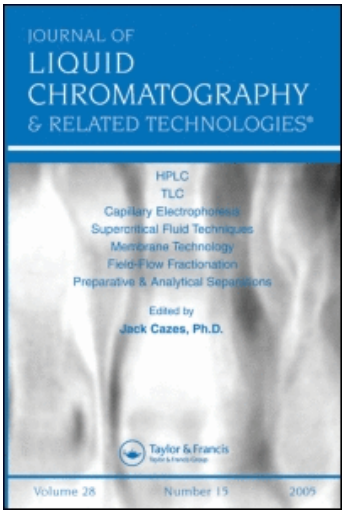

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SILVER ION TLC OF MINOR TRIACYLGLYCEROL COMPONENTS FOR UNAMBIGUOUS DETECTION OF ADULTERATION OF OLIVE OIL WITH VEGETABLE OILS

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SILVER ION TLC OF MINOR TRIACYLGLYCEROL COMPONENTS FOR UNAMBIGUOUS DETECTION OF ADULTERATION OF OLIVE OIL WITH VEGETABLE OILS

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□ *An analytical approach for detection of low levels of adulteration of virgin olive oil with highly unsaturated vegetable oils by Ag-TLC is proposed. It is based on determination of the component TAG classes of the suspicious oil in two stages – analysis of the total oil and detailed analysis of the amount of minor polyunsaturated TAG classes, without the need of fractionation. Virgin olive oils with different linoleic acid content have rather different profile of their minor TAG classes. Marker TAG classes for adulteration with vegetable oils are: for cotton oil – SD_2 and D_3; for sunflower oil – M_2T and D_3; for corn oil – M_2T and D_3; for soybean oil – D_3 and D_2T.*

Keywords adulteration, silver ion thin layer chromatography, triacylglycerols, vegetable oils, virgin olive oil

INTRODUCTION

Olive oil is a major vegetable oil obtained from the mesocarp (soft fleshy fruit) of the fruits of the olive tree. The olive tree is one of the oldest known cultivated trees in the world and olive oil has been used for thousands of years as a staple food for the people of the countries surrounding the Mediterranean Sea. In the early 1950s, the American scientist Dr. Ancel Keys introduced the term Mediterranean diet as a synonym of the healthy dietary patterns of people in these countries. One of the main explanations is thought to be the beneficial effect of olive oil, which is a basic constituent of the Mediterranean diet. In 2004, the U.S. Food and Drug Administration (FDA) announced the availability of a qualified health claim for

[†]This paper is dedicated to the memory of Roumyana Tarandjiiska who initiated and encouraged this study.

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monounsaturated fat from olive oil and reduced risk of coronary heart disease (CHD).[1]

Today it is clear that the biological value of olive oil is due not only to its fatty acid (FA) composition, particularly high of oleic acid, but also to the nature and levels of its many minor constituents such as phenolic compounds, alpha-tocopherol, squalene, pentacyclic triterpenes, and sterols still present in virgin (or natural) oil. Virgin olive oil is the highest quality olive oil; it is obtained from the olive fruits solely by mechanical or other physical means under conditions that do not lead to alteration in the oil, without any steps of extraction and refining, which is important for preservation of its original nutritional and sensory properties and oxidative stability. Then other grades of lower quality olive oil are produced subsequently.

As a result of its beneficial health effects, increasing demand, and high cost of production, virgin olive oil is more expensive than other types of oils, making it a target for adulteration. Adulteration has been a problem in the oil and fat trade for a long time.[2,3] With few exceptions (like the famous "Spanish toxic oil syndrome," when in 1981 consumption of rapeseed oil denatured with aniline, sold as olive oil caused the death of over 300 people in Spain), adulteration of fats and oils is not a threat to public health and just has an economic impact on the rights of consumers. The most common adulterants in virgin olive oil have included refined olive oil, olive pomace oil, and esterified oil prepared by re-esterifying low grade olive oils with glycerol. Other adulterants are less expensive vegetable oils such as corn oil, cottonseed oil, sunflower oil, and soybean oil. Detection of virgin olive oil adulteration is complicated by the fact that the quantity of certain indicators varies due to biological, climatic, agronomical, and temporal factors. Processing of oils can also change greatly the composition of minor constituents. Therefore, too strict specifications can not be set by food inspection as this will eventually increase the number of false positive results.

The evaluation of the quality and the genuineness of olive oils is made on the basis of sets of analytical data of a number of parameters, which must be within limit values established by the International Olive Council (IOC)[4] and other official bodies (European Commission, Codex Alimentarius Commission).

Among the established methods for the control of authenticity of olive oil, the determinations of fatty acids and triglycerides seem to be very useful. The FA composition of olive oil is the most often used indicator of adulteration by other oils, and most often is determined by gas chromatography. If any fatty acid is present in amounts exceeding the postulated by official organizations limits, that would be indicative of adulteration with seed oil. However, although the FA composition of common vegetable oils is different from that of olive oils, the fatty acids could not be satisfactorily

used in most cases as discriminatory parameters between olive oil and the respective vegetable oil.[5] Despite that, a combination of FA analysis and other analytical methods (including minor components analysis) is one of the best suited strategies. Now it is well known that data for triacylglycerol (TAG) composition of oils are more informative (than FA data) and provide a higher level of discrimination between the analyzed samples.[5–15] Methods for analysis and authentication have been reviewed recently.[16]

All these approaches, however, do not account for the natural variations in the contents of FA, either in genuine olive oils or in the vegetable oils used for preparation of the model mixtures. The different techniques used are usually applied to random, undefined samples but not to series of samples covering the limit values tabulated for the measured parameter. Until the mid 1990s, the trilinolein (LLL) content of the olive oil sample (max 0.5%) was used for the detection of adulteration of olive oil with other vegetable oils. Then it became clear that samples with high linoleic acid content (like Tunisian olive oil samples) regularly have higher values for LLL. So, Proto[17] found that some Tunisian samples did not comply with the limit of 0.5%, while some samples of sunflower, safflower, and hazelnut oils had trilinolein contents low enough to permit the adulteration of olive oil without exceeding the 0.5% tolerance for trilinolein. In our study from 1998,[18] we analyzed the TAG composition by silver ion thin layer chromatography (Ag-TLC) of series of olive oil samples with gradually increased linoleic acid content. Four of the samples, with linoleic acid content above 14% showed D_3 higher than 0.5%. Nowadays, the trilinolein content has been replaced by the ΔECN42,[4] the difference between theoretical ECN42 values, calculated from FA composition and the real value obtained by HPLC, where ECN=CN – X.n; ECN – equivalent carbon number; CN – total number of carbon atoms, X – number of double bonds; n-factor for double bond contribution.

Authenticity factor of olive oil samples (Au) adulterated with oils with high linoleic acid content, based on the ECN42 content of the sample has been introduced by El-Hamdy.[13] The authors proposed empirically measured factors for corn, sunflower, and soybean oils. Strangely, the ECN42 value of corn oil was higher than that of sunflower oil, which indicates the occasional choice of the studied oils – typically sunflower oil has higher ECN42 than corn oil.[19]

The aim of this work was, on the basis of the procedure recently developed in our laboratory for quantitative Ag-TLC analysis of highly unsaturated TAG classes in virgin olive oils[18] and common seed oils[20–22] to propose an analytical approach for unambiguous detection of adulteration of olive oil with most common vegetable oils. The variations in the TAG composition of samples caused by the natural variation of the

component FA were considered, with special attention concentrated to the differences between olive oils with different linoleic acid content, in order to avoid erroneous assessment of sample's authenticity.

EXPERIMENTAL

Samples and Reagents

Samples of sunflower, corn, soybean, cotton, and olive oil were purchased from the local supermarkets. One sample representing genetically modified sunflower oil, was kindly donated by Dr. Zlatanov (Plovdiv University, Plovdiv, Bulgaria) and a sample of high oleic sunflower oil (80 + type) 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. [23] on HP 5890 gas chromatograph equipped with a 30 m × 0.25 mm × 0,25 μm 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 Triacylglycerols

The analysis procedure is described in details elsewhere.[18,20,21] 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 varied depending on the TAG profile of the particular analyzed oil – corn, cotton, and sunflower[20,24] olive,[18] and soybean.[21] Sunflower samples with high oleic acid content were analyzed under chromatographic conditions used

for olive oils analysis.[18] Continuous ascending development with the respective predetermined volume of the mobile phase in open cylindrical tanks (24 cm × 5 cm i.d.) was performed, allowing the whole mobile phase volume to pass through the plate. The plate was then dried (1 hour at 110°C), and treated consecutively with bromine and sulfuryl chloride vapors (30 min each, closed tanks, 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 Corporation, 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 2%.

During silver ion chromatography (TLC or HPLC) the following elution order (increasing retention) of the component TAG classes is observed: $S_3 < S_2M < SM_2 < S_2D < M_3 < SMD < M_2D < SD_2 < S_2T < MD_2 < SMT < M_2T < D_3 < SDT < MDT < D_2T < ST_2 < MT_2 < DT_2 < T_3$, where S, M, D and T denote saturated, mono-, di-, and trienoic fatty acyl-moieties, respectively.

Due to the large number of the TAG classes and their unbalanced composition, depending on the oil sample, variation in the conditions (amount of the sample and composition and volume of the mobile phase) is necessary in order to obtain comparable resolution. Analysis of the oils normally takes place under two sets of different conditions. Under the first set of conditions the TAG classes from S_3 to M_2D are separated, while the most unsaturated classes (from SD_2 to T_3), which contained two and more dienoic and/or trienoic FA residues remain unseparated, grouped into one or more peaks expressed as a percentage of the total (denoted as Sum of the Polyunsaturated TAG (SPUTAG), depending on the oil.[18,20–22]

The second set of conditions is used to completely separate the individual components of the SPUTAG group. In olive oil these are minor components (SD_2, MD_2, SMT, M_2T, D_3, MDT and SDT), which are measured as part of SPUTAG (assumed to be 100%). The sample load was 4–6 times higher and thus, minor components had values exceeding the detection limit of the measurement which ensured the reliability of the data. This procedure is illustrated for the case of olive oil analysis in Figure 1. Between 3 and 5 chromatograms of each series were used for analysis of an oil.

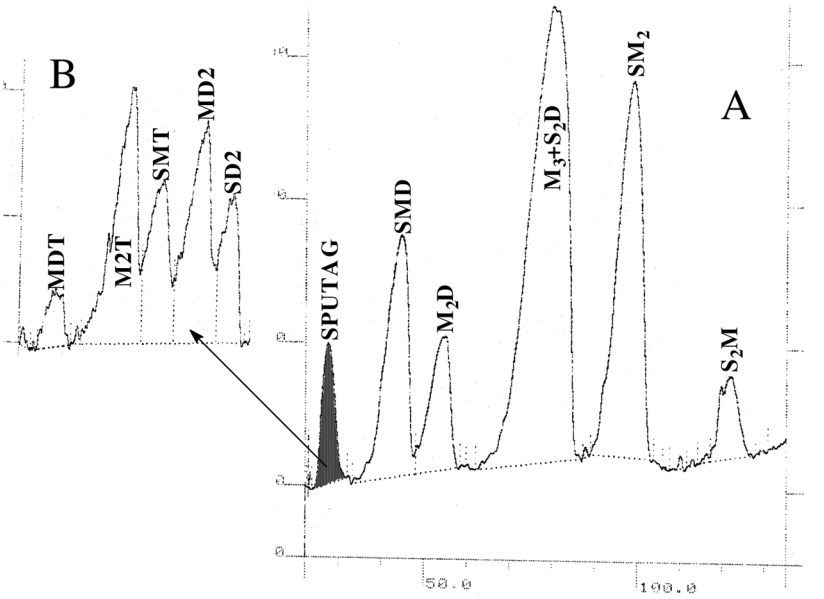

FIGURE 1 Densitometric profiles of TAG classes of typical virgin olive oil separated by Ag-TLC. Plates impregnated with 0.5% $AgNO_3$. (A) Analysis of main TAG classes and SPUTAG, Sample 25 μg, Mobile phase petroleum ether – acetone – ethyl acetate 100:3:2 (v/v/v)/ 6 mL. (B) – separation of SPUTAG components, Sample 120 μg, Mobile phase petroleum ether – acetone – ethyl acetate 100:6:4 (v/v/v)/8 mL. S – saturated, M – monoenoic, D – dienoic, T – trienoic fatty acids.

RESULTS AND DISCUSSION

Determination of the Average Composition of TAG Classes in Vegetable Oils

As was mentioned in the introduction, the natural variation in the composition of FA (and respectively of TAG) of both the virgin olive oils and the common vegetable oils, which are potential adulterants to VOO should also be considered before making decisions about sample authenticity. The FA and TAG composition of series of sunflower, corn, cotton, and soybean oils were analyzed in our lab. Data from the literature for analysis of the four seed oils, where good agreement existed between the directly determined by GC FA data and those calculated from the TAG composition, were also used.[25–32] Table 1 shows the mean values for the TAG composition of the three olive oil types defined by us,[18] as well as sunflower, soybean, cottonseed, and corn oil. After analyzing a series of sunflower oils with decreasing content of linoleic acid, it was found that oils with down to 50% linoleic acid have a TAG composition, characteristical of typical sunflower oil.[22]

TABLE 1 Mean TAG Composition of Different Types of Olive Oil, Sunflower, Soybean, Cottonseed and Corn Oils

	Virgin Olive Oil (Number of Samples)			Vegetable Oils (Number of Samples)			
TAG	Low Linoleic (n = 2)	Typical (n = 7)	High Linoleic (n = 4)	Sun (n = 7)	Soy (n = 7)	Cotton (n = 7)	Corn (n = 7)
S_3	0.0	0.0	0.0	0.0	0.0	0.4	0.0
S_2M	6.2	5.3	6.7	0.6	1.1	4.8	1.8
SM_2	30.3	26.3	21.6	2.2	3.7	3.4	5.6
S_2D	0.0	0.0	0.9	1.7	4.0	13.8	3.7
M_3	44.5	41.2	25.5	1.4	2.6	1.1	5.7
SMD	4.8	7.6	14.7	9.9	12.1	18.5	14.8
M_2D	9.8	13.3	18.5	11.3	7.6	5.4	13.8
SD_2	0.4	1.0	3.3	18.1	14.4	25.5	14.5
S_2T	0.0	0.0	0.0	0.0	0.9	0.0	0.0
MD_2	0.9	1.9	5.6	30.1	16.7	13.1	21.7
SMT	1.0	1.0	1.0	0.0	1.4	0.0	0.0
M_2T	1.9	1.9	1.0	0.0	1.1	0.0	0.4
D_3	0.0	0.0	0.7	24.7	16.9	13.7	16.6
SDT	0.0	0.0	0.0	0.0	4.0	0.1	0.2
MDT	0.3	0.4	0.7	0.0	4.9	0.0	0.2
D_2T	0.0	0.0	0.0	0.0	7.1	0.1	0.9
ST_2	0.0	0.0	0.0	0.0	0.3	0.0	0.0
MT_2	0.0	0.0	0.0	0.0	0.1	0.0	0.0
DT_2	0.0	0.0	0.0	0.0	1.4	0.0	0.0
T_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Reference	[18]	[18]	[18]	[24,25]	[26,21,30,31]	[20,27–29,32]	[20,27,28]

We divided the studied virgin olive oils, according to their linoleic acid content, into three types, assigned as low linoleic olive oil, LLOO (<6% linoleic acid), typical olive oil, TOO (6–10% linoleic), and high linoleic olive oil, HLOO (>10% linoleic acid).[18] The mean content of SPUTAG was 4.5 ± 0.5 for LLOO, 6.2 ± 0.3 for TOO, and 12.1 ± 0.7 for HLOO. When all minor individual TAG classes (components of SPUTAG) were analysed (Figure 1B) it became clear that just the proportions of minor TAG classes in olive oil are particularly sensitive to changes in linoleic acid content and, hence, are the most useful and significant parameters for oils discrimination. The SPUTAG group contains a maximum of six TAG classes – SD_2, MD_2, SMT, M_2T, D_3, and MDT, whereas D_3 is always absent in LLOO samples, appears rarely in TOO, and is always above 0.5% in HLOOs. Actually, the D_3 class is the same as LLL or trilinolein, since the only dienoic fatty acid in olive oil is linoleic acid. MD_2 and SD_2 classes in HLOOs are also present in significantly higher amounts compared with those in TOOs.[18] According to our data (Table 1) the postulated in Ref. [13] values for ECN42 and authenticity factor, Au for olive oil (98.2 ± 3.86) may vary in

much broader limits depending on the linoleic acid content of the oil. According to our TAG data, Au values for LLOO will be above 300, while for HLOO with 18% linoleic acid Au value will be about 60 – corresponding to 3% adulteration according to the approach in Ref. [13].

Calculation of the Changes in the Composition of Minor TAGs of the Three Types of Virgin Olive Oil Caused by Addition of Up to 10% Vegetable Oil

The values listed in Table 1 for the mean TAG compositions of the three postulated types of VOOs and the four plant oils were used for calculation of the changes in TAG classes of olive oil, caused by mixing with increasing amounts (1 to 10%) of each of the four plant oils. Calculations were done easily by elementary Excel® procedure. The output of the calculations for TOO mixed with corn oil is given in Table 2. The upper half of the Table shows the calculated TAG compositions for the whole oil samples, then data of just the target minor TAG classes recalculated as part of SPUTAG are given, and finally, come the absolute changes (with positive or negative sign) in minor TAG classes as part of SPUTAG, caused by mixing with the corn oil. It is seen how the relative content of SPUTAG increases gradually with addition of the more unsaturated corn oil.

The advantage of the SPUTAG values approach is evident from the following comparison: the change in the content of the minor class MDT as part of the total sample during up to 5% adulteration with corn oil is practically 0 (actually it changes from 0.39% to 0.37%), while monitoring the change of the same MDT class at SPUTAG level can register a change from the initial 6.2% in pure olive oil to 4.4% for 5% adulteration with corn oil.

Calculations for each of the rest two olive oil types admixed with each of the four plant oils were done easily by entering in columns 2 and 3 of the spreadsheet the data for TAG composition of the respective oil. At the same time recalculated data appear in the respective cells of the spreadsheet.

Graphically, the results from the calculations are summarized in Figures 2 and 3. The three columns represent the three types of virgin olive oil – LLOO, TOO, and HLOO; rows show the changes in the content of particular TAG class in the three types caused by addition of 1 to 10% of the four plant oils.

As expected, the most significant changes (steepest graphics) are observed in the case of LLOOs, which are with lowest SPUTAG level (about 4.5%) and are, hence, most sensitive to addition of the highly unsaturated plant oils with their major components SD_2, MD_2, and D_3. Contrary, the changes are smallest in the HLLO column (mean SPUTAG content 12.1%), and for the SMT and M_2T classes changes are negligible even at

TABLE 2 Composition of Triacylglycerol Classes of Typical Olive Oil and Statistically Typical Corn Oil and Calculated Compositions of the Admixtures of Olive Oil with Corn Oil

	Typical Olive Oil	Corn Oil	Percentage of the Added Corn Oil									
			1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
S_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S_2M	5.3	1.8	5.3	5.2	5.2	5.2	5.1	5.1	5.1	5.0	5.0	5.0
SM_2	26.3	5.6	26.1	25.9	25.7	25.5	25.3	25.1	24.9	24.6	24.4	24.2
S_2D	0.0	3.7	0.0	0.1	0.1	0.1	0.2	0.2	0.3	0.3	0.3	0.4
M_3	41.2	5.7	40.8	40.5	40.1	39.8	39.4	39.1	38.7	38.3	38.0	37.6
SMD	7.6	14.8	7.7	7.8	7.9	7.9	8.0	8.1	8.1	8.2	8.3	8.4
M_2D	13.3	13.8	13.3	13.3	13.3	13.3	13.3	13.3	13.4	13.4	13.4	13.4
SD_2	1.0	14.5	1.2	1.3	1.4	1.6	1.7	1.8	2.0	2.1	2.2	2.4
S_2T	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MD_2	1.9	21.7	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.9
SMT	1.0	0.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
M_2T	1.9	0.4	1.9	1.9	1.8	1.8	1.8	1.8	1.8	1.8	1.7	1.7
D_3	0.0	16.6	0.2	0.4	0.5	0.7	0.9	1.0	1.2	1.4	1.5	1.7
SDT	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MDT	0.4	0.2	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
D_2T	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1
SUM	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
SPUTAG in the oil, %	6.2	54.5	6.7	7.2	7.7	8.1	8.6	9.1	9.6	10.1	10.6	11.0
SD_2	16.6	26.6	17.4	18.1	18.7	19.2	19.7	20.1	20.5	20.9	21.2	21.5
S_2T	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MD_2	30.8	39.9	31.5	32.2	32.7	33.2	33.7	34.1	34.4	34.7	35.0	35.3
SMT	15.4	0.0	14.1	13.1	12.1	11.3	10.5	9.9	9.3	8.7	8.3	7.8
M_2T	30.3	0.7	27.9	25.8	24.0	22.4	21.0	19.7	18.6	17.5	16.6	15.7
D_3	0.7	30.5	3.1	5.2	7.1	8.7	10.1	11.4	12.6	13.6	14.5	15.4
SDT	0.0	0.4	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2
MDT	6.2	0.4	5.7	5.3	5.0	4.6	4.4	4.1	3.9	3.7	3.5	3.3
D_2T	0.0	1.6	0.1	0.2	0.3	0.4	0.5	0.6	0.6	0.7	0.7	0.8
SPUTAG, %	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Changes in minor TAG classes as part of SPUTAG in mixtures with increased percentage of added corn oil		SD_2	0.8	1.5	2.1	2.7	3.2	3.6	4.0	4.3	4.6	4.9
		S_2T	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		MD_2	0.7	1.4	1.9	2.4	2.9	3.3	3.6	3.9	4.2	4.5
		SMT	−1.3	−2.3	−3.3	−4.1	−4.9	−5.5	−6.1	−6.7	−7.2	−7.6
		M_2T	−2.4	−4.5	−6.3	−7.9	−9.4	−10.6	−11.8	−12.8	−13.8	−14.6
		D_3	2.4	4.5	6.4	8.0	9.4	10.7	11.9	12.9	13.9	14.7
		SDT	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2
		MDT	−0.5	−0.9	−1.2	−1.6	−1.8	−2.1	−2.3	−2.5	−2.7	−2.9
		D_2T	0.1	0.2	0.3	0.4	0.5	0.6	0.6	0.7	0.7	0.8

10% adulteration. The difference between the TAG compositions of the three olive oil types is so large that for some TAG classes even the sign of the changes is opposite – so SD_2 decreases for three of the added oils and MD_2 – for all the added oils in the case of HLOO. Most common is the group of typical olive oils (TOO) and the changes in their TAG classes

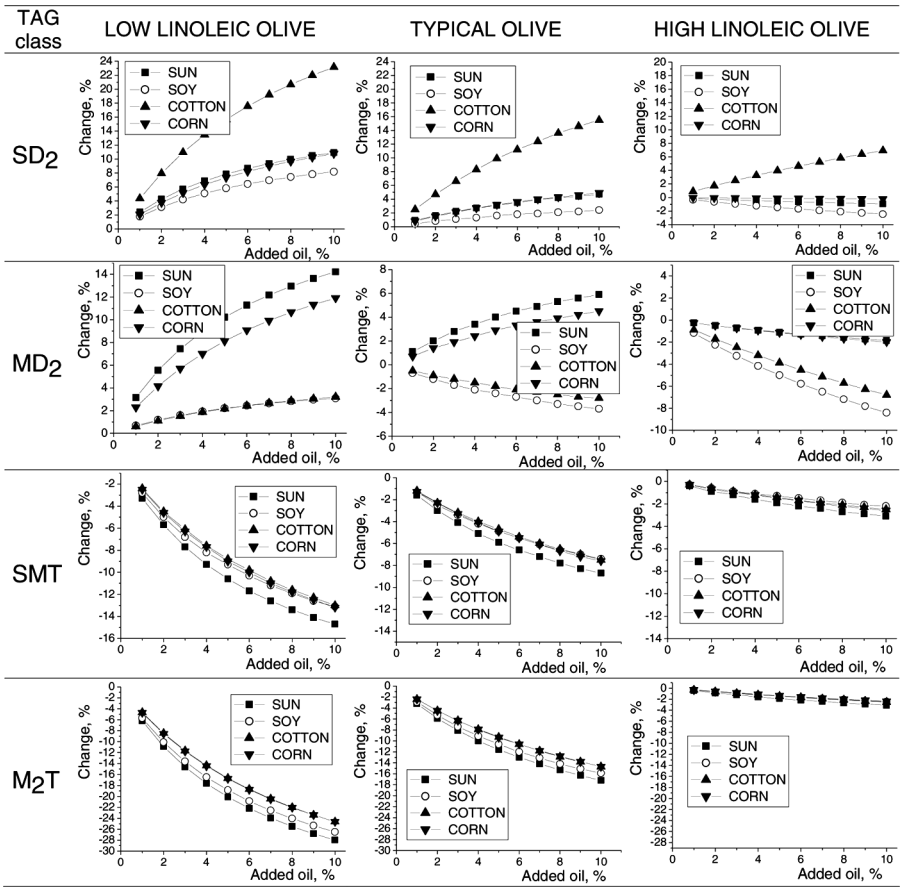

FIGURE 2 Calculated changes in the content of the minor triacylglycerol classes SD_2, MD_2, SMT and M_2T (in %, as part of SPUTAG) in olive oils with various linoleic acid content mixed with the four highly unsaturated plant oils.

are most important. Good markers of potential adulteration would be TAG classes in which the content changes with over 2% with the addition of each 1% of foreign oil. So, the MD_2, SMT, and, particularly MDT change slightly in the whole range of 1–10% adulteration and cannot be used as markers. At the same time SD_2, M_2T, and D_3 classes change notably and seem suitable for markers of adulteration. Analysis of graphics shows that:

For cotton oil – marker components could be SD_2 and D_3 for detection of down to 1% admixture to low linoleic and typical olive oils and down to 3% to high linoleic olive oil.

For sunflower oil – marker components could be M_2T and D_3 for detection of down to 1% admixture to low and typical olive oils and down to 2% to high linoleic olive oil; in HLOO, typical is the SPUTAG profile – with equal amounts of SMT and M_2T, which both gradually decrease during adulteration.

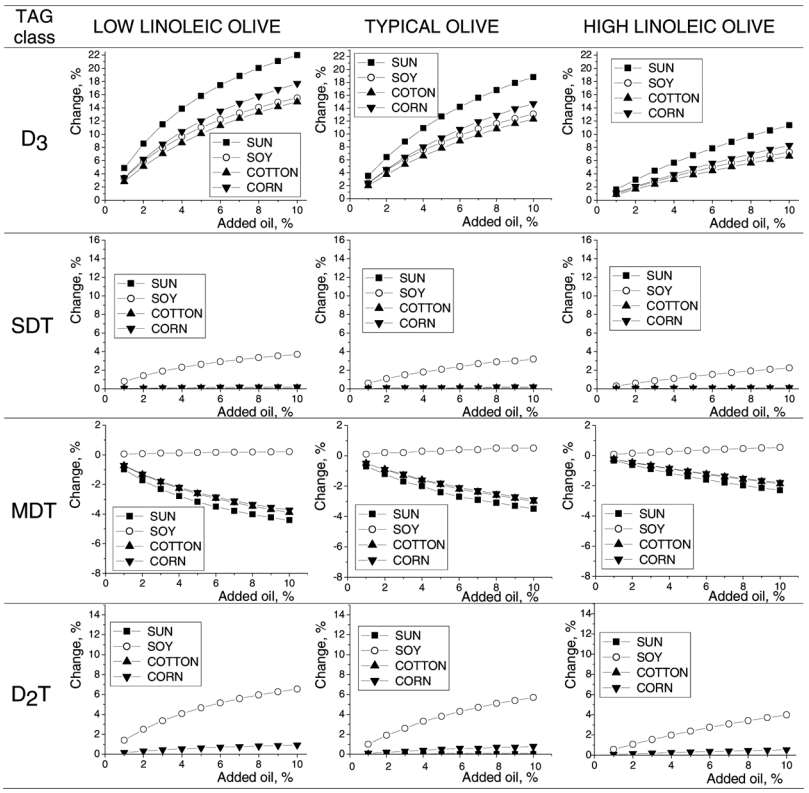

FIGURE 3 Calculated changes in the content of the minor triacylglycerol classes D_3, SDT, MDT and D_2T (in %, as part of SPUTAG) in olive oils with various linoleic acid content mixed with the four highly unsaturated plant oils.

For corn oil – marker components could be M_2T and D_3 for detection of down to 2% admixture to LLOO and TOO and down to 3–4% to HLOO; in HLOO SPUTAG profile – decrease of SMT and M_2T – as for sunflower oil.

For soybean oil – marker components are D_3 and "the new" for olive oils D_2T class for detection of down to 2% admixture to all types of olive oil. Registering of even 0.5% D_2T in SPUTAG is a sure proof for adulteration with soya oil.

Verification of the Ability to Detect Low Levels of Adulteration of Typical Olive Oil

Model mixtures of TOO mixed with 1%, 2%, and 10% of sunflower, corn, cotton, and soybean oils were prepared. The oils were analyzed in advance and it was found that their FA and TAG composition correspond to the values in Table 1. Detailed analysis of SPUTAG components

TABLE 3 Mean Content of the Minor TAG Classes (as Part of SPUTAG) in the Three Types of Olive Oil, in Typical Sunflower, Soybean, Cotton and Corn Oil, and in 8 Samples of Sunflower Oil with Decreasing Content of Linoleic Acid

	Virgin Olive Oils				Sunflower Oils with Decreasing Linoleic Acid Content (%)										
Minor TAG	Low Linoleic	Typical	High Linoleic	Sun Statist.	Sun1 62.8%	Sun2 60.5%	Sun3 59.4%	Sun4 56.8%	Sun5 47.5%	Sun6 21.2%	Sun8 14.5%	Sun7 9.2%	Soy Statist.	Cotton Statist.	Corn Statist.
SD_2	8.1	16.5	27.3	24.8	24.9	24.7	23.6	27.6	26.0	27.6	28.1	26.0	20.8	48.5	26.0
S_2T	–	–	–	–	–	–	–	–	–	–	–	–	1.3	–	–
MD_2	19.1	30.9	45.8	41.3	39.8	37.1	40.4	41.4	42.3	41.9	45.3	38.0	24.2	24.9	39.9
SMT	22.4	15.3	7.6	–	–	–	–	–	–	–	–	–	2.1	–	–
M_2T	42.6	30.3	7.7	–	–	–	–	–	–	–	–	16.0	1.5	–	0.7
D_3	–	0.8	5.5	33.9	35.3	38.2	36.0	31.1	31.7	30.5	26.6	20.0	24.4	26.2	30.5
SDT	–	–	–	–	–	–	–	–	–	–	–	–	5.8	0.2	0.4
MDT	7.6	6.2	6.0	–	–	–	–	–	–	–	–	–	7.1	–	0.4
D_2T	–	–	–	–	–	–	–	–	–	–	–	–	10.3	–	1.6

(Figure 1B) of the model mixtures was performed by at least 3 chromatograms and standard deviation below 1%. The expected quantities of the SPUTAG components in the prepared model mixtures was compared with the experimentally found after TLC and densitometry. The experimental values differ from the calculated theoretical values (Table 2 and Figures 2 and 3) with 5 to 20% (rel.) for blends with 1% and 2% admixtured oil, and with only 5% (rel.) for mixtures with 10% adulterant. Therefore, in the worst case of low adulteration, counterfeiting would be recorded but without distinguishing the added oils. For example, for SD_2 and D_3 classes three of the oils merge (no change to register), and for SMT and M_2T – all four oils merge (Figures 2 and 3). Still, identification can be made by a characteristic marker: for cotton - SD_2, for sunflower – D_3, and for soy – D_2T.

In Table 3 the SPUTAG profile of the three types of olive oil and the four adulterants are compared with the SPUTAG components of eight sunflower oils with decreasing content of linoleic acid, which we analyzed in a previous study.[22] At low levels of adulteration, Sun1 to Sun4 oils will act as typical sunflower oils, Sun5 and Sun6 oils will cause change similar to that of corn oil. The linoleic acid content of samples Sun7 and Sun8 makes them similar to TOO and HLOO, respectively. As is obvious from Table 3 however, due to the unique profile of their SPUTAG group, they can be easily distinguished from olive oils.

CONCLUSION

An analytical approach for detection of low levels of adulteration of virgin olive oil with highly unsaturated vegetable oils by Ag-TLC is proposed. It is based on determination of the component TAG classes in two stages – analysis of the total oil and analysis of the amount of minor polyunsaturated TAG classes, without the need of fractionation. The approach can be accomplished by Ag-HPLC or by reversed phase HPLC. The latter is possible because, due to the low content of stearic acid in olive oil, minor TAG classes are identical with the TAG molecular species: $SD_2 = PLL$; $MD_2 = OLL$; SMT=POLn; M_2T=OOLn; $D_3 = LLL$ and MDT=OLLn. The first four have the same ECN = 44, and the last two TAGs – ECN = 42. The higher separation power of HPLC can contribute to faster and more accurate identification and proof of adulteration of virgin olive oil.

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