Characterization of olive oil by carbon isotope analysis of individual fatty acids: Implications for authentication and palaeoclimatological changes in the Mediterranean Basin

Characterization of Olive Oil by Carbon Isotope Analysis of Individual Fatty Acids: Implications for Authentication

Jorge E. Spangenberg,*,† Stephen A. Macko,‡ and Johannes Hunziker†

Institut de Minéralogie et Pétrographie, Université de Lausanne, BFSH-2,1015 Lausanne, Switzerland, and Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22903

The fatty acids of olive oils of distinct quality grade from the most important European Union (EU) producer countries were chemically and isotopically characterized. The analytical approach utilized combined capillary column gas chromatography−mass spectrometry (GC/MS) and the novel technique of compound-specific isotope analysis (CSIA) through gas chromatography coupled to a stable isotope ratio mass spectrometer (IRMS) via a combustion (C) interface (GC/C/IRMS). This approach provides further insights into the control of the purity and geographical origin of oils sold as cold-pressed extra virgin olive oil with certified origin appellation. The results indicate that substantial enrichment in heavy carbon isotope (^{13}C) of the bulk oil and of individual fatty acids are related to (1) a thermally induced degradation due to deodorization or steam washing of the olive oils and (2) the potential blend with refined olive oil or other vegetable oils. The interpretation of the data is based on principal component analysis of the fatty acids concentrations and isotopic data ($\delta^{13}C_{oil}$, $\delta^{13}C_{16:0}$, $\delta^{13}C_{18:1}$) and on the $\delta^{13}C_{16:0}$ vs $\delta^{13}C_{18:1}$ covariations. The differences in the $\delta^{13}C$ values of palmitic and oleic acids are discussed in terms of biosynthesis of these acids in the plant tissue and admixture of distinct oils.

Keywords: *Olive oil; carbon isotope; isotopic characterization; fatty acids; adulteration*

INTRODUCTION

The olive tree (*Olea europaea*) is the oldest cultivated tree in the soils around the Mediterranean basin. The fruit of this evergreen tree provides a highly esteemed oil, owing to its delicate flavor and nutritional and health benefits (Kiritsakis, 1991). Olive oils are available in different qualities in the EU countries. These include (1) cold-pressed (CP) extra virgin olive oil and virgin olive oil having a maximum of free fatty acids of 1.0% and 2.0% acidity (as wt % oleic acid), respectively, (2) olive oil, consisting of a blend of virgin olive oil and refined olive oil with a maximal acidity of 1.5%, and (3) olive pomace oil which is a blend of refined, solvent-extracted olive−pomace oil and virgin olive oil, having a maximum acidity of 1.5%. Oleic acid (18:1) is the major component of the olive oil (65−85 wt %). This fatty acid is also present at high concentrations in other vegetable oils, including maize, high-oleic variety of sunflower, rape, and hazelnut oil (Gunstone, 1996). Thus, adulteration of a high-quality CP olive oil with refined olive oil or other vegetable oils of lower cost may lead to substantial economic profit. The high consumption of olive oil in the EU countries and the increase observed in other developed countries (Australia, United States, Canada, Japan, and Russia) lead to the expectation that production of this commodity in the Mediterranean region will grow (Kiritsakis, 1991; Demicheli and Bontoux, 1997). Consequently, an increase in the number of cases of adulteration and problems of oil purity may be anticipated. Stable carbon isotope analyses have proven to be a powerful tool for assessing the purity of vegetable products from plants of different photosynthetic pathways (e.g., Doner, 1991; Rossell, 1991, 1994). In fact, isotopic discrimination against the heavier carbon isotope (^{13}C) occurs during photosynthesis and is reflected in the isotopic compositions of the plant tissues and products. The stable carbon isotopic data are expressed in the usual delta (δ) notation as the per mill (‰) deviations of the isotope ratio of a sample relative to that of a standard

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

where $R = {}^{13}C/{}^{12}C$. The standard is the Peedee belemnite limestone (PDB) that has a defined value of 0.0‰ on the δ scale.

The most important atmospheric carbon dioxide-fixing reactions are the C_3 and C_4 pathways (e.g., Farquhar et al., 1989; O'Leary, 1988, 1993). The C_3 plants use the Calvin cycle (ribulose-1,5-biphosphate carboxylase) for CO_2 fixation, and the first metabolite has three carbon. All trees operate with C_3 pathway, and their carbon isotope compositions fall into the range −22 to −34‰. The C_4 plants use the Hatch−Stack cycle and are isotopically heavier (−6 to −23‰). C_4 plants are most plants in the tropic, including tropical grasses, sedge, maize, sugar cane, and salt marsh plants. A further photosynthetic class of plants uses the CAM (Crassulacean acid metabolism) pathway. The isotopic compositions of the CAM plants range between −11 and −33‰. Typical CAM plants are succulents and are of minor importance in the oleochemical industry. The biochemistry and the carbon isotope composition of olive leaves indicate values typical for the C_3 photosynthetic pathway (Bongi et al., 1987). Food chemists are increasingly using the novel instrumentation of GC/C/

* Author to whom correspondence should be addressed (telephone, +41 (0)21-692-4365; fax, +41 (0)21-692-4305; e-mail, Jorge.Spangenberg@imp.unil.ch).
† Université de Lausanne.
‡ University of Virginia.

10.1021/jf980183x CCC: $15.00
Published on Web 09/10/1998

IRMS for carbon isotope analysis of the individual lipids as a tool for assessing adulteration of vegetable oils (e.g., Braunsdorf et al., 1993; Frank et al., 1995; Woodbury et al., 1995; Kelly, 1997; Remaud, 1997). To our knowledge no GC/C/IRMS data of fatty acids from olive oils are available. We report here the chemical and isotopic compositions of the fatty acids of the CP olive oils from the main producer countries of the Mediterranean region and compare them with lower grade olive oils and other vegetable oils (sunflower, hazelnut, groundnut, rape, and maize). The isotopic results enable a distinction between genuine extra virgin olive oil and the other quality olive oils, and also serve as a way of assessing if the oil was blended with refined olive oils or other vegetable oils.

MATERIALS AND METHODS

Samples. Twenty-seven samples of extra virgin olive oil were obtained from the major oil-producing regions of Spain (7), Italy (13), Greece (4), and France (3). All the samples are from the 1996−1997 olive oil season and were obtained from local supermarkets (23) or from local farmers and small oleo cooperatives (4). The former samples represent the virgin olive oil quality sold in the EU market place. The oils labeled "cold pressed virgin olive oils" were compared with the refined olive and pomace oils from Turkey (1), Morocco (1), and Tunisia (2), and other vegetable oils, including maize (1), groundnut (1), sunflower (2), hazelnut (1), walnut (1), rape (1), and a vegetable oil from the United States (1). The samples were stored at +4 °C in the darkness.

Separation and Methylation of Fatty Acids. All the solvents and reagents (Fluka, Switzerland) were of analytical grade or higher purity. The organic solvents were glass distilled, and all the glassware was thoroughly washed, rinsed with deionized water (×4), and ashed (450 °C, 12 h) before use. The oil samples (0.1 mL) were subjected to alkaline hydrolysis by heating (70 °C, 3 h) with 10 mL of aqueous ethanolic (95 v %) potassium hydroxide (1 N) solution. After cooling, the neutral fraction was extracted with hexane (1 × 10 mL and 2 × 5 mL). Acidification of the hydrolysate with 6 N HCl to pH 1 liberates the fatty acids which were extracted with hexane (1 × 15 mL and 2 × 5 mL). The excess hexane was removed by rotoevaporation at 30 °C and carried to dryness in a vacuum desiccator. The fatty acids were derivatized to fatty acid methyl esters (FAMEs) with BF_3 in methanol (60 °C, 8 min), extracted with 10 mL hexane, and washed with saturated KCl solution (2 × 5 mL). The excess solvent was gently evaporated, and the FAMEs were stored with 0.5 mL of hexane in 2 mL vials with PTFE-lined cap at +4 °C until gas chromatographic analysis.

Gas Chromatography/Mass Spectrometry (GC/MS). The chemical characterization of the FAMEs was performed at the Department of Earth Sciences of the University of Lausanne 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 an HP−FFAP fused silica capillary column (50 m × 0.20 mm i.d. × 0.33 μm polyethylene glycol-TPA modified as stationary phase) and helium as carrier gas (1 mL/min flow rate). The injector temperature was 200 °C, to avoid transmerization of the unsaturated FAMEs in the inlet sleeve (Camacho and Cert, 1994). After an initial isothermal period of 2 min at 100 °C, the column was heated to 220 °C at 5 °C/min followed by an isothermal period of 30 min. The EID was operated at 70 eV in the multiple ion detection mode, source temperature of 250 °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 composition of the main fatty acids [palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3)] were calculated as the percentage of total fatty acids.

Bulk Oil Carbon Isotopic Analysis. The carbon isotope composition of the bulk olive oil samples were determined at the Department of Earth Sciences of the University of Lausanne using the on-line elemental analyzer (EA)—continuous flow—isotope ration mass spectrometer (IRMS). The used EA-IRMS system consist of a Carlo Erba 1108 elemental analyzer coupled by a Finnigan Mat continuous flow interface (ConFlo II) to the Delta S IRMS. The EA oxidizes 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 pass through a reduction reactor packed with elemental copper and copper oxide at 640 °C to remove excess of oxygen and to reduce the nitrous (NO_x) products to elemental nitrogen. Water is removed using anhydrous magnesium perchlorate, and the gases enter a chromatographic column for separation of N_2 from CO_2, which are isotopically analyzed on the IRMS. The laboratory reference gas was calibrated against international standards. The reproducibility of the bulk oil analyses, assessed by replicate analyses of a laboratory standard (glycine, working value = −26.1‰ δ^{13}C) was better than 0.1‰ (1 SD).

Isotopic Analysis of Individual Fatty Acids. The isotopic composition of the individual fatty acids were obtained by the use of the novel instrumentation of a GC coupled to an IRMS by a combustion (C) interface (GC/C/IRMS) under a continuous helium flow (e.g., Freedman et al., 1988; Goodman and Brenna, 1992). The GC/C/IRMS analyses were performed at the Department of Environmental Sciences, University of Virginia, using a VG OPTIMA IRMS interfaced to a HP5890 GC with a combustion furnace (Cu−Ni wire, 850 °C) and a cryogenic trap (−90 °C). The GC was equipped with an J & W Scientific fused silica capillary column (30 m × 0.25 mm i.d. × 1.00 μm DB−FFAP as stationary phase) and operated at the following conditions: splitless injection; helium flow rate 1 mL/min; injector temperature 200 °C; initial temperature 150 °C; initial time 5 min; temperature ramp rate 10 °C/min; final temperature 220 °C; final isothermal period 12 min. The performance of the GC/C/IRMS system, including the combustion furnace, was evaluated every 10 analyses by injection of a laboratory standard (deuterated naphthalene-d_8, Cambridge Isotope Laboratories, MA 01810) of known isotopic composition (working value = −26.2 ± 0.4‰ δ^{13}C). For all runs, background subtraction was performed using the parameters supplied by the GC-OPTIMA software. Two to five replicate GC/C/IRMS runs were performed for each sample. The reproducibility ranged between ±0.1 and ±0.5‰ (1SD). The accuracy of the samples' analyses was monitored by co-injection of a FAME laboratory standard (methyl dodecanoate, Supelco) of known isotopic composition (working value = −30.4 ± 0.2‰ δ^{13}C). The isotopic shift due to the carbon introduced in the fatty acids methylation was corrected by the following relationship (Goodman and Brenna, 1992; Ballentine et al., 1996):

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

where $\delta^{13}C_{FAME}$, $\delta^{13}C_{FA}$, and $\delta^{13}C_{MeOH}$ are the carbon isotope composition 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 underivatizad fatty acid and methanol, respectively.

Multivariate Data Analysis. The method of principal component analysis (PCA) was used to reduce to a limited number of independent variables (principal components) the chemical and isotopic parameters. This multivariate data analysis was performed with the software package of Data Desk 3.0.

RESULTS AND DISCUSSION

Variation of Fatty Acid Contents. The relative compositions of fatty acids exhibit a large variability for the studied edible oils (Table 1). Olive oil differs from the other vegetable oils by its high content in monoun-

Table 1. Fatty Acid Composition and Carbon Isotope Ratios of Bulk Oil and Main Individual Fatty Acids in Olive Oil Samples of Different Origin and Grade and Other Common Edible Oils[a]

sample	oil	country	palmitic (16:0, wt %)	palmitoleic (16:1, wt %)	stearic (18:0, wt %)	oleic (18:1, wt %)	linoleic (18:2, wt %)	linolenic (18:3, wt %)	other fatty acids	$\delta^{13}C_{bulk}$
COIL-1	evoo	Spain	15.1	0.9	2.1	74.7	6.0	0.5	<0.1 (t18:1), 0.6 (20:0)	−29.1
COIL-13	evoo	Spain	19.5	0.4	2.2	69.9	6.6	0.4	0.8 (t18:1), 0.2 (20:0)	−27.5
COIL-14	evoo	Spain	14.9	0.4	1.4	72.5	7.4	0.5	0.8 (t18:1), 0.2 (20:0)	−29.6
COIL-15	evoo	Spain	18.3	0.7	3.2	68.0	7.9	0.4	1.8 (t18:1), 0.3 (20:0)	−32.4
COIL-16	evoo	Spain	12.3	0.4	3.5	75.8	4.5	0.7	2.2 (t18:1), 0.5 (20:0)	−32.9
COIL-17	evoo	Spain	12.2	1.0	2.8	76.4	6.6	0.3	0.3 (t18:1), 0.2 (20:0)	−28.3
COIL-22	evoo	Spain	13.6	0.5	3.3	75.4	5.7	0.5	<0.1 (t18:1), 0.3 (20:0)	−30.6
COIL-2	evoo	Italy	16.9	0.9	2.0	74.4	5.1	0.5	<0.1 (t18:1), 0.2 (20:0)	−28.8
COIL-3	evoo	Italy	15.4	0.9	2.3	72.0	7.7	0.5	0.2 (t18:1), 0.3 (20:0)	−30.7
COIL-4	evoo	Italy	18.8	1.7	1.2	71.3	5.5	0.5	0.5 (t18:1), 0.4 (20:0)	−30.9
COIL-5	evoo	Italy	16.3	0.8	1.4	74.8	4.9	0.4	0.3 (t18:1), 0.5 (20:0)	−30.6
COIL-6	evoo	Italy	20.2	1.5	1.7	68.8	6.0	0.5	0.4 (t18:1), 0.2 (20:0)	−31.2
COIL-7	evoo	Italy	17.2	0.9	2.4	73.9	2.9	0.8	0.6 (t18:1), 0.5 (20:0)	−30.0
COIL-8	evoo	Italy	17.4	1.5	2.2	72.7	4.3	0.4	0.8 (t18:1), 0.1 (20:0)	−30.2
COIL-9	evoo	Italy	16.6	1.7	1.8	73.5	5.0	0.2	0.4 (t18:1), 0.1 (20:0)	−29.3
COIL-10	evoo	Italy	16.9	1.5	2.1	75.3	3.7	0.2	<0.1 (t18:1), 0.3 (20:0)	−29.5
COIL-11	evoo	Italy	16.8	3.0	1.3	68.9	7.9	0.5	1.3 (t18:1)	−29.5
COIL-12	evoo	Italy	15.3	1.6	1.5	73.8	5.6	0.4	0.9 (t18:1), 0.3 (20:0)	−30.8
COIL-20	evoo	Italy	17.9	0.9	1.7	71.5	6.4	0.4	1.1 (t18:1), 0.1 (20:0)	−27.7
COIL-21	evoo	Italy	18.4	1.0	1.5	75.2	3.3	0.3	<0.1 (t18:1), 0.2 (20:0)	−30.2
COIL-28	evoo	Greece	16.7	0.9	2.9	72.6	5.9	0.6	<0.1 (t18:1), traces	−28.9
COIL-29	evoo	Greece	16.5	1.2	2.4	74.2	3.8	0.7	<0.1 (t18:1), traces	−29.5
COIL-30	evoo	Greece	15.6	0.6	2.1	71.8	6.6	0.5	1.7 (t18:1), 0.4 (20:0)	−29.4
COIL-27	evoo	Greece	16.5	0.8	1.7	73.7	5.3	1.0	<0.1 (t18:1), 0.5 (20:0)	−29.2
COIL-23	evoo	France	16.7	1.2	2.3	68.9	9.0	0.4	0.6 (t18:1), 0.3 (20:0)	−31.0
COIL-24	evoo	France	17.5	1.0	1.7	73.2	6.1	0.4	<0.1 (t18:1), 0.1 (20:0)	−29.5
COIL-35	evoo	France	16.5	0.9	3.5	71.6	4.0	0.7	2.1 (t18:1), 0.4 (20:0)	−28.8
COIL-31	oo	Turkey	15.9	0.6	2.9	70.6	8.4	0.6	<0.1 (t18:1), 0.3 (20:0)	−28.5
COIL-36	poo	Morocco	13.0	0.9	3.6	67.7	9.3	0.1	4.9 (t18:1), 0.1 (20:0)	−28.8
COIL-39	oo	Tunisia	15.9	0.6	1.8	65.8	13.8	0.3	1.0 (t18:1), 0.1 (20:0)	−27.6
COIL-40	oo	Tunisia	15.6	0.2	2.6	61.1	16.9	0.4	1.5 (t18:1), 0.2 (20:0)	−27.4
COIL-18	voo	United States	12.7	1.1	1.5	73.1	8.3	0.5	0.3 (t18:1), 0.3 other	−28.8
COIL-19	vegetable	United States	10.0	0.1	5.9	29.7	46.5	t	2.9 (t18:1), 4.8 other	−30.7
COIL-26	sunflower	France	12.1	0.2	5.2	25.8	52.7	0.1	0.2 (20:0), 5.7 other	−29.2
COIL-38	sunflower	Switzerland	11.6	0.2	4.6	26.4	54.4	0.2	0.8 (t18:1), 1.6 other	−30.6
COIL-32	hazelnut	France	7.8	0.5	2.8	69.3	14.3	0.1	4.9 (t18:1), 0.2 other	−27.7
COIL-33	walnut	France	11.4	0.2	3.4	17.7	54.3	8.5	0.5 (20:0), 4.1 other	−27.6
COIL-34	groundnut	unknown	14.0	0.1	2.3	44.1	34.8	t	2.8 (20:0), 2.0 other	−28.3
COIL-37	rape	Switzerland	8.4	0.5	1.3	54.0	20.3	6.6	0.5 (t18:1), 0.5 (20:0), 7.9 other	−29.6
COIL-25	maize	France	16.0	0.1	1.9	27.5	49.9	0.4	0.6 (20:0), 1.8 other	−16.5

[a] Evoo = extra virgin olive oil (CP, <1% acidity); voo = virgin olive oil (CP, <2% acidity); oo = blend of virgin olive oil and refined olive oil (<0.5% aicity); poo = blend of refined olive pomace oil and virgin olive oil (<1.5% acidity); CP = cold pressed; t = trace amount of less than 0.05 wt %.

Table 2. Median and Standard Deviation (1 SD) Values For Fatty Acid Composition and Carbon Isotope Ratios of Bulk Oil and Main Individual Fatty Acids of Olive Oil Samples

	fatty acids (wt % of total fatty acids, 1 SD)						δ^{13}C (‰, PDB)		
olive oil class	16:0	16:1	18:0	18:1	18:2	18:3	bulk oil	16:0	18:1
extra virgin olive oil (n = 27)	16.7 (1.9)	0.9 (0.5)	2.1 (0.7)	73.2 (2.3)	5.7 (1.5)	0.5 (0.2)	−29.5 (1.2)	−31.4 (2.0)	−31.5 (2.1)
Spain (n = 7)	14.9 (2.8)	0.5 (0.3)	2.8 (0.8)	74.7 (3.2)	6.6 (1.1)	0.5 (0.1)	−29.6 (2.0)	−31.5 (2.7)	−31.1 (2.6)
Italy (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)	−32.6 (1.7)	−33.9 (1.8)
Greece (n = 4)	16.5 (0.5)	0.8 (0.2)	2.2 (0.5)	73.2 (1.1)	5.6 (1.2)	0.6 (0.2)	−29.3 (0.3)	−30.9 (0.4)	−30.6 (0.7)
France (n = 3)	16.7 (0.5)	1.0 (0.1)	2.3 (0.9)	71.6 (2.2)	6.1 (2.5)	0.4 (0.2)	−29.5 (1.1)	−30.8 (0.5)	−30.4 (0.4)
virgin olive oil (n = 1)	12.7	1.1	1.5	73.1	8.3	0.5	−28.8	−30.5	−32.0
olive oil (n = 3)	15.9 (0.2)	0.6 (0.2)	2.6 (0.6)	65.8 (4.7)	13.8 (4.3)	0.4 (0.1)	−27.6 (0.6)	−30.1 (0.6)	−30.2 (1.1)
pomace olive oil (n = 1)	13.0	0.9	3.6	67.7	9.3	0.1	−28.8	−31.1	−30.8

saturated fatty acids (18:1, 16:1) and relatively low polyunsaturated acids (18:2, 18:3), except for olive oil samples from Tunisia (samples COIL-39 and COIL-40). The significant scatter of the compositions of extra virgin olive of distinct origin (Table 2) likely reflect the variation in variety; climate conditions of the area; water-use efficiency in cultivars; salinity, temperature, and pH of the irrigation water; olive-ripening stage; and other factors (Kiritsakis, 1991). The refining and deodorization (generally at 150−260 °C) of olive oils and drying of the olive pomace before solvent extraction produce (1) transmerization of the *cis* monounsaturated acids (Brühl, 1996) by cleavage of the double-carbon bond in the natural *cis* isomer and rearrangement of the groups into a *trans* configuration, and (2) oxidative degradation of the tryglycerides (Gomes and Caponio, 1997). Therefore, a blend of CP olive oil with thermally refined oils (olive, olive pomace, or other vegetable) leads to an increase of the content in elaidic acid (trans 18:1). The EU regulations place strict processing conditions for the cold-pressed virgin olive oil, and set the maximal legal content of *trans* 18:1 isomer at 0.05 wt % of the total fatty acids (e.g., Brühl,1996). Some samples sold as extra virgin olive oil have high amount (up to 2.2%) of elaidic acid. The olive oil samples (blend of virgin olive oil and refined olive oil) have 1.0−1.5%, and the olive pomace oil (blend of virgin oil and refined pomace olive oil) 4.9% (Table 1). An eventual *trans*-

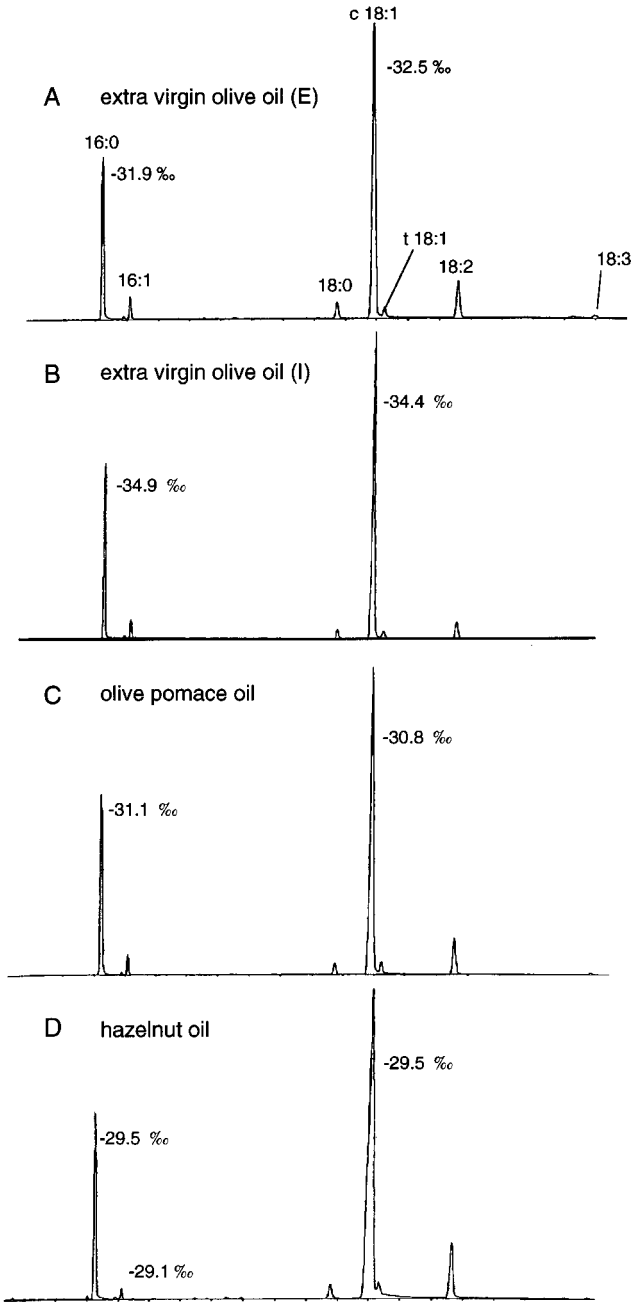

Figure 1. GC/MS chromatograms of the fatty acid methyl esters of extra virgin olive oils from Spain (A) and Italy (B), olive pomace oil (C), and hazelnut oil (D). The isotopic composition of the major acids is given. See text for details on the GC conditions.

merization of the oleic acids during hot (70 °C) alkaline hydrolysis of the oil samples and esterification with methanolic BF_3 (60 °C) cannot be excluded. Thus, the high concentrations for elaidic acid should be considered with some precaution.

Bulk Isotopic Composition. The $\delta^{13}C$ of the bulk olive oils and other nonmaize vegetable oils (−27.4 to −35.5‰) have isotopic compositions typical of C_3 plants (Table 1). The scatter of the $\delta^{13}C$ values of the C3 vegetable oils (4.9‰) may be attributed to variation of the isotope effect during fixation of carbon dioxide. This isotopic shift is at least partially explained by factors affecting the chemical distribution of the fatty acids, and particularly by the physiological processes and enzymatic reactions occurring in the plants cells (see Vogel,

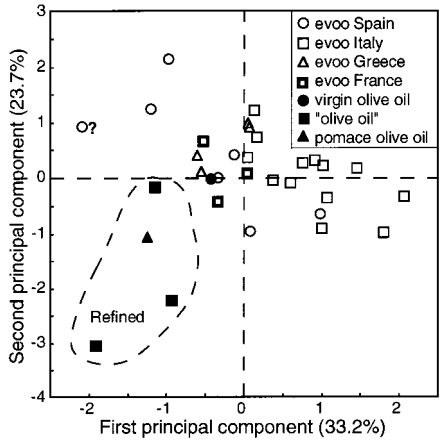

Figure 2. Scatterplot of the scores from the first two principal components for the olive oil samples. Loadings of the principal components are given in Table 3.

Table 3. Principal Component Analysis Performed on the Fatty Acid Composition and Carbon Isotope Ratios of Bulk Oil and Main Individual Fatty Acids of Olive Oil Samples

	principal components				
	1	2	3	4	5
variance proportion	33.2	23.7	12.1	10.8	7.2
loadings					
16:0	0.64	−0.24	−0.13	0.47	0.40
16:1	0.65	0.00	0.43	−0.20	−0.30
18:0	−0.617	0.19	−0.49	−0.30	0.26
18:1	0.13	0.89	0.32	−0.02	−0.10
18:2	−0.42	−0.83	−0.11	−0.09	−0.27
18:3	−0.02	−0.47	−0.45	0.60	−0.44
$\delta^{13}C_{bulk}$	−0.38	−0.48	0.45	0.47	0.12
$\delta^{13}C_{16:0}$	−0.85	0.23	0.26	0.05	−0.13
$\delta^{13}C_{18:1}$	−0.85	0.20	0.24	0.19	0.12

1993; O'Leary, 1988, 1993; Jackson et al., 1993). Some of the variations of the isotopic composition of the olive oil samples may be due to different olive variety and climatic and plant growth conditions. Additionally, the chemical changes (transmerization and oxidation) during heating of the olive oil or olive pomace may cause a further isotopic discrimination. In fact, the preferential cleavage of the $^{13}C-^{12}C$ single or double bonds and loss of light ^{12}C moieties during degradation and transmerization of the fatty acids induce two type of isotopic shifts: (1) an enrichment of heavy (^{13}C) moieties in the residual bulk oil, and (2) higher $\delta^{13}C$ of the oleic acid (cis 18:1). The processes of thermal and chemical refining explain the isotopic discrimination between natural cold-pressed olive oil and virgin olive blended with refined olive oils (Table 2).

GC/C/IRMS Data and Implications for Authentication. The $\delta^{13}C$ values of the virgin olive oil fatty acids vary between −26.5 and −35.5‰. The values for oil of the different C_3 source plants and distinct quality fall in to this broad range (Figure 1, Tables 1 and 2). Olive oil is readily separated from the other oils (especially sunflower, walnut, and hazelnut) by principal component classification combining the fatty acid composition and the carbon isotope data of the bulk oil and the major fatty acids (results not shown for brevity). Virgin olive oils are separated from the lower grade olive oils by a dedicated principal component analysis performed on olive oil samples (Table 3 and Figure 2). The scatterplot of the loadings of the first two principal components separate clearly the refined oils from the

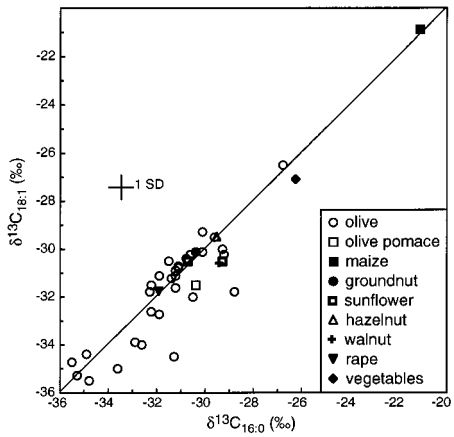

Figure 3. Carbon isotope composition of oleic acid ($\delta^{13}C_{18:1}$) versus palmitic acid ($\delta^{13}C_{16:0}$) of the vegetable oils.

PC oils, and show a trend on the origins of the olive oils. The isotopic composition of the major fatty acids from oils of different origin, particularly maize, some olive, groundnut, sunflower, and rape are similar within the analytical uncertainty (Figure 3). The isotopic compositions of the major fatty acids of pure maize oils was found to be the same (Woodbury et al., 1995). To explain this relationship, one has to consider fatty acid synthesis in plant cells (e.g., Cherif et al., 1979; Jaworski et al., 1990; Lehninger et al., 1993). The reactions of fatty acids biosynthesis are essentially the same in all plants. The fundamental reaction sequence by which the longer chains of fatty acids are assembled is catalyzed by a multienzyme complex. Elongation of carbon chains occurs in a fashion similar to that of synthesis, but differ in enzymes which catalyze the reactions. 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) unsaturated oxidative reactions catalyzed by fatty acyl coenzyme *a* desaturase introduces the unsaturation to the fatty acids. The enzyme that introduces further double bonds, to produce the 18:2 and 18:3 acids do not act on free fatty acids but on a phospholipid, containing at least one oleate (18:1) linked to the glycerol moiety. 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‰) of the GC/C/IRMS measurement. Therefore, 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 suggest admixture of a cold-pressed virgin olive oil with refined olive oils or other vegetable oils of different 18:1/16:0 concentration ratios than the genuine olive oil. This helps to explain the depletion in ^{13}C of the known blend oils, as the olive pomace and vegetable oil, and strongly suggests the adulteration or inappropriate processing of many extra virgin olive oils. The low price of an "extra virgin olive oil" (sample COIL-17) causes a concern about its purity. This sample plots near the vegetable oil in the $\delta^{13}C_{16:0}$ vs $\delta^{13}C_{18:1}$ diagram (Figure 3) and is an outlier in the scatterplot of the ACP loadings (Figure 2).

The effects of environment or physical factors on the carbon isotope composition of plant and trees have revealed long-term trends in the global environment changes, including the increased atmospheric CO_2 excess (e.g., Freyer and Belay, 1983; Walcroft et al., 1997). The Mediterranean region is highly affected by population increases, industrial expansion, and anthropogenic or accidental biomass burning (e.g., forest fires at Southern France and Spain). The subject of a current project is to trace the paleoclimatic changes in the late Holocene (600 B.C. to the present time) in this region by using carbon isotope composition of the major fatty acids in genuine olive oil.

CONCLUSIONS

The $\delta^{13}C$ values of the bulk oil and individual fatty acids can be used for the identification of the sources of the olive oil. The use of $\delta^{13}C_{16:0}$ vs $\delta^{13}C_{18:1}$ covariations holds great promise for assessing cases where impurity or adulteration is suspected. Blending of olive oil with edible oils with slightly different fatty acids composition (olive pomace, sunflower, hazelnut) may be detected with this approach combined with molecular information (GC/MS), and carbon isotope composition of the bulk oil. The results demonstrate the importance of elucidating the metabolism and biosynthesis (chain elongation and unsaturation) of the fatty acids which cause the $^{13}C/^{12}C$ discriminations observed in the individual lipids of the vegetable oils, to better utilize the $\delta^{13}C$ values as indicators of adulteration. We believe that the carbon isotope composition of individual fatty acids in genuine olive oil may be a sensitive molecular trace of paleoclimatic changes in the Mediterranean basin.

ACKNOWLEDGMENT

We thank A. Soler I Gil, F. Ghisetti, G. Ruggieri, S. Schmidt, A. Bacheca, A. Bartolini, N. Khémaies, Z. Sharp, and Y. Vavassis for providing oil samples.

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Received for review February 25, 1998. Revised manuscript received July 28, 1998. Accepted July 30, 1998. The organic geochemical laboratory including the GC/MS facilities at the University of Lausanne were funded under a joint venture of the Swiss National Science Foundation (Grant 2100-047023.96/1) and University of Lausanne. The GC/C/IRMS facilities at the Department of Environmental Science, University of Virginia, were supported by the US NSF.

JF980183X