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Journal of Chromatography A, 976 (2002) 255–263

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Comparative analysis of different plant oils by high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

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Abstract

Different vegetable oil samples (almond, avocado, corngerm, grapeseed, linseed, olive, peanut, pumpkin seed, soybean, sunflower, walnut, wheatgerm) were analyzed using high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. A gradient elution technique was applied using acetone–acetonitrile eluent systems on an ODS column (Purospher, RP-18e, 125×4 mm, 5 μm). Identification of triacylglycerols (TAGs) was based on the pseudomolecular ion $[M+1]^+$ and the diacylglycerol fragments. The positional isomers of triacylglycerol were identified from the relative intensities of the $[M-RCO_2]^+$ fragments. Linear discriminant analysis (LDA) as a common multivariate mathematical–statistical calculation was successfully used to distinguish the oils based on their TAG composition. LDA showed that 97.6% of the samples were classified correctly.

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Keywords: Linear discriminant analysis; Vegetable oils; Triacylglycerols; Glycerols

1. Introduction

Authentication of vegetable oils is of great importance, especially nowadays due to the expanding demand. Determination of adulteration and characterization are based on the analysis of major [1–5], minor [6,7] or both major and minor [8,9] compounds of the oils. Major compounds are triacylglycerols (TAGs) present as 95–98%; and minor compounds are different varieties of compounds present as 5–2%, such as wax esters, hydrocarbons, phenolic derivatives etc. Different chromatographic methods are suitable for determination of adulteration, e.g. GC–FID (gas chromatography–flame ionization detection) [2,3], GC–MS (gas chromatog-

raphy–mass spectrometry) [6], GC–IRMS (gas chromatography–isotope ratio mass spectrometry) [4,5], HPLC–RID (high-performance liquid chromatography–refractive index detection) [1] and HPLC–MS (high-performance liquid chromatography–mass spectrometry) [8].

Analysis of the TAG composition of oil samples by HPLC–MS is a widespread method [10–13]. Few articles deal with the analysis of TAGs by MS using MALDI-MS (matrix-assisted laser desorption ionization mass spectrometry) [14,15], or tandem MS [16] without any previous separations. MS provides detailed information about the molecular mass and the fatty acid composition of the TAG molecules. Combination with HPLC provides identification of non- or partially resolved HPLC peaks. Other HPLC detection techniques including ELSD (evaporative light-scattering detection) [17] and RID [1,18] have

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been also used but the identification of TAGs using these detection methods is more complicated or not useful.

Although many mass spectrometric ionization techniques are available for measuring TAGs, only few are suitable for coupling with HPLC. The atmospheric-pressure chemical ionization (APCI) technique is one of the most often used ionization methods for the analysis of TAGs with HPLC–MS due to the relatively simple mass spectra and the possibility of identifying the positional isomers [19–23]. The identification of TAG is based on the mass of the pseudomolecular $[M+H]^+$ and the diacylglycerol $[M-RCO_2]^+$ fragment ions [24,25]; and the positional isomers are identified from the relative intensities of the diacylglycerol fragment ions [26]. For example, analysis of ABC-type TAG, the least abundant diacylglycerol fragment ion, corresponds to the loss of the fatty acid from the *sn*-2 position, since loss of the fatty acid from the *sn*-2 position is the least favorable energetically. AAB and ABA type of TAGs can also be distinguished, because the ratio of the diacylglycerol fragment ions $[AA]^+/[AB]^+$ is different, ~ 1 and much lower than 1, respectively. This is explained on the same basis as the ratio of the diacylglycerol fragment ions from ABC-type TAG.

Combination of different multivariate statistical methods with results obtained by chromatographic analysis has great importance in food quality control. Linear discriminant analysis (LDA) is a suitable method for classification of different food products (a priori defined or naturally occurring groups)

according to a selected feature, e.g. characterization of different Mozzarella cheese [27], or wines [28,29] according to their age.

The aim of our work was to measure the TAG composition of different kinds of plant oil samples (almond, avocado, corngerm, grapeseed, linseed, olive, peanut, pumpkin seed, soybean, sunflower, walnut, wheatgerm) by HPLC–APCI-MS and classification of the oils by linear discriminant analysis according to their TAG composition.

2. Experimental

2.1. Materials

Different cold-pressed oil samples (almond, avocado, corngerm, grapeseed, linseed, olive, peanut, pumpkin seed, soybean, sunflower, walnut, wheatgerm; 2–5 samples from each) were purchased at local grocery stores and factories (Table 1), and dissolved in HPLC-grade acetone–acetonitrile (2:1) to a concentration of 1%. Acetone and acetonitrile were obtained from Koch-Light (Haverhill, UK) and Riedel-de Haën (Seelze, Germany), respectively.

2.2. Instrumentation

HPLC–MS analyses were carried out using a Shimadzu HPLC instrument (Kyoto, Japan) consisting of high pressure gradient system (LC10-AD, FCV-10AL), autoinjector (SIL-10AD), on-line mem-

Table 1
Type and source of the different oil samples

Sample type	Source of the samples								Number of samples
	Factory 1	Factory 2	Factory 3	Factory 4	Factory 5	Factory 6	Factory 7	Stores	
Almond	2	1		1				1	5
Avocado	1	2							3
Corngerm					3				3
Grapeseed	1	2							3
Linseed			2	2	1				5
Olive								4	4
Peanut		1		1					2
Pumpkin seed			1	1	1				3
Soybean				1		3			4
Sunflower					2		2	1	5
Walnut				2					2
Wheatgerm	2				1				3

brane degasser (DGU-14A) and column oven (CTO-10AS), coupled to a Shimadzu QP2010 fitted with APCI source. This was operated according to the following conditions: APCI capillary temperature of 300 °C, source temperature of 200 °C, block temperature of 200 °C and corona probe high voltage of 4.5 kV. High purity nitrogen was used as nebuliser gas, at a flow-rate of 2 ml min⁻¹. CDL voltage was set to -35 V, Q array voltages were 60 V, Q array radiofrequency (RF) was 150, and the detector gain was 1.5 kV. Spectra were obtained over the range of *m/z* 200–1000, with a scan speed of 1000 amu s⁻¹.

The TAGs presented in the oils were separated on an ODS column (Purospher, RP-18e, 125 × 4 mm, 5 μm, Merck, Darmstadt, Germany) with acetone–acetonitrile eluent system, at a flow-rate of 0.6 ml min⁻¹. Two-stepped linear gradient was applied during the analysis: acetone concentration from 20% to 66% in 3 min, hold at 66% during 13.5 min, then from 66% to 80% in 1 min and finally hold at 80% until 30 min. Autosampler and column oven were set to 20 and 25 °C, respectively. The injection volume was 5 μl. Each sample was analyzed twice.

2.3. Calculations

Comparative analyses of five different oils were performed using linear discriminant analysis (LDA) with the Statistica 5.5 software package (StatSoft, Tulsa, OK, USA). LDA as a pattern recognition method is designed to find explicit boundaries between given classes, in order to discriminate among them. The combined variable (latent variable) calculated in this way is the linear combination of the original variable. These functions are called roots (or canonical varieties). For better visualization of the results these roots can be plotted against one another. In our calculations the relative peak areas of the main TAGs in the oils were considered as variables, and each type of oil was considered as a class.

The TAGs were identified from the mass number of pseudomolecular [M+H]⁺ and diacylglycerol fragment [M-RCO₂]⁺ ions, and from the relative intensities of the diacylglycerol fragment [M-RCO₂]⁺ ion(s). The TAG peak areas were calculated from individual ion chromatograms. In some cases TAG peak area was calculated from the chromato-

Table 2
Ions observed in the HPLC–APCI mass spectra of TAGs in different vegetable oils

TAG	Ions observed in the mass spectra of TAGs ^a						SIC ^b		
	[M+H] ⁺	[M-R ₁₍₃₎ CO ₂] ⁺		[M-R ₃₍₁₎ CO ₂] ⁺		[M-R ₂ CO ₂] ⁺		<i>m</i> ₁	<i>m</i> ₂
	<i>m/z</i>	F ₁	<i>m/z</i>	F ₂	<i>m/z</i>	F ₃	<i>m/z</i>	<i>m/z</i>	
LLLn	877.7	LL	599.5	LLn	597.5	LLn	597.5	877.1	877.9
LLL	879.7	LL	599.5	LL	599.5	LL	599.5	879.1	879.9
LnLP	853.7	LnL	597.5	LP	575.5	LnP	573.5	853.1	853.9
LLO	881.8	LL	599.5	LO	601.5	LO	601.5	881.2	881.9
PLL	855.7	PL	575.5	LL	599.5	PL	575.5	855.1	855.9
OOL	883.8	OO	603.5	OL	601.5	OL	601.5	601.0	601.9
PLO	857.8	PL	575.5	OL	601.5	PO	577.5	577.0	577.9
PLP	831.7	PL	575.5	PL	575.5	PP	551.5	551.0	551.9
OOO	885.8	OO	603.5	OO	603.5	OO	603.5	603.0	603.9
POO	859.8	PO	577.5	OO	603.5	PO	577.5	577.0	577.9
POP	883.3	PO	577.5	PO	577.5	PP	551.5	577.0	577.9
SOO	887.8	SO	605.5	OO	603.5	SO	603.5	605.0	605.9

^a [M+H]⁺ indicates the pseudomolecular ion, [M-R₁₍₃₎CO₂]⁺ and [M-R₃₍₁₎CO₂]⁺ indicate the diacylglycerol fragment ions containing fatty acids in the 2, 3(1) and in the 2, 1(3) position, respectively, [M-R₂CO₂]⁺ indicates the diacylglycerol fragment ion containing fatty acids in the 1(3), 3(1) position.

^b *m*₁ and *m*₂ specify the mass number range used for SICs (single ion chromatograms). TAG peak areas were integrated from the SIC chromatograms.

$[M+H]^+$ and the “diacylglycerol” fragment $[M-RCO_2]^+$ ions did not form any adduct ions (sodiated, ammoniated etc.). The TAG compounds were eluted within 30 min (the retention times were the following: LLLn, 9.35 ± 0.23 ; LLL, 10.21 ± 0.22 ; LnLP, 10.66 ± 0.18 ; LLO, 11.62 ± 0.28 ; PLL, 12.11 ± 0.31 ; OOL, 13.51 ± 0.34 ; PLO, 14.18 ± 0.39 ; PLP, 14.89 ± 0.42 ; OOO, 16.04 ± 0.42 ; POO, 16.95 ± 0.52 ; POP, 18.04 ± 0.62 ; SOO, 20.37 ± 0.67 , the standard deviations were calculated from 58 to 84 measure-

ments). Total ion chromatograms (TIC) of the different oils are shown in Fig. 1. Although in some cases the different TAGs are not baseline separated, MS detection provides baseline separation of different compounds using single ion chromatograms.

Four main types of APCI mass spectra have been distinguished according to the number and the relative intensities of the peaks observed in the TAG spectra, as mentioned in the Introduction. (i) AAA type, homogenous (monoacid) TAG such as OOO,

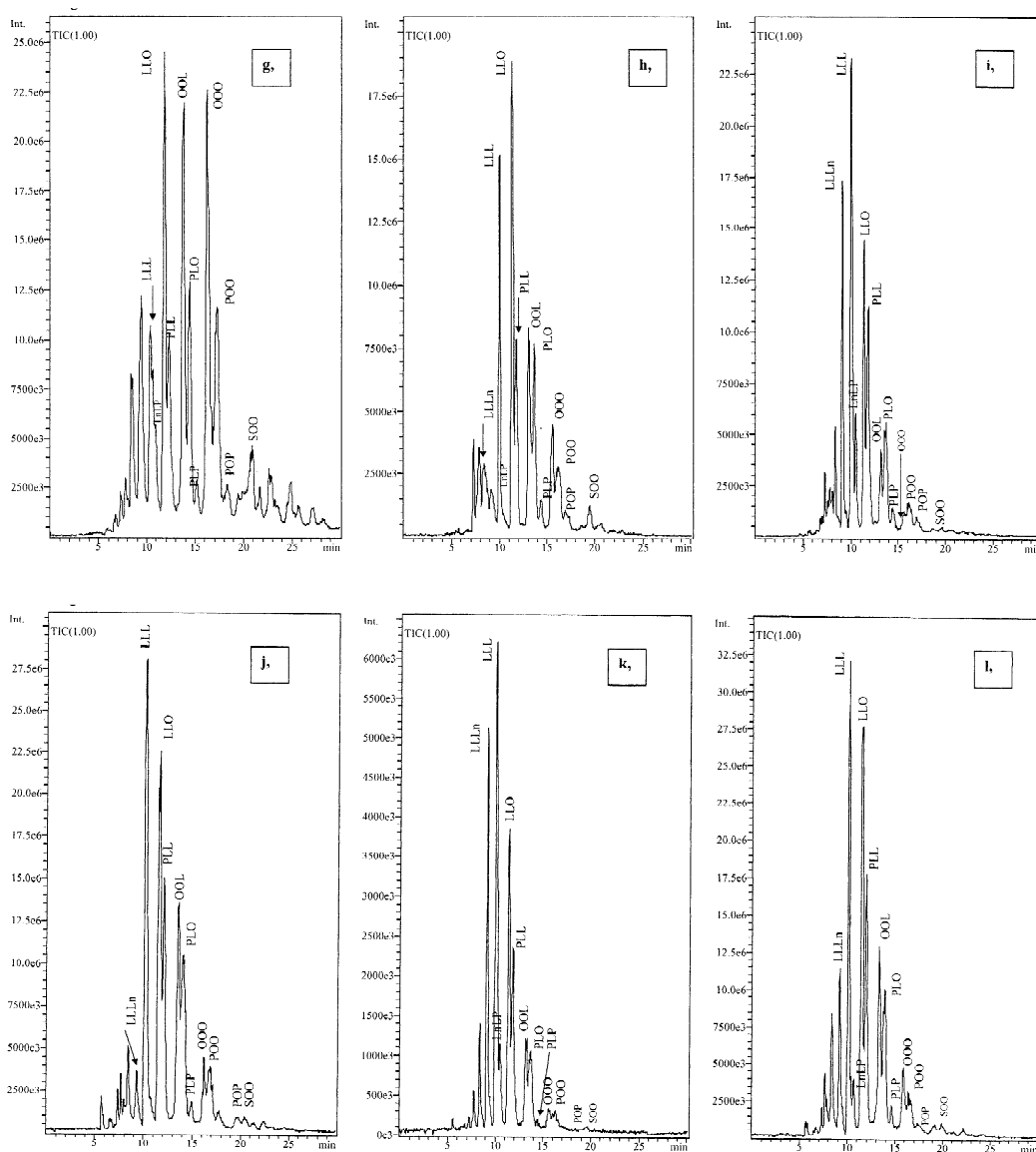


Fig. 1. (continued)

LLL, (ii) ABA type, mixed symmetric TAG containing two different fatty acids such as PLP, (iii) AAB type, mixed asymmetric TAG containing two different fatty acids such as LLLn, LLO, PLL etc. and (iv) ABC type, mixed TAG containing three different fatty acids such as PLO. The intensities of acylium cations in the spectra were not significant using the above mentioned source parameters. The distinction between the positional isomers was not obvious in two cases; between LLO and LOL, and between OOL and OLO. The intensity ratios of $[\text{OL}]^+ / [\text{LL}]^+$ and $[\text{OL}]^+ / [\text{OO}]^+$ are closer to 1, and because of that the structures of these TAGs are probably LLO, and OOL (Fig. 2).

The major TAG compounds (12 different ones) were selected for the statistical calculations (Table 2). The peak areas of selected TAGs were calculated from the single ion chromatogram (SIC). The mass ranges used for SICs are shown in the last two columns of Table 2. The peak areas of different TAGs in oils were integrated from the SIC chromatograms. The individual peak areas of different TAGs were normalized to all (12) TAG peak areas at each oil, resulting in relative peak areas (Table 3). The SDs of relative peak area were around 15% at small peaks and 4% at bigger peaks. (The original data matrix with the SD values is available upon request.)

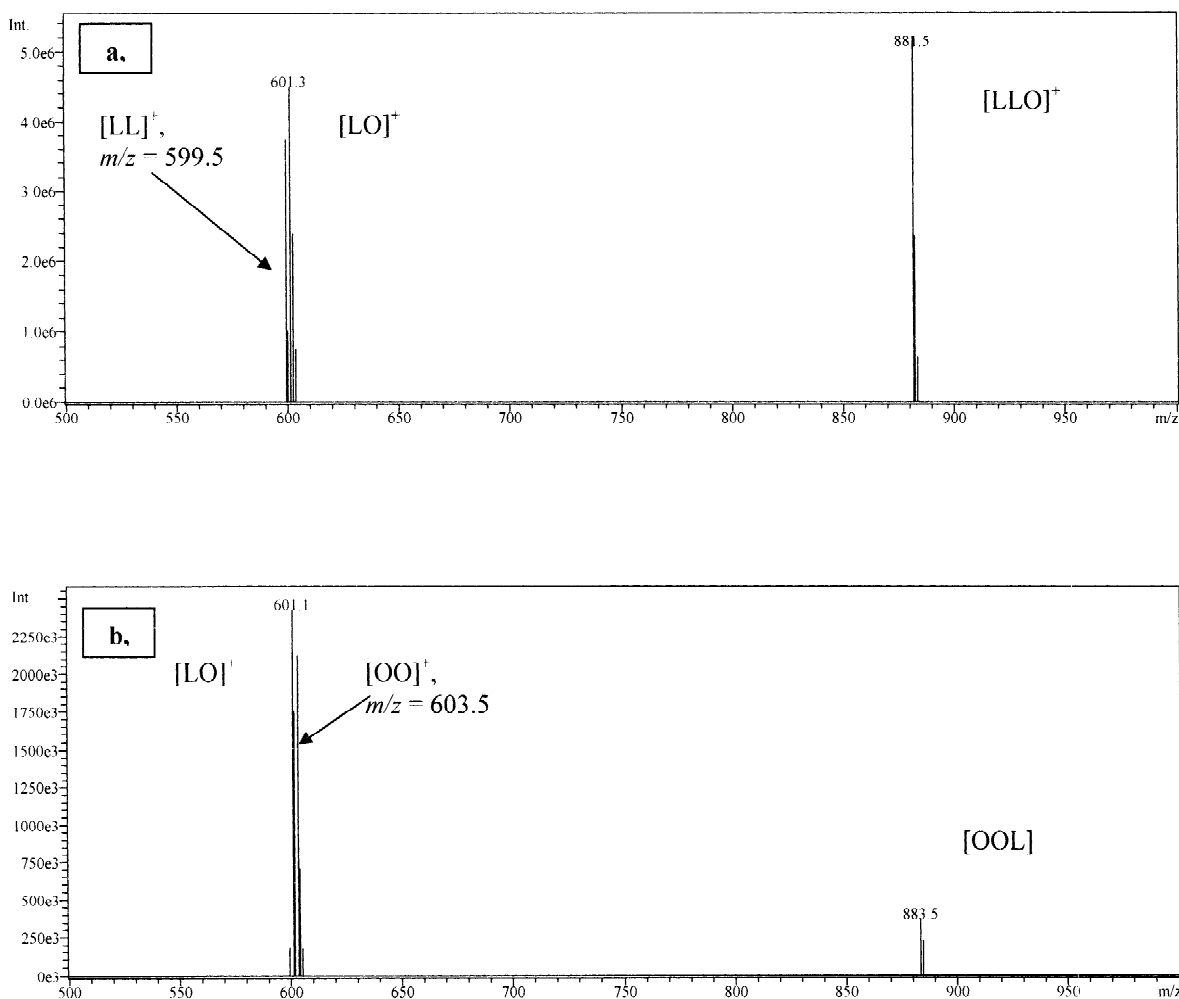


Fig. 2. APCI-MS spectrum of (a) LLO and (b) OOL. For symbols see text.

Table 3

The mean values of the relative peak areas of TAG from different plant oils, calculated from the SIC

Sample	TAG											
	LLLn	LLL	LnLP	LLO	PLL	OOL	PLO	PLP	OOO	POO	POP	SOO
Almond 1	0.58	6.05	0.20	10.41	2.04	21.08	3.87	0.83	40.22	10.26	0.95	3.50
Almond 2	0.49	7.86	0.19	9.35	2.21	18.01	2.95	0.57	43.70	9.96	1.21	3.50
Almond 3	0.53	10.57	<0.1	7.66	2.42	13.57	1.66	0.39	48.59	9.58	0.85	4.18
Almond 4	0.45	11.09	<0.1	7.67	2.57	13.14	1.66	0.36	47.84	10.44	0.93	3.86
Almond 5	<0.1	3.92	<0.1	11.08	2.35	22.43	3.45	0.44	44.40	8.79	0.46	2.69
Avocado 1	<0.5	0.83	1.05	2.46	2.35	10.27	11.14	4.54	32.00	23.39	10.78	1.18
Avocado 2	<0.5	0.84	1.32	2.36	2.60	9.68	12.26	3.94	31.88	24.80	9.28	1.03
Avocado 3	<0.5	0.80	1.29	2.88	2.63	10.71	12.31	3.06	32.80	23.62	9.20	0.72
Corngerm 1	1.05	30.25	0.38	24.91	12.03	12.60	3.83	1.52	8.11	3.33	0.86	1.13
Corngerm 2	1.49	31.74	0.36	20.44	8.95	16.06	4.64	1.89	8.47	3.49	1.16	1.31
Corngerm 3	1.45	32.84	0.52	24.65	11.33	11.73	4.17	1.68	7.38	2.95	0.76	0.73
Grapeseed 1	5.26	39.95	1.22	19.76	14.85	7.23	4.13	0.66	3.68	1.84	0.60	0.82
Grapeseed 2	4.81	41.19	1.34	20.41	14.35	7.09	4.03	0.76	3.05	1.91	0.45	0.60
Grapeseed 3	4.53	38.41	0.99	22.20	16.93	6.52	4.31	0.77	2.92	1.47	0.44	0.50
Linseed 1	23.69	26.37	4.20	18.27	7.66	8.03	2.15	1.03	5.03	1.87	0.38	1.32
Linseed 2	28.67	25.94	4.85	17.07	6.12	6.57	1.67	0.61	4.86	1.74	0.36	1.55
Linseed 3	36.65	26.99	5.04	16.04	5.69	3.81	0.98	0.36	3.08	0.76	0.18	0.42
Linseed 4	41.09	21.10	6.94	17.22	5.28	2.58	0.79	0.37	2.52	1.10	0.24	0.78
Linseed 5	50.56	18.30	6.22	13.22	4.28	1.83	0.47	0.18	3.30	0.85	0.20	0.47
Olive 1	n.d. ^a	0.52	<0.1	2.80	0.76	10.87	2.47	0.45	52.61	18.87	4.61	6.30
Olive 2	0.22	2.15	0.22	6.92	1.95	12.34	2.18	0.58	46.43	18.14	3.85	6.17
Olive 3	0.09	1.49	<0.1	4.82	2.04	11.02	4.17	1.92	42.81	20.49	6.38	5.52
Olive 4	0.06	0.42	0.19	3.75	1.21	10.49	3.11	0.69	49.56	21.10	4.85	4.79
Peanut 1	4.33	8.86	0.74	6.65	2.22	13.14	1.82	0.60	46.10	10.13	1.32	4.08
Peanut 2	n.d. ^a	2.69	0.29	8.54	2.99	17.45	11.87	2.38	34.26	11.84	2.82	4.87
Pumpkinseed 1	<0.1	19.42	<0.1	21.11	9.53	17.20	4.48	3.03	15.14	5.17	1.80	3.14
Pumpkinseed 2	<0.1	20.47	<0.1	17.81	10.29	18.57	4.40	2.64	15.37	5.70	1.35	3.40
Pumpkinseed 3	<0.1	20.56	<0.1	20.95	10.95	17.37	4.08	2.73	14.28	5.22	1.27	2.61
Soybean 1	15.74	27.35	7.05	13.83	11.23	8.47	3.76	3.58	4.02	2.48	1.16	1.33
Soybean 2	16.96	26.35	6.36	14.63	12.13	8.44	3.77	3.77	3.55	2.20	0.92	0.93
Soybean 3	17.86	32.03	5.92	15.40	12.28	5.51	2.93	2.28	2.58	1.85	0.80	0.56
Soybean 4	18.54	33.03	4.62	17.39	9.41	7.18	3.01	1.89	3.70	2.01	0.53	0.64
Sunflower 1	<0.1	29.34	n.d. ^a	21.96	10.90	15.69	3.74	1.68	10.73	2.84	0.63	2.48
Sunflower 2	0.87	31.70	n.d. ^a	21.48	10.40	16.39	4.50	1.88	8.24	2.57	0.59	1.38
Sunflower 3	<0.1	33.59	n.d. ^a	28.96	12.49	11.76	3.65	1.54	4.80	1.68	0.48	1.04
Sunflower 4	<0.1	35.34	n.d. ^a	27.42	11.57	12.55	3.47	1.40	5.68	1.61	0.29	0.68
Sunflower 5	<0.1	30.34	n.d. ^a	25.01	10.66	16.96	4.24	1.61	6.33	2.56	0.60	1.70
Walnut 1	20.92	30.62	6.28	13.75	9.46	8.19	2.80	1.06	4.53	1.42	0.35	0.62
Walnut 2	26.44	38.11	3.36	11.23	6.71	9.22	2.97	0.80	5.16	1.43	0.19	0.05
Wheatgerm 1	5.01	32.99	2.39	23.16	9.41	11.28	3.08	2.04	7.60	1.85	0.38	0.81
Wheatgerm 2	4.43	26.40	1.94	24.26	11.43	12.68	3.68	2.38	8.80	2.25	0.61	1.15
Wheatgerm 3	14.08	25.86	7.79	12.69	15.41	5.03	3.67	5.63	5.27	2.75	1.45	0.36

^a Not detected.

3.2. Linear discriminant analysis (LDA)

LDA was applied to the data matrix. The relative TAG contents of the oils were the variables, and each type of oil was considered as a class (12 different ones). Of the 42 samples, 97.6% were

classified correctly (all samples were included) according to the classification matrix (not presented here), calculated by the LDA. Only one from the two peanut oil samples was not classified correctly. Comparing the relative TAG content of the two peanut oil samples, it can be considered that they

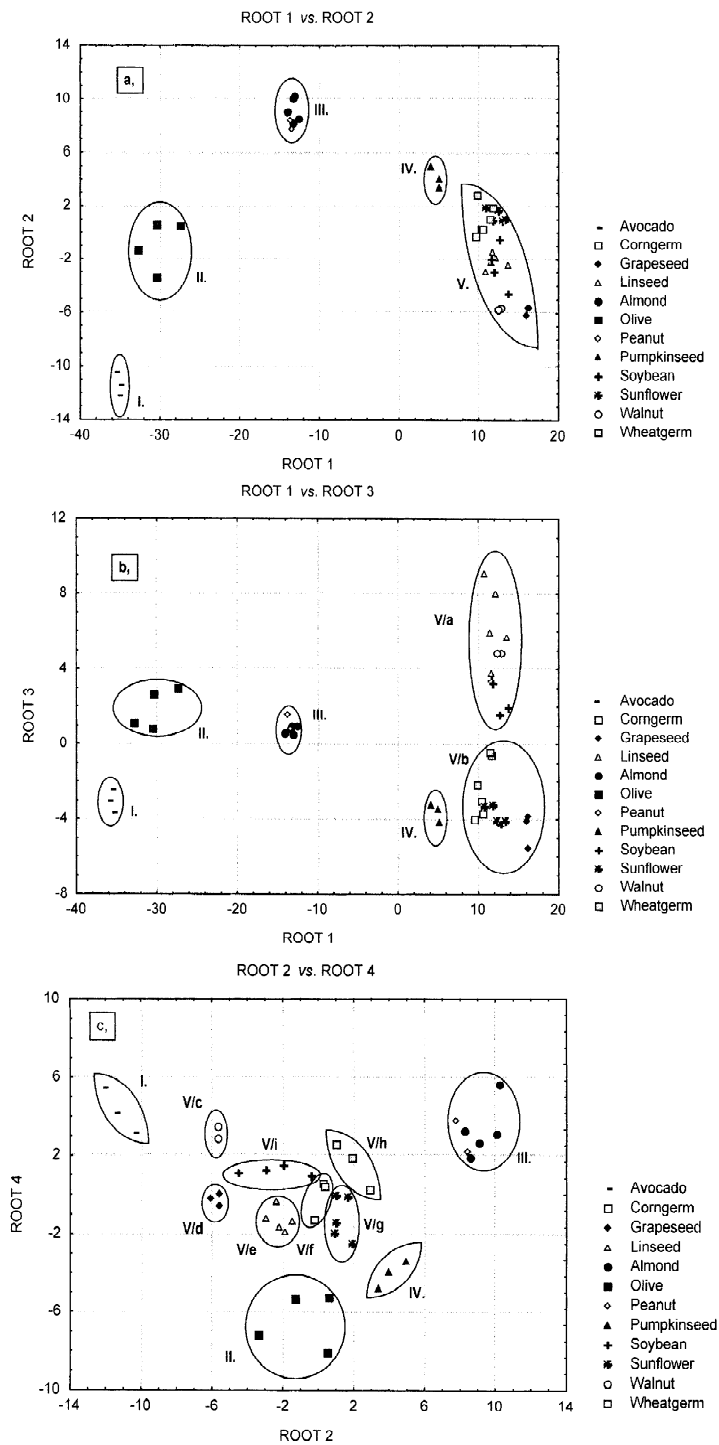


Fig. 3. Results of the discriminant analysis. (a) Root 1 vs. root 2, (b) root 1 vs. root 3, and (c) root 2 vs. root 3.

differ from each other (Table 3). Peanut 1 sample contained 4.33% of LLLn, while LLLn was not detected in peanut 2. There is also a significant difference between the PLO content of the two samples. The content of PLO in peanut 1 and peanut 2 is 1.82% and 11.87%, respectively. Héron et al. also found peanut oil samples containing rather different amounts of TAGs originated from different continents [11]. According to them, it is probable that the oils examined in our study have different origins, although the origins of the peanut samples were not known in our case.

The different canonical roots were plotted against one another in order to visualize the statistical results. Fig. 3 shows impressive two-dimension plots of roots with high discriminant power. The first two roots (Fig. 3a) mostly discriminate between the avocado (cluster I), olive (cluster II), almond together with peanut oil samples (cluster III), pumpkinseed (cluster IV) and the others (cluster V). Cluster V was divided into V/a and V/b by root 1 and 3 (Fig. 3b). These two clusters were separated into smaller groups based on class of the oil by root 2 and 4 (Fig. 3c). Root 2 and 4 have the highest discriminant power to separate cluster V.

4. Conclusion

It can be concluded that HPLC combined with APCI-MS is a suitable tool to analyze triacylglycerols present in different plant oils. HPLC–APCI-MS combined with linear discriminant analysis proved to be a suitable method for the discrimination of different oils according to their TAG composition.

Acknowledgements

We thank the different companies for providing oil samples.

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