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ARTICLE

^{1}H NMR-Based Protocol for the Detection of Adulterations of Refined Olive Oil with Refined Hazelnut Oil

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A ^{1}H NMR analytical protocol for the detection of refined hazelnut oils in admixtures with refined olive oils is reported according to ISO format. The main purpose of this research activity is to suggest a novel analytical methodology easily usable by operators with a basic knowledge of NMR spectroscopy. The protocol, developed on 92 oil samples of different origins within the European MEDEO project, is based on ^{1}H NMR measurements combined with a suitable statistical analysis. It was developed using a 600 MHz instrument and was tested by two independent laboratories on 600 MHz spectrometers, allowing detection down to 10% adulteration of olive oils with refined hazelnut oils. Finally, the potential and limitations of the protocol applied on spectrometers operating at different magnetic fields, that is, at the proton frequencies of 500 and 400 MHz, were investigated.

KEYWORDS: Hazelnut oil; olive oil; NMR; statistical analysis

INTRODUCTION

Adulteration of food products is a relevant problem from different points of view. It impacts quality and safety requirements for consumers and gives rise to a relevant economic loss. It is reported that in Europe, olive oil adulterations with hazelnut oils cause an estimated loss of 4 million euro per year. Therefore, in recent years, the development of official methods for the detection of olive oil adulterations with hazelnut oil at low concentrations has become an important issue for consumers, regulatory agencies, and olive oil suppliers. From a scientific point of view, the main analytical challenge is that hazelnut and olive oils have very similar chemical compositions. Analytical methodologies based on the determination of filberton (*1*, *2*) have given interesting results in the detection of unrefined hazelnut oils, but they do not resolve the real problem of the detection of refined hazelnut oils. Other techniques and methodologies based on free and esterified sterols (*3*−*5*), on tocopherols and tocotrienols (*6*), and on the difference between theoretical and empirical triacylglycerols (*7*) have been proposed to determine the presence of refined hazelnut oil in refined olive oil with diverse success.

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The MEDEO research project (Development and Assessment of Methods for the Detection of Adulteration of Olive Oil with Hazelnut Oil) (*8*, *9*), funded by the European Union, was aimed to develop analytical methodologies to detect adulterations of olive oil with refined hazelnut oils. It was based on the deficiency of an official standard methodology able to detect adulterations of olive oils with refined hazelnut oils within the range of interest of 10−20%. Within the project, interesting results have been obtained using FT-Raman and FT-MIR spectroscopy (*10*), fluorescence spectroscopy (*11*, *12*), mass spectroscopy (*13*, *14*), and various chromatographic techniques (*15*−*19*).

In this paper, results obtained using ^{1}H NMR spectroscopy are discussed. Many studies have been reported on the NMR analysis of vegetable oils and olive oils showing the importance of this technique in their characterization (*20*−*23*) and in the detection of fraudulent adulterations (*24*−*28*) as well. We report a detailed analytical NMR protocol to detect low levels of refined hazelnut oils in refined olive oils. It was developed within a three year study analyzing hazelnut oil and olive oil samples of different origins and their mixtures. The methodology, developed on a 600 MHz spectrometer, was tested on 10 test samples consisting of Tunisian refined olive oils and their admixtures with Turkish refined hazelnut oils (*29*) by two independent laboratories using 600 MHz spectrometers. Preliminary results on the applicability

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of the methodology on 500 and 400 MHz spectrometers are also discussed.

MATERIALS AND METHODS

Details of the analytical protocol, developed within the three year MEDEO project, are reported according to ISO format (*30*). The protocol was optimized to be easily usable by operators with basic NMR knowledge.

Sampling. The protocol was developed using three sets of 92 hazelnut and olive oil samples and their mixtures: a training set for the calibration of the methodology, a test set for the verification of the methodology, and a validation set for peer studies and blind trials. Samples were collected by the International Olive Oil Council (IOOC) according the following considerations: (i) olive oils (monovarietal and blend cultivar) have a chemical composition (fatty acids and sterols) similar to that of hazelnut oils; (ii) the current major problem is the adulteration of refined olive oils with refined hazelnut oils; (iii) the most common blends are made by adding Turkish hazelnut oils, as the cheapest hazelnut oil is produced in Turkey, which accounts for 80% of the world production of hazelnut, to olive oils from the main producer countries, either within the European Union (Greece, Italy, and Spain) or outside the European Union (Morocco, Tunisia, and Turkey).

Therefore, although different fraudulent mixtures of oils from any variety or geographical origin are possible, the adulteration being a secret of fraudulent sellers, the collected commercial hazelnut and olive selected samples can be considered "real" samples covering the main possible kinds of adulteration. Samples from different cultivars and geographical origins were selected using the information on the chemical composition reported previously (*16*).

The oil preparation was carried out according to the procedure hypothetically followed by defrauders, refining lampant virgin olive oil and raw hazelnut oil together or simply spiking virgin and refined olive oils with raw and refined hazelnut oils. Olive oil samples of the training and test sets were of the same cultivar but from different geographical origins. To overcome the influence of the extraction process, virgin and refined olive and hazelnut oils were included in the training set. Some conventional chemical analyses (fatty acids, sterols, triglycerides) were also performed.

Materials and Sample Preparation. Oil sample (50 μL) is introduced directly into a 5 mm NMR tube with 700 μL of $CDCl_3$ and carefully homogenized by hand shaking for 3 min. High-purity (99%) deuterated chloroform ($CDCl_3$) (CAS Registry No. 865-49-6) stabilized on silver foils has to be used. The solvent has to be stored in a refrigerator when not used for the preparation of NMR tubes. Volumetric pipets of appropriate size and calibrated according to standard procedures have to be used for appropriate oil and solvent handling.

The preparation was performed under a fume hood. Refer to the statements corresponding to the following risk and safety numbers before use: R, 22-38-40-48/20/22; S, 36/37.

Sample Storage and Preservation. Samples have to be stored in the dark and in temperature-stable (about 13−18 °C) conditions to preclude oil degradation.

Instruments. This protocol, developed on a 600 MHz instrument and tested on two 600 MHz spectrometers, can be applied on any 600 MHz instrument. Any NMR probe head with a ^{1}H channel can be used provided that the quality requirements described below are met. Interesting results can be also obtained using 500 and 400 MHz instruments.

A Bruker Avance AQS600 instrument (software: XWIN NMR package from Bruker) equipped with a 5 mm probe operating at the ^{1}H frequency of 600.13 MHz (B_o = 14.3 T) was used to develop the protocol.

The NMR spectrometers used by the peer laboratories to test the protocol were a Bruker Avance AQS600 instrument (software: XWIN NMR package from Bruker); a 600 MHz INOVA Varian spectrometer Inova (software: WIN NMR package from Bruker); a 500 MHz Bruker Avance AV500 spectrometer (software: XWINNMR 3.1 from Bruker); a 500 MHz Bruker AMX500 equipped with an autosampler (software: XWINNMR 3.1 package from Bruker); and a 400 MHz Bruker Avance DPX400 instrument equipped with an autosampler (software: XWINNMR 2.6 package from Bruker).

The statistical elaboration of the NMR data was performed by means of SPSS for Windows (version 6.0; 1993) and Statistica package for Windows (version 5.1, 1997). Principal component analysis (PCA), linear discriminant analysis (LDA), and linear multiple regression models, previously reported in olive oil analysis (*31*), were applied to analyze the data.

The PCA provides a global overview of the compositional variability in the samples through the projection of the NMR data into hyperspaces defined by linear combinations, that is, principal components (PCs) of spectroscopic variables. The PCs are calculated to represent the maximum of variance in the NMR data set. The percentage of variance for each specific factor gives the contribution of the factor to the grouping, whereas the variable loadings allow the variables with the highest power to be selected.

The LDA is a classification model that needs a priori knowledge of sample belonging to specific classes. The LDA variable coefficients were used to build equations to predict the percentage of hazelnut oil additions.

Multiple regression models were built on the NMR data obtained on spectrometers operating at different magnetic fields. The results are given as R^2, Durbin−Watson, and p level. The R^2 value is an indicator of how well the model fits the data: an R^2 close to 1.0 indicates that the variables specified in the model account for almost the whole variability. The Durbin−Watson statistic is useful for evaluating the presence or absence of a serial correlation of residuals and therefore for estimating the model reliability. The residual represents the difference between predicted and real values. If the residuals turn out to be independent according to the Durbin−Watson table, the system is extremely reliable with a good prediction capacity. Note that in multiple regression models all statistical significance tests assume that the data consist of a random sample of independent observations. The p level gives the probability of error involved in accepting an observed result as valid: according to conventions on general research experience, results that yield a p level of ≤ 0.05 (probability of error = 5%) are considered to be statistically significant.

The repeatability test, performed on the test samples at 600 MHz, is provided as a percentage of the relative standard deviation (RSD).

To estimate the reliability of the prediction model in the case of 600 MHz data, the root-mean-square errors of prediction (RMSEP) was applied using data from one of 600 MHz spectrometers to build the model and data from the other 600 MHz spectrometer as test set and vice versa.

Acquisition of ^{1}H NMR Spectra. Before the ^{1}H NMR spectrum can be acquired, the field homogeneity has to be optimized through a careful shimming.

The quality of the ^{1}H NMR spectrum has to be evaluated on each sample according to the spectral resolution estimated using the signal at 4.33 ppm due to α' CH_2 resonance of the triglycerol moiety (see the inset in **Figure 1**): the intensity of the minimum between peaks A and B must not exceed 25% of the intensity of the B signal peak.

The ^{1}H NMR spectra have to be acquired using the following conditions: a 90° flip angle; 32K data points; a relaxation delay of 1 s; a spectral width of 12 ppm; 256 scans after 16 dummy scans. Using these conditions, the total experimental time for each sample is about 30 min, including manual or automatic sample changer, lock, tuning, shimming, and acquisition. The temperature of the sample in the probe has to be set at 300 K.

Depending on the actual probe sensitivity, the number of scans can be increased to reach the optimum level of signal-to-noise ratio. The signal-to-noise ratio has to be calculated using the spectral window in the 0.68−0.72 ppm range, which includes the CH_3-18 resonance of β-sitosterol (see **Figure 1**), and the noise spectral region in the 0.30−0.35 ppm range. Using the above acquisition parameters, a signal-to-noise ratio of at least 600 has to be obtained.

Processing of NMR Data. ^{1}H NMR spectra are obtained by the Fourier transformation (FT) of the free induction decay (FID), applying a zero-filling procedure and a line-broadening factor of 0.3 Hz.

Phase Correction. The resulting ^{1}H NMR spectrum has to be manually phased by applying zero- and first-order phase corrections, taking care to achieve good symmetry on all peaks.

Chemical Shift Calibration. To obtain a correct assignment of the ^{1}H NMR signals and to ensure a good reproducibility of the baseline correction, a precise chemical shift calibration is necessary. The resonance of residual light $CHCl_3$ in the deuterated solvent is set at 7.28 ppm, and all chemical shifts are reported with respect to this signal.

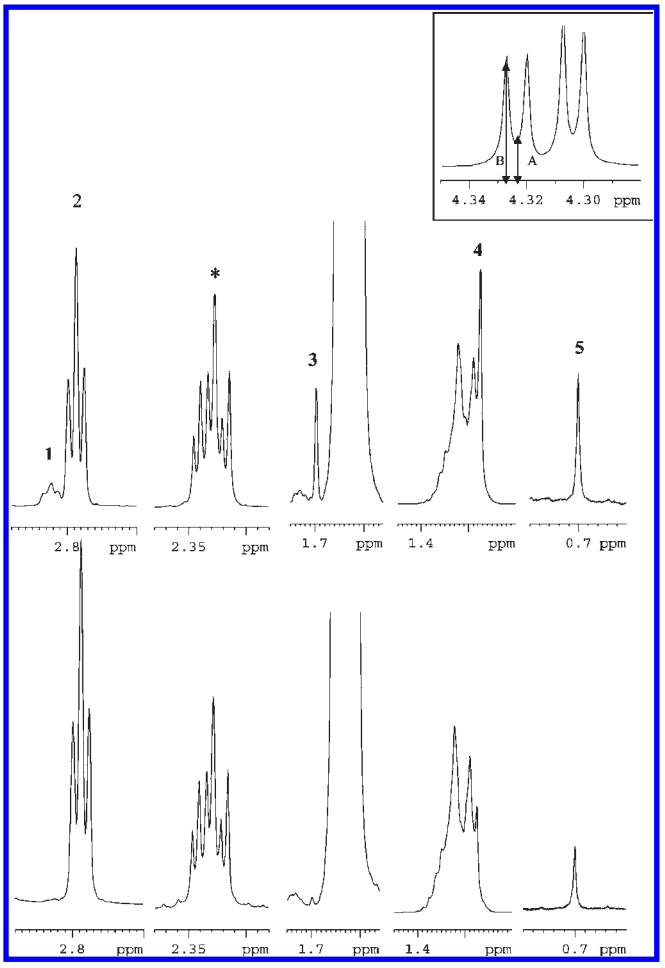

Figure 1. ^{1}H NMR resonances selected for statistical analyses in the 600.13 MHz ^{1}H spectrum of an olive oil (top trace) and an hazelnut oil (bottom trace). Peaks: 1, diallylic protons of linolenic acid, 2.82 ppm; 2, diallylic protons of linoleic acid, 2.78 ppm; 3, a signal due to squalene, 1.69 ppm; 4, methylenic protons of palmitic and stearic fatty chains, 1.27 ppm; 5, methyl-18 of β-sitosterol, 0.70 ppm. The reference peak at 2.32 ppm is also reported (∗). In the inset, the spectral region used to estimate the spectral resolution ppm is reported: the height of the minimum between A and B must not exceed 25% of the B signal intensity.

Baseline Correction. To obtain a quantitative comparability of the spectra, the baseline has to be corrected using a multipoint correction. In particular, the Cubic Spline Baseline Correction routine in the Bruker TOPSPIN software can be used. To use correctly this method and avoid baseline distortions, it is important to choose points close to the signal of interest and to have a uniform distribution of the points in the whole spectrum.

Signal Intensity. The protocol requires the measurement of the intensity of some selected resonances. To propose an easy procedure, only five resonances (**Figure 1**) with the highest discriminant power between hazelnut and olive oils are selected using ANOVA: signal 1, diallylic protons of the linolenic acid at 2.82 ppm; hazelnut oils contain an extremely low amount of linolenic fatty acid with respect to olive oils; signal 2, diallylic protons of the linoleic acid at 2.78 ppm; the linoleic fatty chain is more abundant in hazelnut oils with respect to olive oils; signal 3, CH_3-17 and CH_3-29 of squalene at 1.69 ppm; hazelnut oils contain an extremely low amount of squalene with respect to olive oils; signal 4, methylenic protons from all saturated fatty chains including palmitic and stearic residues at 1.27 ppm; hazelnut oils contain an extremely low amount of saturated chains with respect to olive oils; signal 5, CH_3-18 of β-sitosterol (0.70 ppm); hazelnut oils generally contain a low amount of β-sitosterol with respect to olive oils.

Hereafter, these variables are reported according to the above numeration. The intensity of these five signals has to be measured according to the following procedure. The resonance at 2.32 ppm due to the α-carboxyl protons of all acyl chains is chosen as internal reference, and the intensity of the five selected signals is measured with respect to this reference peak set to 1000. Note that due to the selection of the peak at 2.32 ppm as an internal intensity standard, it is extremely important to correct perfectly the baseline in the 2.19−2.46 ppm range.

It is important to emphasize that, despite the similarity in the chemical composition of hazelnut and olive oils, the intensity of these five signals and therefore the concentration of the related compounds can be considered to be specific of the type of oils. This means that all examined hazelnut oils of different origins contained extremely low amounts of linolenic acid, saturated acid, squalene, and β-sitosterol and a high amount of linolenic acid with respect to the olive oil samples (see **Figure 1**).

RESULTS AND DISCUSSION

Validation and Testing of the Protocol. The protocol, described in detail in the previous section and developed on 92 oil samples and their mixtures, was tested on a set of 10 refined Tunisian olive oil samples with a 0, 10, 15, and 20% addition of refined Turkish hazelnut oils using two independent 600 MHz spectrometers. The intensity of the five selected variables (**Figure 1**) was measured on the ^{1}H spectra of both spectrometers and submitted to PCA (see **Figure 2A**,**B**). An extremely good separation of the samples according to the percentage of hazelnut oil was obtained using both spectrometers. Four groups corresponding to 0, 10, 15, and 20% adulteration levels are easily identifiable. In one case (**Figure 2A**), the first PCA factors 1 and 2 together are responsible for 99.7% of the variance, with factor 1 responsible for 98.9%. Variables on factor 1 have the same discriminant power as depicted by their similar loading values (signal 1, 1.00; signal 2, −0.99; signal 3, 1.00; signal 4, 0.99; signal 5, 0.99). Moreover, the positive signals of 1, 3, 4, and 5 variable loadings and the negative signals of 2 variable loading are consistent with the olive oil composition, which is marked by high amounts of linolenic acid, squalene, saturated fatty acids, and β-sitosterol and a relatively reduced concentration of linoleic acid when compared to the composition of hazelnut oils.

In the second case (**Figure 2B**), the first factors 1 and 2 together are responsible for 97.3% of the variance, with factor 1 responsible for 94.04%. Variables on factor 1 have the same discriminant power as depicted by their similar loading values (signal 1, 0.99; signal 2, −0.94; signal 3, 0.98; signal 4, 0.98; signal 5, 0.96), confirming the same results obtained on the other 600 MHz spectrometer.

The results of the PCA obtained by putting together the results obtained from the two spectrometers are reported in **Figure 2C**. Again, a good classification according to the level of adulteration was obtained. Factors 1 and 2 together are responsible for 96.4% of the variance. The major contribution to this grouping is given by factor 1, which is responsible for 92.8% of the variance. The variables have the same discriminant power as depicted by their similar loading values (signal 1, 0.97; signal 2, −0.96; signal 3, 0.97; signal 4, 0.98; signal 5, 0.94).

To assess the repeatability of the method and to verify the applicability of the protocol, the same 10 samples were analyzed five times using the same spectrometer. The obtained values, reported as RSD, show a very good repeatability (< 2.5%) of the protocol for all signals of the different mixtures (**Table 1**).

Statistical Models. Two types of models are built to predict the percentage of hazelnut oil additions in olive oils. The first model uses LDA and the corresponding equations, whereas the second uses a stepwise regression model. The measure of the intensity of the selected resonances is used as entry variable in the statistical models to predict the amount of hazelnut addition. It is important to specify that all of the models are reliable within

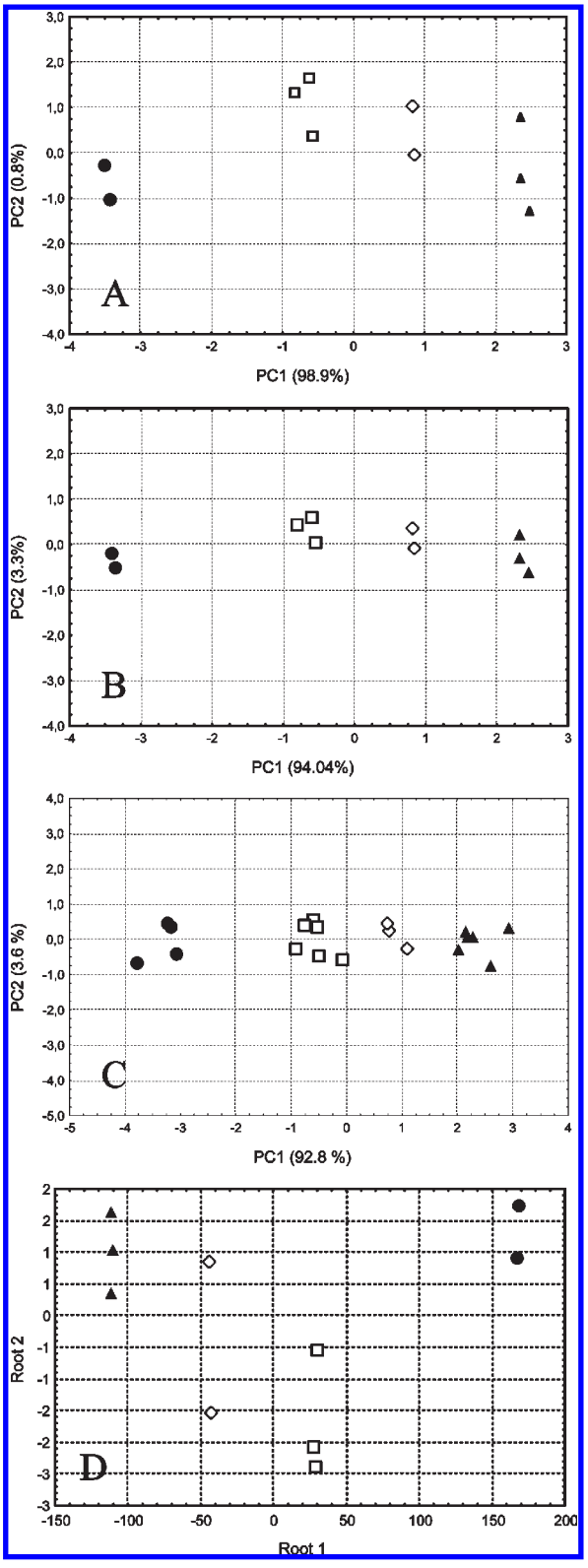

Figure 2. PCA applied to the intensity of five ^{1}H NMR resonances (see **Figure 1**) of 10 test oil samples analyzed on two independent 600 MHz instruments separately (**A**, **B**) and together (**C**). Note that in **C**, two samples corresponding to the hazelnut oil addition of 20% are completely overlapped. (**D**) LDA applied to the intensity of five ^{1}H NMR resonances of the 10 test oil samples. The addition of hazelnut oil to olive oil was 0% (●) 10% (□) 15% (◇), and 20% (▲).

the specific built system; therefore, to obtain the best results, it is important to follow carefully the reported analytical protocol.

Table 1. Mean Intensities and Relative Standard Deviations of the Five Selected Resonances Selected in the Protocol Using a 600 MHz Spectrometer

% of hazelnut oil[a]		signal 1	signal 2	signal 3	signal 4	signal 5
0	intensity mean[b]	8.12	106.31	13.11	3031.79	5.07
	RSD%	1.73	0.47	1.02	0.91	1.60
0	intensity mean	8.12	106.40	13.25	3038.77	5.06
	RSD%	1.86	1.25	2.31	2.02	0.23
10	intensity mean	7.50	111.84	12.05	2926.17	4.91
	RSD%	1.39	0.66	1.43	1.19	1.11
10	intensity mean	7.47	112.15	12.07	2928.87	4.92
	RSD%	1.17	0.79	1.53	0.67	0.71
10	intensity mean	7.57	112.58	12.06	2934.34	4.91
	RSD%	1.73	0.73	1.44	0.50	1.07
15	intensity mean	7.25	115.24	11.13	2872.92	4.81
	RSD%	1.61	0.64	1.52	1.71	1.40
15	intensity mean	7.17	114.58	11.44	2861.30	4.81
	RSD%	1.16	0.20	0.70	0.49	0.72
20	intensity mean	6.81	117.18	10.80	2802.93	4.70
	RSD%	1.24	0.37	1.36	0.89	0.59
20	intensity mean	6.92	117.93	10.90	2816.78	4.71
	RSD%	1.42	0.34	0.88	0.67	0.88
20	intensity mean	6.80	117.20	10.74	2790.22	4.72
	RSD%	1.67	0.34	1.49	0.96	0.47

[a] For each percentage of hazelnut oil, the values obtained on a 600 MHz instrument are reported. [b] Five replicates for each sample. Signal labeling is reported in **Figure 1**.

In the case of samples analyzed using data from a 600 MHz spectrometer, the LDA and the corresponding equation obtained using the five resonances are reported in **Figure 2D** and **Table 2**, respectively. The LDA map shows a good classification of the oils according to the percentage of hazelnut oils.

In the case of the multiple regression model (see model 1 in **Table 3**), it is possible to "predict" the percentage of hazelnut oil in olive oils using only the intensity of variables 2 and 3 due to linoleic fatty chain and to squalene, respectively. The high values of R^2 together with the low values of the p level suggest an extremely good reliability of the model. However, the value of the Durbin−Watson algorithm does not allow the independence of residues to be evaluated.

To estimate the reliability of the prediction model, the RMSEP was applied using data from one 600 MHz spectrometer to build the model and data from the other spectrometer as test set and vice versa. The RMSEPs obtained for two 600 MHz spectrometers are 0.8811 and 0.6541, respectively, which correspond to R^2 values of 0.9860 and 0.9916. These values suggest a good reliability of the model.

Effect of the Magnetic Field. To investigate the potential and restrictions of the protocol when applied on instruments operating at different magnetic fields, the methodology was tested on instruments operating at the proton frequencies of 500 and 400 MHz. The same set of 10 samples used previously was analyzed on 500 and 400 MHz spectrometers. At 500 MHz, the ^{1}H spectrum of any sample shows a good resolution and allows the use of the previously selected five signals. On the other hand, at 400 MHz, the ^{1}H spectrum of an oil does not show enough resolution to allow the measurement of all signals previously selected. In fact, due to strong signal overlapping, the squalene

Table 2. Equation Roots Relative to LDA Obtained at Different Magnetic Fields

Figure	no. of samples	field (MHz)	root	coefficient of variables in the root[a]
2D	10	600	1	= 4.672 [1] − 10.628 [2] + 26.343 [3] + 0.193 [4] + 130.981 [5] − 340.130
			2	= 18.619 [1] + 0.204 [2] + 17.670 [3] − 0.064 [4] − 5.266 [5] + 116.551
3C	10	500	1	= 34.823 [1] − 1.877 [2] + 18.271 [3] − 0.106 [4] − 16.064 [5] + 92.445
			2	= −5.529 [1] + 0.148 [2] + 4.054 [3] − 0.014 [4] − 0.182 [5] + 21.519
3E	10	400	1	= 3.769 [1] − 1.446 [2] + 0.007 [4] + 8.902 [5] + 60.845
			2	= 0.592 [1] + 0.170 [2] − 0.009 [4] + 6.922 [5] − 27.230

[a] In brackets is given the intensity of the selected resonances, 1, 2, 3, 4, and 5 (see **Figure 1**).

Table 3. Multiple Regression Models Obtained at Different Magnetic Fields

model	no. of samples	field (MHz)	dependent variable: % of hazelnut[a]	R^2	*p* level	Durbin−Watson
1	10	600	= 0.727 [2] − 5.057 [3] − 10.387	0.99961	0.0000	2.58595
2	10	500	= −4.370 [1] + 0.571 [2] − 2.528 [3] + 18.710	0.99841	0.0000	1.93668

[a] In brackets is given the intensity of the selected, 1, 2, and 3 signals (see **Figure 1**).

resonance at 1.69 ppm is not measurable. Therefore, to analyze the data together, it was necessary to reduce to 4 the number of variables submitted to the statistical analysis.

The PCA performed on all samples acquired at different magnetic fields is reported in **Figure 3A**.

A clear systematic effect of the magnetic field due to a different resolution and sensitivity of spectrometers operating at different magnetic fields is observable along factor 1, which explains 51.9% of the total variance. The effect of hazelnut oil addition is observable along factor 2, which is responsible for 33.8% of the variance. This result suggests analysis of the data on the two spectrometers, separately.

500 MHz Spectrometer Results. The intensity of the five selected resonances was submitted to PCA (**Figure 3B**). Factors 1 and 2 together are responsible for 96.7% of the variance, factor 1 being responsible for 91.1%. A good classification according to adulteration levels is obtained. The variables have the same discriminant power as depicted by their similar variable loadings (signal 1, 0.97; signal 2, −0.94; signal 3, 0.99; signal 4, 0.96; signal 5, 0.91). Again, the sign of loading values suggests that only linoleic fatty chain is more abundant in hazelnut oils with respect to olive oils.

A preliminary attempt to build statistical models was also performed. It is important to emphasize that this attempt aims only to investigate the potential of the protocol on data from the 500 MHz instrument. The LDA and the corresponding equation obtained at this magnetic field are reported in **Figure 3C** and **Table 2**. A good classification of the oils according to hazelnut presence is obtained.

In the case of samples analyzed using this magnetic field, the multiple regression model (**Table 3**, model 2) requires the measurement of three signals, namely, variables 2 and 3, as in the previous case, and variable 1 due to linolenic fatty chain. It is important to note that a major number of variables is necessary when a lower magnetic field is used. The high value of the R^2 parameter together with the low value of the *p* level suggests a good reliability of the model. Again, according to the Durbin−Watson table, the value of the Durbin−Watson parameter does not allow the independence of residues to be evaluated.

400 MHz Spectrometer Results. At this magnetic field, it was necessary to reduce to 4 the number of variables submitted to statistical analysis due to the squalene signal overlapping. The PCA performed on the four variables (**Figure 3D**) shows four groups consisting of oil samples with 0, 10, 15, and 20% hazelnut oil addition. Factors 1 and 2 together are responsible for 95.4% of the total variance, factor 1 being responsible for 85.8%. The variables have the same discriminant power having a similar variable loading (signal 1:, 0.96; signal 2, −0.96; signal 4, 0.86; signal 5, 0.91). Again, the sign of loading values suggests that only the linoleic fatty chain is more abundant in hazelnut oils with respect to olive oils.

In the case of samples analyzed using this magnetic field, the LDA and the corresponding equation are reported in **Figure 3E** and **Table 2**.

In this case, due to the limited number of variables, it was not possible to build a reliable regression model: in fact, model parameters such as *R*, *p* level, and Durbin−Watson turned out to be not acceptable.

The results reported in this paper show the potential of ^{1}H NMR spectroscopy as an analytical tool to detect adulteration of refined olive oil with refined hazelnut oil. It allows low levels (10%) of refined hazelnut oil in olive oils to be detected. The described NMR methodology is simple, sensitive, fast, and reproducible. It does not require any extraction procedure and can be used to detect olive oil adulteration either as an autonomous technique or, even better, as a complementary test together with other techniques. In addition, with respect to other spectroscopies, it does not have problems in signal quantification, allowing an easy quantification not only of major components present in olive oils, that is, unsaturated and saturated fatty chains, but also of minor components such as squalene, terpenes, and β-sitosterol. The developed methodology, tested by independent peer laboratories on 600 MHz instruments, can be used on any 600 MHz spectrometer and can be easily implemented using spectrometers operating at 500 MHz. The 400 MHz spectrometer providing spectra with a lower resolution does not allow a regression model with acceptable parameters to be obtained.

The main disadvantage of the NMR methodology is the instrumentation cost. However, because NMR spectroscopy is considered to be the most valuable instrument for analytical, inorganic, organic, physical, and medicinal chemistry, as well as for biology and biophysics, it is easily accessible in many laboratories and industrial companies.

It is important again to emphasize that an official method for the detection of adulteration of refined olive oil with refined hazelnut oil has not yet been established and that different methodologies, although giving promising results, are far from being "perfect", each one having advantages and disadvantages. In our opinion, due to the complexity of the problem, the correct way to face this type of fraud is to analyze the potential adulterated sample using one of the most promising methodology and then to confirm the results with the other complementary techniques, such as those reported below, according to the specific problem.

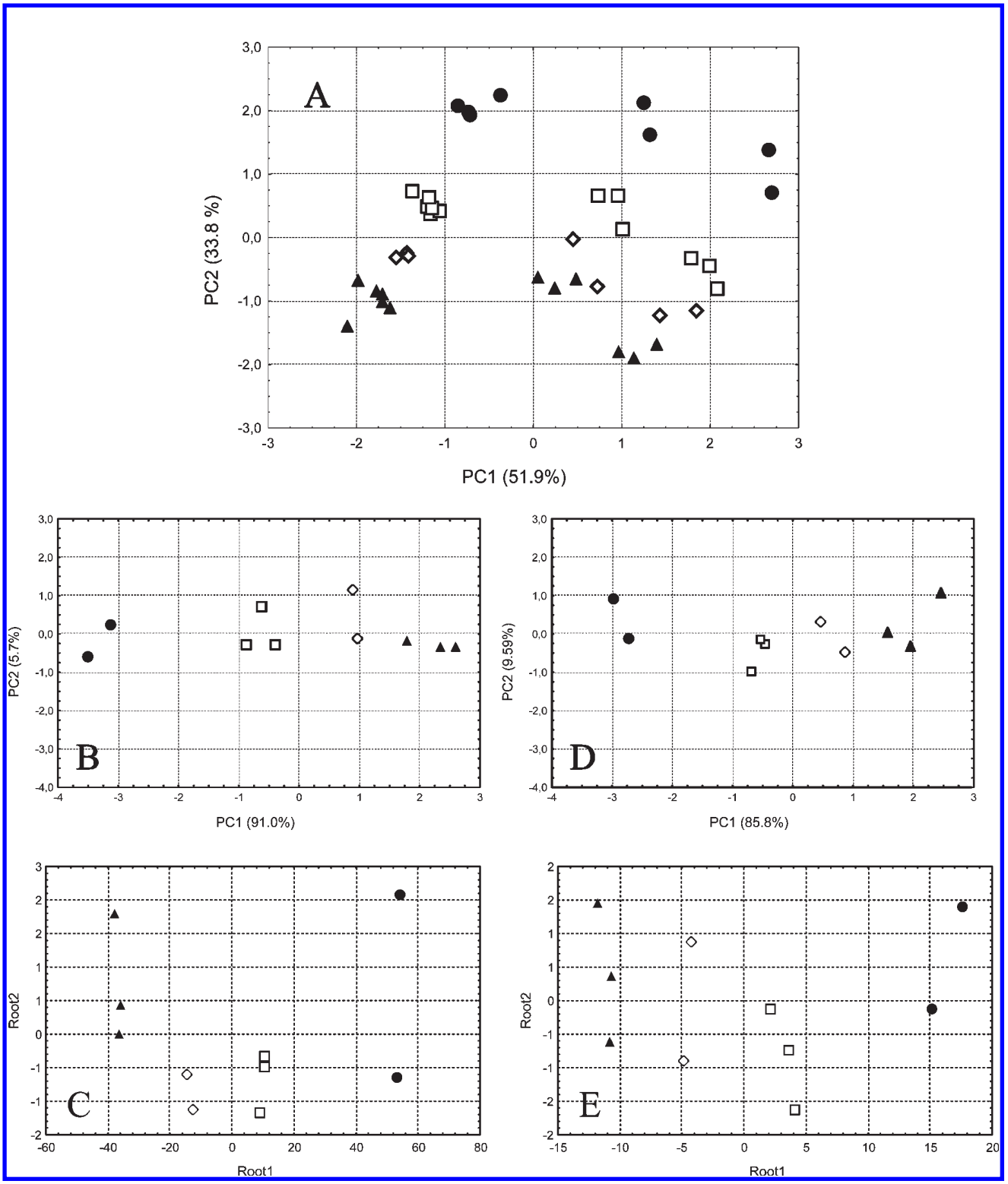

Figure 3. (**A**) PCA applied to selected ^{1}H NMR resonances (see **Figure 1**): intensity of 10 test samples using 600, 500, and 400 MHz spectrometers. PCA and LDA (**B**, **C**) applied on a 500 MHz spectrometer; PCA and LDA (**D**, **E**) on a 400 MHz spectrometer. The addition of hazelnut oil to olive oil was 0% (●) 10% (□) 15% (◇), and 20% (▲).

Analytical methodologies based on the determination of volatile compounds such as filbertone (*1*, *2*) in spiked olive oil samples using headspace-program temperature vaporization−gas chromatography−mass spectrometry are extremely promising, but they give good results only in the detection of unrefined hazelnut oils in olive oil, volatile compounds being easily removed upon gentle deodorization of the oils.

Some interesting studies (*32*) have proposed γ-lactones as chiral markers to detect adulterated olive oils, but further research is still needed to establish if these markers are useful to improve the reliability of the declaration of an oil as genuine or adulterated with hazelnut oil.

Extremely good results have been obtained using the chromatographic methodology proposed by Mariani et al. (*3*, *4*) and based

on the determination of esterified sterols in olive oils. This methodology allows even a 6−8% olive oil admixture with hazelnut oil to be detected. Similar levels of detection of hazelnut oil in olive oil were also attained with the method based on triglycerides (*7*), the so-called Global Method, although it shows better repeatability and reproducibility values.

Finally, some spectroscopies such as Fourier transform infrared (FT-IR) and Raman spectroscopy (*10*) have been also used for the detection of adulteration of olive oil with hazelnut oils, revealing an 8% of hazelnut oil addition for blends obtained by mixing Turkish hazelnut and olive oils.

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