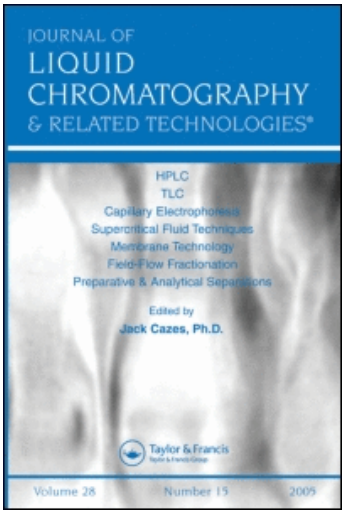

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Quantitative Silver Ion Thin Layer Chromatography of Triacylglycerols from Sunflower Oils Differing in the Level of Linoleic Acid

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Abstract: Eight samples of sunflower oil with different linoleic acid contents (9–63%) were subjected to a triacylglycerol (TAG) analysis by silver-ion thin-layer chromatography with densitometric quantification. In spite of this substantial change in the fatty acid composition, the relative content of the component TAG classes in the sum of polyunsaturated triacylglycerols remains constant. Thus, a characteristic fingerprint of sunflower oil TAG has been outlined. It is not affected by the linoleic acid content and might be of use in authenticity and adulteration control of sunflower and olive oils.

Keywords: Fatty acids, Gas chromatography, High oleic sunflower oil, Thin-layer chromatography, Triacylglycerols

INTRODUCTION

Sunflower seed oil is a major vegetable oil produced from the seeds of the plant Helianthus annuus. Today, it is the fourth most common vegetable oil in the world with annual consumption of about 9 million tons. The oil has a specific fatty acid (FA) composition characterized by the high content (60–75%) of the biologically active linoleic acid (*cis*9, *cis*12–18:2),

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15–45% of oleic and virtually no linolenic acid. The linoleic acid is an essential component of the human diet, being a precursor for the synthesis of biologically important eicosanoids. On the other hand, due to the high level of linoleic acid, the oil is highly susceptible to oxidation. Attempts to apply partial hydrogenation in order to produce more stable oily products based on sunflower oil often lead to unacceptably high content of the "claimed as unhealthy" *trans* fatty acids. It is, therefore, that the selection of new Helianthus annuus varieties was considered to be more prospective with the idea to produce plants with high oleic acid (*cis*9–18:1) content and, indeed, high oleic sunflower oil was created for the first time in 1976.[1] The seed oil of this variety contained only about 10% of linoleic acid and over 80% of oleic acid (higher percentage of oleic acid than in olive oil itself). Hence, this variety was more stable towards oxidation, both at ambient temperatures (i.e., under storage) and at high cooking/frying temperatures. There are now Helianthus annuus varieties with seed oils that approach, or even exceed, 90% of oleic fatty acid content. A sunflower oil variety with middle oleic acid content like, for example, NuSun, with 65% oleic and 26% linoleic acid is now available in the USA. It is considered to be a good frying oil, low in saturated fatty acid content.

Triacylglycerols (TAG) and their component FAs are the main components of vegetable oils, accounting for almost 100% of the weight. Each particular oil has a characteristic TAG pattern that determines the physical and chemical properties of the oil and its nutritional value.

Data on the FA and TAG composition of high oleic sunflower oil (HOSO) were collected gradually during the last 20 years. FA composition was reported most often since the gas chromatographic determination of FAs as methyl esters (FAME) is a common and robust analytical procedure. The more informative TAG analysis which requires specific skills and instrumentation was rarely performed. The application of high temperature gas chromatography (GC)[2] or high performance liquid chromatography (HPLC)[3] was reported. Andrikopoulos[4] compared the analytical potential of GC and HPLC, presenting the analysis of nine less common edible vegetable oils, including HOSO. There are some data on the FA distribution in the TAG molecule (stereospecific analysis).[2,3,5,6] Most comprehensive was the analysis performed in Ref[3] where TAG composition of genetically modified sunflower oils was studied by HPLC/evaporative mass detector and confirmed by GC of the component fatty acids, while stereospecific analysis was accomplished by a combination of chemical and enzymatic approaches.

The aim of this work was to determine the FA and TAG composition of a series of commercial sunflower oils, with gradually decreasing linoleic acid content, available in the local market. Further,

the TAG profiles of these samples were compared with those of three virgin olive oil samples with the intent to examine whether TAG analysis can be used to detect the adulteration of olive oil with low linoleic sunflower oils.[7]

EXPERIMENTAL

Samples and Reagents

Eight samples of sunflower oil were examined altogether. Six samples were purchased from the local market. Five of these were manufactured locally and one (No 2) originated from Turkey. Another sample (No 7), representing genetically modified sunflower oil, was kindly donated by Dr. Zlatanov (Plovdiv University, Plovdiv, Bulgaria) and sample No 8 was donated by the German company Narocon.

All solvents, reagents, and sorbents were of analytical grade or better and were purchased from Merck (Darmstadt, Germany).

The analyses were performed with the pure TAG fraction preliminary isolated by preparative thin layer chromatography (TLC) on silica gel G with mobile phase hexane:acetone, 100:8 by volume.

Gas Chromatographic (GC) Analysis of Fatty Acids

The FA composition of the samples was determined by GC of the corresponding methyl esters, prepared according to Ref[8] on HP 5890 gas chromatograph equipped with a 30 m × 0.25 mm i.d. capillary INNOWax column (cross-linked PEG, Hewlett Packard, G.m.b.H, Austria). The oven temperature was programmed from 165°C to 240°C at 4°C/min and held at this temperature for 10 min. The detector and injector temperatures were maintained at 260°C. Nitrogen was the carrier gas at a flow rate of 0.8 mL/ min, split 80:1. Components were quantified by electronic integration (integrator Shimadzu DR-3).

Silver Ion-TLC Analysis of TAGs

The quantitative Ag-TLC procedure has been developed and successfully applied to common sunflower oil in silver ion[9] and reversed phase[10] mode years ago. The analytical protocol is described in details elsewhere.[11] Briefly, TAG classes differing in unsaturation were separated on 19 × 4 cm glass plates, coated with ca. 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 fume-cupboard) to ensure the correct quantitative charring (at 180–200°C) of the separated TAG classes. Recording of Ag-TLC chromatograms and quantitative measurement of peak areas were performed with a CS-930 densitometer (Shimadzu, Kyoto, Japan) equipped with a DR-2 Shimadzu integrator. Scanning was carried out in the zigzag reflection mode at 450 nm. Beam-slit was varied from 0.4 × 0.4 to 1.2 × 1.2 mm depending on the separation achieved. The quantity of each spot was presented as relative area percentage, as derived from the integrator. Each sample was analyzed at least three times. Depending on the content of the respective TAG component the standard deviation varied from 0.1% to 1%.

RESULTS AND DISCUSSION

Fatty Acid Composition

With the intention to cover as large range of HOSO as possible, we screened a large number of commercial sunflower oils, choosing only

Table 1. Chromatographic conditions for the separation of TAG classes by silver ion TLC

	Samples 1 to 5	Samples 6 to 8
Separation of S_2M and SM_2		
Sample (μg)	80–100	30–40
Mobile phase (v/v)	PE:A 100:4	PE:A:EA 100:3:2
Vol. (mL)	5	6
Separation of TAGs from M_3 to D_3		
Sample (μg)	20–30	30–40
Mobile phase (v/v)	PE:A 100:4	PE:A:EA 100:3:2
Vol. (mL)	6	6
Separation of SPUTAG components		
Sample (μg)	5–10	60–200
Mobile phase (v/v)	PE:A 100:4.5	PE:A:EA 100:6:4
Vol. (mL)	7	8

S – saturated; M – monoenoic; D – dienoic fatty acid residues; SPUTAG – sum of the polyunsaturated TAG; PE – petroleum ether (b.p. 40–60°C); A – acetone; EA – ethylacetate.

Table 2. Fatty acid composition (wt%) of sunflower oils with different linoleic acid content

	Sample No.							
Fatty acid	1	2	3	4	5	6	7	8
14:0	0.1	0.1	0.1	0.1	0.1	–	0.1	–
16:0	5.9	6.4	6.6	7.1	6.4	4.9	4.2	12.3
16:1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	1.0
17:0	–	–	–	–	–	–	0.4	0.1
18:0	3.7	3.4	3.3	4.0	3.3	3.2	3.0	2.2
18:1	24.8	28.2	28.8	29.7	41.2	69.3	76.3	74.0
18:2	62.8	60.5	59.4	56.8	47.5	21.2	14.5	9.2
18:3	–	–	–	–	–	–	–	0.5
20:0	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.3
20:1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3
22:0	1.2	0.5	0.9	1.0	0.7	0.7	0.7	0.1
22:1	0.7	0.2	0.2	0.5	–	–	–	–
24:0	0.2	0.2	0.2	0.2	0.3	0.2	0.2	–

samples differing in the oleic acid content. The fatty acid compositions of the eight samples chosen for the present study are shown in Table 2.

Evidently, the samples are characterized by a gradual decrease of the content of 18:2 and, correspondingly, by a gradual increase in the content of 18:1 (varying between 24.8% and 76.3%). In samples 1 to 6, the profile of the rest of the FA is almost constant. Practically constant is the sum of the saturated FA (palmitic acid, 16:0, is the main component), varying in the range 10–13%. Sample 8 differs with 12.3% 16:0 and with the presence of 18:3 (linolenic) acid which is not common for sunflower oils.

TAG Composition

The mobile phase and the sample loads used for correct Ag-TLC/densitometric determination of the oil samples are shown in Table 1. Two sets of conditions were employed and these were required because of the differences in the fatty acid composition of the samples. Thus, samples 1 to 5 having similar FA composition were analyzed as described previously for sunflower oils.[9,10] Due to the increased oleic acid content, samples 6 to 8 were analyzed under chromatographic conditions used for olive oils analysis.[12] Most important for the resolution and the correct quantitation of TAG was the sample load. Thus, higher sample load (80–100 μg) was required for correct quantitation of S_2M and SM_2, moderate sample load (20–30 μg) was the

best for M_3 to M_2D classes and just 5–10 μg of the sample were applied on the plate to ensure for correct measurement of S_2D and M_2D TAG classes (S – saturated; M – monoenoic; D – dienoic fatty acid residues).

Table 3 presents the TAG classes composition of the eight HOSO samples as determined by analytical Ag-TLC and densitometry.

The accuracy of the TAG composition was confirmed by comparison between the FA content as determined by GC and as calculated from TAG data, Table 4. We consider the agreement between the two sets of results to be a good evidence for the reliability of the present results. Comparison with literature data was considered unnecessary since the FA compositions were subjected to differences due to the various origin.

In our previous studies on highly unsaturated vegetable oils[13,14] TAG classes which contained two and more dienoic and/or trienoic (T) FA residues were denoted as Sum of the Polyunsaturated TAG (SPUTAG). Here we use the same designation to define the group of three major TAG classes – S_2D, M_2D and D_3 that characterize the sunflower oil (Table 3).

With the intention to outline the impact of the different 18:2 content on the TAG composition the SPUTAG content and the respective TAG profiles of the eight samples were compared. Expectedly, the SPUTAG content (as a percent of the total TAG) decreases (from 67.9% in sample 1 to 5.0% in sample 8) with the decreasing of 18:2 content (Table 3). Unexpectedly, however, the relative proportions of the component TAG

Table 3. Triacylglycerol classes[a] composition (rel.%)[b] of sunflower oils with different linoleic acid content

	Sample No.							
TAG class	1	2	3	4	5	6	7	8
S_2M	0.3	0.5	0.5	0.8	1.0	1.1	1.5	5.6
SM_2	2.7	3.1	4.1	3.9	7.8	16.5	18.6	28.1
M_3	3.0	4.5	5.1	4.8	16.3	44.5	54.6	40.6
S_2D	1.5	1.9	1.7	2.6	1.5	0.4	0.5	0.9
SMD	11.4	10.8	12.2	11.1	9.5	6.2	3.8	7.0
M_2D	13.2	14.8	15.3	13.7	13.5	10.3	8.2	12.8
SD_2	16.9	15.9	14.4	17.4	13.1	5.8	3.6	1.3
MD_2	27.0	23.9	24.7	26.1	21.3	8.8	5.8	1.9
M_2T	–	–	–	–	–	–	–	0.8
D_3	24.0	24.6	22.0	19.6	16.0	6.4	3.4	1.0
SPUTAG	67.9	64.4	61.1	63.1	50.4	21.0	12.8	5.0

[a]For the abbreviations see the footnote to Table 1.
[b]Mean of at least three measurements by analytical Ag-TLC.

Table 4. Comparison between the fatty acid compositions of the sunflower oil samples as determined directly by GC (a) and as calculated from TAG data (b)

	Sample No.															
	1		2		3		4		5		6		7		8	
Fatty acids	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
S	11.3	11.5	10.7	11.5	11.2	11.7	12.6	13.1	10.9	11.8	9.2	10.5	8.7	10.0	15.0	16.5
M	25.8	26.5	28.7	28.2	29.3	30.5	30.5	29.2	41.5	41.1	69.6	67.7	76.7	76.2	75.3	73.2
D	62.8	62.0	60.5	60.3	59.4	57.8	56.8	57.7	47.5	47.1	21.2	21.8	14.5	13.8	9.2	10.0
T	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.3

[a]For the abbreviations see the footnote to Table 1.

Table 5. Comparison between the relative TAG[a] proportions (rel.%) in SPUTAG of virgin olive oil[b] and sunflower oil[c] samples

	Virgin olive oils			Sunflower oils, Sample No.							
SPUTAG components	Low linoleic	Typical	High linoleic	1	2	3	4	5	6	7	8
SD_2	8.1	16.5	27.3	24.9	24.7	23.6	27.6	26.0	27.6	28.1	26.0
MD_2	19.1	30.9	45.8	39.8	37.1	40.4	41.4	42.3	41.9	45.3	38.0
SMT	22.4	15.3	7.6	–	–	–	–	–	–	–	–
M_2T	42.6	30.3	7.7	–	–	–	–	–	–	–	16.0
D_3	–	0.8	5.5	35.3	38.2	36.0	31.1	31.7	30.5	26.6	20.0
SDT	Traces	Traces	6.0	–	–	–	–	–	–	–	–
MDT	7.6	6.2		–	–	–	–	–	–	–	–

[a]For the abbreviations see the footnote to Table 1.
[b]as defined and determined in[14].
[c]as determined in the current study.

in this sum remain very close as is evident from Table 5. In this table, the TAG profile in SPUTAG of the examined here sunflower oils are compared with the TAG profile in the SPUTAG of the three types of olive oils determined earlier.[12] These three olive oils were also characterized by different linoleic acid content and were, therefore, denoted as low linoleic, typical and high linoleic type. The SPUTAG content of these oils changes from 4.5% to 6.2% and 12.1%, respectively, and in general, it is by far lower than those of sunflower oils. Moreover, as seen, the component TAG profiles of olive oil and sunflower oils differ strongly. While the TAG profile of sunflower oil SPUTAG remains the same irrespective of the changes in 18:2 content (sample 8 is an exception because of the presence of 18:3 FA), the SPUTAG profile of olive oil is influenced strongly by this change. Thus, S_2D, MD_2 and D_3 contents increase sharply with the increasing 18:2 content, while SMT and M_2T contents decrease in the same way and MDT remains practically constant (the content of SDT was not determined as it forms mixed with MDT zone under the experimental conditions employed).

It is evident, from the results presented in Table 5, that determination and analysis of SPUTAG content of oils containing linoleic acid is very informative for the origin of the oil and is a promising indicator for both authenticity and adulteration of the oil.

CONCLUSION

A unique TAG profile of sunflower oil SPUTAG was established in this study. It is not affected by the linoleic acid content; may be used as a fingerprint of sunflower oil and is of certain practical interest.

The results confirm the importance of detailed chromatographic analysis of TAG composition of edible oils in order to examine their authenticity and possible adulteration.

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