Characterization of Italian Extra Virgin Olive Oils Using ^{1}H-NMR Spectroscopy

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High-field (600-MHz) proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy was applied to the analysis of 55 extra virgin olive oil samples from four Italian regions (Campania, Lazio, Sicily, and Umbria) and obtained from diffrent olive varieties. The multivariate statistical analysis (PCA, hierarchical clustering) performed on the normalized intensities of ^{1}H- NMR resonances due to minor components (β-sitosterol, *n*-alkenals, *trans*-2-alkenals, and other volatile compounds) allows a good classification of oil samples obtained from traditional varieties with respect to the region of origin (96% of oils correctly classified). Samples obtained from one new experimental cultivar (FS-17) were not correctly classified, indicating a strong contribution of olive variety on the chemical composition of virgin olive oils. The potential contribution and limits of NMR in the authentication of virgin olive oil geographical origin and variety are discussed.

Keywords: *Proton NMR spectroscopy; virgin olive oil; hierarchical clustering; geographical origin; olive variety; authentication*

INTRODUCTION

In the world market, due to their sensory and nutritional quality, there is a growing interest for extra virgin olive oils. Extra virgin olive oil can be in fact considered a "natural fruit juice", since it is obtained from olive fruit only by physical operations (milling, pressing, centrifugation, filtration).

Recent international regulations have established analytical criteria to define olive oil genuineness (detection of adulterations with seed oils or other solvent-extracted oils) and quality grade (extra virgin, virgin, "lampante", "refined", "pure", etc.) (European Communities, 1991; International Olive Oil Council, 1995).

The definition of the geographical origin of extra virgin olive oils is a question only recently introduced as far as the denomination of origin is concerned. The authentication of olive oil by means of objective analytical parameters is the actual goal of several research efforts to certify the declared geographical origin. The composition of extra virgin olive oils is the result of complex interactions among olive variety, environmental conditions, fruit ripening (Solinas et al., 1987; Solinas, 1987; Patumi et al., 1992; Fontanazza et al., 1993), and extraction technology. Therefore, for the careful definition of the origin based on chemical composition many factors need to be taken into account, thus making difficult the definition of the necessary markers.

In recent years, several attempts have been made to define olive oil origin by means of multivariate analysis of chemical parameters. Forina and Tiscornia (1982), using the principal component analysis (PCA) of fatty acid composition, obtained a first classification of Italian olive oils from different regions. Using an expert system (so-called SEXIA), Aparicio et al. (1987, 1988, 1990) have studied data from different chemical analyses to classify Spanish oils with respect to their origin and variety. PCA of fatty acid and triacylglycerol profiles have been also applied for the geographical classification of Greek oils (Tsimidou et al., 1987).

No data are present in the literature about the use of high-resolution NMR spectroscopy applied to the geographical characterization of virgin olive oil. ^{1}H- and ^{13}C-NMR have been already applied in the analysis of virgin olive oil to detect adulterations with refined, esterified, and pomace oils (Sacchi et al., 1989, 1990, 1991, 1992; Zamora et al., 1994) and to evaluate virgin olive oil quality (Sacchi et al., 1990, 1994, 1996).

The aim of this work was to evaluate the possible contribution of high-field proton NMR to the geographical characterization of virgin olive oils. For this purpose, 600-MHz ^{1}H-NMR spectra were recorded on 55 extra virgin olive oil samples from four Italian regions. Quantitative data of selected resonances due to minor components (sterols, *n*-alkanals, *trans*-2-alkenals, and other volatile compounds) were analyzed by multivari-

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Table 1. Characteristics of Virgin Olive Oil Samples

code[a]	place	date[b]	variety	RD[d]	ES[e]
A1	Marcellina	Nov 12	Carboncella-Frantoio	U	P
A2	Marcellina	Nov 7	Carboncella-Frantoio	U	P
A3	Marcellina	Nov 16	Leccino-Rosciola	N	P
A4	Marcellina	Nov 14	S.Vitese-Rosciola	U	P
A5	Marcellina	Nov 23	Carboncella-Leccino	N	P
A6	Marcellina	Nov 9	Leccino-Rosciola	U	P
A7	Marcellina	Nov 12	Carboncella-Frantoio	U	P
A8	Marcellina	Nov 27	S. Vitese-Rosciola	N	P
A9	Marcellina	Nov 8	S. Vitese-Rosciola	U	P
A10	Marcellina	Nov 11	Leccino-Rosciola	U	P
A11	Marcellina	Nov 11	Carboncella-Frantoio	U	P
A12	Marcellina	Nov 26	Leccino-Frantoio	N	P
B1	Vallo Lucania	Dec 15	Pisciottana	N	P
B2	Polla	Dec 14	Gentile-Romanella	O	P
B3	Battipaglia	Dec 18	Leccino-Pendolino	N	S
B4	Montecorvino	Nov 23	Leccino	N	P
B5	Vallo Lucania	Jan 21[c]	Pisciottana	O	C
B6	Vallo Lucania	Dec 15	Rotondella	N	C
B7	Montecorvino	Dec 15	Rotondella	N	P
B8	Polla	Nov 27	Gentile-Romanella	N	P
B9	Montecorvino	Nov 29	Leccino-Frantoio	N	P
B10	Montecorvino	Nov 29	Pisciottana	N	P
B11	Vallo Lucania	Nov 20	Frantoio-Leccino	N	C
B12	Serre	Nov 30	Frantoio-Leccino	N	P
B13	Vallo Lucania	Jan 17[c]	Pisciottana	O	C
B14	Campagna	Nov 18	Leccino-Coratina	N	P
B15	Montecorvino	Nov 29	Frantoio	U	P
B16	Polla	Dec 4	Gentile-Romanella	N	P
C1	Perugia	Oct 27	Moraiolo	U	C
C2	Amelia	Oct 27	FS-17	U	C
C3	Amelia	Oct 27	Frantoio	U	C
C4	Perugia	Nov 30	Moraiolo	N	C
C5	Amelia	Nov 30	FS-17	N	C
C6	Amelia	Nov 30	Frantoio	N	C
C7	Perugia	Dec 14	Moraiolo	O	C
C8	Amelia	Dec 14	FS-17	O	C
C9	Amelia	Dec 14	Frantoio	O	C
C10	Perugia	Nov 16	Moraiolo	N	C
C11	Amelia	Nov 16	FS-17	N	C
C12	Amelia	Nov 16	Frantoio	N	C
C13	Perugia	Nov 18	FS-17	N	C
C14	Perugia	Nov 15	Frantoio	N	C
C15	Perugia	Nov 30	Frantoio	N	C
D1	Modica	Nov 10	Tonda Iblea-Moresca	N	P
D2	Modica	Nov 5	Moresca	N	C
D3	Modica	Nov 3	Moresca-Verdese-Tonda	N	P
D4	Modica	Nov 5	Tonda Iblea-Moresca	N	P
D5	Modica	Nov 6	Moresca-Verdese-Tonda	N	P
D6	Modica	Oct 27	Moresca-Verdese	N	P
D7	Modica	Nov 4	Moresca-Verdese	N	P
D8	Modica	Nov 7	Moresca-Verdese-Tonda	N	P
D9	Modica	Oct 28	Verdese-Moresca-Tonda	N	C
D10	Modica	Nov 10	Verdese-Moresca-Tonda	N	P
D11	Modica	Nov 5	Verdese	N	C
D12	Modica	Nov 2	Nocellara-Moresca	N	P

[a] Regions: A, Lazio; B, Campania; C, Umbria; D, Sicily. [b] Olives harvested in 1994. [c] 1995. [d] Ripening degree. [e] Extraction system.

ate statistical analysis (PCA, hierarchical clustering) with the aim of evaluating the possible contribution of proton NMR to the characterization of virgin olive oil and authetication of its geographical origin.

MATERIALS AND METHODS

Sampling. In Table 1 and Figure 1 the basic characteristics and geographical origin are shown for 55 extra virgin olive oil samples produced in extraction plants located in four Italian regions: Campania, Lazio, Sicilia, and Umbria. Extra virgin olive oils were extracted from olive batches of known place, date of production (October 1994−January 1995), and variety. Oils coming from some typical varieties of different regions (Table 1), as well as oil samples from the FS-17, a new experimental variety produced from breeding of Frantoio (Patent IRO-CNR no. 245 NV/88), were studied. Ripening degree was evaluated on the basis of the percent of green,

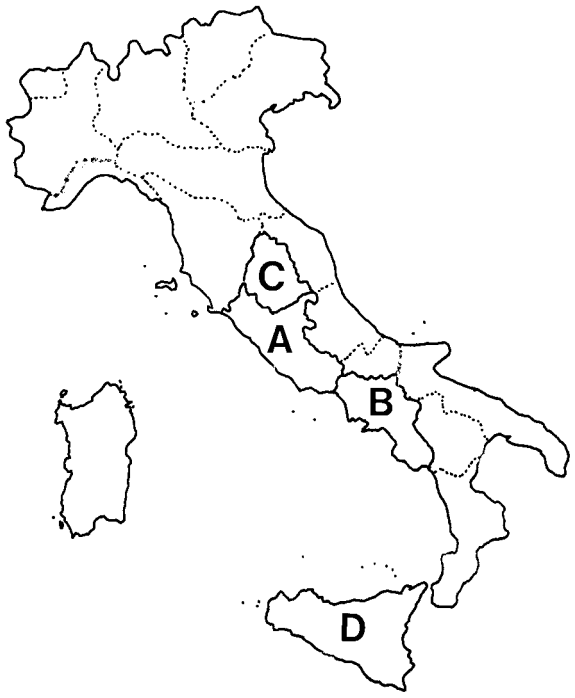

Figure 1. Italian regions from which extra virgin olive oil samplings were made: (A) Lazio; (B) Campania; (C) Umbria; (D) Sicily.

aging, and over-ripe olives. Oil samples were classified in three ripening classes, defined as unripe (U), normal ripe (N), and over-ripe (O), corresponding to the values of the ripening index proposed by Uceida and Frías (1975) of 1−2, 3−5, and 6−7, respectively. Extra virgin olive oils were extracted within 24 h after olive harvesting. Three phases of centrifugation or traditional pressing were used as extraction systems with process temperatures <35 °C. Two Spanish samples (Ojiblanca and Picual varieties) were also analyzed to obtain a preliminary comparison with oils of a quite different origin. Italian regions were selected on the basis of their latitude variation (two central regions, a southern region, and the island of Sicily) (Figure 1). Samples A1−A12 were produced in the Lazio region (Province of Roma, Sabina), B1−B15 in the Campania region (Province of Salerno, from two areas of Colline Salernitane and Cilento), C1−C15 in Umbria, and D1−D12 in Sicily (Modica). All samples considered in this study were extra virgin olive oils according to the official analytical methods and limits (European Communities, 1991; International Olive Oil Council, 1996).

NMR Analysis. Oils (20 μL) were placed into 5-mm NMR tubes and dissolved in chloroform-d (0.7 mL) and DMSO-d_6 (20 μL). One-dimensional spectra were recorded on a Bruker AMX600 (Karlsruhe, Germany) instrument operating at 600.13 MHz. Spectra were acquired and processed in the phase-sensitive mode (TPPI) according to the conditions previously described (Sacchi et al., 1996).

Statistical Methods. NMR data analysis was performed by using the S-plus statistical system (Venables and Ripley, 1994). The aims of the statistical analysis were twofold: (a) to select the most important frequency bands, that is, those which have the highest discrimination power to distinguish between oils of different regions; and (b) to classify oils with respect to the selected frequency bands.

Variable Selection. To satisfy the first requirement, a PCA was performed, and nonrendundant variables (frequency bands) were selected according to the method described by Mardia et al. (1979). The number $n = N$ of selected variables, among the N measured ones, was chosen by the following procedure: (i) let $n = N - 1$; (ii) choose the n most important variables; (iii) classify the oils with the methods described, applied to the n variables chosen, and (iv) if the classification is the same as that given retaining all of the variables, then

Table 2. Chemical Shift Assignment of Selected ^{1}H-NMR Resonances Used in the Multivariate Data Analysis

peak	δ (ppm)	group	multiplicity	compound
1	9.70	−CHO	broad singlet	*n*-alkanals
2	9.61	−CHO	doublet	branched alkanals[a]
3	9.54	−CHO	doublet	branched alkenals[a]
4	9.45	−CHO	doublet	*trans*-2-alkenals
5	8.00	−CH	singlet	unknown volatile compound
9	4.53	nd	doublet	unknown volatile compound
8	4.627	nd	singlet	unknown volatile compound
7	4.654	nd	singlet	unknown volatile compound
6	4.886	nd	singlet	unknown volatile compound
10	0.622	$-CH_3$	singlet	β-sitosterol

[a] Tentative assignment.

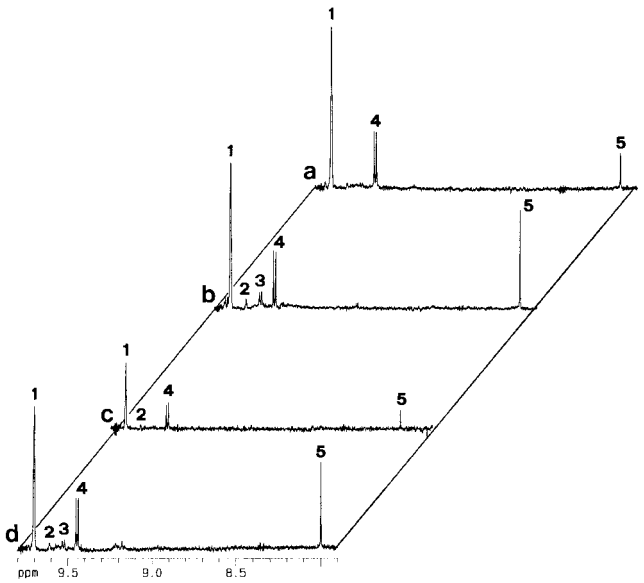

Figure 2. Expansion of the 7.8−9.8-ppm region from the 600-MHz ^{1}H-NMR spectra of four Italian extra virgin olive oil samples: (a) Umbria; (b) Sicily; (c) Campania; (d) Lazio. Labeled peaks are assigned in Table 2.

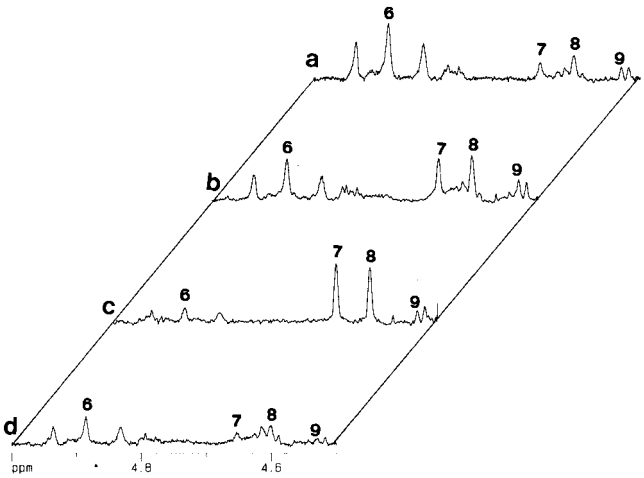

Figure 3. Expansion of the 4.5−5-ppm region from the 600-MHz ^{1}H-NMR spectra of four Italian extra virgin olive oil samples: (a) Umbria; (b) Sicily; (c) Campania; (d) Lazio. Labeled peaks are assigned in Table 2.

put $n = n - 1$ and go to (ii); otherwise put $n = n + 1$ and stop. In the present case, all of the $n = 10$ variables were used for classifying the oils.

Classification. Given $n = 10$ variables (see Table 2), the oils were classified by using the single-linkage method of cluster analysis (Mardia et al., 1979). Specifically, a dissimilarity matrix was computed by measuring the distance between two oils O_1 and O_2 by the following expression (Manhattan metric):

$$\sum_{j=1}^{n} |O_1(j) - O_2(j)|$$

Then, for a given threshold Δ, a graph $G\Delta$ was built, the vertices of which are associated with the oils and an arc connects two vertices, if the distance between the corresponding oils was less than Δ. The clustering associated to Δ was the set of the connected components of $G\Delta$ (clusters). Therefore, two oils, O_1 and O_2, were in the same cluster, at level Δ, if there exists a path in the graph $G\Delta$ connecting them. The values Δk, $K = 1, \ldots, M$ of Δ, that give rise to distinct clusterings were univocally determined using the dissimilarity matrix. It turns out that the clusterings are nested. Therefore, the final result of the single-linkage cluster analysis was the set of all M clusterings, which can be represented as a dendrogram (see Figure 6). The value of Δk can be read on the vertical axis.

The procedure described above has been implemented in the S language (Becker et al., 1988) and makes use of macros (PCA, hierarchical clustering) belonging to the S-Plus system (Venables and Ripley, 1994).

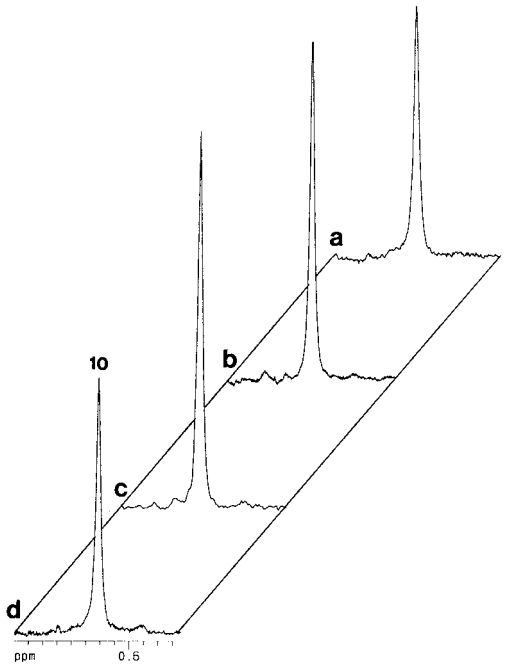

Figure 4. Expansion of the 0.56−0.70-ppm region from the 600-MHz ^{1}H-NMR spectra of four Italian extra virgin olive oil samples: (a) Umbria; (b) Sicily; (c) Campania; (d) Lazio. Peak 10 corresponds to methyl protons (C18) of β-sitosterol (Table 2).

RESULTS AND DISCUSSION

In a previous work we have studied the high-field ^{1}H-NMR spectra of virgin olive oils and assigned some minor resonances to *n*-alkanals, *trans*-2-alkanals, and other volatile and steroidal compounds (Sacchi et al., 1996).

In the present study, minor resonances from the ^{1}H-NMR spectra of 55 Italian extra virgin olive oils were carefully measured and quantitative data were submitted to multivariate statistic analysis. NMR data were analyzed on the basis of their ability to differentiate the four groups of samples from corresponding different Italian regions (PCA coupled to a variable selection

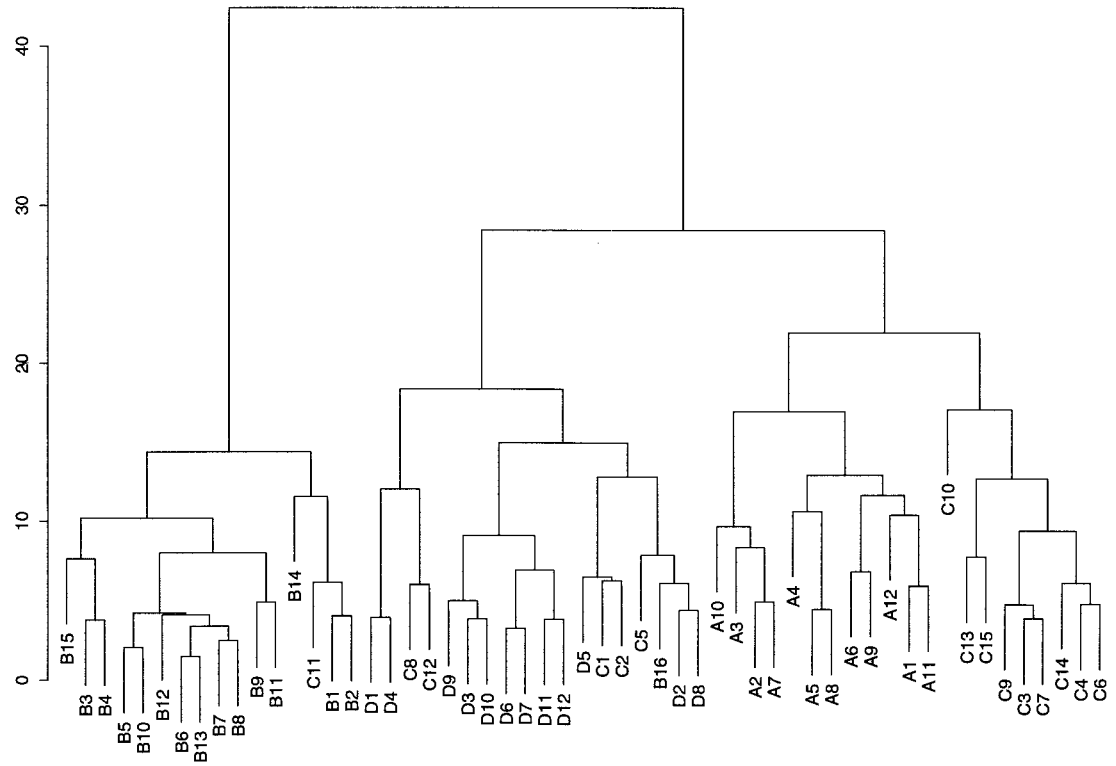

Figure 5. Dendrogram showing the clustering of the 55 extra virgin olive oil samples based on NMR dataset. Samples labeled with the same letter come from the same Italian region: (A) Lazio; (B) Campania; (C) Umbria; (D) Sicily. Details on samples are reported in Figure 1 and Table 1.

method to select the independent variables most useful in classifying oils).

Among >30 resonances considered, only 10 variables (peaks labeled in Figures 2−4 and assigned in Table 2) resulted in significant discrimination between virgin olive oils of different geographical origin. The selected resonances do not show a significant variability with the degree of ripening and the extraction system.

A cluster analysis was then performed using the intensity of peaks 1−10 to reveal natural grouping of oil samples. Figure 5 shows the dendrogram that exhibits the clustering of the olive oil samples grouped on the basis of their origin (A−D). The distance between oils was measured in the Manhattan metric, and the distance between clusters was given by the average of the distances between members of the clusters (*X*-axis).

A reliable classification can be observed with four groups corresponding to the four geographical regions examined, suggesting that intensity patterns from ^{1}H-NMR might be useful in determining compositional similarities linked to virgin olive oil origin. The dendrogram shows very little overlap among groups with the exception of samples labeled C2, C8, C11, C12, C1, C5, and B16 (Figure 5). Samples C2, C5, C8, C11, and C12 were oils obtained from the FS-17 variety, as indicated under Materials and Methods, a new experimental variety produced by breeding of the Frantoio variety, which cannot be considered a typical geographical variety.

Among typical varieties, 96% of samples are correctely classified with the exception of samples C1 and B16 (Figure 5). The plot of 49 samples (excluding FS-17 oil samples) on the plane defined by the first two principal variables is shown in Figure 6 from which different groups can be easily identified.

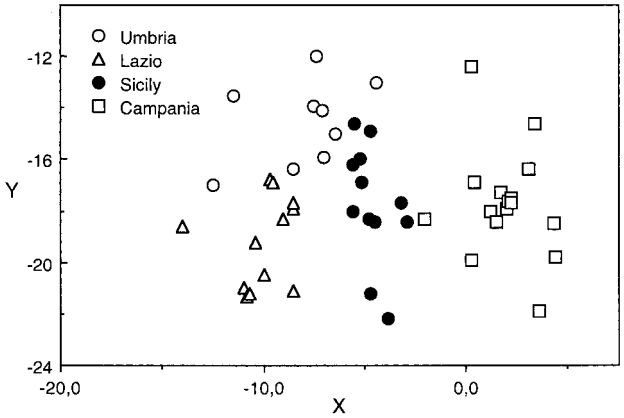

Figure 6. Plot of Italian extra virgin olive oils from different geographical regions on the plane defined by the two first principal components obtained with the selected variables (NMR intensities of peaks reported in Figures 2−4 and Table 2).

It is difficult to distinguish the effect of the variety from the effect of the environment: every Italian region has its natural, characteristic cultivars, and, to assess the relative contribution of region and variety, oil samples from the same variety obtained in different regions have to be tested. This point will constitute the main argument of a further study.

Two Spanish samples from two typical olive varieties of the Andalusia region (Picual and Ojiblanca) were also studied, and the NMR profiles were found to be significantly different from those of the 55 Italian samples studied (data not shown).

Sampling made in the present work, certainly not representative of the Italian production, offers, in our opinion, a suitable model to be expanded in future studies. The results presented, on the other hand,

suggest a possible contribution of ^{1}H-NMR in the characterization of virgin olive oils from different varieties and geographical origin.

To confirm the usefulness and limits of proton NMR in the authentication of extra virgin olive oil, an extension of the sampling to different Italian regions and Mediterranean countries is needed, as is the validation of the method in different years of harvesting.

A combined approach using multivariate statistics of analytical data from different spectroscopic and chromatographic techniques should be a way to increase the prediction ability of the whole analytical system.

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