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Analysis

Statistical evaluation of triacylglycerol composition by HPLC/APCI-MS

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Summary

The statistical evaluation of triacylglycerol profiles in plant oils based on HPLC/MS analysis permits differentiation based on the multidimensional data matrix. The data set of 93 oils from 60 plants composed from 355 triacylglycerols is evaluated using the principal component analysis (PCA). Similarities among some plant oils are visualized by the formation of clusters in the PCA plot. The authentication of plant oils is tested with model samples of olive oil adulterated with sunflower oil at different concentration levels. Our HPLC/MS method using the statistical multivariate data analysis of large data matrix enables a clear identification of adulterated olive oils from 1% of added sunflower oil as an adulterant.

Introduction

Plant oils are important commodities in world markets due to their widespread utilization in many branches of industry, cosmetics and nutrition (*1*). They are produced from oil plants representing almost 10% of world production of all crops. Edible plant oils are mixtures of lipids composed mainly from triacylglycerols (TAG) with a content up to 95%. Higher prices of high-quality plant oils can lead to adulteration by cheaper oils of lower quality and less beneficial nutritional properties. Therefore their authentication is of great interest. Many authentication methods use the measurement of oil fingerprints without any sample pretreatment and separation steps, e.g., infrared/Raman spectroscopy, NMR or MS (*2*). Although fingerprint methods are fast and simple, some plant oils have similar fingerprints differing only in trace components not detectable this way. TAG are suitable for the authentication of plant oils, because they are main components of plant oils with several tens of species occurring at different concentrations resulting from fatty acids (FA) esterified on the glycerol skeleton. These differ in carbon number (CN), double bond (DB) number, the configuration and position of DBs and the stereochemical position of FA. TAG profiles differ for each plant oil which is used for authentication based on the chromatographic separation. The highest number of identified TAG in plant oils have been reported using non-aqueous reversed-phase (NARP) HPLC with APCI-MS detection (*3*). In this mode, TAG are separated according to the equivalent carbon number (ECN) defined as ECN = CN − 2DB. The separation of almost all TAG within one ECN group (*3*) or of TAG with different DB positions (*4*) is possible.

Simple comparison of TAG concentrations of pure and adulterated samples is not convenient for the authentication of plant oils due to the complexity of the data. Multivariate statistical methods are used for the discrimination of different samples and the detection of adulteration, e.g. PCA, partial least square analysis, linear discriminant analysis and hierarchical cluster analysis. PCA uses a simple mathematical procedure for easy transformation of a high number of possibly correlated (covariant) variables into the smaller number of uncorrelated variables called principal components (PC). PCA is mathematically defined as an orthogonal linear transformation that transforms the data to a new coordinate system in such a way that the greatest variance shows on the first coordinate (PC1) and the second greatest variance on the second coordinate (PC2).

Analytical methods

Samples for this study were either extracted in our laboratory according to a simple procedure (3-5) or purchased from Augustus Oils (UK). Oil samples were dissolved in a mixture of acetonitrile/2-propanol/hexane (1:1:1). The liquid chromatograph Waters 616 (USA) was coupled to Esquire 3000 ion trap mass analyzer (Bruker Daltonics, Germany) using positive-ion atmospheric pressure chemical ionization (APCI). HPLC conditions: two NovaPak C_{18} columns (300×3.9 and 150×3.9 mm, 4 μm, Waters) connected in series, flow rate 1 mL/min, injection volume 10 μL, column temperature 25°C, gradient 0 min − 100% acetonitrile, 106 min − 31% acetonitrile − 69% 2-propanol. The data set for multivariate statistical analysis was processed using Simca-P (Umetrics, Sweden). Data annotation: 1 − kiwi; 2 − macadamia nut; 3 − hemp; 4 − Brazil nut; 5, 6 − mango; 7 − dog rose; 8, 9, 10 − hazelnut; 11 − sweet chestnut; 12 − pumpkin; 13 − lemon; 14 − bell pepper; 15 − grapefruit; 16 − cucumber; 17, 18 − blackcurrant; 19 − mandarin orange; 20 − blueberry; 21 − melon cantaloupe; 22 − papaya; 23 − buckwheat; 24 − pistachio; 25, 26 − peanut; 27, 28 − camellia; 29 − rice; 30 − coffee butter; 31 − apricot kernel; 32 − raspberry; 33 − argan; 34 − black cumin; 35 − moringa; 36 − tamanu; 37, 38, 39 − soya; 40, 41, 42 − rapeseed; 43, 44 − 51 − sunflower; 52, 53 − 67 − olive; 68 − palm; 69 − cotton; 70 − coconut palm; 71 − corn; 72 − sesame; 73 − almond; 74 − safflower; 75 − grape wine white; 76 − grape wine red; 77 − linseed;

Table1. Number of identified TG and FA together with the calculation of average equivalent carbon number (aECN), average carbon number (aCN), average double bond (aDB) number, the relative weight percentage of essential FA (L + Ln), C18 + C16, saturated (Sat), monounsaturated (Mono) and polyunsaturated (Poly) FA.

Oil	No.	No. of TG/FA	aECN	aCN	aDB	Essential FA [%]	C18+C16 FA [%]	Sat [%]	Mono [%]	Poly [%]
Kiwi	**1**	47/11	13.59	17.83	2.12	70.7	99.4	11.7	17.3	71.0
Macadamia nut	**2**	45/13	15.82	17.53	0.85	2.9	91.0	17.5	79.6	2.9
Hemp	**3**	70/18	14.03	17.87	1.89	70.7	98.0	12.9	11.8	75.3
Brazil nut	**4**	26/7	15.38	17.66	1.14	39.9	99.9	24.9	35.2	39.9
Mango	**6**	53/13	16.57	17.86	0.65	8.2	96.5	44.2	47.6	8.2
Dog rose	**7**	51/14	14.27	17.93	1.83	69.7	98.4	8.2	22.0	69.8
Hazelnut	**9**	30/10	15.62	17.83	1.10	21.3	99.2	11.1	67.6	21.3
	10	30/10	15.72	17.84	1.06	17.6	99.1	11.4	71.0	17.6
Sweet chestnut	**11**	49/16	15.04	17.72	1.34	44.2	98.7	16.4	39.3	44.3
Pumpkin	**12**	31/9	15.01	17.71	1.35	56.0	99.0	19.9	24.1	56.0
Lemon	**13**	58/12	14.90	17.62	1.36	46.9	99.3	23.0	30.1	46.9
Bell pepper	**14**	44/16	14.61	17.77	1.58	74.5	98.4	15.9	9.6	74.6
Grapefruit	**15**	51/14	15.07	17.46	1.19	44.7	99.2	29.9	25.5	44.7
Cucumber	**16**	45/13	14.54	17.62	1.54	72.1	99.1	20.0	7.9	72.1
Blackcurrant	**18**	80/14	13.76	17.85	2.05	57.5	99.3	8.9	15.2	75.9
Mandarin orange	**19**	56/14	15.03	17.55	1.26	48.1	98.8	26.4	25.5	48.1
Blueberry	**20**	37/9	13.94	17.86	1.96	70.0	99.7	7.9	22.0	70.0
Melon cantaloupe	**21**	37/11	14.70	17.76	1.53	68.6	99.5	15.6	15.8	68.6
Papaya	**22**	55/17	15.93	17.66	0.87	9.4	97.9	22.4	68.2	9.4
Buckwheat	**23**	59/16	15.51	17.92	1.20	39.4	92.0	21.5	39.0	39.5
Pistachio	**24**	40/11	15.28	17.81	1.27	38.5	99.1	11.5	50.0	38.5
Peanut	**26**	60/16	15.60	17.94	1.17	37.9	92.9	21.2	40.8	37.9
Camellia	**27**	26/12	15.87	17.80	0.97	9.5	99.1	12.5	78.0	9.5
	28	26/12	15.88	17.82	0.97	8.8	99.2	11.6	79.6	8.8
Rice	**29**	48/12	15.30	17.66	1.18	38.1	97.9	20.6	41.3	38.1
Coffee butter	**30**	68/14	14.05	15.01	0.48	20.5	57.6	72.3	7.2	20.5
Apricot kernel	**31**	27/10	15.40	17.86	1.23	31.1	99.8	7.3	61.7	31.1
Raspberry	**32**	51/13	13.91	17.94	2.02	79.7	99.4	4.8	15.5	79.7
Argan	**33**	60/16	15.45	17.71	1.13	33.3	98.8	19.5	47.2	33.3
Black cumin	**34**	35/9	14.89	17.80	1.45	58.8	96.6	15.0	23.5	61.5
Moringa	**35**	33/16	16.65	18.24	0.80	0.9	86.4	22.1	77.0	0.9
Tamanu	**36**	43/12	15.40	17.79	1.19	41.7	98.6	21.7	36.6	41.7
Soya	**38**	66/14	14.86	17.79	1.47	56.9	98.5	16.8	26.3	56.9
Rapeseed	**41**	55/13	15.29	17.90	1.31	30.8	97.9	9.6	59.6	30.8
Sunflower	**44**	50/16	14.91	17.88	1.49	61.9	97.9	13.3	24.8	61.9
Olive	**53**	37/15	15.90	17.75	0.92	7.5	98.5	15.8	76.7	7.5

78 – poppy seed; 79 – walnut; 80 – avocado pear; 81 – redcurrant; 82 – borage; 83 – cacao butter; 84 – evening primrose; 85 – kukui nut; 86 – wheat germ; 87 – cashew nut; 88 – yellow melon; 89 – fig; 90 – date; 91 – European larch; 92 – Norway spruce; 93 – European silver fir.

Results and discussion

The separation of TAG from plant oils is a challenging task due to the presence of numerous TAG species with similar physicochemical properties. The NARP-HPLC separation mode is used for the separation of TAG complex mixtures of plant oils based on our optimized method (Figure 1). Individual TAG are identified based on their APCI mass spectra using $[M+H]^+$ ions for the molecular weight determination and $[M+H-R_iCOOH]^+$ fragment ions for the identification of individual FA. The cleavage of FA from *sn*-2 position is less preferred than from the *sn*-1/3 positions resulting in a lower abundance of the corresponding $[M+H-R_iCOOH]^+$ fragment used for the determination of prevailing FA in the *sn*-2 position. We have found the preference of unsaturated FAs (mainly linoleic) in *sn*-2 position for plant oils in agreement with the literature. 355 TAG are identified in 93 plant oils composed from 35 FA with 6 to 26 carbons and 0 to 4 DB. TAG are quantified using APCI-MS response factor approach described previously (6). Table 1 lists average parameters and

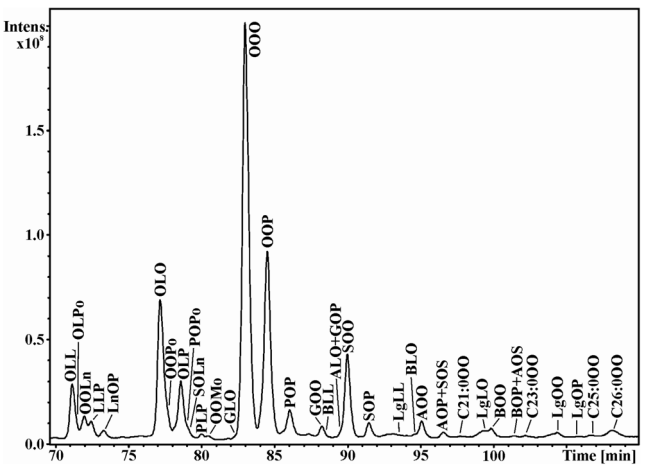

Figure 1. HPLC/APCI-MS chromatogram of the analysis of olive oil.

Table 2 shows FA composition of individual plant oils calculated from the HPLC/MS results.

Multivariate data analysis using PCA is used for the evaluation of TAG composition in the samples studied. The data set contains 93 plant oils (objects) of 60 different types characterized by relative peak areas of 355 identified TAG species (variables). 13 variables are excluded due to zero variability corresponding to

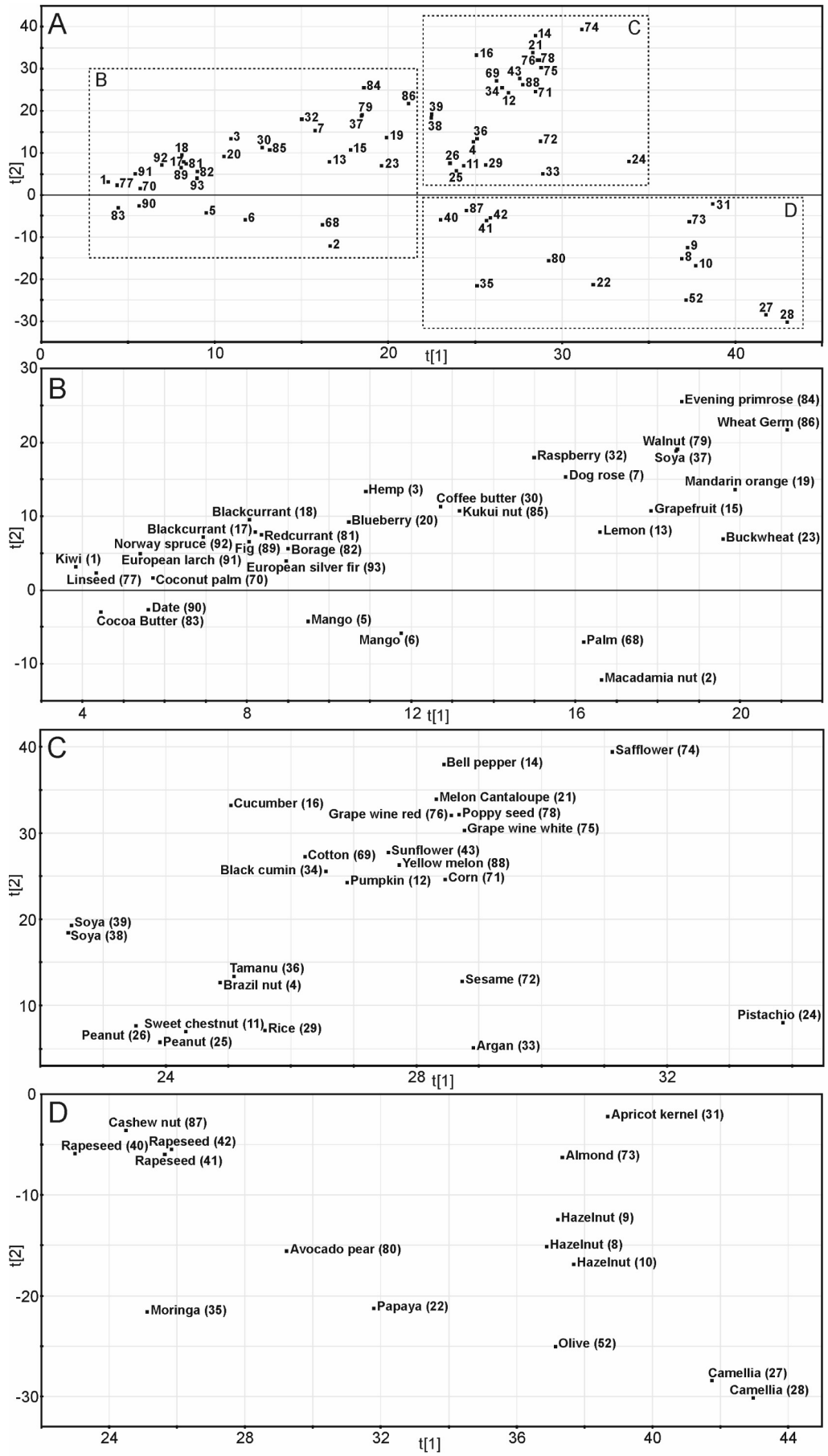

Figure 2. Projection of principal components t[1] and t[2] in two dimensional scatter plot (A) and annotated zooms of individual regions (B, C and D).

Table 2. Relative weight percentage of main FA calculated from HPLC/APCI-MS of triacylglycerols for individual oils. The remaining part to 100% is formed by odd carbon number and unusual FA.

Oil	No.	P 16:0	Po 16:1	S 18:0	O 18:1	L 18:2	Ln 18:3	γLn 18:3	St 18:4	A 20:0	G 20:1	B 22:0	Lg 24:0
Kiwi	**1**	8.5		3.1	17.1	17.1	53.6			0.04	0.2		
Macadamia nut	**2**	8.0	18.9	3.3	57.9	2.9				2.8	2.6	1.3	0.5
Hemp	**3**	8.6		2.6	11.5	49.5	21.2	3.2	1.4	1.0	0.3	0.5	0.1
Brazil nut	**4**	16.3	0.08	8.5	35.1	39.9				0.1	0.02		
Mango	**6**	10.7		30.3	47.3	7.4	0.8			1.7	0.3	0.5	0.6
Dog rose	**7**	4.6		2.4	21.7	47.9	21.8			1.0	0.3	0.1	0.04
Hazelnut	**9**	7.8	0.6	3.0	66.5	20.8	0.5			0.2	0.3		
	10	7.5	0.3	3.5	70.2	17.3	0.3			0.3	0.3		
Sweet chestnut	**11**	14.3	0.08	1.5	38.6	37.1	7.1			0.3	0.6	0.1	0.03
Pumpkin	**12**	14.5		4.5	24.0	56.0				0.6	0.06	0.2	0.04
Lemon	**13**	18.8		3.5	30.1	33.4	13.5			0.3	0.03	0.08	0.2
Bell pepper	**14**	12.5		2.1	9.3	73.7	0.8			0.4	0.07	0.3	0.3
Grapefruit	**15**	25.9	0.8	3.2	24.6	38.6	6.1			0.3	0.05	0.02	0.2
Cucumber	**16**	17.5		1.7	7.8	68.3	3.8			0.2	0.09	0.04	0.05
Blackcurrant	**18**	7.7		1.1	14.7	40.9	16.6	14.6	3.7	0.1	0.5	0.01	<0.01
Mandarin orange	**19**	22.1	0.4	3.3	24.9	42.6	5.5			0.3	0.2	0.1	0.3
Blueberry	**20**	6.6		1.1	22.0	35.7	34.3			0.2	0.06		
Melon cantaloupe	**21**	11.8		3.4	15.7	67.8	0.8			0.2	0.09	0.06	0.02
Papaya	**22**	16.6	0.9	4.5	66.5	8.9	0.5			0.6	0.7	0.2	0.07
Buckwheat	**23**	14.7	0.2	2.0	35.7	36.9	2.5			1.1	3.0	2.0	1.4
Pistachio	**24**	9.7		1.5	49.4	38.2	0.3			0.1	0.5	0.1	0.05
Peanut	**26**	12.6		2.7	39.7	37.6	0.3			1.1	1.0	3.1	1.5
Camellia	**27**	10.0	0.08	2.3	77.2	9.2	0.3			0.06	0.6		0.07
	28	9.1	0.07	2.4	78.8	8.5	0.3			0.04	0.6		0.03
Rice	**29**	16.5	0.7	2.5	40.1	36.1	2.0			0.5	0.5	0.2	0.4
Coffee butter	**30**	17.6		12.3	7.2	19.7	0.8			0.9	0.03	0.3	0.06
Apricot kernel	**31**	6.2	0.3	1.0	61.2	31.0	0.05			0.06	0.06		
Raspberry	**32**	3.4		0.8	15.5	52.9	26.8			0.3	0.04	0.2	0.02
Argan	**33**	14.0	0.4	4.7	46.4	33.1	0.2			0.3	0.4	0.2	0.07
Black cumin	**34**	12.4		2.3	23.1	58.8				0.2	0.4	0.06	0.04
Moringa	**35**	6.4	1.2	4.7	73.2	0.9				3.4	2.5	5.8	1.6
Tamanu	**36**	11.2	0.03	9.3	36.4	41.7				0.8	0.1	0.3	0.04
Soya	**38**	12.1		3.3	26.2	49.7	7.2			0.4	0.1	0.6	0.2
Rapeseed	**41**	7.1	0.2	1.6	58.2	21.2	9.6			0.4	1.1	0.3	0.1
Sunflower	**44**	7.6		3.8	24.6	61.5	0.4			0.4	0.1	0.8	0.3
Olive	**53**	12.5	1.3	2.3	74.9	6.5	1.0			0.5	0.4	0.3	0.1

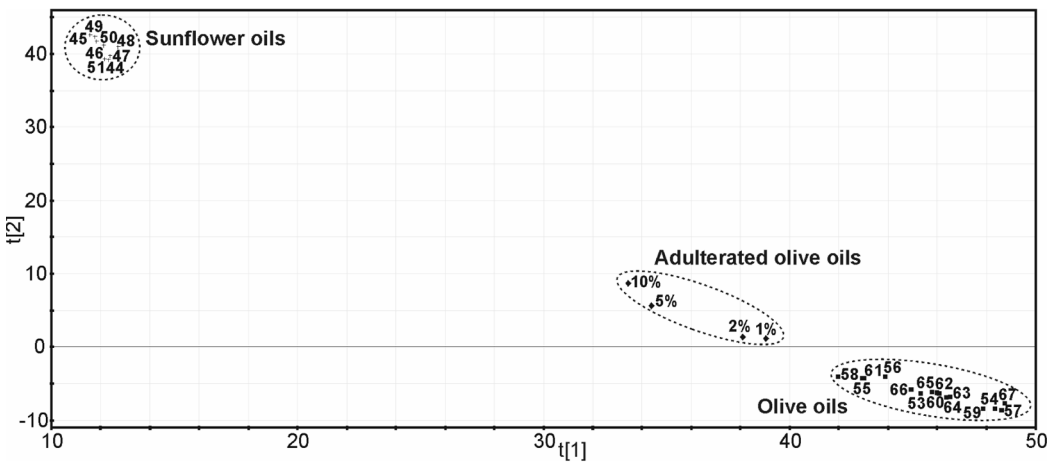

Figure 3. Projection of principal components t[1] and t[2] in two dimensional scatter plot for sunflower (44–51) and olive (53–67) oils and olive oil adulterated by 1%, 2%, 5%, or 10% of sunflower oil.

the content of this variable in all samples lower than LOD. Values of other 342 variables range between 0.01% and 49.32% (3.5 orders of magnitude).

Figure 2 shows score plots of the first (t[1]) and second (t[2]) PC with a good resolution of analyzed samples. Two variables describe 82% of the total variability in the data set, where the first PC t[1] describes 52% and second PC t[2] 30% of the total variability. Other PCs describe significantly lower variability, e.g., t[3] has 4% and t[4] 3% of the total variability. Samples of one type of plant oil form narrow clusters, e.g., different samples of hazelnut and camellia oils (Figure 2D). Samples with similar TG profiles are grouped in small regions in PCA plot, e.g., blackcurrant and redcurrant oils (Figure 2B). Similar positions of samples in PCA plot indicate their similar properties, e.g., Brazil nut and tamanu oils in Figure 2C. Their similar properties can be confirmed by the comparison of average parameters and sums of FA shown in Table 1.

Olive oil is one of the most expensive dietary plant oils which may lead to its illegal falsification. Most favorable oils for the adulteration are plant oils with low price, geographical availability and sufficient production, e.g., sunflower oil. The set of 8 sunflower and 15 olive oils and 4 model samples of olive oil adulterated by 1%, 2%, 5% or 10% of sunflower oil is tested to develop a method to identify the adulteration even at very low amounts of adulterant. Figure 3 shows the scores plot of the first (t[1]) and second (t[2]) PCs. This data set is represented by 27 objects (oil samples) and 62 variables (TG concentrations) with significant variability. PCs t[1] and t[2] account 99.6% of total variability (t[1] = 73.5% and t[2] = 26.1%). Samples of sunflower oil have small differences in TG composition and form a small cluster clearly distinguished from other samples in a PCA plot. Samples of olive oil have a wider distribution due to slightly different TG composition of different types (virgin vs. pomace oil) and origin of samples. Samples of adulterated olive oil with increasing concentration of sunflower oil have an increasing distance from the olive oil cluster in PCA plot.

Conclusions

The results presented here demonstrate the utilization of HPLC/MS analysis and PCA statistical evaluation in the quality control of plant oils. Carefully optimized HPLC/MS method is used for detailed characterization of TG profiles. PCA evaluation of multidimensional data matrix of the TG profiles enables the comparison of all samples. PCA analysis is used for the authentication of expensive olive oil with only 1% of added sunflower oil.

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References

1. Gunstone, F. (Ed.) (2006) *Modifying lipids for use in food*, Woodhead Publishing Ltd, Cambridge, England.
2. Jakab, A. *et al.* (2002) Differentiation of vegetable oils by MS combined with statistical analysis, *Rapid Commun. Mass Spectrom.* 16: 2291–2297.
3. Lísa, M. and Holčapek, M. (2008) TGs profiling in plant oils important in food industry, dietetics and cosmetics using HPLC/APCI–MS, *J. Chromatogr. A* 1198: 115–130.
4. Lísa, M. et al. (2007) HPLC/APCI–MS and GC/FID characterization of 5–polyenoic FAs in TGs from conifer seed oils, *J. Chromatogr. A* 1146: 67–77.
5. Lísa, M. *et al.* (2009) Statistical evaluation of TG composition in plant oils based on HPLC/APCI–MS data, *J. Agric. Food Chem.* 57: 6888–6898.
6. Holčapek, M. et al. (2005) Quantitation of TGs in plant oils using HPLC with APCI–MS, evaporative light–scattering, and UV detection, *J. Sep. Sci.* 28: 1315–1333.

