 ± 0.4	2.1 ± 0.2	1.9 ± 0.1	—	—
18:0	17.4 ± 0.9	16.9 ± 0.7	18.4 ± 0.6	18.0 ± 0.7	—	—
cis 9–18:1	34.6 ± 1.3	35.3 ± 1.1	34.3 ± 1.0	35.1 ± 1.1	—	—
cis 9,12–18:2	16.8 ± 0.6	16.2 ± 0.5	16.4 ± 0.5	16.0 ± 0.6	—	—
cis 9,12,15–18:3	0.1 ± 0.03	0.1 ± 0.04	tr.	tr.	—	—
20:0	13.7 ± 0.4	13.4 ± 0.5	n.d.	n.d.	—	—
U/S	1.5	1.5	1.1	1.1	—	—
Triacylglycerols						
14:0	tr.	tr.	tr.	tr.	tr.	tr.
16:0	6.0 ± 0.3	5.6 ± 0.2	6.7 ± 0.3	6.8 ± 0.2	6.0 ± 0.3	6.3 ± 0.2
16:1	tr.	tr.	tr.	0.2 ± 0.05	0.4 ± 0.1	0.5 ± 0.1
18:0	2.0 ± 0.1	2.1 ± 0.2	2.9 ± 0.2	2.8 ± 0.1	2.2 ± 0.2	2.0 ± 0.1
cis 9–18:1	14.1 ± 0.5	14.4 ± 0.4	79.2 ± 1.7	78.8 ± 1.9	65.8 ± 1.1	66.2 ± 1.2
18:1 isom.	0.7 ± 0.1	0.7 ± 0.1	n.d.	n.d.	1.0 ± 0.1	1.1 ± 0.1
cis 9,12–18:2	63.8 ± 1.5	64.0 ± 1.2	10.9 ± 0.6	11.1 ± 0.7	24.6 ± 0.5	23.9 ± 0.7
cis 9,12,15–18:3	13.4 ± 0.7	13.2 ± 0.5	0.2 ± 0.05	0.2 ± 0.04	tr.	tr.
20:0	tr.	tr.	0.1 ± 0.03	0.1 ± 0.02	n.d.	n.d.
U/S	11.5	12.0	9.3	9.3	11.2	11.1

(*continued*)

Table 5. Continued

Lipid class	Walnut		Hazelnut		Almond	
Fatty acid	Raw	Roasted	Raw	Roasted	Raw	Roasted
Unidentified						
12:0	n.d.	n.d.	tr.	tr.		
14:0	n.d.	n.d.	0.3 ± 0.1	0.4 ± 0.1		
16:0	9.1 ± 0.6	9.4 ± 0.7	8.1 ± 0.5	8.0 ± 0.3		
16:1	n.d.	n.d.	0.5 ± 0.1	0.7 ± 0.2		
18:0	3.4 ± 0.5	3.9 ± 0.4	4.0 ± 0.2	4.1 ± 0.3		
cis 9-"18:1	18.8 ± 0.6	18.5 ± 0.5	74.8 ± 1.2	74.2 ± 1.1		
cis 9,12-18:2	58.1 ± 1.1	57.8 ± 0.9	12.3 ± 0.3	12.1 ± 0.4		
cis 9,12,15-18:3	10.6 ± 0.4	10.3 ± 0.4	n.d.	n.d.		
U/S	7.0	6.5	7.1	7.0		
Diacylglycerols						
14:0	0.2 ± 0.05	0.1 ± 0.05	tr.	tr.	tr.	tr.
16:0	13.7 ± 0.4	13.0 ± 0.5	8.8 ± 0.2	9.1 ± 0.3	7.3 ± 0.3	8.0 ± 0.4
16:1	0.4 ± 0.1	0.6 ± 0.1	0.2 ± 0.05	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
18:0	4.2 ± 0.2	4.8 ± 0.2	2.5 ± 0.1	2.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.1
cis 9-18:1	11.1 ± 0.6	11.8 ± 0.5	69.9 ± 1.3	68.9 ± 1.2	64.9 ± 0.8	64.4 ± 0.7
18:1 isom.	1.5 ± 0.2	1.6 ± 0.2	n.d.	n.d.	1.7 ± 0.3	2.0 ± 0.2
cis 9,12-18:2	61.6 ± 1.5	61.1 ± 1.4	18.7 ± 0.7	19.1 ± 0.6	23.9 ± 0.5	23.1 ± 0.4
cis 9,12,15-18:3	6.6 ± 0.4	6.2 ± 0.3	tr.	tr.	n.d.	n.d.

18:3 isom.	0.3 ± 0.1	0.4 ± 0.1	n.d.	n.d.	n.d.	n.d.
20:0	0.4 ± 0.05	0.3 ± 0.1	tr.	tr.	n.d.	n.d.
U/S	4.4	4.5	7.9	7.6	9.2	8.6
Polar lipids						
12:0	n.d.	n.d.	tr.	tr.	n.d.	n.d.
14:0	1.8 ± 0.2	1.5 ± 0.1	1.9 ± 0.1	2.1 ± 0.2	tr.	tr.
16:0	20.4 ± 0.8	20.8 ± 0.9	21.2 ± 1.0	20.2 ± 0.9	18.4 ± 0.6	18.0 ± 0.6
16:1	2.2 ± 0.3	2.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	tr.	tr.
18:0	8.9 ± 0.4	8.5 ± 0.5	12.5 ± 0.8	11.6 ± 0.9	8.3 ± 0.5	8.9 ± 0.5
cis 9-18:1	19.8 ± 0.8	20.6 ± 0.9	50.7 ± 1.8	51.5 ± 1.9	54.5 ± 1.3	53.8 ± 1.3
18:1 isom.	1.2 ± 0.1	0.8 ± 0.1	tr.	tr.	tr.	tr.
cis 9,12-18:2	40.8 ± 1.1	39.2 ± 1.3	12.9 ± 0.7	13.8 ± 0.8	18.9 ± 0.7	19.7 ± 0.6
cis 9,12,15-18:3	5.0 ± 0.1	5.9 ± 0.2	tr.	tr.	tr.	tr.
U/S	2.2	2.3	1.8	1.9	2.8	2.7

[a]Determined by GC on the fatty acid methyl esters.
For the abbreviations see Table 4.

Table 6. Triacylglycerol composition (rel.%)[a] of walnut, hazelnut and almond oils

TAG classes	TAG species	Walnut	Hazelnut	Almond
S_2M	PPO	tr.	1.1	tr.
	PStO	tr.	1.1	tr.
	StStO + APO	n.d.	0.4	n.d.
SM_2	POO	0.6	15.0	8.3
	StOO	0.3	6.8	3.6
M_3	OOO	1.6	48.5	31.0
S_2D	PPL	0.2	tr.	tr.
	PStL	0.1	tr.	tr.
	StStL	tr.	tr.	tr.
SMD	POL	2.9	2.8	8.8
	StOL	1.1	1.8	3.1
	AOL	n.d.	0.1	n.d.
M_2D	OOL	4.9	15.1	23.8
D_2S	PLL	11.8	0.9	2.1
	StLL	3.5	0.2	0.6
	ALL	n.d.	0.1	n.d.
D_2M	OLL	11.4	4.7	16.3
D_3	LLL	26.5	1.4	2.4
M_2T	OOLn	tr.	n.d.	n.d.
SDT	PLLn	4.5	n.d.	n.d.
	StLLn	1.6	n.d.	n.d.
MDT	OLLn	4.8	n.d.	n.d.
D_2T	LLLn	19.9	n.d.	n.d.
MT_2	OLnLn	1.1	n.d.	n.d.
DT_2	LLnLn	3.2	n.d.	n.d.

[a]Determined by consecutive applying of analytical Ag-TLC, preparative Ag-TLC and RP-TLC; a mean of four independent measurements with relative standard deviation below 12%.

For the abbreviations see Tables 1 and 2.

Almond oil contained 10 TAG molecular species with OOO, OOL, OLL and POL being the main components (about 70% of the total content). A monoacid TAG was the major component in the oils: LLL in walnut oil, OOO in hazelnut and almond oils.

The results about TAG molecular species in walnut, hazelnut, and almond oils were in agreement with those obtained by RP-HPLC with either evaporative light-scattering detection[20,21,31,32] or mass spectrometry.[33–35] The consecutive application of silver ion- and reversed-phase chromatography demonstrated here ensured complete separation of TAG with equal PN forming critical pairs (such as PStO/StOO/StStL/AOL with PN = 50, PPO/POO/PStL/StOL/ALL with PN = 48, PPL/POL/StLL with PN = 46 and PLL/StLLn with PN = 44; P - palmitic; St - stearic; A - arachidic;

Table 7. Comparison between the fatty acid compositions (rel.%) of walnut, hazelnut and almond TAGs measured directly by GC and calculated from TLC analyses data[a]

	Walnut		Hazelnut		Almond	
Fatty acids	Measured	Calculated	Measured	Calculated	Measured	Calculated
16:0	6.0	6.8	6.7	7.3	6.0	6.4
16:1	tr.	—	tr.	—	0.4	—
18:0	2.0	2.3	2.9	3.7	2.2	2.4
18:1	14.8	12.7	79.2	77.2	66.8	64.3
18:2	63.8	65.3	10.9	11.8	24.6	26.9
18:3	13.4	12.9	0.2	—	tr.	—
20:0	tr.	—	0.1	—	—	—

[a]After consecutive applying of analytical Ag-TLC, preparative Ag-TLC and RP-TLC.

O - oleic; L - linoleic; Ln - linolenic fatty acyl residues) thus avoiding the main problem in RP-HPLC of TAG when used as a single separation method.

Finally, the quantitative results for TAG composition achieved by a combination of different TLC methods were verified comparing the FA proportions as calculated from TAG and as determined directly by GC (Table 7). The good agreement between the two sets of results confirms the accuracy of the TLC/densitometric determinations.

Sterol Composition

Composition of free sterols and sterols derived from sterol esters in walnut, hazelnut, and almond oils is presented in Table 8. As with all high terrestrial plants, sitosterol was the main component, higher than 90% in walnut and hazelnut oils, and higher than 80% in almond oil. Campesterol in an amount lower than 6% was found in all three oils. Almond oil contained about 15% fucosterol and a small amount of stigmasterol, whereas about 3% cholesterol was detected in walnut oil (Table 8). Sitosterol was the single component of sterol esters. No significant changes in sterol composition were observed after roasting of the kernels (Table 8).

CONCLUSIONS

The lipid classes, their fatty acid profiles, and the triacylglycerol and sterol compositions of raw and microwaved roasted walnut, hazelnut, and almond kernels were determined by consecutive application of different

Table 8. Main sterols in walnut, hazelnut and almond kernel oils (rel.%)[a]

	Walnut		Hazelnut		Almond	
Free sterols	Raw	Roasted	Raw	Roasted	Raw	Roasted
Campesterol	5.0 ± 0.1[b]	5.4 ± 0.1	5.1 ± 0.1	5.7 ± 0.2	2.3 ± 0.2	2.4 ± 0.3
Stigmasterol	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.1	0.4 ± 0.1
Sitosterol	91.9 ± 0.7	92.6 ± 0.8	94.9 ± 0.5	94.3 ± 0.3	81.6 ± 0.8	80.7 ± 0.7
Fucosterol	n.d.	n.d.	n.d.	n.d.	15.7 ± 0.4	16.5 ± 0.3
Cholesterol	3.1 ± 0.2	2.0 ± 0.1	n.d.	n.d.	n.d.	n.d.

[a]Normalized.
[b]Mean ± S.D. (S.D., standard deviation), n = 3.

chromatographic methods. The combination of TLC techniques employed for identification and quantification of triacylglycerols ensured results comparable with those obtained using reversed phase liquid chromatography-mass spectrometry (LC-MS). No changes in kernel lipids were detected after microwave roasting at full power for 3 min.

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