Stable Isotope Characterization of Olive Oils: II-Deuterium Distribution in Fatty Acids Studied by Nuclear Magnetic Resonance (SNIF-NMR)

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ABSTRACT: Site-specific isotope fractionation of hydrogen was investigated, at natural abundance, by deuterium nuclear magnetic resonance (SNIF-NMR) on nearly two hundred olive oil samples. Owing to the complexity of the ^{2}H-NMR spectra of the mixtures of fatty acids obtained after hydrolysis of the oils, the different signals were gathered into six clusters. Knowing the contribution to the clusters of each of the four fatty acids considered ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$) and the composition of the fatty acids in the mixture, it is possible to compute the site-specific isotope ratios of the clusters from the molar fractions obtained from the ^{2}H-NMR-spectra and from the total isotope ratio of the mixture, determined by isotope ratio mass spectrometry (IRMS). The results are discussed in terms of geographical (country, region and elevation) and temporal (year) parameters and they are tentatively explained on a climatic basis.

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In the preceding article (1), gas chromatography (GC) and online GC-isotope ratio mass spectrometry (GC-C-IRMS) were applied to characterize fatty acids from olive oils produced in different regions during different years and therefore subjected to different climatic conditions. These analytical results constitute the first elements of a data bank on olive oils which could be used to tentatively authenticate the oil in terms not only of botanical genuineness, but also of geographical and temporal origin and of variety. Since isotopic fractionation depends on conversion rates and on kinetic and thermodynamic isotope effects intervening in the complex set of enzymatic reactions which govern the formation of fatty acids (2), variations in the hydrogen isotope ratios as a function of the composition may be expected. Moreover, the environmental conditions, which exert some influence on carbon fractionation, are also likely to modify the deuterium distribution. Hydrogen isotope ratios, $(D/H)_i$, at specific sites or clusters of sites in a molecule are accessible by nuclear magnetic resonance (SNIF-NMR) (3). The climatic dependence of these parameters is most pronounced in molecules such as cellulose, starch, and sugars, which are produced in the first reaction steps following photosynthesis (4). However the effect is still observable in metabolites situated farther along the biosynthetic pathway. For example, climatic dependence of the $(D/H)_i$ values was detected on nicotine molecules extracted from tobacco leaves harvested in different regions (5) and on caffeine samples extracted from coffee beans cultivated in South America and in Africa (6). The purpose of this work was primarily to elaborate an analytical procedure for determining site-specific isotope ratios, $(D/H)_i$, in order to characterize the fatty acids present in olive oil triglycerides, and secondly to investigate the dependence of the isotopic data on the region and year of production of olives.

MATERIALS AND METHODS

188 samples of olive oils mainly of extra virgin quality oil (Olea Europeae L.), four kernel olive oils, three commercial hazelnut oils, four sunflower oils (two products of a high oleic acid content), two soybean oils, and two maize oils were investigated. Among the 188 olive oils, 166 were produced in Greece, 5 in Italy, 11 in Spain, and 6 in France. Different temporal (year of production), botanical (variety and maturity of the fruit), and geographical (region of production and elevation) factors were defined for the Greek olive oil sample set in order to study their influence on the physico-chemical parameters.

Fatty acid preparation. About 3 g of oil was mixed in a flask with 30 mL of potassium hydroxide (1 mol/L in ethanol) and refluxed at 100°C for 30 min. At the end of the reaction, the ethanol was evaporated. The aliquot was 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, the solution was acidified to pH 1 with 5 mL of hydrochloric acid (18 mol/L). 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. The solution of free fatty acid was directly used in the NMR measurements.

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Stable isotope determinations. Isotope Ratio—Mass Spectrometry (IRMS): The overall, $[(D/H)_{tot}]$ ratios of the fatty acid mixtures were measured using a VG Instrument SIRA IRMS. They were computed in absolute values (ppm) by resorting to the V.SMOW reference (8) which is characterized by an isotope ratio R_{ref} = 155.16 ppm. They could be converted to the δ scale (%) by means of Equation 1:

$$\delta D_j = 1000\left(\frac{R_j}{R_{ref}} - 1\right) \quad [1]$$

The internal repeatability of the (*D*/*H*) determinations is better than 0.3 ppm, but the reproducibility measured on different samples and at different periods of time is only of the order of 0.5 ppm in terms of mean of the Mean Standard Deviations (MMSD).

NMR Spectroscopy (SNIF-NMR). The 2H-NMR spectra were recorded on a Bruker DPX 400 spectrometer operating at 61.4 MHz and fitted with a 19F field-frequency-locking device. The acquisition parameters were as follows: number of scans, 6000; sweep width, 1200 Hz; acquisition time, 3.42 s; pulse width, 90°, 16 µs; T = 303 K.

3.4 g of $CHCl_3$ were added to 1.5 g of the fatty acid mixture. Each sample was measured three times. The signal intensities were calculated using a dedicated software, adapted to oil samples, based on a complex total least-squares analysis (9). This curve-fitting adjustment in both real and imaginary domains integrates all phase, frequency, and damping parameters in a rigorous and fully automatic treatment (Eurospec program from Eurofins Scientific, Nantes, France). For spectra with reasonable signal-to-noise ratios this approach was shown to optimize the repeatability and reproducibility of the signal area determinations.

Statistical evaluation of the data. Variance analyses (ANOVA) and computation of the means, standard deviations, coefficients of variation (CV in %), and distances between centroids of groups were performed for the different sets of data classified according to the considered natural factors (1,10).

RESULTS AND DISCUSSION

Determination of site-specific isotope ratios. Since we were dealing with a mixture of fatty acid methyl esters, knowing precisely the molar composition of the mixture was a pre-requirement for computing isotope ratios. Moreover, even at high magnetic field, it is impossible to achieve a complete resolution of the individual signals of the monodeuterated isotopomers present in the hydrolysis products of triglycerides. Therefore it was first necessary to carefully assign the ^{2}H-NMR signals to appropriate clusters of isotopomers. This could be done by examing the literature (11) and by multi-dimensional NMR experiments. A limited number of pseudolorentzian signals is observed (Fig. 1) and only ten isotopic clusters containing contributions of the different fatty acids may be quantified (Table 1). As a consequence of their very small concentration in the mixtures derived from olive oils, and taking into account the overall experimental precision of the measurement, palmitoleic and linolenic acids may be safely ignored in the computation. Moreover, because of its low intensity and low signal/noise ratio, the deuterium cluster 2 (2.75–2.80 ppm/TMS), assigned to the methylene groups of linoleic and linolenic acids located between double bonds, was not computed. Therefore we are left with six deuterium clusters, j = 1,3,4,5,6,7, situated at the following chemical shifts: 5.25, 2.25, 1.95, 1.56, 1.25, and 0.85ppm/TMS. A matrix $[M_T]_{(4,6)}$ of theoretical cluster populations may be constructed from the stoichiometric populations (defined in Table 1) associated with the four remaining fatty acids (stearic, palmitic, oleic and linoleic acids) contributing to the six clusters selected (Fig. 2). A compositional matrix denoted $[M_C]_{(m,4)}$ describes the composition in the four fatty acids retained, for the i = 1 to m olive oil samples investigated. On this basis a weighted contribution $[M_W]$(m,6) of the different fatty acids to the 6 observed clusters, can be computed (12):

$$[M_W]_{(m,6)} = [M_C]_{(m,4)} * [M_T]_{(4,6)} \quad [2]$$

The isotope ratios of the six clusters considered, $(D/H)_j$, can be calculated from the molar fractions, f_j, measured by ^{2}H-NMR and the overall isotope ratio, $(D/H)_{tot}$, measured by IRMS on the whole mixture of fatty acids (13). For a given oil sample, i, the isotope ratio associated with cluster, j, $(D/H)_j$ can be computed from Equation [3] :

$$(D/H)_j^i = \frac{f_j^j}{F_j^i}(D/H)_{tot} \quad [3]$$

where f^i_j and F^i_j are the real and statistical contributions of deuterium at site j.

In order to estimate the statistical contributions it is necessary to take into account both the site populations (Table 1) and the proportions of the fatty acids in the mixture :

$$F_j^i = \frac{W_{ij}}{\sum_j W_{ij}} \quad [4]$$

where W_{ij} is the element of the matrix $[M_W]_{m,6}$ corresponding to sample i and cluster j (Eq. 2).

The $(D/H)^i_j$ values calculated on the V.SMOW scale may be transformed in δ per mL units by means of Equation 1.

Since the isotope ratio of a cluster depends on three experimental parameters, i.e. the composition in fatty acids measured by GC, the molar fractions obtained by ^{2}H-NMR, and the overall isotope ratio of the hydrolysate determined by IRMS, it is necessary to investigate the influence of experimental errors on these three parameters before any discussion in terms of authentication of oils.

Precision of the analytical determinations. The internal repeatability of the NMR measurements is defined as the Mean Standard Deviation by column (MSD) of the data matrices which contain the variables in columns and the observations in rows. For example, the variances calculated on the three

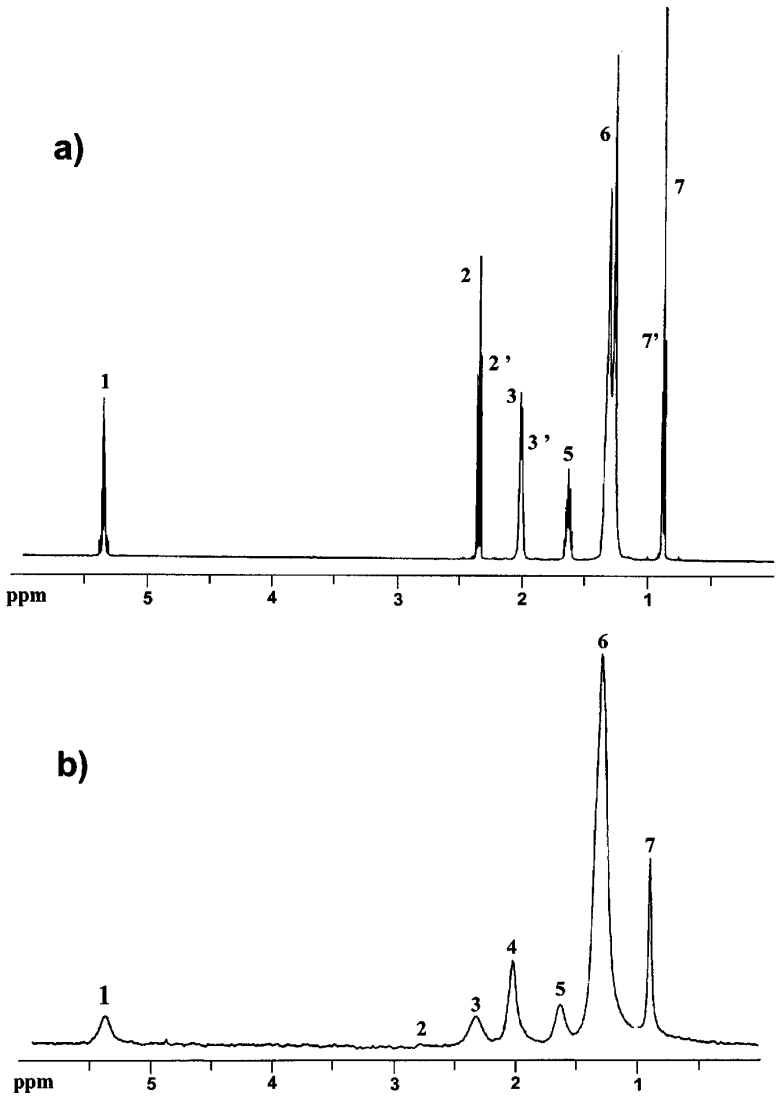

FIG. 1. Hydrogen NMR spectra of a sample of Greek olive oil. a) ^{1}H-NMR spectrum obtained at 500.1 MHz; b) ^{2}H-NMR spectrum obtained at 61.4 MHz. The assignments are described in Table 1 and Figure 2.

replicates performed for each different oil sample are pooled and the square root of the mean variance is defined as the MSD. The mean of the MSD computed for the six clusters (MMSD) is equal to 0.0035 in ^{2}H molar fraction units. However, this value conceals different situations. For example, clusters 4, 6, and 7 are measured with a precision of about 2%, whereas clusters 1, 3, and 5 are less precisely determined (6%). This loss of precision for clusters 1, 3, and 5 is due not only to a lower signal-to-noise ratio but also to more or less pronounced distortions with respect to the lorentzian shape. However, the experimental ranges of precision remain largely lower than the natural dispersion of the isotopic values.

TABLE 1
Contribution of the 4 fatty Acids Considered to the 10 Clusters Observed in the ^{2}H-NMR Spectra of Oil Hydrolysates[a]

NMR cluster	1	2	2′	3	3′	4	5	6	7′	7	
δ(ppm/TMS)	5.35	2.80	2.75	2.25	2.00	1.95	1.55	1.25	0.85	0.90	Total
Fatty acid											
Palmitic $_{C16:0}$	0	0	0	2	0	0	2	24	0	3	31
Stearic $C_{18:0}$	0	0	0	2	0	0	2	28	0	3	35
Oleic $C1_{8:1}$	2	0	0	2	0	4	2	20	0	3	33
Linoleic $C_{18:2}$	4	2	0	2	0	4	2	14	0	3	31

[a]δ is ^{1}H chemical shift of the clusters identified in Figure 2. Since an optimal resolution was not obtained in all cases, some contributions of the less abundant fatty acids were merged subsequently with that of the closest cluster: 2′ with 2, 3′ with 7′ with 7.

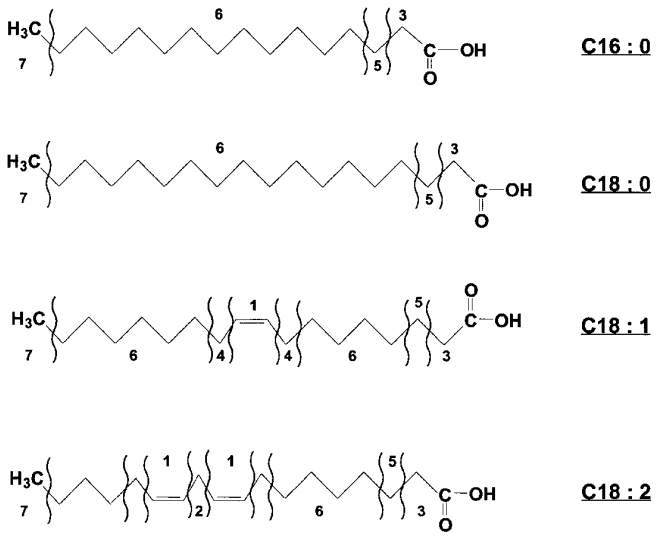

FIG. 2. Structural identification of the clusters of signals described in Figure 1 and Table 1.

The compositional study of the oil samples performed by GC has been described in the previous study (1). It was shown that only four fatty acids have concentrations that are significantly higher than 2% w/w. Since the precision of the GC and IRMS determinations is better than 0.5% (1), the main source of imprecision in the calculation of the isotope ratios of the clusters lies in the 2H-NMR determinations. It may therefore be considered that the precision of the $(D/H)_j$ values is governed by that of the molar fractions f_j.

Molecular distribution of deuterium in olive oil fatty acids. A mean value of the average isotope ratio, $(D/H)_{tot}$, associated with the whole set of fatty acids contained in the mixtures extracted from the oils has been estimated to 134.8 ppm (SD = 1.3ppm) from determinations performed on 50 olive oils. The mean values and standard deviations (SD) of the isotope ratios of clusters 1, 3, 4, 5, 6, and 7 computed for the 188 oils from different origins (Table 2a, b, and c) elicit some comments

	CH=CH	CH_2-CO	CH_2-C=	CH_2-(CH_2CO)	(CH_2)n	CH_3
Cluster	**1**	**3**	**4**	**5**	**6**	**7**
Mean	106.1	133.8	138.2	127.5	140.0	129.1
SD	4.9	10.0	5.7	10.2	2.8	5.0.

A significant depletion in deuterium is observed at the olefinic sites, **1**. This fractionation probably results from enzymatic kinetic isotope effects intervening during the oxidation reaction of the saturated precursors. The two clusters, **3** and **5**, which are related to different moieties of the acetyl CoA involved in the lipid biosynthesis, have different deuterium contents. This behavior is reminiscent of the alternation observed in the ^{13}C contents (2). Cluster **4** groups the methylenic hydrogens in an a position to one double bond. These methylene groups correspond either to the methyl or to the carbonyl position in AcCoA. The $(D/H)_4$ values remain relatively close to $(D/H)_6$, which also cumulates signals from both parts of AcCoA. It is interesting to note that, for the whole population of samples investigated, correlations are observed between the isotope ratio $(D/H)_3$ and either $(D/H)_5$ or $(D/H)_6$ (Fig. 3). The slopes b of these two correlations are of opposite signs: $b_5 = 0.78(0.04)$ and $b_6 = –0.24$ (0.01). Considering the high value of the degree of freedom, these correlations are highly significant. Since pairs of consecutive carbons of the fatty acid skeleton share common parents in the biosynthetic pathway, such correlated behaviour is not surprising.

Natural factors of deuterium discrimination in olive oils. In order to analyze the origin of the isotopic variations, the data obtained on the 188 different oils has been classified in several ways according to the specific factor considered. Table 2a shows the influence of the geographical situation and identifies the isotopic values of oils produced in different countries, in different regions of a given country, and at different altitudes. We have considered four European countries (Greece, France, Italy, and Spain), four regions of Greece (Centre, Crete, Islands, and Peloponese) and three altitudes (mountains, hills, and plains). Table 2b illustrates the influence of the year of production of the oil. Four years were investigated (1993, 1994, 1995, and 1996) and as a further refinement, the data concerning 1995 and 1996 were split into two groups of oils prepared from the same olive variety (Koroneiki) but harvested in two regions (Crete and the Peloponese) characterized by well-defined climatic conditions. Finally, Table 2c groups the data according to botanical properties of the oils: variety (Chondrolia and Koroneiki) and maturity of fruits (ripe, half-ripe, and unripe).

Geographical factors. Whereas the natural variance of the ^2H molar fractions represented by the standard deviation of the mean of each group is of the same order of magnitude as the experimental mean standard deviation for French, Italian, and Spanish oils, the dispersion observed for Greek oils is much larger. This behavior, which may be explained by the heterogeneity of the geographical and botanical situations represented in the very large data set of Greek oils, will be further discussed later on.

Although the weight of the Greek population largely exceeds that of the three other countries, typical trends may be pointed out. Clusters 3, 4, 6, and 7 are the most efficient criteria for distinguishing olive oils from the four countries considered. The differences between groups, expressed by the distances between the gravity centers are maximum for France and Greece or Italy and minimum for Greece and Italy and Italy and Spain. The discriminating potential of clusters 3 and 4, for instance, is illustrated in Figure 4. About 95% of Greek olive oils are correctly classified by discriminant analysis, but the proportion is significantly smaller (65%) for Italy and Spain (Fig. 4a). Oils from France are distinguished from those of Italy, and at 95% confidence level from those of Spain (Fig. 4b). The discriminations observed are probably explained largely by an influence of the climatic parameters on the deuterium distribution (4,14). Thus a canonical correlation has been determined between a set of two isotope ratios, $(D/H)_3$ and $(D/H)_7$, which are nearly un-

TABLE 2a
Influence of Geographical, Year of Production, and Botanical characteristics on Deuterium Distribution in Olive Oil Fatty Acids

		Nb	$(D/H)_1$	$(D/H)_3$	$(D/H)_4$	$(D/H)_5$	$(D/H)_6$	$(D/H)_7$
Country	Greece	166	105.7	135.1	139.3	128.2	139.7	130.1
			3.9	7.0	4.1	7.4	1.6	3.9
	France	6	108.2	117.7	124.7	118.9	142.1	119.5
			6.0	20.7	7.8	25.4	6.4	3.7
	Italy	5	110.5	126.7	133.6	124.1	143.5	123.8
			8.2	18.1	11.0	21.3	7.1	8.9
	Spain	11	109.0	126.1	131.3	123.3	142.7	121.9
			11.3	20.9	7.2	21.4	6.3	6.0
	All	188	106.1	133.8	138.2	127.5	140.0	129.1
			4.9	10.0	5.7	10.2	2.8	5.0
Region	Center	14	107.5	135.6	141.6	127.6	139.1	131.9
(Greece)			4.6	3.8	2.5	3.5	0.7	2.6
	Crete	50	106.0	135.0	139.0	127.5	139.7	130.0
			4.0	6.7	3.3	5.2	1.4	4.0
	Islands	21	105.4	133.3	140.7	127.3	140.0	130.8
			3.9	3.6	3.6	8.0	0.9	3.2
	Peloponese	76	105.4	136.4	139.5	129.9	139.4	129.8
			3.4	6.6	3.5	7.7	1.1	3.7
	All	161	105.8	135.5	139.7	128.6	139.5	130.2
			3.8	6.2	3.4	6.8	1.2	3.7
Elevation	Mountain	15	106.1	134.3	140.1	126.8	139.6	130.4
(Greece)			3.8	7.3	3.0	5.1	0.7	3.4
	Medium	102	106.0	135.5	139.9	128.6	139.5	130.2
			3.9	6.0	3.8	6.9	1.2	3.9
	Plain	42	105.3	135.8	139.1	129.0	139.5	130.1
			3.3	6.4	2.5	6.9	1.2	3.4
	All	159	105.8	135.5	139.7	128.6	139.5	130.2
			3.7	6.2	3.4	6.8	1.2	3.7
Year	1993	10	102.1	131.1	134.3	129.3	141.5	129.7
			5.9	11.1	5.1	9.4	2.9	4.2
	1994	21	103.0	136.9	137.6	139.2	139.1	129.1
			2.9	6.4	2.8	7.1	0.6	3.6
	1995	65	107.5	137.6	141.1	128.5	139.2	128.8
			3.3	6.3	2.7	5.2	1.0	3.9
	1996	65	105.4	133.6	139.8	125.2	139.7	132.2
			3.0	3.7	2.8	3.5	0.7	2.5
	All	161	105.8	135.5	139.7	128.6	139.5	130.2
			3.8	6.2	3.4	6.8	1.2	3.7
1995	Crete	16	108.1	140.0	139.5	129.4	139.3	126.8
(Greece)			3.6	7.5	1.8	5.7	1.1	3.8
(Koroneiki)	Peloponese	16	106.1	136.3	141.2	129.8	139.0	128.9
			2.4	5.2	1.5	4.9	0.8	4.4
	All	32	107.1	138.2	140.3	129.6	139.1	127.8
			3.2	6.6	1.9	5.2	1.0	4.2
1996	Crete	20	105.0	133.3	139.3	125.3	139.8	131.9
(Greece)			3.3	3.6	2.0	2.7	0.6	2.6
(Koroneiki)	Peloponese	19	104.5	134.0	138.1	126.2	139.6	132.6
			2.6	3.3	1.2	3.2	0.6	2.2
	All	39	104.8	133.6	138.7	125.7	139.7	132.2
			3.0	3.4	1.7	3.0	0.6	2.4

[a]The results, standardized with respect to the V. SMOW reference, are expressed in ppm.
Nb is the number of samples investigated. Each pair of entries corresponds to the mean and standard deviation of the considered set of data.

correlated (r = 0.10), and a set of climatic normals, mean temperature, T (°C), and mean monthly precipitations, P (mm) (Fig. 5). For the five regions of production, the climatic normals used in the correlation were the following: France (14.2°C, 62 mm), Italy (15.8°C, 77 mm), Spain (18°C, 47mm), Peloponese (18.1°C, 67 mm) and Crete (18.9°C, 35 mm).

A similar tendency is observed for $(D/H)_3$ and $(D/H)_5$ as a function of the elevation of the region of production: olive trees cultivated in the mountains give oils which are relatively depleted in deuterium. This behavior is somewhat similar to that discussed for nicotine extracted from tobacco leaves (5).

Temporal factors: The analysis of variance performed on the isotope ratios of olive oils collected in Greece over four

TABLE 2 (Continued)
nfluence of Geographical, Year of Production, and Botanical characteristics on Deuterium Distribution in Olive Oil Fatty Acids

		Nb[b]	$(D/H)_1$[c]	$(D/H)_3$	$(D/H)_4$	$(D/H)_5$	$(D/H)_6$	$(D/H)_7$
Maturity	Ripe	78	105.5	135.1	138.6	129.0	140.0	129.0
			4.3	8.3	4.7	8.3	2.0	3.9
	Unripe	15	105.9	136.1	140.1	130.1	139.1	130.2
			3.4	5.5	2.4	7.9	0.8	4.1
	Half-ripe	54	105.3	134.7	140.0	127.0	139.5	131.7
			3.0	4.8	2.8	4.4	0.8	3.1
	All	147	105.4	135.1	139.3	128.3	139.7	130.1
			3.8	6.9	3.9	7.1	1.6	3.8
Variety	Chondrolia	13	107.7	135.2	142.1	124.7	139.3	132.0
			3.8	4.1	3.6	4.3	1.0	2.0
	(Koroneiki)	100	104.8	134.7	138.2	128.7	139.8	129.9
			3.9	7.4	3.9	7.8	1.9	4.2
	All	113	105.2	134.8	138.6	128.2	139.8	130.1
			4.0	7.1	4.0	7.6	1.8	4.0

[a]The results, standardized with respect to the V. SMOW reference, are expressed in ppm.
Nb is the number of samples investigated. Each pair of entries corresponds to the mean and standard deviation of the data set.

consecutive years shows that most of the clusters have a high degree of discrimination. Although they behave differently, clusters 1, 3, 4, and 5 in particular enable the four years of production to be partly distinguished. The greatest differences between years are observed for 1993 and 1995 (Fig. 6a) but the distinction is more difficult between 1995 and 1996. The temporal and geographical effects can be decoupled by investigating samples produced in a given year but in different regions. The number of oils available was sufficient to enable a comparison between Crete and the Peloponese in 1995 and 1996. In this case the climatic features of the regions are not very different and it is observed that the distinction between olives harvested in a given region (either Crete or the Peloponese) during the two different years, 1995 and 1996, is better than the distinction between oils produced during the same year, 1995 or 1996, in the two regions considered (Fig. 6b). As discussed for wines (4,15) and for orange juices (16,17), the deuterium distribution in sugars from a given plant species is mainly governed by the climatic conditions of the region of production of the fruit. When the regions to be compared have very different climates, such as the Loire Valley (France) and New South Wales (Australia) in the case of wines, or Argentina and Israel in the case of orange juices, the geographical effect prevails over the temporal effect. However, for regions having relatively similar climates, such as Greece, South of Italy, and Spain, the yearly variation may overcome the regional differences.

Botanical factors: The maturity of the fruit has nearly no influence on the deuterium distribution in the fatty acids. The variety Chondrolia seems to give oils that are relatively enriched

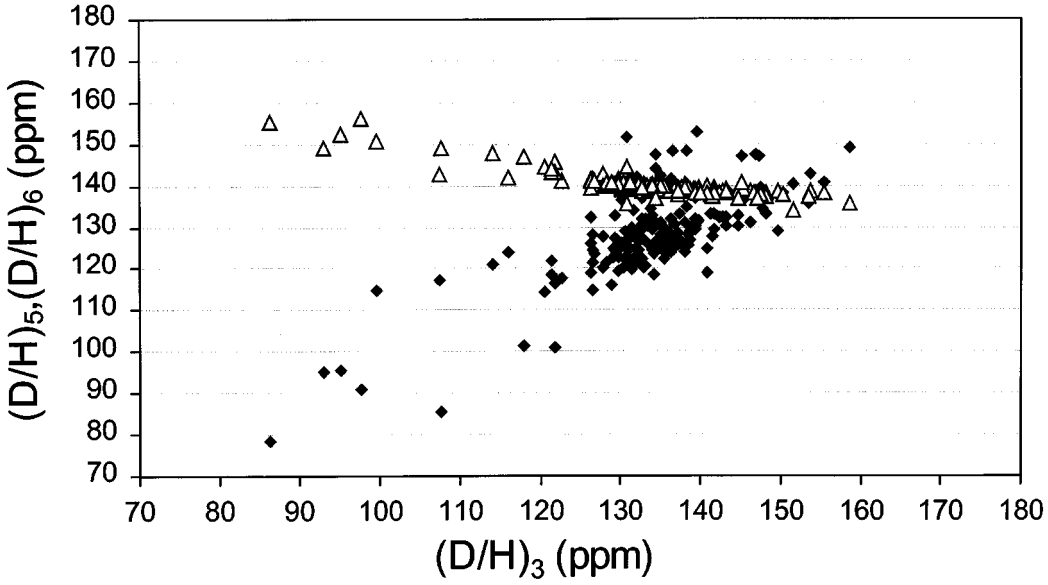

FIG. 3. Correlations between the isotopic ratios (in ppm) of clusters 5 (◆) and 6 (Δ) and that of cluster 3. Linear relationships are computed (r = 0.73 for 5 and r = 0.86 for 6).

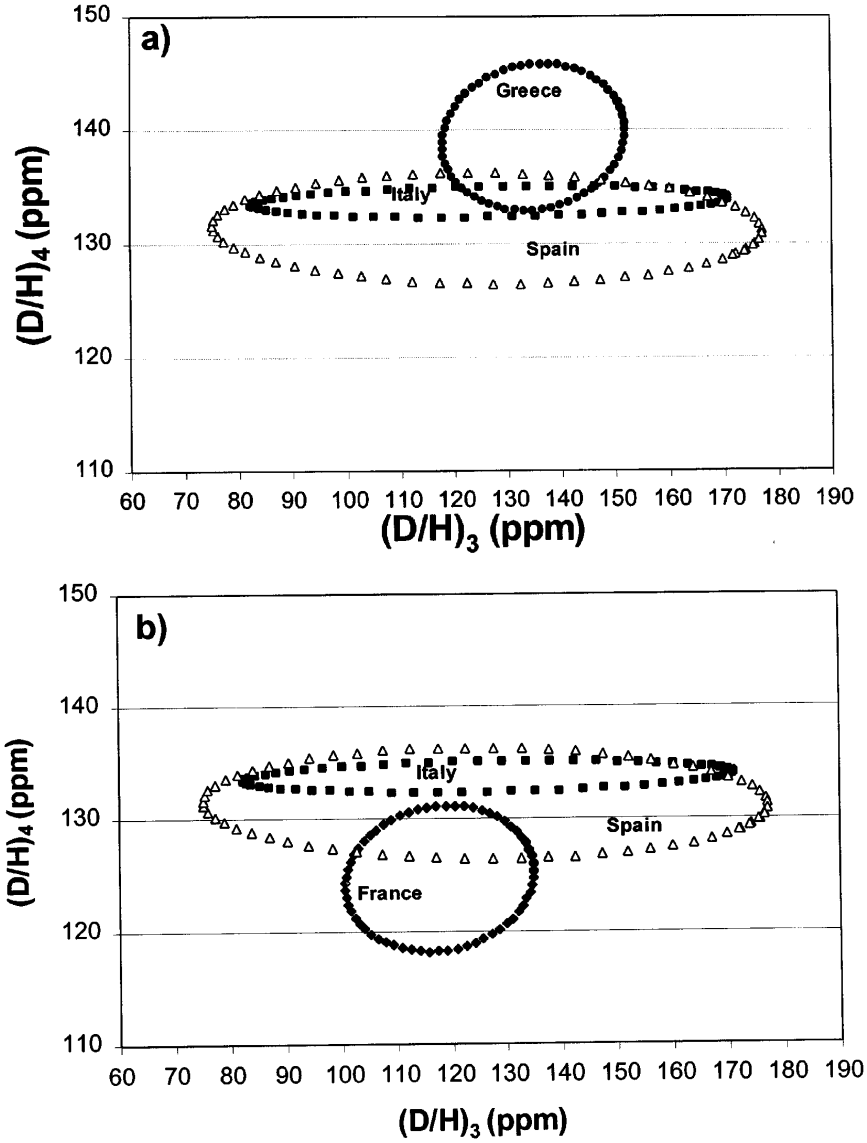

FIG. 4. Representation of olive oils produced a) in Greece, Italy, or Spain and b) in France, Italy, or Spain in the plane of the isotope ratios of clusters 3 and 4. The populations are described by their bivariate 95% confidence domains.

in deuterium with respect to the variety Koroneiki, however, the number of Chondrolia products is insufficient to fully take into account the regional and temporal effects. In this respect it should be noted that noticeable variety effects have only been observed in cases of very different precocities (4,17).

CONCLUSION

A great deal of effort has been devoted to the analytical study of edible oils (18–21). In this context, isotopic analysis is particularly well suited for characterizing the product in terms of botanical and geographical factors. For a given region of production the deuterium distribution in lipids may enable oils from different plant species to be unambiguously characterized (12). In a number of cases this distinction is possible whatever the geographical origin of the product. Thus the $(D/H)_1$, $(D/H)_3$, $(D/H)_4$, $(D/H)_5$, and $(D/H)_7$ isotope ratios of hazelnut oils present large differences from those of olive oils, and this behavior seems very promising even if the number of products investigated is not statistically significant. On the other hand, sunflower oil, even genetically modified, can be distinguished from olive oil especially by considering the isotope ratios $(D/H)_4$ and $(D/H)_5$. This observation might be very valuable from a basic point of view when it becomes possible to correlate the specific gene responsible to the increase of oleic content in genetically modified sunflower to the enzymic equipment of the lipidic elongation mechanism.

However, in order to improve the authentication potential of the method the natural dispersion of the isotopic parameters associated with the geographical and temporal factors must be estimated. In this work the relative sensitivity of isotope ratios, $(D/H)_j$, associated with different clusters of lines

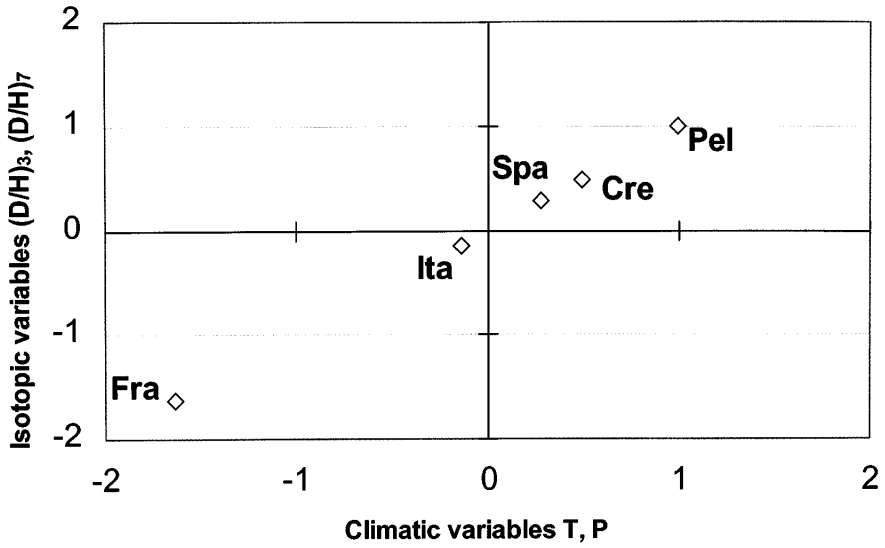

FIG. 5. Canonical correlation between the isotope ratios of clusters 3 and 7 and the climatic parameters, temperature (T) and precipitation (P) for the five regions of production considered. The climatic parameters used are the climatic normals defined by the World Meteorological Organization (Geneva). Fra (France), Ita (Italy), Spa (Spain), Pel (Peloponese), Cre (Crete).

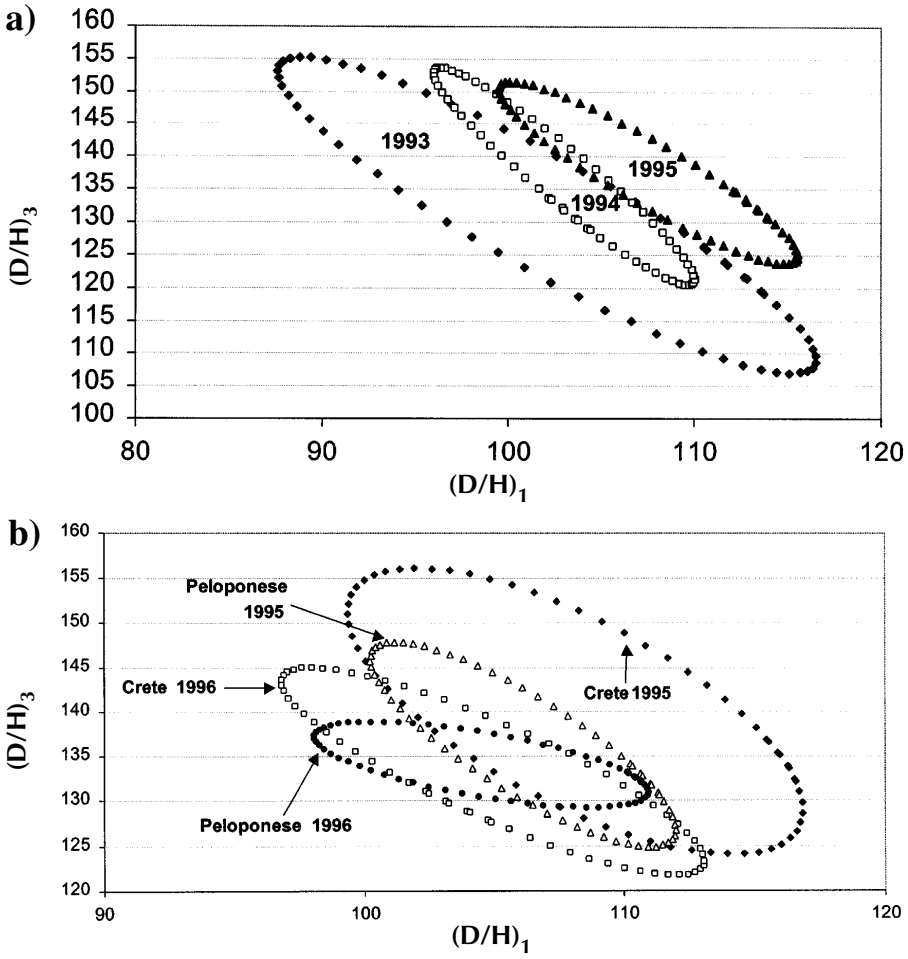

FIG. 6. Representation in the plane of the two variables $(D/H)_1$ and $(D/H)_3$ of the bivariate 95% confidence domains of olive oils produced a) during different years, 1993, 1994, and 1995 in Greece; and b) in 1995 or 1996 in Crete or Peloponese.

in the ^{2}H spectra of olive oil fatty acids has been estimated on a statistical basis. Although lipids are more remote than sugars from the first photosynthetic steps involving plant water, their isotopic contents are still influenced by climatic variations. The ^{2}H and ^{13}C distribution therefore depends on the region and on the year of production, and factors such as variety and degree of maturity exert a less important role. It has been shown recently (22) that glycerol extracted from oils, meats, and fermented beverages is a good probe for determining the origin of the products. A systematic study of glycerol from olive oils would probably improve the characterization of these oils, and work is in progress in this perspective. However, the present results provide a substantial database for origin recognition of olive oil with respect to other types of oils and for the detection of adulteration of this high-value product.

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