Authentication of Vegetable Oils by Bulk and Molecular Carbon Isotope Analyses with Emphasis on Olive Oil and Pumpkin Seed Oil

Jorge E. Spangenberg*

Institut de Minéralogie et Géochimie, Université de Lausanne, BFSH-2, 1015 Lausanne, Switzerland

Nives Ogrinc

Department of Environmental Sciences, J. Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

The authenticity of vegetable oils consumed in Slovenia and Croatia was investigated by carbon isotope analysis of the individual fatty acids by the use of gas chromatography−combustion−isotope ratio mass spectrometry (GC/C/IRMS), and through carbon isotope analysis of the bulk oil. The fatty acids from samples of olive, pumpkin, sunflower, maize, rape, soybean, and sesame oils were separated by alkaline hydrolysis and derivatized to methyl esters for chemical characterization by capillary gas chromatography/mass spectrometry (GC/MS) prior to isotopic analysis. Enrichment in heavy carbon isotope (^{13}C) of the bulk oil and of the individual fatty acids are related to (1) a thermally induced degradation during processing (deodorization, steam washing, or bleaching), (2) hydrolytic rancidity (lipolysis) and oxidative rancidity of the vegetable oils during storage, and (3) the potential blend with refined oil or other vegetable oils. The impurity or admixture of different oils may be assessed from the $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:1}$ covariations. The fatty acid compositions of Slovenian and Croatian olive oils are compared with those from the most important Mediterranean producer countries (Spain, Italy, Greece, and France).

Keywords: *Olive oil; pumpkin seed oil; vegetable lipids; carbon isotope; CSIA; authenticity*

INTRODUCTION

Olive oil (*Olea europaea*) is a premium vegetable oil because of its health and nutritional benefits and distinctive flavor. Because of its elevated price as compared to other edible oils, there is an economic interest in blending genuine olive oil with cheaper vegetable oils. Olive oil is characterized by its high concentration (65−85%) of oleic acid (18:1). Other vegetable oils, including high-oleic sunflower (70−75%), rape (55−65%), hazelnut (about 69%), and maize (about 31%) oils also are rich in oleic acid (*1*). Pumpkin (*Cucurbuta pepo*) seed oil is one of the gourmet specialty oils available in Europe, and in the Adriatic countries it is a highly esteemed salad oil. Its major acids, including palmitic (16:0, 7−13%), stearic (18:0, 3−13%), oleic (18:1, 21−47%), and linoleic (18:2, 36−61%), are accompanied by small amounts (up to 1.3%) of saturated and monounsaturated C_{20}−C_{24} acids (*2*). Linoleic acid is the major component of soybean (45−60%) and maize (35−60%) oils. Sesame oil is the only single-seed gourmet oil that contains moderate levels of both oleic (45 to 47%) and linoleic (33 to 35%) acid, and lower content of palmitic (12 to 14%), stearic (5 to 6%), and arachidic (1%) acids (*2, 3*). This overlap in fatty acid composition of different vegetable oils makes the detection of adulteration and authenticity frauds difficult. Furthermore, the natural variations in fatty acid composition may mask the adulteration of premium or gourmet oils attained by adding small amounts (up to <10%) of cheaper varieties. Consequently, comparison of the fatty acid composition of different vegetable oils is not used to distinguish samples of single vegetable oils from blended oils. Stable carbon isotope analysis is a powerful technique for assessing the authenticity of vegetable food products from plants of different photosynthetic pathways (*4*−*7*). The carbon isotope compositions of plants and their products are linked to the processes of photosynthetic atmospheric CO_2 fixation. During photosynthetic carbon fixation, plant cells discriminate against the heavier stable carbon isotope ^{13}C. The most important atmospheric CO_2-fixing reactions are the C_3 and C_4 pathways (*8*−*10*). C_3 plants use the Calvin cycle for CO_2 fixation, and most of the isotopic fractionation occurs in the enzymatic carboxylation of CO_2 by ribulose-1,5-biphosphate carboxylase. The carbon isotope compositions of C_3 plants fall into the range of −34 to −22‰ (*11*). The C_4 plants use the Hatch-Stack cycle, and produce much less isotopic fractionation than do C_3 plants. C_4 plants comprise most plants in the tropics, including maize and sugar cane, and are isotopically heavier (−16 to −9‰) (*11*). Some succulents utilize the crassulacean acid metabolism (CAM) pathway, and have intermediate carbon isotope ratios, between −33 and −11‰. Factors other than the CO_2-fixation pathway, however, may also have some impact on the isotopic composition of plants. These include local atmospheric CO_2 concentration, plant variety, and factors affecting the plant physiology and the nutritional status of the cell, such as changes in stromatal aperture, enzyme levels, plant growth rate, water-use efficiency, and cultivation practices (*10, 12, 13*). Therefore, the carbon isotopic composition of bulk vegetable oils and their individual lipids may record their source (C_3 or C_4 plant)

* Corresponding author. Fax: +41 (0)21-692-4305. Tel.: +41 (0)21-692-4365. E-mail: Jorge.Spangenberg@imp.unil.ch.

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and their geographical origin. The novel technique of compound-specific isotope analysis of individual lipids by the use of on-line gas chromatography−combustion−stable isotope ratio mass spectrometry (GC/C/IRMS) helps to distinguish between the natural variations of $^{13}C/^{12}C$ isotope ratios ($\delta^{13}C$) of genuine C_3 or C_4 oils and admixtures of oils of different varieties of C_3 plants (*14*−*18*). Recently, we reported our investigations of olive oils from the main producing regions in the Mediterranean basin (*19*). Twenty-seven samples of extra virgin olive oil from Spain, Italy, Greece, and France, and olive oil samples from Turkey, Morocco, and Tunisia, were characterized by GC/MS and GC/C/IRMS of individual fatty acids, and the results were evaluated by principal component analysis. The combined chemical and isotopic data were used to distinguish the geographical origin of the Mediterranean olive oils. We concluded that the $^{13}C/^{12}C$ isotope ratios of the bulk oil and individual fatty acids can be used for the identification of the sources of the olive oil (*19*). By using carbon and oxygen isotope analyses of the bulk oil and lipids fractions, Angerosa et al. (*20*) detected some trends in the geographical origin of olive oil samples from Greece, Morocco, Spain, Italy, Tunisia, and Turkey. To our knowledge, no chemical and isotopic data of fatty acids of olive oils from Adriatic countries are available. We report here the chemical and isotopic composition of fatty acids from olive oils from Slovenia and Croatia, and other vegetable oils commonly consumed in these countries. The advantage of the analytical approach (GC/MS and GC/C/IRMS) should be reflected in reliable results for the chemometric characterization of the oil variety, and provide some insights into the geographical origin of vegetable oils. The impurity or admixture of different oils may be assessed from the $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:1}$ covariations. The new data set was used to compare the Adriatic olive oils with those from Spain, Italy, Greece, and France.

MATERIALS AND METHODS

Vegetable Oil Samples. Thirteen samples of olive oil were obtained from the major growing areas of Slovenia (Koper region, $n = 6$) and Croatia (Istria, Dalmatia, $n = 7$). All the samples, except one, were from the 1997−1998 olive growing season and all were homemade cold-pressed olive oils obtained from local farmers. The samples COIL-58 and COIL-59 are olive oils produced in the same farm from fruits from the same olive cultivars harvested from the 1996−1997 and 1997−1998 olive-seasons, respectively. These samples (COIL-58 and COIL-59) differ only in a one-year storage at ambient temperature in the farm. The olive oils were compared with other vegetable oils consumed in Slovenia and Croatia, including pumpkin ($n = 4$), sunflower ($n = 2$), soybean ($n = 1$), sesame ($n = 1$), maize ($n = 1$), and rape ($n = 1$) seed oils. The seed oil samples were obtained from local supermarkets. There could be no assurance that these oils which were labeled as pure single-seed products were actually from a single vegetable source. All the samples were stored at +4 °C in the darkness prior to analyses at the Department of Earth Sciences of the University of Lausanne.

Sample Preparation. All of the solvents used were of a quality suitable for chromatography (Fluka, Switzerland) and were glass-distilled shortly before use. Separation and methylation of the fatty acids from the oil samples were performed by procedures previously described (*19*). Briefly, the fatty acids were separated from 0.1-mL oil samples by alkaline hydrolysis (70 °C, 3 h) with aqueous ethanolic potassium hydroxide and extracted with hexane (1 × 10 mL, 2 × 5 mL). The fatty acids were sterified with approximately 0.5 mL of 10% methanolic BF_3 (60 °C, 8 min). The fatty acid methyl esters (FAMEs) were extracted with 10 mL of hexane, and the hexane extract was washed with saturated aqueous potassium chloride solution (2 × 5 mL). The FAMEs were stored with 0.5 mL of hexane in 2-mL vials with PTFE-lined caps at +4 °C until gas chromatographic analysis. The reason we used this procedure (hot hydrolysis of the oil samples and sterification with BF_3) was to permit the comparison of Adriatic olive oils with those from Mediterranean countries studied in a previous work (*19*).

Gas Chromatography/Mass Spectrometry (GC/MS). GC/MS was performed by using a Hewlett-Packard G1800A GCD system based on a HP 5890 Series II gas chromatograph with an electron ionization detector (EID). The system was equipped with a HP-FFAP fused-silica capillary column (50 m × 0.20 mm i.d.) coated with poly(ethylene glycol)-TPA modified as stationary phase (film thickness 0.33 μm). Helium was used as carrier gas (1 mL/min flow rate), and the injection was made splitless manually. Injector temperature was 200 °C to prevent potential isomerization of the unsaturated fatty acids (*21*). After an initial period of 2 min, the column was heated to 250 °C at 5 °C/min followed by an isothermal period of 10 min. The EID was operated at 70 eV in the multiple ion detection mode, with source temperature of 280 °C, emission current of 1 mA, and a scan range from 45 to 450 amu. Data were processed with a HP Chemstation data system. The relative compositions of the fatty acids were calculated as the percent of the total fatty acids.

Isotopic Analysis of Bulk Oil by EA/IRMS. The bulk oils were analyzed for carbon isotope composition by combustion isotope-ratio-monitoring mass spectrometry using an on-line Carlo Erba 1108 elemental analyzer (EA) connected to a Finnigan MAT Delta S isotope ratio mass spectrometer (IRMS) via a Conflo II split interface (EA/IRMS). The EA oxidized all the organic compounds under a stream of helium and oxygen by flash combustion in a quartz tube packed with oxidizing catalyst (chromium oxide, silver-coated cobaltous oxide) at 1020 °C. The oxidation products passed through a reduction reactor packed with elemental copper and copper oxide at 640 °C to remove excess of oxygen and to reduce the nitrous products (NO_x) to elemental nitrogen. Water was removed using anhydrous magnesium perchlorate, and the gases entered a chromatographic column for separation of N_2 from CO_2. The gases were then analyzed on the IRMS for their isotopic composition. The stable carbon isotope ratios are reported in the delta (δ) notation as the per mil (‰) deviations relative to the Pee Dee Belemnite limestone (PDB) standard

$$\delta^{13}C = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3$$

where $R = {}^{13}C/{}^{12}C$. The reproducibility of the EA/IRMS, assessed by replicate analyses of a laboratory standard material (glycine) with established working $\delta^{13}C$ value (−26.1‰) was better than 0.1‰.

Isotopic Analysis of Individual Fatty Acids by GC/C/IRMS. The compound-specific carbon isotope analyses of the fatty acids were obtained by using a Hewlett-Packard 6890 GC coupled to a Finnigan MAT Delta S IRMS by a combustion (C) interface III (GC/C/IRMS) under a continuous helium flow (*22*, *23*). The combustion interface consisted of a ceramic furnace with copper oxide and platinum catalyst at a temperature of 840 °C. A He-flushed Nafion membrane prevented water from reaching the ion source of the IRMS. The IRMS ion source pressure was 6×10^{-6} bar. The GC was operated with the same type of column and temperature program used for GC/MS analyses. The performance of the GC/C/IRMS system, including the GC and combustion furnace, was evaluated every 10 analyses by injection of a mixture of FAMEs of known $\delta^{13}C$ values. The background subtraction and $\delta^{13}C$ values were calculated using the ISODAT 7.4 software. The reproducibility assessed from six replicate analyses of the samples ranged between ±0.1 and ±0.4‰. The accuracy of the GC/C/IRMS analyses was monitored by co-injection of a FAME laboratory standard of known isotopic composition. The isotopic shift due to the carbon introduced in the fatty acid

Table 1. Fatty Acid Composition of Vegetable Oils Consumed in Slovenia and Croatia

sample	oil	country	palmitic (16:0, wt %)	palmitoleic (16:1, wt %)	stearic (18:0, wt %)	oleic (9c18:1, wt %)	linoleic (18:2, wt %)	linolenic (18:3, wt %)	other fatty acids[a]
COIL-58	olive	Slovenia	20.6	1.5	1.1	70.1	4.7		2.0 (6c18:1), traces (9t18:1)
COIL-59	olive	Slovenia	21.2	0.8	3.1	68.3	4.1	0.3	1.6 (6c18:1), 0.3 (9t18:1), 0.3 (20:0)
COIL-60	olive	Slovenia	13.9	1.1	2.4	73.8	5.9	0.4	1.7 (6c18:1), 0.5 (9t18:1), 0.1 (20:0)
COIL-75	olive	Slovenia	17.7	1.2	1.9	70.4	6.7	0.3	1.6 (6c18:1), 0.2 (9t18:1), 0.1 (20:0)
COIL-76	olive	Slovenia	22.3	0.9	1.9	62.9	8.2	0.5	1.8 (6c18:1), 0.9 (9t18:1), 0.1 (20:0), 0.1 (20:1)
COIL-79	olive	Slovenia	23.4	1.3	1.8	67.1	3.5	0.1	1.3 (6c18:1), 1.0 (9t18:1), 0.5 others
COIL-65	olive	Croatia	16.1	0.8	1.8	69.1	10.1	0.3	1.7 (6c18:1; 9t18:1), 0.1 (20:0)
COIL-66	olive	Croatia	16.3	1.0	2.3	68.4	8.4	0.4	2.6 (6c18:1; 9t18:1), 0.2 (20:0), 0.1 (22:0)
COIL-67	olive	Croatia	18.3	0.9	1.7	66.5	10.3	0.3	1.9 (6c18:1; 9t18:1), 0.1 (20:0), traces
COIL-68	olive	Croatia	24.9	2.2	1.7	61.3	7.5	<0.1	2.2 (6c18:1; 9t18:1)
COIL-77	olive	Croatia	24.7	3.3	1.0	63.1	4.4	<0.1	2.6 (6c18:1), 1.0 (9t18:1)
COIL-78	olive	Croatia	25.2	1.4	1.5	62.7	6.7	0.1	1.5 (6c18:1; 1.1 (9t18:1)
COIL-80	olive	Croatia	23.0	0.8	2.1	65.1	6.1	<0.1	1.9 (6c18:1), 0.9 (9t18:1), 0.2 (20:0)
COIL-70	pumpkin	Slovenia	13.6	0.3	4.8	30.2	47.9	0.4	0.7 (6c18:1), 0.8 (9t18:1), 0.1 (20:0), 1.2 other
COIL-71	pumpkin	Slovenia	49.2	0.9	11.2	31.6	4.9	0.9	1.3 other
COIL-72	pumpkin	Slovenia	25.4	0.5	8.5	31.7	24.5	0.4	1.7 (6c18:1), 0.9 (9t18:1), 0.5 (20:0), 6.4 other
COIL-73	pumpkin	Slovenia	16.7	0.2	7.0	33.9	34.3	0.5	0.8 (6c18:1), 0.3 (9t18:1), 8.7 other
COIL-63	sunflower	Slovenia	9.3	0.1	3.3	22.7	63.7	0.2	0.6 (6c18:1), <0.1 (9t18:1), 6.3 other
COIL-64	sunflower	Slovenia	28.0	0.7	6.5	28.0	33.7	0.4	2.7 other
COIL-61	soybean	unknown	13.6	0.1	3.2	27.1	49.5	4.8	1.2 (9t18:1), traces (6c18:1)
COIL-62	sesame	unknown	15.4	0.2	4.6	38.5	40.4	0.2	0.2 (6c18:1)
COIL-69	maize	Slovenia	17.5	0.1	1.8	25.4	51.8	1.5	1.8 (6c18:1), 0.1 (20:0)
COIL-74	rape	Slovenia	12.8	0.3	3.8	33.8	47.5	1.6	0.4 (6c18:1; 9t18:1)

[a] Petroselinic (6c 18:1), elaidic (9t 18:1), arachidic (20:0) acid.

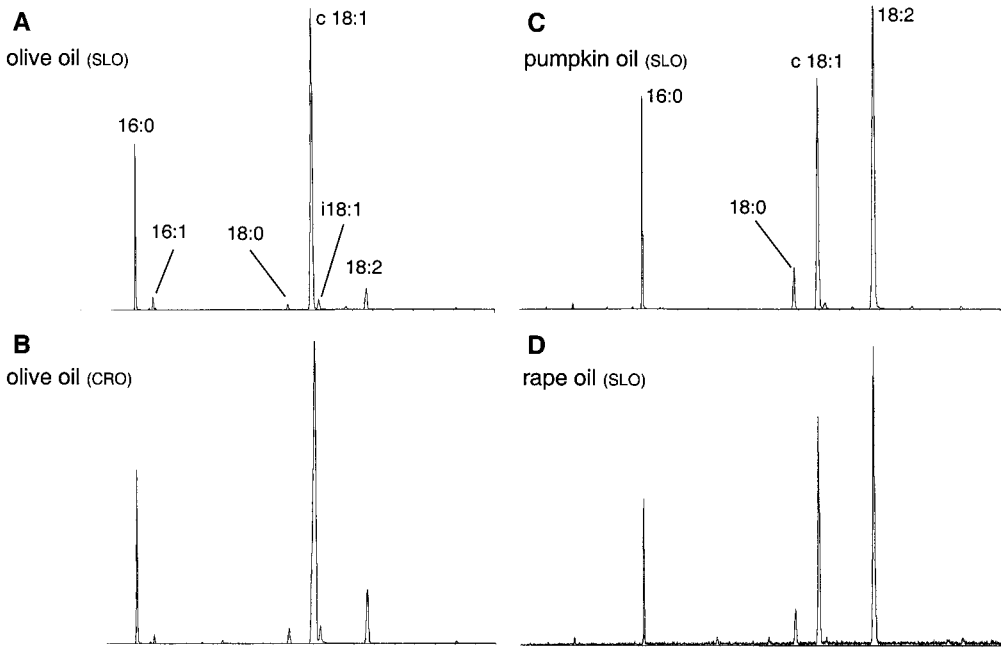

Figure 1. GC/MS chromatograms of the fatty acid methyl esters of olive oils from Slovenia (A) and Croatia (B), pumpkin oil (C), and rape oil (D).

methylation was corrected by a mass balance equation (*19*):

$$\delta^{13}C_{FAME} = f_{FA}\,\delta^{13}C_{FA} + f_{MeOH}\,\delta^{13}C_{MeOH}$$

where $\delta^{13}C_{FAME}$, $\delta^{13}C_{FA}$, and $\delta^{13}C_{MeOH}$ are the carbon isotope compositions of the fatty acid methyl ester, the fatty acid, and the methanol used for methylation of the fatty acid, respectively, and f_{FA} and f_{MeOH} are the carbon fractions in the fatty acid methyl ester due to the underivatized fatty acid and methanol, respectively.

Statistical Analysis. The GC/MS, GC/C/IRMS, and bulk oil $\delta^{13}C$ data set were reduced by the technique of multivariate statistical analysis using the software package of Data Desk 3.0. The method of principal component analysis (PCA) was used to reduce the chemical and isotopic measurements to a limited number of independent variables (principal components).

RESULTS AND DISCUSSION

Fatty Acid Contents. The compositions of the main fatty acids of the analyzed vegetable oils are given in Table 1. Olive oils are easily distinguished from the other vegetable oils by their high content of monounsaturated fatty acids (16:1 and 18:1) and low concentrations of polyunsaturated acids (18:2 and 18:3) (Figure 1). Sunflower (33.7−63.7%), soybean (49.5%), and pumpkin (24.5−47.9%, excluding sample COIL-71) seed oils contain high concentrations of linoleic acid, and the sesame oil contains moderate concentrations of both oleic (38.5%) and linoleic (40.4%) acid. High amounts of palmitic acid were measured in a sunflower oil (sample COIL-64, 28%) and maize oil (sample COIL-69, 17.5%). These values are abnormally high compared

Table 2. Carbon Isotope Ratios of Bulk Oil and Individual Fatty Acids of Vegetable Oils

			δ^{13}C (‰, PDB)						
sample	oil	country	bulk oil	palmitic (16:0)	palmitoleic (16:1)	stearic (18:0)	oleic (18:1)	linoleic (18:2)	linolenic (18:3)
COIL-58	olive	Slovenia	−30.1	−34.6	−32.7	−33.5	−34.4	−36.5	−[a]
COIL-59	olive	Slovenia	−28.4	−31.5	−28.5	−31.4	−30.2	−32.1	−
COIL-60	olive	Slovenia	−29.4	−32.3	−31.3	−32.4	−30.2	−32.8	−
COIL-75	olive	Slovenia	−29.1	−33.0	−30.5	−32.4	−31.7	−32.2	−
COIL-76	olive	Slovenia	−30.0	−33.8	−	−	−33.9	−32.7	−
COIL-79	olive	Slovenia	−29.2	−32.6	−31.3	−33.0	−32.1	−32.4	−
COIL-65	olive	Croatia	−29.7	−33.1	−30.3	−32.6	−31.1	−33.9	−
COIL-66	olive	Croatia	−29.4	−32.0	−31.0	−32.2	−30.4	−32.7	−32.4
COIL-67	olive	Croatia	−29.1	−30.7	−32.1	−31.6	−30.8	−35.5	−
COIL-68	olive	Croatia	−29.7	−33.6	−32.0	−32.9	−31.4	−32.5	−
COIL-77	olive	Croatia	−30.6	−35.4	−32.3	−	−33.3	−33.5	−
COIL-78	olive	Croatia	−27.7	−32.0	−	−	−32.8	−30.9	−
COIL-80	olive	Croatia	−28.0	−32.6	−	−31.3	−32.5	−32.5	−
COIL-70	pumpkin	Slovenia	−28.7	−31.3	−32.4	−32.8	−30.9	−30.4	−32.8
COIL-71	pumpkin	Slovenia	−28.1	−31.3	−	−28.9	−30.6	−34.8	−
COIL-72	pumpkin	Slovenia	−29.0	−33.4	−	−	−33.2	−	−
COIL-73	pumpkin	Slovenia	−29.1	−33.4	−	−	−33.5	−31.7	−
COIL-63	sunflower	Slovenia	−30.0	−33.6	−	−32.8	−33.4	−31.7	−
COIL-64	sunflower	Slovenia	−30.3	−32.4	−	−31.2	−31.2	−30.4	−
COIL-61	soybean	unknown	−30.8	−34.0	−	−34.1	−31.7	−31.7	−
COIL-62	sesame	unknown	−26.5	−29.7	−	−29.5	−27.5	−28.5	−
COIL-69	maize	Slovenia	−20.8	−24.0	−	−	−23.1	−23.2	−
COIL-74	rape	Slovenia	−29.5	−32.9	−	−	−32.2	−32.9	−

[a] − = not analyzed.

to those reported by other researchers for genuine sunflower and maize seed oils (sunflower, 4−6% 16:0; maize, 13% 16:0) (*1*). The low price of these oils sold as single seed oils causes a concern about their authenticity. The scatter of the compositions of fatty acids for the olive oils probably reflects the variation in oil variety, climatic conditions of the area, water-use efficiency in cultivars, salinity, temperature and pH of the irrigation water, olive-ripening stage, and other factors (*24*). Oleic acid (9c-octadecenoic) is often accompanied by low levels of other 18:1 isomers, mainly the *cis*-isomer petroselinic (6c-octadecenoic) and the trans-isomer elaidic (9t- octadecenoic). The deodorization and steam washing (generally between 150° to 260 °C) of vegetable oils may induce isomerization of the cis monounsaturated acids by cleavage of the double carbon bond in the natural cis isomer which permits rotation around a single C−C bond and rearrangement of the atoms into a trans configuration (*25*). Furthermore, chemical treatment (e.g., degummation with phosphoric acid; neutralization of free fatty acids with sodium hydroxide; or bleaching) and inadequate storage of the oils may induce oxidative degradation (rancidity) of the tryglycerides (*24*, *26*−*29*). Therefore, the refinement of cold-pressed vegetable oil or mixing with thermally refined oils leads to an increase in the content of the trans fatty acids (*25*). The European Economic Union (EEU) regulation set the maximal legal content of trans isomers of oleic and linoleic acid in cold-pressed olive oil at 0.05%. The refined soybean oil contains 1.2% trans isomers. Some samples of cold-pressed oil have significant amounts of trans isomers, which suggest that the oils were thermally degraded or blended with refined products (Table 1). Isomerization of the monounsaturated acids during the procedure of hot (70 °C) hydrolysis of the oil samples followed by sterification with BF_3 (60 °C) may not be excluded. Therefore, the high concentration of trans isomers in some oil samples should be considered with some precaution. No trans isomers were detected in the sesame oil (sample COIL-61), which is sold as cold-pressed not-refined gourmet oil in local supermarkets.

Bulk Isotopic Composition. The δ^{13}C of the bulk olive oils (−27.7 to −30.6‰), pumpkin seed oils (−28.1 to −29.1‰), and nonmaize vegetable oils show isotopic compositions typical of C_3 plants (Table 2). The scatter of the δ^{13}C values of the C_3 oils (2.9 ‰) may be attributed to factors affecting the chemical distribution of the fatty acids, and particularly by the physiological processes and enzymatic reactions occurring in the plant cells. Additionally, the chemical changes (isomerization of oleic acid and oxidation) during thermal degradation (natural or induced during steam washing or other refining procedures) of the vegetable oils, or blending of the oil with refined oil or other vegetable oil, may cause a further isotopic discrimination. The homemade olive oil sample from 1996/1997 (COIL-59, −28.4‰) is isotopically heavier than the oil from the 1997/1998 season (COIL-58, −30.1‰). The one-year-older oil became enriched in ^{13}C during thermal and oxidative alteration during the storage, because of preferential release of isotopically lighter, thermally and chemically less stable lipid moieties. The δ^{13}C value (−26.5‰) of bulk sesame seed oil is less negative than other C_3-vegetable oils. Rossell (*7*) reported similar values for Sudanese sesame seed oils (−26.1 to −25.1‰). The δ^{13}C value of maize oil (sample COIL-69, −20.8‰) is very negative for carbon from a C_4 plant (see below).

Isotopic Composition of Individual Fatty Acids. The δ^{13}C values of the olive oil fatty acids vary from −36.5 to −28.5‰ and those of the pumpkin oil range from −34.8 to −28.9‰ (Table 2). The values for oil of the different C_3 source plants vary from −36.5 to −27.5‰ (Table 2). Olive oils are readily separated from the seed oils (especially pumpkin, sunflower, soybean, and sesame) by principal component analysis combining the fatty acid composition and the carbon isotope data of the bulk oil and the major fatty acids (Table 3, Figure 2). The first four principal components explain 92.2% of the total variance (Table 3). The first principal component correlates better than 0.7 with the concentrations of the monounsaturated fatty acids and negatively with the concentrations of the polyunsaturated

Table 3. Principal Component Analysis Performed on the Fatty Acid Composition and Carbon Isotope Ratios of Bulk Oil and Main Individual Fatty Acids of Vegetable Oils Consumed in Slovenia and Croatia

	principal components				
olive oil class	1	2	3	4	5
variance proportion	42.1	25.1	17.2	7.8	4.7
unrotated factor loadings					
16:0	0.08	0.14	−0.97	−0.08	−0.06
16:1	0.78	−0.23	−0.29	0.09	−0.47
18:0	−0.47	0.67	−0.40	−0.32	0.14
18:1	0.79	−0.51	0.02	0.14	0.30
18:2	−0.77	0.33	0.48	−0.01	−0.28
18:3	−0.45	0.45	−0.25	0.73	0.05
$\delta^{13}C_{bulk}$	−0.68	−0.65	−0.180	−0.08	−0.07
$\delta^{13}C_{16:0}$	−0.76	−0.62	−0.15	−0.04	0.05
$\delta^{13}C_{18:1}$	−0.70	−0.61	−0.16	0.14	−0.01

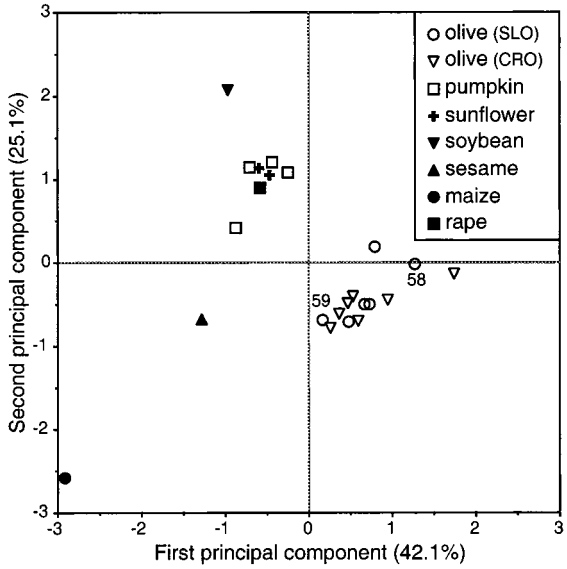

Figure 2. Scatterplot of the scores from the first two principal components for the vegetable oil samples. Loadings of the principal components are given in Table 3.

fatty acids and the isotope ratios. The second principal component has an opposite correlation between the stearic acid and the oleic acid and isotope ratios. Two well-defined clusters can be seen in addition to the maize oil in the scatterplot of the loadings of the first two principal components (Figure 2). One cluster belongs to the olive oil samples, and does not show a clear indication of the geographical origin of the oil. However,

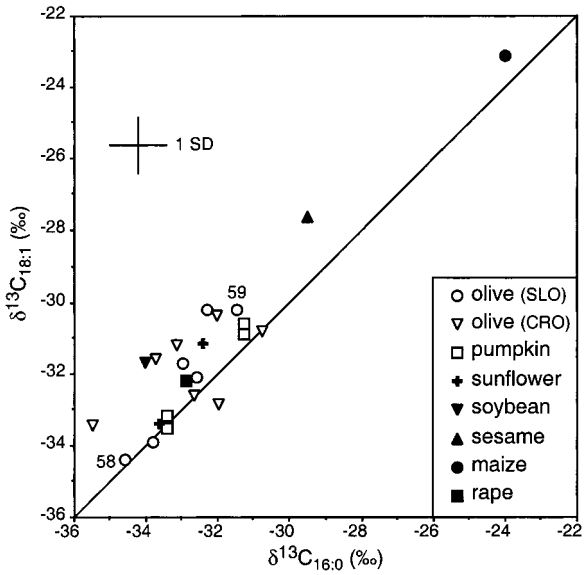

Figure 3. Carbon isotope composition of oleic acid ($\delta^{13}C_{18:1}$) versus palmitic acid ($\delta^{13}C_{16:0}$) of the vegetable oils.

PCA clearly separates the samples from the same olive cultivars and different season (COIL-58, COIL-59). The other cluster contains the C_3 seed oils. Pumpkin, sunflower, and rape seed oils form a close sub-group. The outliers are the sesame and soybean seed oils. The olive oils from Slovenia and Croatia have lower fatty acid concentration ratios, 18:1/16:0 about 3.3 and 3.5 respectively, than those from Spain (5.0), Italy (4.5), Greece (4.4), and France (4.3, Table 4).

The distribution of the samples in the $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:1}$ diagram suggests the adulteration or inappropriate processing or storage of some vegetable oils (Figure 3). Spangenberg et al. (*19*) demonstrated that a substantial separation of the oils from the 1:1 line in the $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:1}$ diagram could be used as an indication of adulteration or inappropriate processing of cold-pressed oils. The differences in the $\delta^{13}C$ values of palmitic and oleic acids are discussed in terms of acids biosynthesis and admixtures of distinct vegetable oils. The reactions of fatty acids biosynthesis are essentially the same in all plants (*30*−*32*). A multienzyme complex catalyzes the key reaction sequence by which the longer chains of fatty acids are assembled. Elongation of carbon chains occurs in the same way as that of synthesis, but differs in the enzymatic set which catalyzes the reac-

Table 4. Minimum, Maximum, Median, and Standard Deviation Values (1 SD) for Fatty Acid Compositions and Carbon Isotope Ratios of Bulk Oil and Main Fatty Acids of Olive Oils from Slovenia, Croatia, and Main Producer Mediterranean Countries

	fatty acids (wt % of total fatty acids)						$\delta^{13}C$ (‰, PDB)		
country	palmitic (16:0)	palmititoleic (16:1)	stearic (18:0)	oleic (18:1)	linoleic (18:2)	linolenic (18:3)	bulk oil	palmitic (16:0)	oleic (18:1)
Slovenia	14.6 to 24.5	0.8 to 1.5	1.1 to 3.1	62.4 to 73.1	3.2 to 8.0	0.1 to 0.4	−30.1 to −28.4	−34.6 to −31.5	−34.4 to −30.2
(n = 6)	20.9 (3.7)	1.1 (0.3)	1.8 (0.7)	68.8 (3.5)	5.2 (1.7)	0.3 (0.1)	−29.3 (0.6)	−33.0 (1.1)	−31.9 (1.8)
Croatia	16.3 to 27.1	0.8 to 2.8	1.1 to 2.3	58.8 to 68.0	4.5 to 10.2	0.1 to 0.4	−30.6 to −27.7	−35.4 to −30.7	−33.3 to −30.4
(n = 7)	18.8 (4.1)	1.1 (0.8)	1.7 (0.4)	66.2 (3.2)	8.0 (2.1)	0.2 (0.2)	−29.4 (1.0)	−32.6 (1.5)	−32.7 (1.4)
Spain[a]	12.2 to 19.5	0.4 to 1.0	1.4 to 3.5	68.0 to 76.4	4.5 to 7.9	0.3 to 0.7	−32.9 to −27.5	−35.5 to −26.8	−34.7 to −26.5
(n = 7)	14.9 (2.8)	0.5 (0.2)	2.8 (0.8)	74.7 (3.2)	6.6 (1.1)	0.5 (0.1)	−29.6 (2.0)	−31.5 (2.8)	−31.1 (2.6)
Italy[a]	15.3 to 20.2	0.7 to 3.0	1.2 to 2.5	68.8 to 75.3	2.9 to 8.7	0.2 to 2.2	−31.2 to −27.7	−35.3 to −29.3	−32.5 to 30.0
(n = 13)	16.9 (1.3)	1.5 (0.6)	1.7 (0.4)	73.5 (2.1)	5.1 (1.5)	0.4 (0.1)	−30.2 (1.0)	−32.9 (1.5)	−33.4 (1.6)
Greece[a]	15.6 to 16.7	0.6 to 1.2	1.7 to 2.9	71.8 to 74.2	3.8 to 6.6	0.5 to 1.0	−29.5 to −28.9	−31.2 to −30.4	−31.6 to 30.1
(n = 4)	16.5 (0.5)	0.8 (0.2)	2.2 (0.5)	73.1 (1.1)	5.6 (1.2)	0.6 (0.2)	−29.3 (0.3)	−30.9 (0.4)	−30.5 (0.7)
France[a]	16.5 to 17.5	0.9 to 1.2	1.7 to 3.5	68.9 to 73.2	4.0 to 9.0	0.4 to 0.7	−31.0 to −28.8	−31.1 to −30.1	−30.8 to −30.1
(n = 3)	16.7 (0.5)	1.0 (0.2)	2.3 (0.9)	71.6 (2.1)	6.1 (2.5)	0.4 (0.2)	−29.5 (1.1)	−30.8 (0.5)	−30.4 (0.4)

[a] Values calculated from data in Spangenberg et al. (*19*).

tions. The product formed by addition of one acetyl group to palmitic acid (16:0) is stearate (18:0). At the same site of the plant tissue (the endoplasmic reticulum) oxidative reactions catalyzed by fatty acyl-coenzyme A desaturase introduces the unsaturation to the fatty acids. One can safely assume that the isotopic discrimination between the first biosynthesized fatty acid (16:0) and the first elongation and unsaturated product (18:1) will be less than the analytical error (±0.5‰). Loss of isotopically light moieties during thermal or oxidative alteration (e.g., conversion of 18:1 to 16:0, and 16:0 to 14:0 acid) explains the shift toward less negative $\delta^{13}C_{18:1}$ and $\delta^{13}C_{16:0}$ values. The olive oil samples after one year of storage clearly show this isotopic shift (samples COIL-58 and COIL-59, Figure 3). Therefore, a substantial deviation of some oil samples from the 1:1 line in the $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:1}$ diagram, toward less negative $\delta^{13}C$ values, suggests thermal or oxidative degradation during processing or storage, or admixture of a cold-pressed oil with refined oils. The maize oil commercialized in Slovenian supermarkets (sample COIL-69) with very negative bulk and molecular $\delta^{13}C$ values for a C_4 plant, plot far from the 1:1 line in the $\delta^{13}C_{16:0}$ vs $\delta^{13}C_{18:1}$ diagram (Figure 3). This strongly suggests mixing of maize and a C_3 vegetable oil. Furthermore, blending of a C_3 vegetable oil with other C_3 oil of slightly different fatty acid composition (18:1/16:0 concentrations ratio) will also induce a significant deviation from the 1:1 line in the $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:1}$ diagram. The results demonstrate that carbon isotope composition of the bulk oil and individual fatty acids combined with the fatty acids composition can be used for a reliable chemometric classification of vegetable oils. Ongoing projects on the $^{18}O/^{16}O$ isotopic analysis of individual lipids will enhance the sensitivity of this approach as further tests for authenticity controls.

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