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ARTICLE

Statistical Evaluation of Triacylglycerol Composition in Plant Oils Based on High-Performance Liquid Chromatography−Atmospheric Pressure Chemical Ionization Mass Spectrometry Data

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The statistical evaluation of triacylglycerol profiles in plant oils based on high-performance liquid chromatography mass spectrometry (HPLC/MS) analysis enables the differentiation of various plant oils on the basis of the multidimensional data matrix. A data set of 93 oil samples from 60 varieties of plants composed from 355 triacylglycerols is evaluated using the principal component analysis. Analyzed samples are resolved in the principal component analysis plot, and similarities among some types of plant oils are visualized by the formation of clusters. 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 a large data matrix enables a clear identification of adulterated olive oils already from 1% of added sunflower oil as an adulterant.

KEYWORDS: High-performance liquid chromatography; mass spectrometry; atmospheric pressure chemical ionization; triacylglycerol; plant oil; adulteration; authentication; statistics; principal component analysis

INTRODUCTION

Plant oils are an important commodity in world markets because of their widespread utilization in many branches of industry, cosmetics, and nutrition. They are produced from oil plants representing almost 10% of the world production of all crops according to the Food and Agriculture Organization of the United Nations (*1*). The annual production of edible plant oils has increased in the past decade by more than 50% to 127 million tonnes a year (*1*) and is still increasing annually. Edible plant oils are mixtures of lipids composed mainly from triacylglycerols (TGs) with the content up to 95%. They serve as an important source of fatty acids in the human diet, mainly essential fatty acids necessary for the biosynthesis of long-chain polyunsaturated fatty acids important for the synthesis of cell membranes in the human body. A diet with 70 g of fat per day for female adults and 90 g for male adults corresponding to 30−35% of daily energy coming from fats is now considered as consistent with good health (*2*). In reality, the consumption of oils and fats in USA and EU is about 130 g per day per person (*3*).

Prices of plant oils are given by many parameters, mainly by the production cost and the quality of plant oils. Higher prices of high-quality plant oils can lead to the effort of falsification by cheaper oils with a lower quality and less beneficial nutritional properties (e.g., expensive virgin olive oil adulterated by cheaper sunflower oil); therefore, their authentication is of great interest nowadays. Many authentication methods use the measurement of oil fingerprints without any separation and sample pretreatment steps, e.g., Raman spectroscopy (*4*, *5*), infrared spectroscopy (*6*, *7*), nuclear magnetic resonance spectroscopy (*8*, *9*), matrix-assisted laser desorption/ionization mass spectrometry (MS) (*10*, *11*), electrospray ionization (ESI) MS (*12*, *13*), atmospheric pressure photoionization MS (*13*), and so forth. Although the fingerprint methods are fast and simple, some plant oils have similar fingerprints differing only in low concentration components not detectable this way. TGs are compounds suitable for the authentication of plant oils because they are the main components of plant oils with several tens of different species occurring at different concentration levels. They are characterized by fatty acids esterified on the glycerol skeleton and their properties, i.e., carbon number (CN), double bond (DB) number, the configuration and position of DBs in acyl chains, and the stereochemical position of fatty acids on the glycerol skeleton. TG profiles differ for each type of plant oil which is used for authentication based on chromatographic separation, i.e., gas chromatography/isotopic ratio mass spectrometry (*14*), gas chromatography/flame ionization detection (GC/FID) (*15*, *16*), high-performance liquid chromatography (HPLC)/refractive-index detection (*17*), HPLC/atmospheric pressure chemical ionization (APCI) MS (*10*, *18*−*20*), and off-line two-dimensional HPLC/MS (*21*).

The highest number of identified TGs in plant oils have been reported using nonaqueous reversed-phase (NARP) HPLC with APCI-MS detection (*22*, *23*). In NARP-HPLC mode, TGs are separated according to the equivalent carbon number (ECN)

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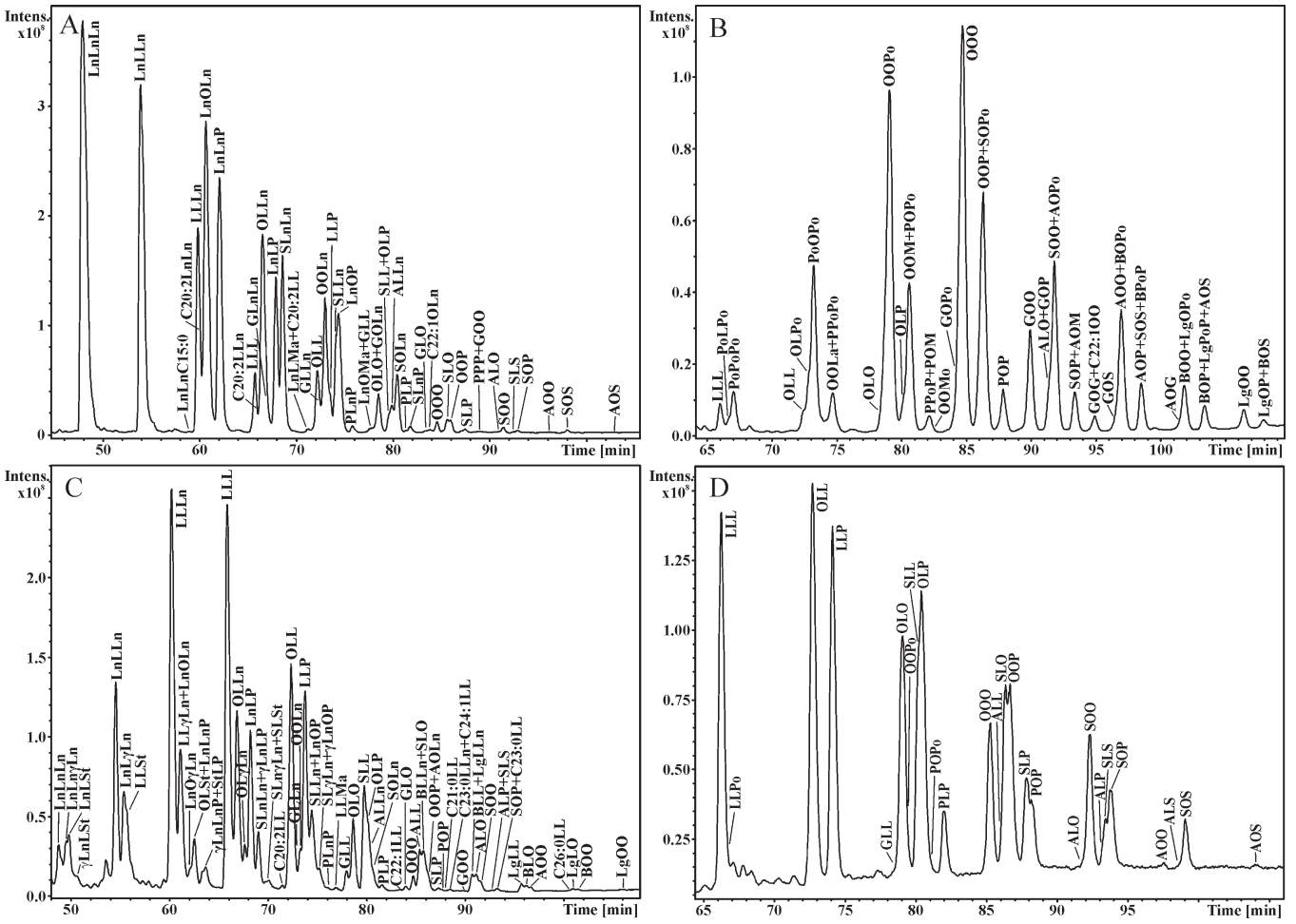

Figure 1. NARP-HPLC/APCI-MS analysis of plant oils: (**A**) kiwi seed (*Actinidia deliciosa*), (**B**) macadamia nut (*Macadamia integrifolia*), (**C**) hemp (*Cannabis sativa*), and (**D**) Brazil nut (*Bertholletia excelsa*).

defined as ECN = CN − 2DB. The separation of almost all TGs within one ECN group (*22*, *23*) or TGs with different positions of DBs (*24*) have been reported. The complementary separation mode, silver-ion chromatography, is based on the formation of weak complexes of silver ions with DBs, which is used for the separation of unsaturated TGs differing in the number and position of DBs. Silver-ion HPLC suffers from a lower reproducibility of retention times and a lower selectivity for saturated TGs in comparison to that in NARP-HPLC, but it enables the separation of TG regioisomers ($R_1R_1R_2$ vs $R_1R_2R_1$) (*25*, *26*). APCI is the most suitable ionization technique for the HPLC/MS analysis of TGs because of the excellent sensitivity and the structural information based on protonated molecules $[M+H]^+$ and $[M+H-R_iCOOH]^+$ fragment ions observed already in full-scan APCI mass spectra. Low abundance of protonated molecules in APCI mass spectra of saturated TGs can be improved by the formation of ammonium adducts $[M + NH_4]^+$ due to the postcolumn addition of ammonium acetate (*27*). Ratios of fragment ions $[M + H - R_iCOOH]^+$ are used for the determination of prevailing fatty acids esterified in the *sn*-2 position because of the lower abundance of fragment ions corresponding to the neutral loss of fatty acid from this position (*28*−*32*). ESI can be also used for the detection of TGs, but $[M + H]^+$ ions in the spectra are replaced by adducts with alkali metal ions $[M+Na]^+$ and $[M+K]^+$ or ammonium adducts $[M + NH_4]^+$ depending on the mobile phase composition (*30*, *33*). Fragment ions $[M + H - R_iCOOH]^+$ are also present in full-scan ESI mass spectra but with lower relative abundances in comparison to that in APCI. Moreover, ESI is less convenient for NARP systems typical for HPLC analysis of TGs.

Simple comparison of TG concentrations of pure and adulterated samples is not often sufficient proof for the authentication of plant oils because of the complexity of the data matrix. The statistical evaluation is a powerful tool for processing of large data sets, which enables the discrimination of different samples. Different multivariate statistical methods are used for the evaluation of TG composition and the detection of adulteration of plant oils, such as principal component analysis (PCA) (*9*, *13*), partial least-squares analysis (*6*, *7*), linear discriminant analysis (*10*, *19*, *20*), hierarchical cluster analysis (*15*), etc. PCA (*34*) 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 (PCs). 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 by any projection of the data shows on the first coordinate (PC1), the second greatest variance on the second coordinate (PC2), etc. PCA is theoretically the optimum transform for a given data set in the least-squares terms. Unlike standard multiple linear regression methods, PCA is not sensitive to any covariance in the data, which is quite common for MS based data sets.

The main goal of this work is the statistical evaluation of full TG profiles in a wide range of natural plant oils and the application of an elaborated PCA method for the identification of adulteration of expensive olive oils by cheaper sunflower oils

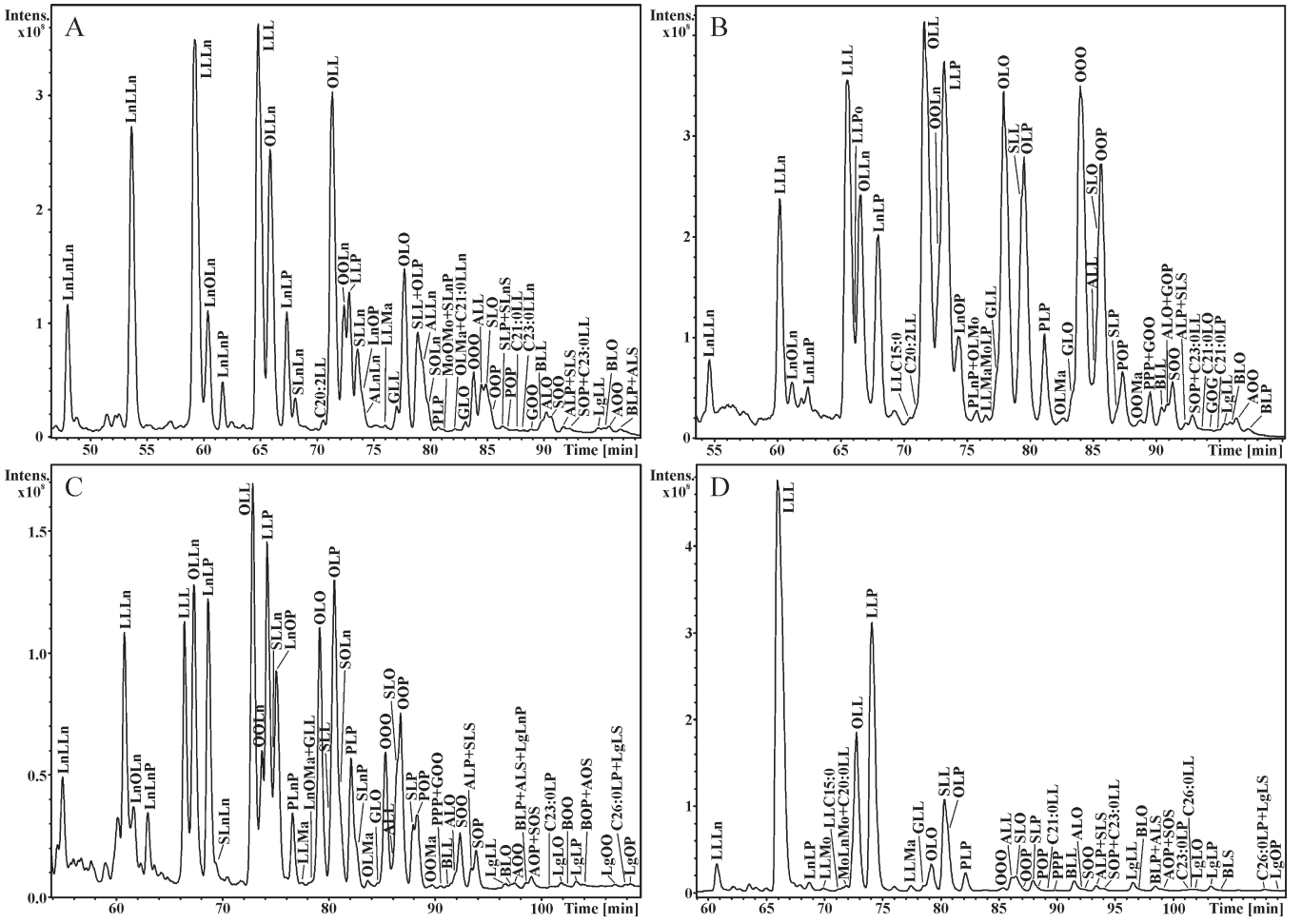

Figure 2. NARP-HPLC/APCI-MS analysis of plant oils: (**A**) dog rose (*Rosa canina*), (**B**) sweet chestnut (*Castanea sativa*), (**C**) lemon (*Citrus limon*), and (**D**) bell pepper (*Capsicum annuum*).

already at low concentration levels of added adulterant. TG concentrations are obtained by our previously developed NARP-HPLC method and precise quantitation with APCI-MS detection and response factor approach (*22*). To our best knowledge, TG profiles of such high numbers of plant oil samples of different types and origin are reported and statistically evaluated for the first time resulting in a robust method for the authentication of olive oils.

MATERIALS AND METHODS

Materials. Acetonitrile, 2-propanol (both solvents are of HPLC gradient grade), and hexane (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents were degassed by continuous stripping with helium during the analysis. Samples of camellia oil (*Camellia sinensis*), rice oil (*Oryza sativa*), and coffee butter (a mixture of *Coffea arabica* seed oil and hydrogenated vegetable oil) were purchased from Augustus Oils (Bordon, UK). Samples of apricot kernel oil (*Prunus armeniaca*), camellia oil (*Camellia sinensis*), raspberry oil (*Rubus idaeus*), argan oil (*Argania spinosa*), black cumin oil (*Nigella sativa*), macadamia nut oil (*Macadamia integrifolia*), moringa oil (*Moringa ovalifolia*), and tamanu oil (*Calophyllum tacamahaca*) were purchased from Fragrant Earth (Glastonbury, UK). Plant oils from mango (*Mangifera indica*), kiwi (*Actinidia deliciosa*), dog rose (*Rosa canina*), hazelnut (*Corylus avellana*), sweet chestnut (*Castanea sativa*), pumpkin (*Cucurbita pepo*), Brazil nut (*Bertholletia excelsa*), lemon (*Citrus limon*), bell pepper (*Capsicum annuum*), grapefruit (*Citrus paradisi*), cucumber (*Cucumis sativus*), blackcurrant (*Ribes nigrum*), mandarin orange (*Citrus reticulata*), hemp (*Cannabis sativa*), blueberry (*Vaccinium myrtillus*), melon cantaloupe (*Cucumis melo cantalupensis*), papaya (*Carica papaya*), buckwheat (*Fagopyrum esculentum*), pistachio (*Pistacia vera*), and peanut (*Arachis hypogaea*) were prepared in our laboratory according to the following procedure (*22*−*24*). Ten to 15 g of seeds were carefully crushed in a mortar to fine particles. Then 15 mL of hexane was added, and this mixture was stirred occasionally for 15 min. The solid particles were filtered out using a course filter paper, and the extract was filtered again using a fine filter (0.45 μm). From the filtered extract, hexane was evaporated using a mild stream of nitrogen to yield pure plant oil. Samples of cooking oils, i.e., 2 soybean oils (*Glycine max*), 2 rapeseed oils (*Brassica napus*), 8 sunflower oils (*Helianthus annuus*), and 15 olive oils (*Olea europaea*), were purchased at local stores and used without any modification. Four model samples of adulterated olive oil were prepared by addition of 1, 2, 5, or 10% (weight) of sunflower oil to olive oil. Oil samples were dissolved in an acetonitrile/2-propanol/hexane mixture (1:1:1, v/v/v) to prepare the initial solution of plant oil with the concentration 10 g/L. Then initial solutions were diluted with the same solvent mixture to prepare the working solution at the concentration of all TGs within the calibration range. Ten microliters of working solution was injected for the HPLC analysis in triplicate.

HPLC/MS Conditions. The chromatographic apparatus consisted of a Model 616 pump with a quaternary gradient system, a Model 996 diode-array UV detector, a Model 717+ autosampler, a thermostatted column compartment, and a Millennium chromatography manager (all from Waters, Milford, MA, USA). The HPLC conditions were used according to ref *22*: two chromatographic columns Nova-Pak C_{18} (300 × 3.9 and 150×3.9 mm, 4 μm, Waters) connected in series, a flow rate of 1 mL/min, an injection volume of 10 μL, and a column temperature of 25 °C, and a mobile phase gradient with a slope of 0.65%/min with 0 min, 100% acetonitrile; 106 min, 31% acetonitrile/69% 2-propanol; 109 min, 100% acetonitrile. The injector needle was washed with the mobile phase before each injection. The column hold-up volume, t_M, was 3.20 min for the system with 300+150 mm Nova-Pak C_{18} columns. The UV detection at

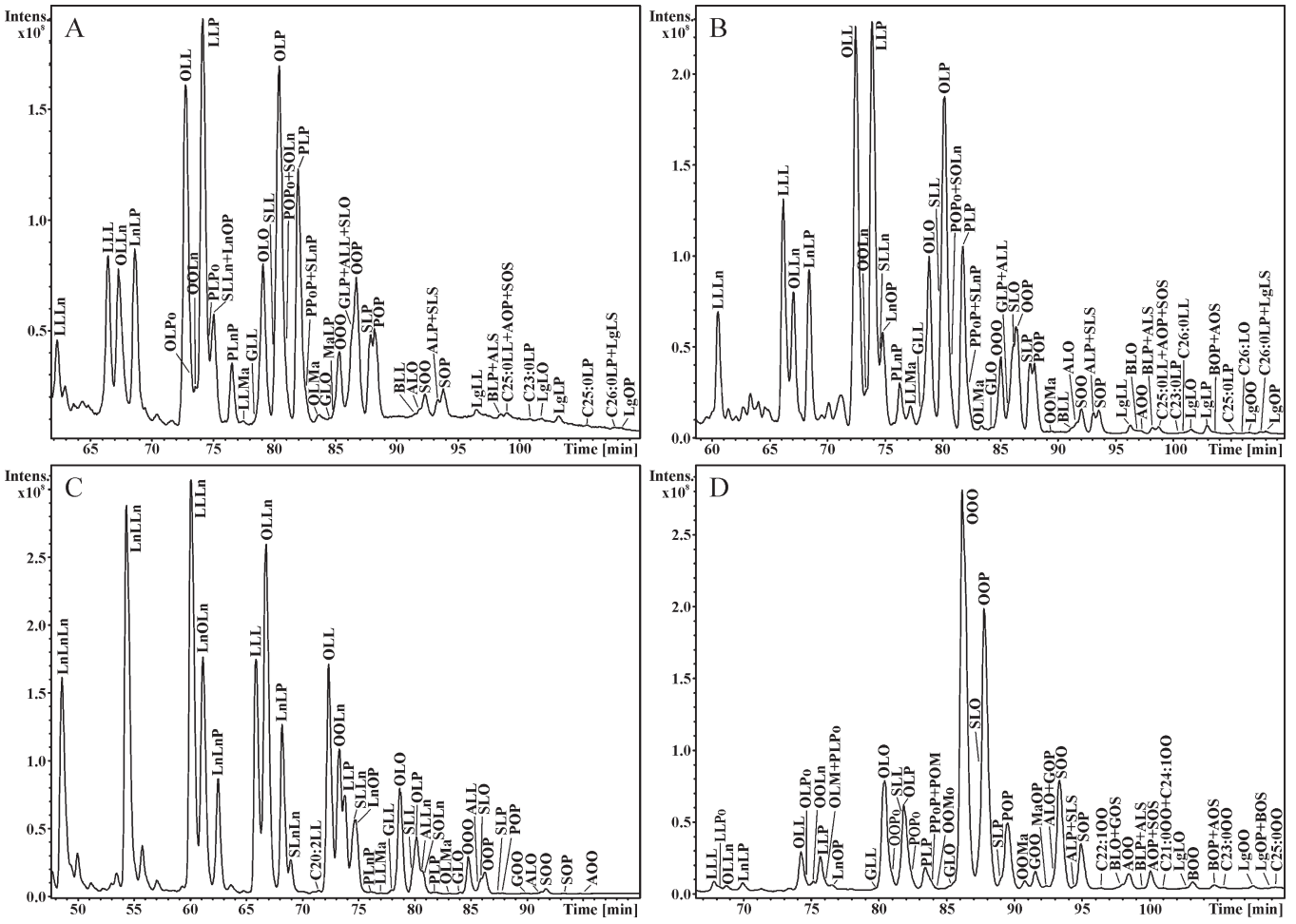

Figure 3. NARP-HPLC/APCI-MS analysis of plant oils: (**A**) grapefruit (*Citrus paradisi*), (**B**) mandarin orange (*Citrus reticulata*), (**C**) blueberry (*Vaccinium myrtillus*), and (**D**) papaya (*Carica papaya*).

205 nm and positive-ion APCI-MS were coupled in series. The Esquire 3000 ion trap analyzer (Bruker Daltonics, Bremen, Germany) in the mass range m/z 50−1200 was used with the following setting of tuning parameters: the pressure of the nebulizing gas was 70 psi, the drying gas flow rate was 3 L/min, and temperatures of the drying gas and APCI heater were 350 and 400 °C, respectively. Reconstructed ion current chromatograms in the region m/z 300−1200 were used for the peak integration. Presented peak areas correspond to averaged values from three consecutive chromatographic runs. Individual reconstructed ion current chromatograms were used to support the identification and quantitation of coeluting peaks.

Multivariate Data Analysis. In our calculations, 93 plant oils and 4 adulterated olive oils were objects (rows), and relative peak areas of 355 identified TGs were variables (columns). The data set for multivariate statistical analysis was processed using multivariate statistical package Simca-P (Umetrics, Umea, Sweden) without any additional pretreatment. The variability of data values was tested, and 13 columns were automatically excluded from original 355 variable columns because of their zero variability. Fifteen samples of olive oils, 8 sunflower oils, and 4 samples of adulterated olive oils were used for the authentication of plant oils using the same procedure. The final data set for the authentication of plant oils consisted of 27 objects and 62 variables. The following samples were processed in this paper: 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; 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. The data on new samples are shown in Tables, and the remaining data are taken from our previous works (*22*−*24*).

RESULTS AND DISCUSSION

NARP-HPLC/APCI-MS Analysis of Plant Oils. The separation of TGs from plant oils is quite a challenging task because of the presence of numerous TG species with similar physicochemical properties. NARP-HPLC separation mode is used for the separation of TG complex mixtures of plant oils based on our previously optimized conditions (*22*), i.e., the column coupling in the total length of 45 cm, the mobile phase of acetonitrile/2-propanol, and column temperature of 25 °C. TGs are resolved according to the ECN, and most TGs are clearly separated within individual ECN groups according to esterified fatty acids, i.e., saturation, DB position, and fatty acid chain lengths. **Figure 1** illustrates four examples of HPLC/MS separation of TGs in plant oils with high (kiwi seed oil, **Figure 1**A) and low (macadamia nut oil, **Figure 1**B) concentrations of polyunsaturated fatty acids, and the separation of plant oils with high (hemp oil, **Figure 1**C) and low (Brazil nut oil, **Figure 1**D) number of TG species. **Figures 2**−**4** show HPLC/MS chromatograms of some unusual plant oils, whose chromatograms have not been reported in the literature so far. Other HPLC/MS chromatograms are available in Supporting Information (Figures S1−S5). Individual TGs are identified

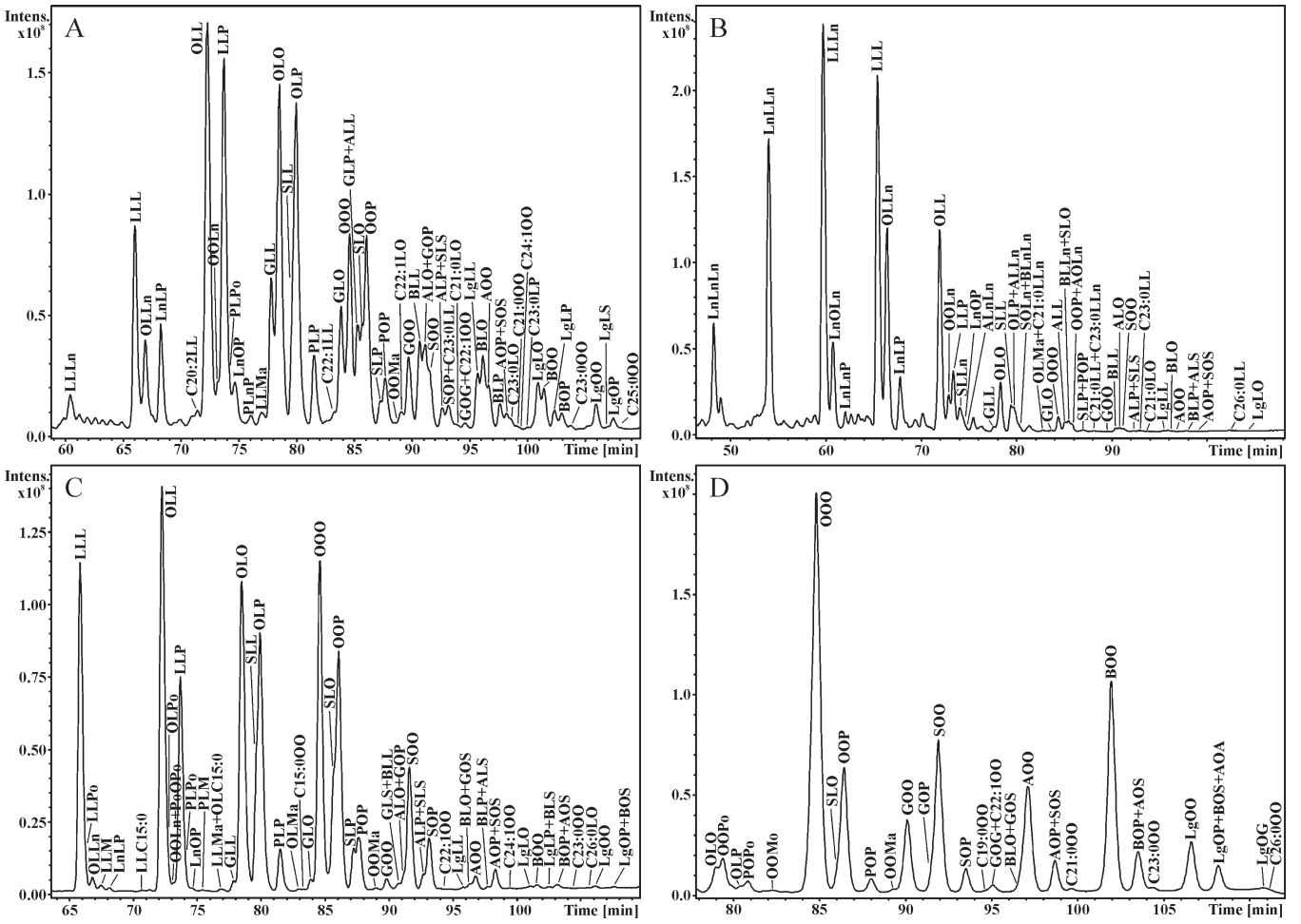

Figure 4. NARP-HPLC/APCI-MS analysis of plant oils: (A) buckwheat (*Fagopyrum esculentum*), (B) raspberry (Rubus idaeus), (C) argan (Argania spinosa) and (D) moringa (Moringa ovalifolia).

on the basis of their positive-ion APCI mass spectra using $[M + H]^+$ ions for the molecular weight determination and $[M + H - R_iCOOH]^+$ fragment ions for the identification of individual fatty acids. It is well known (*28*−*32*) that the cleavage of fatty acid from the *sn*-2 position on the glycerol skeleton is less preferred in comparison to *sn*-1/3 positions resulting in a lower abundance of corresponding $[M + H - R_iCOOH]^+$ fragment ions, which is used for the determination of prevailing fatty acid in the *sn*-2 position. We have found a preference of unsaturated fatty acids (mainly linoleic acid) in the *sn*-2 position for analyzed plant oils, in agreement with the literature data. Fatty acids in *sn*-1/3 positions cannot be resolved using NARP-HPLC/APCI-MS, and they are considered as equivalent in this work. Fatty acids in these positions are arranged according to their decreasing masses.

TG composition of various plant oils has been characterized using optimized NARP-HPLC separation with APCI-MS detection. APCI-MS is applicable also for the identification of trace and chromatographically nonresolved species based on the high sensitivity and possibility of utilization of extracted ion chromatograms, which results in the identification of the highest number of TG species in individual plant oils ever reported (**Table 1**). The number of identified TGs ranges from 26 TGs in Brazil nut and camellia oils as examples of relatively simple oils up to 80 TG species identified in blackcurrant oil as the example of a rather complex oil. The number of TG species in individual oils partially corresponds with the number of fatty acids present in TGs, e.g., 18 fatty acids are identified in hemp oil as one of the most complex oils containing 70 TGs. In total, 355 TG species are identified in 93 plant oils composed from 35 fatty acids with 6 to 26 carbon atoms and 0 to 4 DBs.

TGs in analyzed plant oils are quantified using the APCI-MS response factor approach for the quantitation of TGs in natural samples described previously (*22*). Briefly, the response factors of individual fatty acids are calculated as the ratio of calibration slopes of corresponding single-acid TG standards (type $R_1R_1R_1$) to the calibration slope of triolein as one as the most common TGs in nature. Response factors of mixed TGs (type $R_1R_2R_3$) are calculated as the arithmetic mean of response factors of presented fatty acids in TGs. Concentrations of individual TGs in analyzed plant oils are listed in Supporting Information (Tables S1 and S2). The quantitation of coeluting peaks is supported by reconstructed ion chromatograms of protonated molecules and individual diacylglycerol fragment ions. Precise concentrations of TGs in individual plant oils using HPLC/MS response factor quantitation approach can be used for the calculation of their fatty acid composition (**Tables 2** and S3 (Supporting Information)) and nutritional parameters (**Tables 1** and S4 (Supporting Information)), as confirmed previously (*22*) by the comparison with fatty acid composition determined with validated GC/FID analysis of fatty acid methyl esters prepared by the transesterification of TGs. **Table 1** lists average parameters calculated from HPLC/MS results of TGs, i.e., average equivalent carbon number (aECN), average carbon number (aCN), double bond (aDB) number, and sums of essential fatty acids (linoleic and linolenic), 18 and 16 carbon fatty acids, and saturated, monounsaturated, and polyunsaturated fatty acids. aECN ranges from 13.59 to 16.65, aCN

Table 1. Number of Identified Triacylglycerols (TGs) and Fatty Acids (FAs), Average Equivalent Carbon Number (aECN), Average Carbon Number (aCN), Average Double Bond (aDB) Number, the Relative Weight Concentration [%] of Essential Fatty Acids (Linoleic and Linolenic Acids), Fatty Acids with 18 (C18) and 16 (C16) Carbon Atoms, and Saturated (Sat), Monounsaturated (Mono), and Polyunsaturated (Poly) Fatty Acids in Analyzed Plant Oils Calculated from NARP-HPLC/APCI-MS of Triacylglycerols

oil	no.	number of TGs/FAs	aECN	aCN	aDB	essential FAs [%]	C18 + C16 FAs [%]	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

from 17.46 to 18.24, aDB from 0.48 to 2.12, and the sum of C18 and C16 fatty acids from 86.4% to 99.8%, showing that plant oils are composed almost exclusively from TGs containing fatty acids with 16 and 18 carbon atoms and 0 to 4 DBs, i.e., palmitic (ECN; CN; DB-16; 16; 0), stearic (18; 18; 0), oleic (16; 18; 1), linoleic (14; 18; 2), and linolenic (12; 18; 3) acids (**Tables 2** and S3 (Supporting Information)). Remaining fatty acids with low or usually trace concentrations represent long or short-chain acids, odd-number acids, and acids with unusual DB positions. Higher differences are found among the sums of essential (from 0.9% to 79.7%), saturated (from 4.8% to 72.3%), monounsaturated (from 7.2% to 79.6%), and polyunsaturated (from 0.9% to 75.9%) fatty acids differing significantly for individual oils, and therefore, these parameters can be used for fast consideration of nutritional values or possible industrial applications.

PCA of TG Composition. The evaluation of TG profiles is an important step in the quality control of plant oils. The concentration of individual TG species can be used for simple comparison of various plant oils, but such comparison is not practical due to a high number of detected TGs. For detailed characterization, the comparison of all TG species in all analyzed samples is necessary, which leads to the complex multidimensional data set. Multivariate data analysis using PCA is used for the evaluation of TG composition in all analyzed samples. First, PCA analysis using TG concentrations based on APCI-MS response approach and TG relative peak areas are compared. No significant differences in resulting PCA plots are found, and therefore, relative peak areas are used for further PCA analysis of all samples. The final data set contains 93 plant oils (i.e., objects) of 60 different types characterized by relative peak areas of 355 identified TG species (i.e., variables). Thirteen variables are excluded from the data set because of their zero variability corresponding to the content of this variable in all plant oils lower than the limit of detection (0.01%). Data values of other 342 variables range between 0.01% and 49.32%, i.e., in the range of 3.5 orders of magnitude. Hence, no scaling, normalization, or centering is applied, and the data set without any modification is taken for the direct PCA analysis. Multivariate data set of 342 nonredundant variables is visualized as a set of coordinates in a multidimensional data space with $N = 342$ (one axis per variable) dimensions.

Figure 5 shows the score plots of the first (t[1]) and second (t[2]) PCs of the general PCA model with a good resolution of analyzed samples. These 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. The projection of PCs t[3] and t[4] (Figure S6 (Supporting Information)) shows only a small variance among analyzed samples, and most of samples are grouped around the

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Table 2. Relative Weight Concentrations [%] of Individual Fatty Acids in Analyzed Plant Oils Calculated from HPLC/APCI-MS of Triacylglycerols

		Cy	C	La	M		P	Po	Ma	Mo	S	O	L	Ln	γLn	St		A	G			B			Lg			
oil	no.	C8:0	C10:0	C12:0	C14:0	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C18:3	C18:4	C19:0	C20:0	C20:1	C20:2	C21:0	C22:0	C22:1	C23:0	C24:0	C24:1	C25:0	C26:0
Kiwi	**1**					0.01	8.5		0.03		3.1	17.1	17.1	53.6				0.04	0.2	0.3			0.02					
Macadamia nut	**2**			0.2	1.4		8.0	18.9		0.1	3.3	57.9	2.9					2.8	2.6			1.3	0.1		0.5			
Hemp	**3**						8.6		0.02		2.6	11.5	49.5	21.2	3.2	1.4		1.0	0.3	0.03	<0.01	0.5	0.01	0.03	0.1	0.01		<0.01
Brazil nut	**4**						16.3	0.08			8.5	35.1	39.9					0.1	0.02									
Mango	**6**						10.7		0.3		30.3	47.3	7.4	0.8			0.03	1.7	0.3			0.5		0.04	0.6		0.03	
Dog rose	**7**						4.6		0.04	0.01	2.4	21.7	47.9	21.8				1.0	0.3	0.07	0.01	0.1		0.03	0.04			
Hazelnut	**9**						7.8	0.6	0.1	0.2	3.0	66.5	20.8	0.5				0.2	0.3									
	10						7.5	0.3	0.1	0.2	3.5	70.2	17.3	0.3				0.3	0.3									
Sweet chestnut	**11**					0.04	14.3	0.08	0.1	0.04	1.5	38.6	37.1	7.1				0.3	0.6	0.06	0.01	0.1		0.04	0.03			
Pumpkin	**12**						14.5		0.1		4.5	24.0	56.0					0.6	0.06			0.2			0.04			
Lemon	**13**						18.8		0.08		3.5	30.1	33.4	13.5				0.3	0.03			0.08		<0.01	0.2			0.01
Bell pepper	**14**					0.06	12.5		0.1	0.2	2.1	9.3	73.7	0.8				0.4	0.07	0.08	0.01	0.3		0.03	0.3			0.05
Grapefruit	**15**						25.9	0.8	0.2		3.2	24.6	38.6	6.1				0.3	0.05			0.02		0.01	0.2		0.01	0.01
Cucumber	**16**				0.4		17.5		0.1		1.7	7.8	68.3	3.8				0.2	0.09			0.04			0.05		0.01	0.01
Blackcurrant	**18**						7.7		0.02		1.1	14.7	40.9	16.6	14.6	3.7		0.1	0.5	0.07		0.01		<0.01	<0.01			
Mandarin orange	**19**						22.1	0.4	0.2		3.3	24.9	42.6	5.5				0.3	0.2			0.1		0.02	0.3		0.03	0.05
Blueberry	**20**						6.6		0.02		1.1	22.0	35.7	34.3				0.2	0.06	0.02								
Melon cantaloupe	**21**					0.05	11.8		0.08		3.4	15.7	67.8	0.8				0.2	0.09			0.06			0.02			
Papaya	**22**				0.2		16.6	0.9	0.2	0.06	4.5	66.5	8.9	0.5				0.6	0.7		0.02	0.2	0.03	0.01	0.07		0.01	
Buckwheat	**23**						14.7	0.2	0.06		2.0	35.7	36.9	2.5				1.1	3.0	0.08	0.04	2.0	0.1	0.2	1.4		0.02	
Pistachio	**24**						9.7		0.05	0.1	1.5	49.4	38.2	0.3				0.1	0.5			0.1			0.05			
Peanut	**26**						12.6		0.09	0.08	2.7	39.7	37.6	0.3				1.1	1.0	0.04	0.01	3.1	0.05	0.03	1.5			0.1
Camellia	**27**						10.0	0.08	0.08	0.08	2.3	77.2	9.2	0.3				0.06	0.6				0.03		0.07			
	28						9.1	0.07	0.06	0.07	2.4	78.8	8.5	0.3				0.04	0.6				0.03		0.03			
Rice	**29**				0.4		16.5	0.7			2.5	40.1	36.1	2.0				0.5	0.5			0.2			0.4			0.1
Coffee butter	**30**	5.1	1.5	24.4	10.1		17.6		0.01		12.3	7.2	19.7	0.8				0.9	0.03			0.3			0.06			
Apricot kernel	**31**						6.2	0.3	0.03	0.1	1.0	61.2	31.0	0.05				0.06	0.06									
Raspberry	**32**						3.4		0.02		0.8	15.5	52.9	26.8				0.3	0.04		0.01	0.2		0.01	0.02			<0.01
Argan	**33**				0.1	0.05	14.0	0.4	0.05		4.7	46.4	33.1	0.2				0.3	0.4			0.2	0.01	0.01	0.07			0.01
Black cumin	**34**						12.4				2.3	23.1	58.8					0.2	0.4	2.7		0.06			0.04			
Moringa	**35**						6.4	1.2	0.05	0.04	4.7	73.2	0.9				0.02	3.4	2.5		0.06	5.8	0.07	0.05	1.6			0.01
Tamanu	**36**						11.2	0.03	0.08	0.03	9.3	36.4	41.7					0.8	0.1		0.02	0.3			0.04			
Soya	**38**						12.1		0.04	0.03	3.3	26.2	49.7	7.2				0.4	0.1	0.02		0.6		0.1	0.2			0.01
Rapeseed	**41**						7.1	0.2	0.1		1.6	58.2	21.2	9.6				0.4	1.1			0.3		0.01	0.1	0.09		
Sunflower	**44**				0.3		7.6		0.05	0.08	3.8	24.6	61.5	0.4			<0.01	0.4	0.1	<0.01		0.8		0.06	0.3			0.01
Olive	**53**						12.5	1.3		0.1	2.3	74.9	6.5	1.0				0.5	0.4		0.03	0.3		0.05	0.1		0.01	0.01

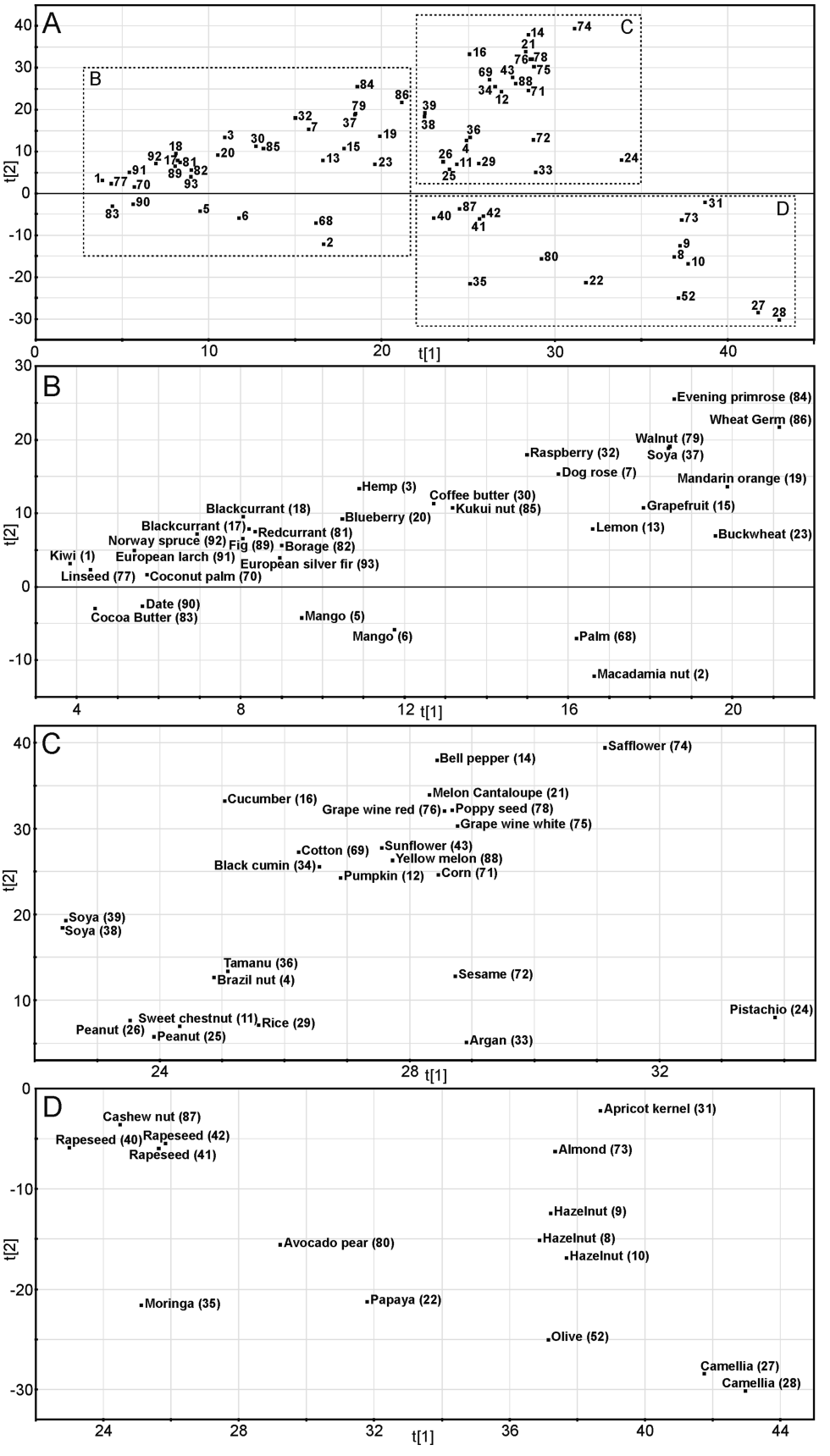

Figure 5. Projection of principal components t[1] and t[2] in two-dimensional scatter plot for all measured samples (**A**) and zooms of individual regions (**B**, **C**, and **D**).

zero of both PCs. Only samples with significantly different composition containing high concentrations of TGs with highly unsaturated (linseed, kiwi and blueberry oils) or saturated (cacao butter and mango oils) fatty acids are clearly distinguished from other samples in Figure S6 (Supporting Information). The projection of analyzed samples using t[1] and t[2] PCs provides

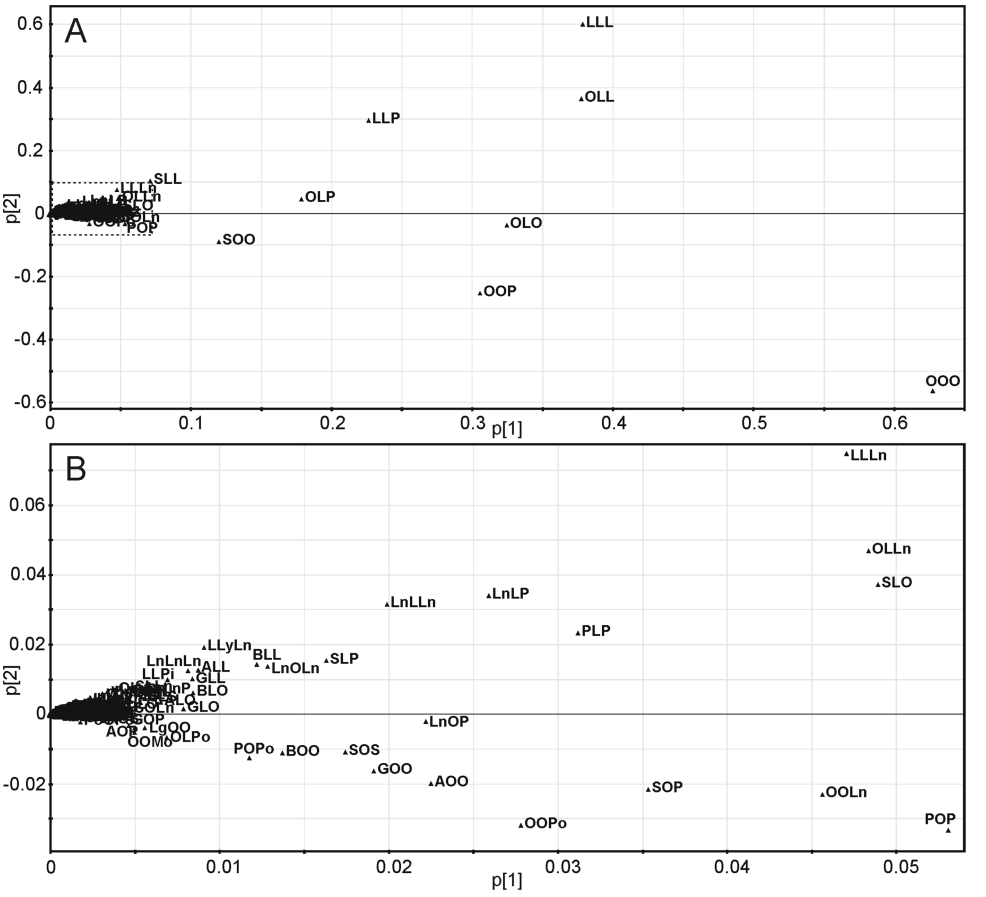

Figure 6. Projection of variables p[1] and p[2] in two-dimensional loadings plot for all measured samples showing the major variables representing TG concentrations (**A**) and zoomed area (**B**).

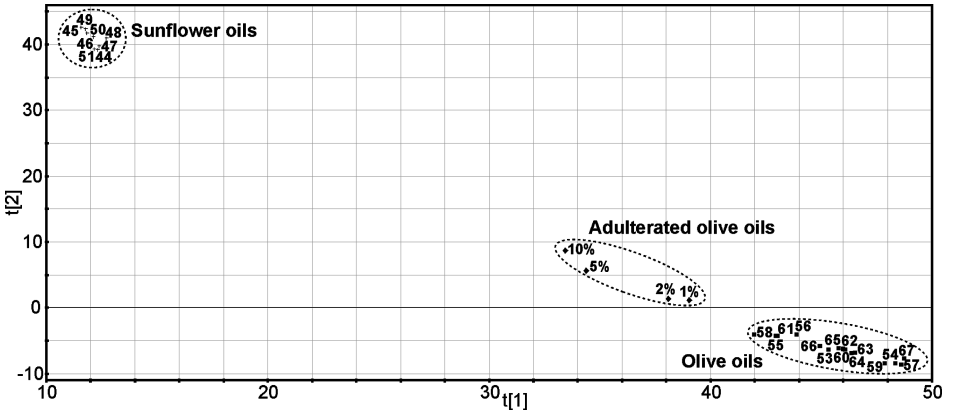

Figure 7. Projection of principal components t[1] and t[2] in two-dimensional scatter plot for analyzed sunflower (44−51) and olive (53−67) cooking oils and four samples of adulterated olive oil by 1%, 2%, 5%, or 10% of sunflower oil.

significantly better resolution of analyzed samples for the comparison of their properties. Samples of one type of plant oil having similar TG composition form narrow clusters, e.g., different samples of hazelnut and camellia oils (**Figure 5D**). Samples with similar TG profiles are grouped in small regions in the PCA plot, e.g., samples of blackcurrant and redcurrant oils (**Figure 5B**). Similar positions of various samples in the PCA plot indicate their similar properties, e.g., Brazil nut and tamanu oils in **Figure 5C**. Their similar properties can be confirmed by the comparison of their average parameters and sums of fatty acids for individual plant oils calculated from TG composition (**Table 1**), i.e., aECN of Brazil nut oil/tamanu oil = 15.38/15.40, aCN = 17.66/17.79, aDB = 1.14/1.19, C18 + C16 fatty acids = 99.9%/98.6%, polyunsaturated fatty acids = 39.9%/41.7%, etc. **Figure 6** shows variables (TG concentrations) in our PCA, and their variance model mostly affects the variability of samples. The most significant variable is the concentration of OOO with more than 60% positive effect on t[1], while −55% effect on t[2]. Other important variables are LLL, OLL, OLO, OOP, LLP, OLP, and SOO. These eight variables are the most significant parameters for the statistical differentiation among plant oils.

Authentication of Olive Oil. Olive oil is one of the most expensive plant oils used in dietetics. For its healthy properties, it is an important ingredient in the so-called Mediterranean diet of southern nations. High prices of olive oils can lead some merchants to illegal falsification by cheaper plant oils, which decrease

their nutritional value. Most favorable oils for adulteration are plant oils with similar TG composition, which are difficult to distinguish using common analytical techniques. The TG composition of hazelnut, camellia, or papaya oils is relatively close to olive oil composition, but they are still clearly distinguished using our HPLC/MS method and PCA analysis (**Figure 5D**). Moreover, their prices and small quantity production in comparison to those of the most common plant oils are not favorable for falsification. The utilization of low-price plant oils produced in large quantities in the same geographical region is more favorable, e.g., sunflower oil. The set of 8 sunflower and 15 olive cooking oils, and 4 model samples of adulterated olive oil by 1%, 2%, 5%, or 10% of sunflower oil (Table S2, Supporting Information) is tested to develop an unambiguous method to identify adulteration even at very low amounts of adulterant. **Figure 7** shows the scores plot of the first (t[1]) and second (t[2]) PCs of all cooking and model adulterated oil samples. 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 for 99.6% of total variability, where t[1] represents 73.5% and t[2] represents 26.1% of total variability. Samples of sunflower oil have small differences in TG composition and form a small cluster clearly distinguished from other samples in the PCA plot. Samples of olive oil have a wider distribution in comparison to the cluster of sunflower oils because of slightly different TG composition of different types (virgin oil, pomace oil, etc.) and different origin of samples, which are not differentiated in this study. Anyway, a clear resolution of sunflower and olive oil samples and their grouping into small clusters enable the resolution of model samples of adulterated olive oil by sunflower oil (**Figure 7**). Samples of adulterated olive oil with increasing concentrations of sunflower oil have an increasing distance from the olive oil cluster in the PCA plot. Even the adulteration of olive oil by 1% of sunflower oil can be clearly visualized in a PCA plot regardless of the fact that different types and origin of olive oils are neglected. This approach is well suitable for the detection of possible adulteration in tested samples.

The presented results demonstrate the utilization of HPLC/MS analysis and statistical evaluation in the quality control of plant oils. A carefully optimized HPLC/MS method is used for detailed characterization of TG profiles of plant oils. PCA evaluation of multidimensional data matrix of TG profiles enables the comparison of all analyzed samples and the resolution of samples with similar properties. PCA analysis is used for the authentication of expensive olive oil. PCA enables the detection of adulterated olive oil starting from 1% of added sunflower oil as an adulterant.

ABBREVIATIONS USED

CN, carbon number; DB, double bond; ECN, equivalent carbon number; ESI, electrospray ionization; MS, mass spectrometry; NARP-HPLC, nonaqueous reversed-phase high-performance liquid chromatography; PC, principal component; PCA, principal component analysis; TG, triacylglycerol; fatty acid abbreviations, Cy, caprylic (CN:DB, C8:0); C, capric (C10:0); La, lauric (C12:0); M, myristic (C14:0); C15:0, pentadecanoic; P, palmitic (C16:0); Po, palmitoleic (Δ9-C16:1); Ma, margaric (C17:0); Mo, margaroleic (Δ9-C17:1); S, stearic (C18:0); O, oleic (Δ9-C18:1); L, linoleic (Δ9,12-C18:2); Ln, α-linolenic (Δ9,12,15-C18:3); γLn, γ-linolenic (Δ6,9,12-C18:3); St, stearidonic (Δ6,9,-12,15-C18:4); C19:0, nonadecanoic (C19:0); A, arachidic (C20:0); G, gadoleic (Δ9-C20:1); C20:2, eicosadienoic (Δ11,14-C20:2); C21:0, heneicosanoic (C21:0); B, behenic (C22:0); C22:1, erucic (Δ13-C22:1); C23:0, tricosanoic (C23:0); 24:1, nervonic (Δ15-C24:1); Lg, lignoceric (C24:0); C25:0, pentacosanoic (C25:0); C26:0, hexacosanoic (C26:0).

Supporting Information Available: HPLC/MS chromatograms of analyzed plant oils (Figures S1−S5), projection of PCs t[3] and t[4] (Figure S6), relative weight concentrations of triacylglycerols (Tables S1 and S2) and fatty acids (Tables S3), average parameters (Table S4) of analyzed plant oils. This material is available free of charge via the Internet at http://pubs.acs.org.

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