Stable Isotope Characterization of Olive Oils. I—Compositional and Carbon-13 Profiles of Fatty Acids

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ABSTRACT: Nearly 200 olive oils produced in the Mediterranean basin, mainly in Greece, during 4 yr from 1993 to 1996, were studied by gas chromatography (GC) and on-line GC-isotope ratio mass spectrometry (GC-C-IRMS). The composition of the oils in the more abundant fatty acids ($C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$) was obtained by GC after transesterification of the triglycerides into methyl esters. Using the hyphenated GC-C-IRMS technique, the ^{13}C contents of the three most abundant acids, $C_{16:0}$, $C_{18:1}$, and $C_{18:2}$, were measured with satisfactory accuracy. The results, analyzed in terms of geographical, temporal, and botanical factors, provide new criteria for the authentication of olive oils.

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World production of olive oil is much smaller than that of soybean, sunflower, and peanut oils. Although it is mainly consumed in the Mediterranean Basin, the beneficial dietary effects of olive oil have in recent years contributed to its increasing popularity among consumers in Western Europe and North America. Its very high added value, however, is an incentive for unscrupulous traders to adulterate or mislabel olive oil and, as a consequence, there have been numerous studies to devise methods of authenticating the product. Compositional analysis is the most widely used technique for the authentication of olive oil; hundreds of components have been determined, mainly by chromatography, and analyzed by chemometrics (1). Even limited to the case of fatty acids, compositional analysis can provide a very useful means of olive oil characterization (2). Unfortunately there are potential adulterants, such as genetically modified sunflower oil, with a similar composition to olive oils, and in these cases other techniques are needed for authentication.

Stable isotope analysis of fatty acids was developed in the 1970s to study the pathways of lipid biosynthesis (3–5) but the isotope ratio mass spectrometry (IRMS) technique has been applied only recently to the authentication of olive oils. Bianchi *et al.* (6) and Angerosa *et al.* (7) investigated the efficiency of the average $\delta^{13}C$ parameter of raw olive oils for characterizing the addition of heterogeneous components. Kelly *et al.* (8), using the same technique, tried to assess the authenticity of oils extracted from plants with a C_3 photosynthetic metabolism. On the other hand, Woodbury *et al.* (9) have shown that the adulteration of C_4 oil (maize) by C_3 adulterants (rapeseed, groundnut) can be detected at the 5% level by gas chromatography-IRMS (GC-C-IRMS). However, no study concerning the combined compositional and ^{13}C analyses of the main fatty acids in olive oils has been published. The purpose of this work is to investigate the natural factors which govern these two parameters, with a view to developing a new authentication method (10).

MATERIALS AND METHODS

Olive oil samples (n = 188), mainly of extra virgin quality (*Olea europeae* L.), were studied. In addition, four kernel olive oils, three commercial hazelnut oils, four sunflower oils (two products with high oleic acid content), two soybean oils, and two maize oils were considered for obtaining a brief survey of their isotopic properties. However, the number of oils studied in each class is too small to have any statistical significance. Among the 188 olive oils studied, 166 were produced in Greece, 5 in Italy, 11 in Spain, and 6 in France. Different botanical (variety and maturity of the fruit), temporal (year of production), and geographical (region of production and elevation) factors were considered for the Greek olive oil population in order to investigate their influence on the physico-chemical parameters studied.

Sample preparation. Fatty acid methyl esters (FAME) preparation. FAME were prepared using BF_3-methanol (14:86, w/w), according to the derivatization method ISO 5509 (11). About 350 mg of oil was saponified at 100°C with 6 mL of a methanolic sodium hydroxide solution (0.5 mol/L) for 10 min. A methanolic boron trifluoride solution (7 mL) was then added and boiling was continued for 2 min. When the reaction was complete, 3 mL of heptane was added to the mixture, and boiling was continued for 1 min. After cooling to room temperature, a saturated sodium chloride solution

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was added. The FAME were extracted twice with 20 mL of hexane. The organic solution was washed with 20 mL of distilled water and two drops of methyl red indicator were added. The organic phase was dried over sodium sulfate, filtered, and the solvent was evaporated. The concentration of the resulting FAME was 10 mg per mL of hexane.

Fatty acid preparation. About 3 g of oil was mixed (1 mol/L) in ethanol with 30 mL of potassium hydroxide and refluxed at 100°C for 30 min. After the end of the reaction, the ethanol was evaporated and the aliquot taken up in 25 mL of distilled water and heated in a double boiler at 60°C for 5 min with stirring. After cooling to room temperature, this solution was acidified with 5 mL of hydrochloric acid (18 mol/L) to pH 1. The free fatty acids were extracted twice with 50 mL of diethyl ether. The extract was dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated.

Fatty acid composition. The FAME composition of oils was obtained using a High Resolution Hewlett-Packard 5890 Series II (Wilmington, DE) gas chromatograph equipped with a Carbowax 20 M (J.W. Scientific, Folsom, CA), 60 m × 0.32 mm i.d., 0.1 μm capillary column.

The helium (N55) carrier gas had a flow rate of about 2mL/min. The temperature program was: 100°C (initial temperature), increase to 200°C at a rate of 30°C/min, 30 min at 200°C and increase to 220°C at a rate of 10°C/min for 10 min. The injector and detector temperature was 230°C. An autosampler was used for the injections. The split ratio was around 1:15. The injection volume was 1 μL. The FAME composition is expressed as percentages, calculated, following the ISO 5508 method (12), by referring, without correction, the area of the considered peak to the sum of the areas of all peaks, with the exception of that of the solvent. Each sample was analyzed three successive times and the overall experimental uncertainty on the fatty acid composition is discussed later.

Stable isotope determinations. The overall carbon isotopic parameters ($^{13}C/^{12}C$) of whole oils and commercial FAME were obtained using a Finnigan Mat Delta E (Bremen, Germany) mass spectrometer coupled to a Carlo Erba NA 1501 Thermo Quest (Franklin, MA) micro-elemental analyzer.

The carbon isotopic parameters ($^{13}C/^{12}C$) of each fatty acid were measured using a Delta S mass spectrometer from Finnigan-MAT, coupled with a Varian 3400 (Palo Alto, CA) gas chromatograph and a combustion interface. The carrier gas was helium (N55). The helium flow in the column was about 2.8 mL/min (19 psi column head pressure). The gas chromatograph was equipped with a DBwax (J&W Scientific, Folsom, CA) capillary column, 60 m × 0.32 mm, 0.1 μm. The temperature program was the following: 100°C, increase to 200°C at a rate of 30°C/min, 15 min at 200°C and increase to 230°C at a rate of 5°C/min for 5 min. The injector temperature was 220°C. The injections were made manually or via an A200S Fisons (Manchester, United Kingdom) autosampler. The split ratio was around 1:7. The injection volumes were 1 and 5 μL according to the fatty acid considered (oleic or palmitic and linoleic acids).

The GC column was connected to the combustion interface through a Valco (Finnigan) connector. This crosspiece comprises one entry, where the GC column is connected, and two exits, a capillary connected to the combustion furnace and a vent valve.

The combustion interface is constituted of a ceramic tube containing a platinum wire and two copper wires, which are oxidized *in situ* once a week, and operates either in straight or in back-flush mode. In straight mode, the compounds separated at the exit of the GC can be oriented toward the mass spectrometer. In back-flush mode, a helium flow, around 3 mL/min, is created in the opposite direction to the helium flow from the GC. When this mode is activated, all the separated compounds coming out of the GC are vented. After chromatographic separation, the compounds are burnt in the combustion oven, at a temperature of 840°C, in order to produce CO_2 for the $^{13}C/^{12}C$ determination. The combustion water was removed using a Nafion (Finnigan) semipermeable membrane.

The sample was compared to a CO_2 standard isotopically calibrated vs. NBS19 which is related to the international reference, Vienna-PDB (13,15) by the equation:

$$\delta^{13}C_{NBS19/V.PDB} = 1.95‰ \quad [1]$$

This reference gas was directly introduced into the source of the mass spectrometer *via* a capillary tube and could be switched on or off with a pneumatic valve. The intensity of the major peak signal was usually between 1.5 and 2 V with a background noise usually below 10 mV. Peak centering using reference CO_2 was carried out before each injection.

The isotope ratios were computed from the signals recorded in channels corresponding to masses 44, 45, and 46. The ^{17}O contribution to mass 45 was corrected for according to Santrock *et al.* (14). The isotopic composition is expressed on the δ scale (‰) which refers the isotope ratio of the sample, *S*, to that of the international reference, V-PDB (13,15):

$$\delta^{13}C = 1000\left[\frac{(^{13}C/^{12}C)S - (^{13}C/^{12}C)_{PDB}}{(^{13}C/^{12}C)_{PDB}}\right] \quad [2]$$

During routine measurements, a mixture of commercial FAME is injected at regular intervals as a working reference to check the accuracy of the results.

Each sample was generally analyzed three times successively. The results were validated if the deviation was below 0.4 ‰.

Statistical evaluation of the data. The means, standard deviations, and coefficients of variation (CV in %) were computed for the different groups of data corresponding to the natural factors considered.

In addition, the analysis of variance and the computation of both the least significant differences (LSD) between the means (16) and the Mahalanobis distances between centroids (Md) (17) were carried out with the two sets of parameters studied (GC composition and IRMS isotope ratios) for the different factors considered: botanical factors (variety and maturity) and geographical factors (country, region, and ele-

vation of the production area). The LSD was computed at the 99% confidence level and, in order to achieve a robust evaluation of the discriminating power of the variables, the confidence levels of the Fischer, F, and of the Mahalanobis, chi2, variables were estimated. In some typical cases, the bivariate existence domains of the different groups were also computed.

RESULTS AND DISCUSSION

Precision of the analytical determinations. The yield of fatty acid derivatization, determined on commercial fatty acids (purity ≥ 99%), was 100% except for linoleic (80%) and linolenic (50%) acids. The CV of the derivatization, calculated on six commercial solutions (three injections by solution), was less than 5%.

The internal precision of the GC or IRMS measurement is defined here as the mean standard deviation by column (MSD) of the data matrices which contain the variables in columns and the observations (olive oils studied) in rows. For each of the seven fatty acids determined, the MSD is better than 0.04% w/w and the mean MSD for the set of fatty acids is equal to 0.02% w/w. In relative values, the precision of the determinations varies between 0.05% for the most abundant compound, $C_{18:1}$, to the range 0.1–0.4% for $C_{16:0}$, $C_{18:0}$, $C_{18:2}$, and $C_{18:3}$. Only $C_{16:1}$ is poorly determined (1.8%). As regards the $\delta^{13}C$ values of oils measured by off-line IRMS, the precision is of the same order of magnitude (0.1‰) as that demonstrated for sugars in an interlaboratory comparison (18). In the case of GC-^{13}C-IRMS, where the esters of the individual fatty acids are separated beforehand on a chromatographic column, the overall precision is poorer (0.2‰) but still satisfactory.

GC fatty acid composition. Thirteen fatty acids are found in olive oils as triglycerides (19), but only seven represent more than 97% of the oils. All the triglycerides of olive oil contain oleic acid, and triolein is the most abundant component (≈50%). As shown in Table 1, the $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ fatty acids have concentrations higher than 2% w/w and can be used relatively safely in authentication processes. The three most abundant components are $C_{18:1}$ (≈75%), $C_{16:0}$ (≈11%), and $C_{18:2}$ (between 7 and 13%). Stearic acid has a lower concentration (≈2%), and palmitoleic and linolenic acids represent less than 1% each. These results are in agreement with data reported in Codex Alimentarius (19). As previously observed (1) a statistical analysis of the data of Table 1 demonstrates that the composition in fatty acids depends on geological and climatic factors characterizing the geographical situation. By using the five most abundant fatty acids, olives harvested in France can be distinguished from the other countries investigated. In particular, the French oils have a lower palmitic acid content. However, although the centers of gravity of the French and Spanish groups are differentiated at the 95% confidence level (Mahalanobis distance) there is significant overlap between the two populations. The same behavior is observed between French and Greek, Spanish, and Greek, or French and Italian oils. The sample size for Greece is sufficiently large to investigate more specific regional effects. $C_{18:0}$, $C_{18:2}$, and $C_{18:3}$ contents are significantly different in the Aegian Islands and in Crete or the Peloponese. In the comparison of French and Italian oils, the influence of the country of production includes that of the variety, since the samples investigated were produced by only one variety in each country (Tanche for France and Coratina for Italy). The roles of variety and maturity are disassociated in the results of Table 1. Among these two factors, only variety exhibits a significant influence on the fatty acid composition. The populations of Koroneiki and Chondrolia olives are identified at the 100% confidence level (Mahalanobis distance) on the basis of their content in $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$. Variations in the contents of $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, and $C_{18:3}$ are observed as a function of the year of production. Taking into consideration only $C_{16:0}$ and $C_{18:1}$, the centers of gravity of years 1993 and 1994 are differentiated at a 100% confidence level on the basis of the Mahalanobis distance. However, a substantial overlap of the existence domains is observed.

^{13}C isotope ratios of olive oil fatty acids. The results and their statistical evaluation are given in Table 2. The mean ^{13}C content of the oil determined by IRMS for the whole population of samples is –28.7‰ with a standard deviation of 0.9‰. The means of the isotope ratios of the three main fatty acid esters have very close values: oleic acid (C18:1) –28.5, palmitic acid (C16:0) –28.8, and linoleic acid (C18:2) –29.0‰. However, as will be shown, this deceptive behavior masks some characteristic differences. First of all, the consistency was checked between the ^{13}C isotopic profiles of fatty acid esters obtained by GC-C-IRMS and the mean $\delta^{13}C$ values measured on the oil by IRMS. Since $C_{16:0}$, $C_{18:1}$, and $C_{18:2}$ acids represent at least 93–94% of the whole fatty acid content of olive oils, the overall ^{13}C parameter of the oil may be approximately computed from the individual parameters of these three components using the mass and isotopic balance equation.

The mean standard deviation (MSD) between the experimental and computed values is less than $2.5 \cdot 10^{-6}$ in abundance units. In δ units (Eq. 2) the MSD is less than the experimental precision, 0.2‰. This result shows that GC-^{13}C-IRMS and IRMS give consistent and accurate values. On the other hand, since the derivatization does not induce significant isotopic fractionation (20), the true carbon isotopic composition of the free fatty acid (FA) could be estimated from the isotopic abundance measured on the methyl esters (FAME); but from an analytical point of view, considering $\delta^{13}C$ (FAME) instead of $\delta^{13}C_{(FA)}$ does not induce any bias when the same methanol pool is used and the data in Table 2 may be safely discussed in terms of origin recognition.

Mean $\delta^{13}C$ values, corresponding to groups of samples classified according to different geographical, temporal, or botanical criteria, are given in Table 2. To attempt to explain the origin of the variations in the $\delta^{13}C$ values of fatty acids, it is more convenient to consider differences between the three acids rather than absolute values. Thus it is calculated that the

TABLE 1
Influence of the Geography, Year of Production, Botanical Conditions, and Maturity on the Fatty Acid Composition of Olive Oils, Expressed in %w/w

Geographical situation		Number[a]	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Fatty acids								
Country	Greece	166	11.14	0.67	2.82	77.29	7.42	0.66
			1.22	0.16	0.31	2.84	2.29	0.10
	France	6	8.39	0.50	2.75	82.10	5.64	0.63
			0.05	0.10	0.02	0.07	0.06	0.01
	Italy	5	10.26	0.57	2.65	76.81	9.05	0.66
			1.35	0.23	0.12	3.14	1.41	0.03
	Spain	11	10.69	0.89	3.22	77.85	6.74	0.61
			2.05	0.42	0.90	3.82	2.03	0.05
	All	188	11.00	0.68	2.83	77.46	7.37	0.66
			1.35	0.19	0.37	3.00	2.26	0.09
Region (Greece)	Center	14	11.19	0.67	2.59	76.57	8.32	0.67
			1.45	0.17	0.46	3.20	2.06	0.13
	Crete	50	11.16	0.64	2.93	77.52	7.10	0.64
			0.69	0.10	0.27	1.83	1.43	0.06
	Islands	21	10.62	0.61	2.73	76.06	9.25	0.73
			1.50	0.23	0.22	3.16	3.10	0.12
	Peloponnesus[b]	76	11.30	0.71	2.80	77.47	7.06	0.66
			1.33	0.16	0.30	3.18	2.34	0.10
	All	161	11.16	0.67	2.81	77.23	7.47	0.67
			1.21	0.16	0.31	2.85	2.31	0.10
Elevation (Greece)	Mountain	15	10.53	0.58	2.82	78.40	7.03	0.64
			1.25	0.20	0.36	2.82	2.27	0.09
	Medium	102	11.11	0.67	2.78	76.96	7.81	0.67
			1.23	0.17	0.32	3.09	2.53	0.11
	Plain	42	11.51	0.70	2.90	77.37	6.86	0.67
			1.10	0.12	0.28	2.14	1.57	0.09
	All	159	11.16	0.67	2.81	77.20	7.48	0.67
			1.22	0.16	0.32	2.86	2.32	0.10
Year of production								
Year (Greece)	1994	10	10.79	0.70	2.96	77.00	7.86	0.69
			0.77	0.10	0.30	2.15	1.63	0.05
	1995	21	11.99	0.80	2.76	76.19	7.50	0.76
			0.67	0.11	0.25	1.97	1.69	0.12
	1996	65	11.50	0.64	2.87	76.54	7.81	0.65
			1.21	0.14	0.37	2.86	2.46	0.09
	1997	65	10.60	0.66	2.75	78.28	7.05	0.65
			1.15	0.18	0.26	2.84	2.40	0.09
	All	161	11.16	0.67	2.81	77.23	7.47	0.67
			1.21	0.16	0.31	2.85	2.31	0.10
1996 (Greece) (Koroneiki)	Crete	16	11.18	0.63	3.08	76.94	7.57	0.61
			0.58	0.06	0.25	1.06	0.80	0.05
	Peloponnesus	16	12.09	0.66	2.94	77.48	6.17	0.66
			0.93	0.06	0.36	1.47	1.00	0.08
	All	32	11.64	0.65	3.01	77.21	6.87	0.63
			0.89	0.06	0.31	1.28	1.14	0.07
1997 (Greece) (Koroneiki)	Crete	20	10.99	0.64	2.85	78.46	6.40	0.66
			0.58	0.09	0.24	1.72	1.21	0.05
	Peloponnesus	19	10.75	0.75	2.63	79.60	5.57	0.70
			0.63	0.07	0.21	1.08	0.77	0.06
	All	39	10.87	0.69	2.75	79.01	5.99	0.68
			0.61	0.10	0.25	1.51	1.09	0.06
Botanical conditions								
Maturity (Greece)	Ripe	78	11.10	0.66	2.87	77.07	7.62	0.67
			0.96	0.14	0.28	2.60	2.31	0.11
	Unripe	15	11.08	0.61	2.89	77.78	7.00	0.63
			1.01	0.10	0.33	2.31	1.77	0.08
	Half-ripe	54	10.95	0.66	2.72	77.74	7.25	0.68
			1.35	0.16	0.35	2.57	2.28	0.08
	All	147	11.04	0.66	2.82	77.39	7.42	0.67
			1.12	0.15	0.32	2.56	2.25	0.10
Variety	Chondolia	13	11.16	0.65	2.63	76.07	8.85	0.64
			1.51	0.22	0.43	7.21	3.15	0.14
	Koroneiki	100	11.28	0.69	2.87	77.91	6.57	0.68
			0.89	0.10	0.29	1.83	1.25	0.09
	All	113	11.27	0.69	2.84	77.69	6.83	0.67
			0.97	0.12	0.32	2.29	1.73	0.09

[a]Number, number of samples investigated. Each pair of entries in the table corresponds to the mean and standard deviation of the considered set of data.

TABLE 2
Influence of Geography Year of Production, Species, and Maturity on the Carbon-13 Distribution in the Oil, Expressed in ‰/Vienna-PDB (13,15)

δ^{13}C of Fatty acid / Geographical situation		Number[a]	Oil	Palmitic $C_{16:0}$	Oleic $C_{18:1}$	Linoleic $C_{18:2}$
Country	Greece	166	–28.7	–28.8	–28.5	–29.1
			0.8	0.8	1.0	1.2
	France	6	–29.7	–29.7	–29.2	–30.2
			0.2	0.3	0.3	0.7
	Italy	5	–27.7	–27.4	–28.0	–27.7
			0.2	0.2	0.4	0.8
	Spain	11	–29.0	–29.1	–28.5	–28.9
			1.3	1.3	2.1	1.4
	All	188	–28.7	–28.8	–28.5	–29.0
			0.9	0.9	1.1	1.2
Region (Greece)	Center	14	–29.2	–29.4	–28.8	–30.0
			0.6	0.6	1.0	1.3
	Crete	50	–28.4	–28.5	–28.3	–28.6
			0.7	0.7	0.7	1.0
	Islands	21	–28.7	–29.0	–28.6	–28.8
			0.9	1.0	1.3	1.4
	Peloponnesus	76	–28.7	–28.9	–28.5	–29.2
			0.9	0.9	1.1	1.2
	All	161	–28.6	–28.8	–28.5	–29.0
			0.8	0.8	1.0	1.2
Elevation (Greece)	Mountain	15	–28.6	–28.8	–28.3	–28.8
			0.8	0.9	1.1	1.6
	Medium	102	–28.6	–28.8	–28.4	–29.1
			0.8	0.8	1.0	1.2
	Plain	42	–28.7	–28.9	–28.6	–28.9
			0.9	0.8	1.1	1.1
	All	159	–28.6	–28.8	–28.5	–29.0
			0.8	0.8	1.0	1.2
Year of production						
	1993	10	–27.8	–28.4	–28.1	–27.5
			0.8	0.8	0.8	0.7
	1994	21	–28.5	–28.8	–29.1	–28.9
			0.8	0.9	0.9	0.9
	1995	65	–28.5	–28.7	–27.9	–29.0
Greece			0.8	0.9	0.9	1.4
	1996	65	–29.0	–29.1	–28.9	–29.3
			0.7	0.8	0.9	1.0
	All	161	–28.6	–28.8	–28.5	–29.0
			0.8	0.8	1.0	1.2
1995	Crete	16	–28.1	–28.2	–28.0	–28.3
			0.7	0.7	0.6	0.7
(Koroneiki)	Peloponnesus	16	–28.3	–28.6	–27.2	–29.4
			0.8	0.7	0.8	1.6
	All	32	–28.2	–28.4	–27.6	–28.8
			0.7	0.7	0.8	1.3
1996	Crete	20	–28.7	–28.8	–28.5	–29.1
			0.7	0.7	0.7	1.0
(Koroneiki)	Peloponnesus	19	–29.3	–29.5	–29.4	–29.8
			0.7	0.8	0.8	0.9
	All	39	–29.0	–29.1	–28.9	–29.4
			0.8	0.8	0.9	1.0
Botanical conditions						
Maturity (Greece)	Ripe	78	–28.4	–28.6	–28.4	–28.7
			0.8	0.8	1.0	1.1
	Unripe	15	–28.8	–28.9	–28.2	–29.4
			0.8	0.8	1.4	1.3
	Half-ripe	54	–28.9	–29.1	–28.7	–29.5
			0.8	0.9	0.9	1.3
	All	147	–28.6	–28.8	–28.5	–29.0
			0.8	0.9	1.0	1.3
Variety (Greece)	Chondrolia	13	–28.8	–28.9	–28.5	–28.8
			0.8	0.8	1.1	1.6
	Koroneiki	100	–28.6	–28.8	–28.5	–29.0
			0.9	0.8	1.0	1.2
	All	113	–28.6	–28.8	–28.5	–29.0
			0.9	0.8	1.0	1.2

[a]Number, number of samples investigated. Each pair of entries of the table corresponds to the mean and standard deviation of the considered set of data.

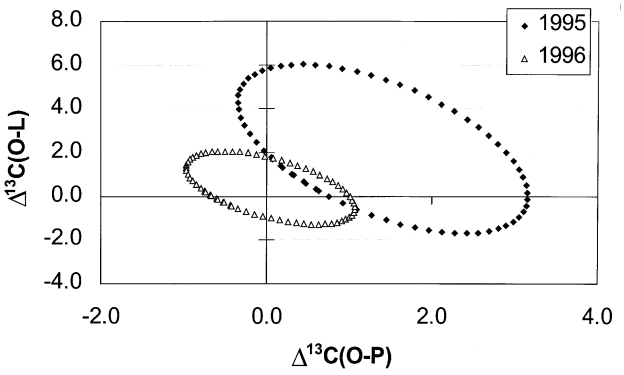

FIG. 1. Representation of the 95% bivariate domains of olive oil samples, produced in Peloponese in 1995 and 1996, on the basis of the isotopic parameters △(O–P) and △(O–L), which denote the differences in the $\delta^{13}C$ contents of oleic ($C_{18:1}$) and palmitic ($C_{16:0}$) acids and of oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids, respectively.

differences of mean values between oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids or between oleic ($C_{18:1}$) and palmitic ($C_{16:0}$) acids are frequently significant at the 99% confidence level. In contrast, nearly no meaningful differences between $C_{18:2}$ and $C_{16:0}$ can be found in most cases. The mean $\delta^{13}C$ values given in Table 2 exhibit some differences as a function of the region of production. However, only French and Italian oils are safely recognized at the 99.9% confidence level. For the geographical situations considered, oleic acid is usually enriched in ^{13}C with respect to linoleic or palmitic acid, and the differences are highly significant for the Peloponese [△(O–L) = 0.7‰ and △(O–P) = 0.4‰] and the Center of Greece [△(O-L) = 1.2‰ and △(O–P) = 0.7%]. This trend is observed whatever the elevation, and is particularly marked for moderately elevated regions [△(O–L) = 0.7‰ and △(O–P) = 0.5‰]. An influence of the climatic conditions, and especially of the amount of precipitation, is apparent in the results of Table 2. In particular, $C_{16:0}$ and $C_{18:2}$ exhibit significantly different $\delta^{13}C$ values in the Center of Greece and in Crete or the Peloponese (the Mahalanobis distances have a probability of 98.5%). In 1995, for instance, the mean differences for all regions of Greece were, respectively, equal to △(O–L) = 1.1‰ and △(O–P) = 0.8‰, and they were nearly twice as large for Peloponese [△(O–L) = 2.2‰ and △(O–P) = 1.4‰] (Table 2). For this region of production, the two parameters considered enable the 2 yr of production of olive oils, 1995 and 1996, to be differentiated at the 95% confidence level, as illustrated in Figure 1. A dependency of the ^{13}C contents on the amount of precipitation in the region of production has already been observed for grapes and wines (21). In the Peloponnesus, where the monthly mean precipitation in 1996 (110.3 mm) was about twice as high as in 1995 (57.8 mm), the $\delta^{13}C$ values for the 1995 oils are 1‰ higher than for those of 1996. A significant dependence of $\delta^{13}C$ on the maturity of the fruit is also observed (Table 2). This behavior may be compared with the results of Salmon *et al.* (22) obtained on banana aromas, which consist of aliphatic esters or alcohols resulting from the degradation of lipids. Ripe products show very small, if any, differences between oleic and the two other acids, but the differences increase with the state of unripeness: △(O–L)‰ = 0.3, 0.8, 1.3 and △(O–P)‰ = 0.2, 0.4, 0.7 for, respectively, ripe, half-ripe, and unripe olives.

From a fundamental point of view, differences in the ^{13}C contents of fatty acids are expected as a result of fractionation effects accompanying the different steps of the biosynthesis. An elongation step followed by the action of stearyl-CoA desaturase is involved in the transformation of palmitic to oleic acid (23,24), and the ^{13}C depletion of linoleic acid compared to oleic acid is consistent with studies of kinetic isotope effects on lipids (5,25,26).

The compositional and isotopic characterization of edible oils in terms of botanical and geographical factors is a considerable challenge for the analytical chemist. A study of the molecular composition and ^{13}C profile of olive oils shows that the country, the region of production, the species, and the state of maturity of the fruit may exert a significant influence. The present work delineates the ranges of natural dispersion exhibited by these two types of analysis, and the results provide a basis for investigating the distinction between olive oil and other, less expensive, oils. Indeed, olive oil is not easily differentiated from products such as olive kernel oil, "high oleic content" sunflower oil, or hazelnut oil, which exhibit similar overall and individual fatty acid carbon-13 contents. In this respect the distinction between maize and C_3 oils (olive, sunflower, hazelnut, soybean) is trivial, since the difference between the photosynthetic metabolisms induces drastic changes in $\delta^{13}C$. Consideration of the fatty acid composition improves, in some cases, the discriminating potential, since common sunflower and soybean oils are significantly depleted (enriched) in oleic (linoleic) acid with respect to olive and kernel olive oils. On the other hand, genetically modified sunflower and olive have a similar oleic acid content but differ in their palmitic acid composition. The present results, which estimate the influence of the environmental conditions on the compositional and carbon isotope parameters of olive oils, form the basis for an authentication procedure. In addition, based on the work of Quemerais *et al.* (27) on ^{2}H content in lipids, it may be expected that a determination of the deuterium distribution in olive oils will further improve the efficiency of the isotopic characterization.

In this perspective the following article of this series is devoted to an investigation of the influence of the same natural factors as those considered here on site-specific hydrogen isotope parameters.

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