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Quality Assessment of Olive Oil by ^{1}H-NMR Fingerprinting

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1. Introduction

Olive oil is the oil extracted exclusively from the fruit of *Olea europea* L. only by means of mechanical methods or other physical procedures that do not cause any alteration of the glyceric structure of the oil thus preserving its characteristic properties. Olive oil is a highly appreciated edible oil, which is an important component of the Mediterranean diet, and is recognized for its potential health benefits.

The International Olive Council (IOC) establishes the definitions and classes of olive oils, based on methods of production and the free acidity of the oil, as well as the trade standard for their commercialization (International Olive Council, 2011). Much analytical work has been done on the authentication and quality assessment of this high added value agricultural product, as well as on the detection of its adulteration for both economic and health considerations (Frankel, 2010; Guillen & Ruiz, 2001). However, these issues continue to be major analytical challenges. In this context, the European Commission launched the TRACE project (http://www.trace.eu.org/) through the Sixth Framework Program under the Food Quality and Safety Priority with the aim of providing reliable analytical strategies to address this kind of problem.

This chapter reports research work on the use of ^{1}H-NMR fingerprinting, combined with pattern recognition techniques, for the quality assessment of olive oil. Two major issues have been studied, the geographical origin of virgin olive oil (VOO) and the stability of VOO at room temperature.

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1.1 Geographical origin of virgin olive oil

At present, 75% of the global production of olive oil takes place in the Mediterranean basin, mainly in Spain, Italy and Greece (International Olive Council, 2011). VOO is permitted to be marketed under a Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), or Traditional Specialty Guaranteed (TSG) label, on the basis of its area and method of production [Council Regulations (EC) No 510/2006 and No 509/2006]. In this context, the characterization of the geographical origin of VOO is becoming increasingly important. According to the EU definition, PDO products are most closely linked to the concept of *terroir* — a sense of place discernible in the flavor of the food. PDO products must be produced, processed and prepared in a specific region using traditional production methods. The raw materials must also be from the defined area whose name the product bears. The quality or characteristics of the product must be due essentially or exclusively to its place of origin, i.e., climate, the nature of the soil and local know-how. Food products with a PGI status must have a geographical link in at least one of the stages of production, processing or preparation. The European Commission has already registered in the "Register of protected designations of origin and protected geographical indications" 86 PDO and 15 PGI olive oils, produced in Italy, Greece, Spain, France, Portugal and Slovenia. As can be expected, given the financial benefits associated with these prestigious labels, it is very likely that economic fraud occurs (e.g. labeling a non-PDO product as a PDO one or adulteration with olive oils that do not fulfill the PDO requirements).

Another fraudulent practice is the mislabeling of the geographical origin of olive oils. The EU established new labeling rules that make origin labeling compulsory for virgin and extra virgin labeled olive oil [Commission Regulation (EC) No 182/2009]. So, oil produced from olives from just one EU Member State or third country has to be labeled with the name of the country of origin. VOO produced from olives from more than one EU Member State has to be labeled as a 'blend of Community olive oils', while oil produced using olives from outside the EU would be labeled as a 'blend of non-Community olive oils' or 'blend of Community and non-Community olive oils', or a reference to the EU and/or third countries of origin. Therefore, analytical methods are urgently needed to guarantee the authenticity and traceability of PDO and PGI olive oils, as well as the country of provenance, to help prevent illicit practices in this sector, and to support the antifraud authorities dealing with these issues.

VOO is made up of triglycerides (more than 98%) and minor components (about 1-2%) such as squalene, α-tocopherol, phytosterols, phenolic compounds, carotenoids, and aliphatic and terpenic alcohols, which constitute the unsaponifiable fraction of the oil (Bortolomeazzi et al., 2001; Harwood & Aparicio, 2000). The chemical composition of this fraction may vary both qualitatively and quantitatively depending on vegetal species, climatic conditions, extraction and refining procedures and storage conditions (Canabate-Diaz et al., 2007; Harwood & Aparicio, 2000), which also greatly influence the organoleptic quality and stability of the oil. The diversity and interdependence between all these factors makes it highly unlikely that these influences would be the same in different regions. Hence, the geographical characterization of VOO addresses all these agronomic, pedoclimatic and botanical aspects which are unique to the oil of each origin (Aparicio et al., 1994).

A considerable number of sensorial (Pardo et al., 2007), physical (F. Marini et al., 2004; Federico Marini et al., 2006) and chemical (Aguilera et al., 2005; Boggia et al., 2002; Lanteri et al., 2002; Federico Marini et al., 2006; Federico Marini et al., 2007) approaches combined with

statistical analysis have been used to distinguish olive oils from different types, botanical, geographical origins and pedoclimatic conditions. For this purpose, fatty acids (Matos et al., 2007; Ollivier et al., 2006), triglycerides (Aranda et al., 2004), sterols (Alves et al., 2005; Matos et al., 2007; Temime et al., 2008), phenolic compounds (Lopez Ortiz et al., 2006; Vinha et al., 2005), and pigments (Cichelli & Pertesana, 2004) have been analyzed by conventional methods that usually require time-consuming pre-treatment methods (solvent extraction, isolation and/or derivatization) followed by chromatographic techniques (Aparicio & Aparicio-Ruiz, 2000) such as GC-MS and/or GC-FID (Bechir Baccouri et al., 2007; Haddada et al., 2007; Temime et al., 2006; Vichi et al., 2005) and HPLC-MS (Canabate-Diaz et al., 2007; Lopez Ortiz et al., 2006). PDO olive oils were distinguished using physicochemical parameters of the oils and chemometric class-modeling tools (Federico Marini et al., 2006), sensory parameters and fatty acid profiles of the oils (Ollivier et al., 2006), or the oil sterol composition (Alves et al., 2005). IRMS measurements of the alcohol and sterol fractions of olive oil also proved to be useful for its geographical characterization (Angerosa et al., 1999).

Fingerprinting techniques such as NMR (Mannina & Segre, 2002), NIR (Mignani et al., 2011), MIR (Reid et al., 2006), fluorescence (Kunz et al., 2011), FT-IR, FT-MIR and FT-Raman (Baeten et al., 2005; Lopez-Diez et al., 2003; Yang et al., 2005) spectroscopies, MS (Vaclavik et al., 2009), GC (Pizarro et al., 2011; Vaz-Freire et al., 2009) and DNA fingerprinting (Martins-Lopes et al., 2008; Ranalli et al., 2008) have been used for the determination of food authenticity (Reid et al., 2006). These types of techniques are particularly attractive since they are non selective, require little or no sample pre-treatment; use small amounts of organic solvents or reagents; and the analysis takes only a few minutes per sample. Chemometric analysis of NIR spectra of virgin olive oils allow us to determine its composition and geographical origin (Galtier et al., 2007). ^{1}H, ^{13}C and/or ^{31}P-NMR analysis of the bulk oil (Rosa M. Alonso-Salces et al., 2010b; Rosa M. Alonso-Salces et al., 2011b; Mannina et al., 2010; Petrakis et al., 2008; Rezzi et al., 2005) or the unsaponifiable fraction of olive oil (R. M. Alonso-Salces et al., 2010), in combination with multivariate techniques, have been used to distinguish VOOs from certain geographical origins. In section 3.2., the achievements of ^{1}H-NMR fingerprinting and chemometrics for the geographical characterization of VOO is reported. ^{1}H-NMR fingerprints of a statistically significant number of authentic VOOs from seven countries, namely Italy, Spain, Greece, France, Turkey, Cyprus and Syria and from three different harvests (2004/05, 2005/06 and 2006/07) were analyzed by pattern recognition and classification techniques, such as principal component analysis (PCA), linear discriminant analysis (LDA) and partial least square discriminant analysis (PLS-DA), to evaluate the best approach to identify the geographical origin at the national, regional and/or PDO level.

1.2 Stability of virgin olive oil

Another matter of major concern regarding the quality of edible oils is their oxidation, not only from the technological and economic point of view but also for safety reasons, due to the undesirable properties of some compounds produced during this process (Guillen & Ruiz, 2001).

The high resistance to oxidative deterioration of VOO is due to both its triglyceride composition, which is low in polyunsaturated fatty acids, and its antioxidant constituents, i.e. polyphenols and tocopherols. The oxidative stability of VOO has been evaluated by

several methods: Rancimat test (Di Lecce et al., 2009; Esquivel et al., 2009; Kamvissis et al., 2008; Mateos et al., 2006; Platero-López & García-Mesa, 2007), oxygen stability index (OSI) (Carrasco-Pancorbo et al., 2007; Ceci & Carelli, 2010; Cercaci et al., 2007; Gómez-Caravaca et al., 2007; Márquez-Ruiz et al., 2008), peroxide value (Carrasco-Pancorbo et al., 2007; Di Lecce et al., 2009; Márquez-Ruiz et al., 2008), AOCS method (Diraman, 2008), conjugated dienes (Deiana et al., 2002) and conjugated trienes (Hrncirik & Fritsche, 2005) analyses, K_{232} and K_{270} UV indexes (Antolin & Meneses, 2000; Cañizares-Macías et al., 2004a; Márquez-Ruiz et al., 2008; Platero-López & García-Mesa, 2007), thermogravimetry analyses (Coni et al., 2004; García Mesa et al., 1993; Gennaro et al., 1998; Santos et al., 2002; Vecchio et al., 2009), differential scanning calorimetry analyses (Vecchio et al., 2009), ^{13}C NMR on chromatographically enriched oil fractions (Hidalgo et al., 2002), high-resolution chromatographic techniques coupled with UV spectrometry or mass spectrometry (B. Baccouri et al., 2008; Gallina-Toschi et al., 2005; Tena et al., 2009), capillary electrophoresis (Carrasco-Pancorbo et al., 2007; Gallina-Toschi et al., 2005), ultrasound-assisted method (Cañizares-Macías et al., 2004b; Platero-López & García-Mesa, 2007), microwave-assisted method (Cañizares-Macías et al., 2004a), chemiluminescence (Navas & Jiménez, 2007), electron paramagnetic resonance (Papadimitriou et al., 2006), and ORAC assay (Ninfali et al., 2002), among others. Fingerprinting techniques such as NMR (Alonso-Salces et al., 2011a; Guillen & Ruiz, 2001, 2006), FTIR (Guillen & Cabo, 2000), and fluorescence (Guimet et al., 2005; Tena et al., 2009) spectroscopies, DNA fingerprinting (Spaniolas et al., 2008), electronic nose (Lerma-García et al., 2009), and Oxitest method (Kamvissis et al., 2008; Mora et al., 2009) have been also used successfully to study edible oil stability.

Most of the studies on the oxidative stability of olive oil employed questionably high-temperatures which, unfortunately, cannot be considered reliable to predict the stability of olive oils at room temperature (Frankel, 2010), i.e. under normal storage conditions. This is due to the fact that the mechanism of lipid oxidation changes at the elevated temperatures at which these experiments were run. In this sense, the rate of lipid oxidation is independent of O_2 pressure at ambient temperatures; whereas it does become dependent on O_2 pressures at elevated temperatures due to the decrease in solubility of O_2. This causes the O_2 concentration to become a significant limiting factor that increases with the degree of oxidation. For this reason, in oxidative stability studies the use of several temperatures, in a range as low as practical, preferably at or below 60 ^{o}C, is an important consideration. Moreover, polymerization and cyclization of PUFA, which mainly occurs at elevated temperatures, are not significant at room temperature. Furthermore, volatile acids that are measured by the Rancimat and OSI methods are produced only at elevated temperatures (Frankel, 2010). For all these reasons, the results of the studies on the oxidative stability of olive oil at high temperatures are neither relevant nor can be extrapolated to normal storage conditions at room temperature. Olive oil stability at room temperature is of great interest, for instance, to know its storage shelf-life. Because VOO is relatively stable to oxidation due to its particular chemical composition, there has been apparently little or no control of its stability under normal storage conditions in the past. To provide some knowledge on this issue, the stability of olive oil at room temperature while protected from light by ^{1}H-NMR fingerprinting was studied (Alonso-Salces et al., 2011a). The ^{1}H-NMR spectra of the VOO aliquots kept under these conditions, over a certain period of time, were analyzed by principal component analysis (PCA).

2. Experimental

2.1 Chemicals and plant material

Deuterated chloroform for NMR analysis (99.8 atom %D) was provided by Sigma-Aldrich Chemie (Steinheim, Germany).

Virgin olive oils (963 samples) from seven countries of the Mediterranean basin, namely Italy (661 VOOs), Spain (144 VOOs), Greece (97 VOOs), France (39 VOOs), Turkey (14 VOOs), Cyprus (6 VOOs) and Syria (2 VOOs), were collected directly from the producers (olive oil mills) from most of the main producing regions of these countries during three harvests (2004/05, 2005/06 and 2006/2007). The sample collection was carried out with the collaboration of Dipartimento di Chimica e Technologie Farmaceutiche ed Alimentari - Università degli Studi di Genova (Italia), Laboratorio Arbitral Agroalimentario (Ministry of Agriculture and Fishery, Spain), General Chemical State Laboratory D'xy Athinon (Greece), General State Laboratory (Ministry of Health, Cyprus), Departamento de Química Orgánica - Universidad de Córdoba (Spain), Istituto di Metodologie Chimiche - Laboratorio di Risonanza Magnetica Annalaura Segre – CNR (Italy), Fondazione Edmund Mach - Istituto San Michele all'Adige (Italy), and Eurofins Scientific Analytics (France), in the framework of the EU TRACE project. The true type (virgin or extra virgin) and origin of the olive oils at the national, regional and PDO level were assured. The Italian samples were representative of the olive oil producing areas, which are markedly influenced by pedoclimatic factors from the North to the South of the country.

For the study of VOO stability, about a liter of VOO was divided into aliquots contained in dark glass 40mL-vials completely filled and kept at -20°C in a freezer. Each month, over a period of more than 3 and half years (samples for the months 20th, 32nd, 38th and 42nd are missing), one vial was taken from the freezer and stored at room temperature (r.t.) in a closed box. A preliminary supposition was made; this considered that the degradation of VOO at -20°C is not significant and thus the last aliquot taken out of the freezer was time 0. All aliquots were analyzed by ^{1}H-NMR once the last sample was taken from the freezer.

2.2 NMR analysis

Aliquots of 40 μL of each VOO were dissolved in 200 μL of deuterated chloroform, shaken in a vortex, and placed in a 2 mm NMR capillary. The ^{1}H-NMR experiments were performed at 300K on a Bruker (Rheinstetten, Germany) Avance 500 (nominal frequency 500.13 MHz) equipped with a 2.5 mm broadband inverse probe. The spectra of the samples used for the study of the geographical origin of VOOs were recorded using a 7.5 μs pulse (90°), an acquisition time of 3.0 s (32k data points) and a total recycling time of 4.0 s, a spectral width of 5500 Hz (11 ppm), 64 scans (+ 4 dummy scans), with no sample rotation. The spectra of the samples used for the VOO stability study were recorded using a 6.7 μs pulse (90°), an acquisition time of 3.5 s (50k data points) and a total recycling time of 7.0 s, a spectral width of 7100 Hz (14 ppm), 32 scans (+ 4 dummy scans), with no sample rotation. Prior to Fourier transformation, the free induction decays (FIDs) were zero-filled to 64k and a 0.3 Hz line-broadening factor was applied. The chemical shifts are expressed in δ scale (ppm), referenced to the residual signal of chloroform (7.26 ppm) (Hoffman, 2006). The spectra were phase- and baseline-corrected manually, binned with 0.02 ppm-wide buckets, and normalized to total intensity over the region 4.10-4.26 ppm (glycerol signal). TopSpin 1.3 (2005) and Amix-Viewer 3.7.7 (2006) from Bruker BioSpin GMBH (Rheinstetten, Germany)

were used to perform the processing of the spectra. The region of the NMR spectra studied was 0-7 ppm for the geographical origin determination of VOOs, and 0-10 ppm in the VOO stability study. The data tables generated with the spectra of all samples, excluding the eight buckets in the reference region 4.10-4.26 ppm, were submitted to multivariate data analysis.

2.3 Data analysis

The data matrices, consisting of the ^{1}H-NMR buckets (variables) arranged in columns and VOO samples in rows, were firstly analyzed by univariate procedures (ANOVA, Fisher index and Box-Whisker plots), and afterwards, by the following multivariate techniques, already described in bibliography (Berrueta et al., 2007): unsupervised ones as principal component analysis (PCA); and supervised as linear discriminant analysis (LDA) and partial least squares discriminant analysis (PLS-DA). Statistic and chemometric data analysis were performed by means of the statistical software packages Statistica 6.1 (StatSoft Inc., Tulsa, OK, USA, 1984-2004), The Unscrambler 9.1 (Camo Process AS, Oslo, Norway, 1986-2004) and SIMCA-P 11.0 (Umetrics AB, Umea, Sweden, 1992-2005). Strategies used for variable selection in LDA and selection of the optimum number of PLS components in PLS-DA are described elsewhere (Rosa M. Alonso-Salces et al., 2010b).

For the geographical characterization of VOOs, the supervised techniques were applied to the autoscaled (or standardised) or Pareto-scaled data matrix of the VOO profiles following these steps: (*i*) the data set was divided into a training-test set and an external data set; (*ii*) the training-test set was subsequently divided into a training set and a test set several times in order to perform cross-validation; (*iii*) the training-test set was used for the optimization of parameters characteristic of each multivariate technique by cross-validation, for instance for variable selection in LDA or the number of PLS components in PLS-DA; (*iv*) a final mathematical model was built using all the samples of the training-test set and the optimized parameters; (v) this model was validated using an independent test set of samples (external data set), i.e. performing an external validation. During the parameter optimization step, the models were validated by 3-fold cross-validation (3-fold CV) or leave-one-out cross-validation (LOO). The reliability of the classification models achieved in the cross-validation was studied in terms of recognition ability (percentage of the samples in the training set correctly classified during the modeling step) and prediction ability (percentage of the samples in the test set correctly classified by using the models developed in the training step). The reliability of the final model was evaluated in terms of classification ability (percentage of the samples of the training-test set correctly classified by using the optimized model) and the prediction ability in the external validation (percentage of the samples of the external set correctly classified by using the optimized model) (Berrueta et al., 2007).

3. Results and discussion

3.1 ^{1}H-NMR spectra of virgin olive oil

^{1}H-NMR spectra of VOOs were recorded (Fig. 1). Olive oil is mainly made up of triglycerides, differing in their substitution patterns in terms of length, degree and kind of unsaturation of the acyl groups (Harwood & Aparicio, 2000). The chemical shifts of their ^{1}H signals are well known (Mannina & Segre, 2002; Sacco et al., 2000). However, the ^{1}H signals of the minor oil components, such as mono- and di-glycerides, sterols, tocopherols, aliphatic alcohols,

hydrocarbons, fatty acids, pigments and phenolic compounds (Harwood & Aparicio, 2000), are only observed by ^{1}H-NMR when their signals do not overlap with those of the main components and their concentrations are high enough to be detected (R. M. Alonso-Salces et al., 2010a; Rosa M. Alonso-Salces et al., 2010b; D'Imperio et al., 2007; Guillen & Ruiz, 2001; Mannina et al., 2003; Sacchi et al., 1996). Table 1 gathers the common ^{1}H-NMR signals of the major and some minor compounds together with their chemical shifts and their assignments to protons of the different functional groups. Several signals of minor compounds were found in ^{1}H-NMR spectra recorded because they were not overlapped by those of the triglyceryl protons: cycloartenol at 0.318 ppm and 0.543 ppm, β-sitosterol at 0.669 ppm, stigmasterol at 0.687 ppm, squalene at 1.662 ppm, sn-1,2 diglyceryl group protons at 3.71 ppm and 5.10 ppm, and three unknown terpenes at 4.571 ppm, 4.648 ppm and 4.699 ppm.

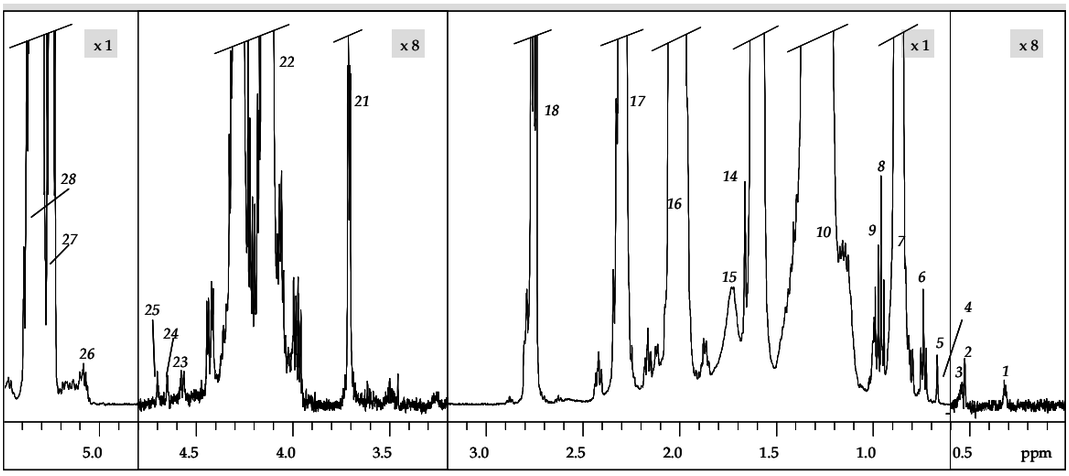

Fig. 1. ^{1}H-NMR spectra of a VOO (signal numbering, see Table 1).

3.2 Geographical origin of virgin olive oil

The large dataset of VOOs was studied regarding the situations that the antifraud authorities and regulatory bodies face. The PDO "*Riviera Ligure*", some Italian regions and the main countries that produce VOOs were used as examples to prove the potential of the tools to detect the mislabeling of non-PDO oils as PDO VOOs, or the mislabeling of the provenance of VOOs at the regional or national level. With this purpose in mind, several multivariate data analysis techniques, datasets, types of data scaling and cross-validation were evaluated to attain the best classification models for each case study.

After removing 28 extreme samples, the dataset (935 x 342) was analyzed by PCA. The four first principal components, accounting for 63% of total system variability (TSV), showed that samples were distributed in a compact cluster. However, some overlapping sub-clusters due to the harvest year were observed in the score plot of the samples in the space defined by PC2 (13% TSV), PC3 (11% TSV) and PC4 (7% TSV). Taking into account that 70% of the samples were Italian and the rest from countries in the Mediterranean region, seasonal aspects seem to affect all samples in the same way, independently of their geographical origin. Therefore, in the modeling for the authentication of agricultural food products, it is important to include chemical data of several harvests in order to obtain general classification models that allow for the seasonal variability.

#	Chemical shift (ppm)	Multiplicity[a]	Functional group	Attribution
1	0.318	d	-C*H*$_2$- (cyclopropanic ring)	cycloartenol
2	0.527	s		
3	0.543	d	-C*H*$_2$- (cyclopropanic ring)	cycloartenol
4	0.669	s	-C*H*$_3$ (C18-steroid group)	β-sitosterol
5	0.687	s	-C*H*$_3$ (C18-steroid group)	stigmasterol
6	0.740	t	-C*H*$_3$ (^{13}C satellite of signal at 0.87 ppm)	
7	0.866	t	-C*H*$_3$ (acyl group)	saturated, oleic (or ω-9) and linoleic (or ω-6)
8	0.960	t	-C*H*$_3$ (acyl group)	linolenic (or ω-3)
9	0.987	t	-C*H*$_3$ (^{13}C satellite of signal at 0.87 ppm)	
10	1.19-1.37		-(C*H*$_2$)$_n$- (acyl group)	
11	1.243		-(C*H*$_2$)$_n$- (acyl group)	saturated (palmitic, stearic)
12	1.256		-(C*H*$_2$)$_n$- (acyl group)	oleic
13	1.288		-(C*H*$_2$)$_n$- (acyl group)	linoleic and linolenic
14	1.51-1.65		-OCO-CH$_2$-C*H*$_2$- (acyl group)	
15	1.662	s	-C*H*$_3$	squalene
16	1.96-2.07		-C*H*$_2$-CH=CH- (acyl group)	
17	2.26-2.32	m	-OCO-C*H*$_2$- (acyl group)	
18	2.72-2.82		=CH-C*H*$_2$-CH= (acyl group)	
19	2.754	t	=CH-C*H*$_2$-CH= (acyl group)	linoleic
20	2.789	t	=CH-C*H*$_2$-CH= (acyl group)	linolenic
21	3.69-3.73	d	-C*H*$_2$OH (glyceryl group)	*sn* 1,2-diglycerides
22	4.09-4.32		-C*H*$_2$OCOR (glyceryl group)	triglycerides
23	4.571	d		terpene
24	4.648	s		terpene
25	4.699	s		terpene
26	5.05-5.15	m	>C*H*OCOR (glyceryl group)	*sn* 1,2-diglycerides
27	5.22-5.28	m	>C*H*OCOR (glyceryl group)	triglycerides
28	5.28-5.38	m	-C*H*=C*H*- (acyl group)	

[a] Signal multiplicity: s, singlet; d, doublet; t, triplet; m, multiplet.

Table 1. Chemical shift assignments of ^{1}H-NMR signals of the main components in VOOs.

3.2.1 PDO virgin olive oils

Under the PDO of *Riviera Ligure*, only extra virgin olive oils produced in Liguria (Italy) that fulfill the PDO requirements related to olive varieties, farming practices, oil extraction procedures, bottling and labeling (Dossier Number: IT/PDO/0017/1540, Official Journal L22 24.01.1997) can be marketed. The ^{1}H-NMR dataset of VOOs from different geographical origins and PDOs was studied to create a classification model that differentiates between VOOs belonging to the PDO *Riviera Ligure* and those not belonging to this PDO.

Univariate data analysis (ANOVA, Fisher index and Box-Whisker plots) disclosed that any single variable could distinguish between Ligurian (belonging to the PDO *Riviera Ligure*) and non-Ligurian (not belonging to the PDO) samples. So, it was necessary to apply supervised pattern recognition methods to build classification models that can distinguish VOOs of this PDO from the rest. Several multivariate approaches (LDA and PLS-DA) were tested using balanced or unbalanced data sets, different cross-validation methods (LOO and 3-fold CV), different data scaling techniques (auto-scaling and Pareto-scaling) to find the best approach for the authenticity and traceability of PDO olive oils (Rosa M. Alonso-Salces et al., 2010b; Rosa M. Alonso-Salces et al., 2011b). Table 2 summarized the results of the best classification models achieved. Both supervised pattern recognition techniques performed better if using a balanced training-test set than an unbalanced data set (Rosa M. Alonso-Salces et al., 2010b; Rosa M. Alonso-Salces et al., 2011b). PLS-DA outperformed LDA. LDA achieved classifications of around 85% of hits for both categories. PLS-DA provided a model with 5 PLS components and the boundary at 0.540, that achieved slightly better results for the Liguria class (prediction ability in the cross-validation, 86-88%; classification ability of the final model, 92%; and prediction ability of the final model in the external validation, 88%) than for the non-Liguria VOOs (86-87%, 90% and 86% respectively). These results, together with the facts that in the cross-validation the recognition ability was higher but close to the prediction ability and the classification ability of the final model was also higher but close to prediction ability in the external validation, disclosed that the model achieved was feasible and not random, as well as being well-represented by the samples in the dataset.

Regarding the most important NMR variables on the classification models provided by these pattern recognition techniques, the variables selected in LDA were among the variables that presented the highest weighted regression coefficients (Esbensen et al., 2002) in the PLS-DA models (the larger the regression coefficient, the higher the influence of the variable on the PLS model). Thus, both pattern recognition techniques arrived at consistent results, and provided information about the most important features for the characterization of PDO *Riviera Ligure* VOOs. In this sense, the variables selected for the LDA model were five NMR buckets centered at the following chemical shifts: 6.61 ppm; 5.11 or 5.09 ppm; 4.57 ppm; 4.05 ppm; and 0.33 ppm. These buckets correspond to signals of the following VOO components: phenolic compounds and unsaturated alcohols, which present characteristic resonances in the spectral region 6-7.5 ppm (Owen et al., 2000) and 4.5-5 ppm respectively; sn-1,2-diglycerides (5.09-5.11 ppm) and sn-1,3-diglycerides (4.05 ppm), due to their CH glycerol protons; and cycloartenol (0.33 ppm), to the methylene proton of its cyclopropanoic ring (Sacchi et al., 1996).

In the PLS-DA models, the variables that presented the highest weighted regression coefficients were: 6.85-6.83 ppm, 6.75 ppm, 6.67 ppm, 6.59 ppm, and 6.23 ppm belonged to

signals of phenolic compounds; 5.15-5.07 ppm were due to the CH glycerol protons of *sn*-1,2-diglycerides; 4.99 ppm to unsaturated alcohols; 4.71 ppm, 4.65 ppm and 4.57 ppm, to terpenes; 2.79 ppm, to diallylic proton of linolenic acyl group; 1.29 ppm, to methylene proton of linoleic and linolenic acyl group; and 0.33 ppm, to cycloartenol.

		Cross-validation					Model		External Validation	
			% Recognition		% Prediction		% Classification		% Prediction	
	N						132	135	67	601
	prior prob						0.49	0.51		
Technique	Miscellaneous	Validation	Lig	Non-Lig	Lig	Non-Lig	Lig	Non-Lig	Lig	Non-Lig
LDA[b]	5 NMR buckets selected: 6.61, 5.11, 4.57, 4.05 and 0.33 ppm; autoscaling	3-fold CV	84.1	85.9	84.1	83.7	82.6	85.2	86.6	79.7
PLS-DA[b]	5 PLS components selected; boundary: 0.540; autoscaling	3-fold CV	91.3	92.6	87.9	86.7	91.7	90.4	88.1	85.5
PLS-DA[c]	5 PLS components selected; boundary: 0.540; autoscaling	3-fold CV	-	-	86.4	85.9	91.7	90.4	88.1	85.5
PLS-DA[c]	5 PLS components selected; boundary: 0.540; autoscaling	LOO	-	-	87.1	85.9	91.7	90.4	88.1	85.5
PLS-DA[d]	5 PLS components selected; boundary: 0.540; autoscaling	3-fold CV/	-	-	-	-	91.7	90.4	88.1	85.5
PLS-DA[d]	4 PLS components selected; boundary: 0.520; Pareto scaling	3-fold CV/	-	-	-	-	87.1	83.0	80.6	81.0

[a] Abbreviations: N, number of samples; prior prob, prior probability; Lig, Liguria; Non-Lig, Non-Liguria; LDA, linear discriminant analysis; PLS-DA, partial least square discriminant analysis; Class codes: Liguria, 1; non-Liguria, 0.
[b] Statistica.; [c] The Unscrambler.; [d] SIMCA-P.

Table 2. Classification results obtained by supervised pattern recognition techniques for the authentication of VOO of the PDO *Riviera Ligure* using ^{1}H-NMR spectral data (balanced data set).[a]

3.2.2 Virgin olive oils from different regions

The large sample set of VOOs available was also studied from the point of view of the authentication of VOOs at the regional level, in particular, VOOs produced in certain Italian regions. The regions selected were those best represented in the dataset: Umbria (which is also a registered PDO: PDO *Umbria*), Sicily (6 PDOs: *Monte Etna*, *Val di Mazara*, *Valli Trapanesi*, *Valle del Belice*, *Valdemone* and *Monti Iblei*), Puglia (4 PDOs: *Terra d'Otranto*, *Collina di Brindisi*, *Dauno* and *Terra di Bari*), Lazio (3 PDOs: *Tuscia*, *Canino* and *Sabina*), Garda (3 PDOs: *Garda*, *Laghi Lombardi* and *Veneto Valpolicella, Veneto Euganei e Berici, Veneto del Grappa*), Campania (3 PDOs: *Peninsola Sorrentina*, *Colline Salernitane* and *Cilento*) and Calabria (3 PDOs: *Lametia*, *Alto Crotonese* and *Bruzio*). The binary classification models created for these regions were developed using an auto-scaled balanced training-test set by PLS-DA and LOO cross-validation (Rosa M. Alonso-Salces et al., 2010b). The final models were also evaluated by external validation. The results are summarized in Table 3.

Origin	Cross-validation			Model	External Validation		
Binary model[b]	***N***	***prior prob***	**% Prediction**	**% Classification**	***N***	***prior prob***	**% Prediction**
Umbria	*35*	*0.45*	71.4	82.9	*12*	*0.014*	50.0
Non-Umbria	*43*	*0.55*	74.4	79.1	*845*	*0.986*	74.8
Sicily	*54*	*0.47*	92.6	98.1	*24*	*0.029*	87.5
Non-Sicily	*62*	*0.53*	85.5	88.7	*795*	*0.971*	85.8
Puglia	*47*	*0.42*	68.1	72.3	*22*	*0.027*	81.8
Non-Puglia	*64*	*0.58*	62.5	71.9	*802*	*0.973*	65.1
Lazio	*40*	*0.49*	80.0	97.5	*19*	*0.022*	73.7
Non-Lazio	*41*	*0.51*	68.3	90.2	*835*	*0.978*	69.3
Garda	*36*	*0.46*	72.2	91.7	*13*	*0.015*	69.2
Non-Garda	*43*	*0.54*	74.4	90.7	*843*	*0.985*	80.1
Campania	*21*	*0.43*	71.4	81.0	*7*	*0.008*	57.1
Non-Campania	*28*	*0.57*	64.3	78.6	*879*	*0.992*	62.9
Calabria	*17*	*0.38*	70.6	94.1	*5*	*0.006*	60.0
Non-Calabria	*28*	*0.62*	85.7	96.4	*885*	*0.994*	79.9

[a] See abbreviations: Table 2; Models obtained by PLS-DA using autoscaling, LOO and The Unscrambler; Class codes: "Region", 1; "non-Region, 0.

[b] Binary models: Umbria *vs.* non-Umbria: 2 PLS components, boundary at 0.525; Sicily *vs.* non-Sicily: 3 PLS components, boundary at 0.460; Puglia *vs.* non-Puglia: 2 PLS components, boundary at 0.4435; Lazio *vs.* non-Lazio: 4 PLS components, boundary at 0.515; Garda *vs.* non-Garda: 3 PLS components, boundary at 0.555; Campania *vs.* non-Campania: 2 PLS components, boundary at 0.430; Calabria *vs.* non-Calabria: 3 PLS components, boundary at 0.445.

Table 3. Classification results obtained by supervised pattern recognition techniques for the authentication of VOO from certain Italian regions using ^{1}H-NMR spectral data.[a]

The model obtained to authenticate VOOs from Sicily recognized 98% of the Sicilian oils and 89% of the non-Sicilian ones and managed to correctly predict in the cross-validation step 93% and 86% of Sicilian and non-Sicilian oils respectively. Since this model achieved similar predictions in the external validation (higher than 85% of hits for both categories) to those in the modeling step, it can be considered stable and robust. In contrast, the models created for other regions such as Lazio, Garda and Calabria, were not so satisfactory: although the classification abilities were close to 90% of correct hits or even higher, the prediction abilities in the cross-validation were from 10 to 24% lower, which meant that the classification results were very dependent on the samples included in the training set in the modeling step. This also occurred for Umbria and Campania, but the models achieved about 80% of correct classification for the training set, and predictions on the test set were more than 10% lower, except for the oils belonging to the non-Umbria category (5% less). The external validation

of some models (only 50% of Umbria, 57% of Campania, and 60% of Calabria VOOs were correctly predicted) confirmed that the classes were not well represented in the modeling step. Puglian VOOs, as well as non-Garda VOOs, were much better predicted in the external data set (82% of hits) than in the cross-validation (68% and 72% of hits respectively). This was probably due to the way samples were divided into the training-test set and the external set: the PCA scores of all the VOOs were regarded to select samples from the whole cloud of points including the borders. This procedure assured that the training-test set was representative of all the samples (at least of the 3 harvests studied), however the predictions on the external set could be overoptimistic.

Regarding the most influential variables, i.e. those with the highest weighted regression coefficients, on the binary PLS-DA models achieved for each region are the following. The signals due to cycloartenol (0.31-0.33 ppm) and *sn*-1,2-diglycerides (5.07-5.15-ppm) were important for all models except for Garda, as well as the resonances in the phenolic region at 6.73-6.79 ppm, which only did not influence the model for Sicily. The acyl group methylene protons of saturated fatty acids (1.23 ppm), ^{13}C satellite of signal at 4.09-4.32 ppm (α-methylene protons of the glyceryl group of triglycerides) at 3.97 ppm and the signal at 5.57 ppm were important specifically for the Umbria model; the signals at 0.53 ppm and 0.79 ppm, for the Sicily model; the methylic proton of the C18-steroid group of β-sitosterol (0.67 ppm) and the terpene signal at 4.57-4.59 ppm, for the Puglia model; the signal of the cycloartenol at 0.55 ppm, ^{13}C satellite of signal at 2.26-2.32 ppm (α-methylene protons of the acyl group) at 2.15 ppm, the glycerol proton of *sn*-1,2-diglycerides (3.71 ppm) and signals at 6.19 ppm and 6.15 ppm in the phenolic region, for the Lazio model; signals in the region 1.35-1.43 ppm, 2.35-2.39 ppm, and 4.33-4.35 ppm, the α-methylene protons of the acyl group (2.29 ppm and 2.33 ppm), the signal at 3.75 ppm, the α-methylene protons of the glyceryl group of triglycerides (4.27 ppm) and the signal at 6.15 ppm in the phenolic region for the Campania model; and the signal at 5.93 ppm for the Calabria model. The glyceryl protons of *sn*-1,3-diglycerides (4.05-4.07 ppm) and triglycerides (5.25 ppm, 5.29 ppm) were influent for the models of Umbria, Lazio, Umbria and Campania respectively; signals in the phenolic region at 6.25-6.29 ppm for the models of Puglia and Calabria; signals in the phenolic region at 6.63-6.65 ppm and 6.69-6.71 ppm for the models of Umbria and Campania ; signals in the phenolic region at 6.45-6.47 ppm for the models of Umbria and Garda.

These results disclosed that ^{1}H-NMR spectra of VOOs contained information related to the region of provenance of the oil, nonetheless further studies should be carried out with a considerably larger sample set for each region, and even for each of their PDOs, in order to guarantee the detection of counterfeit VOOs. In this regard, Sicily, which is an island at the southernmost part of Italy, produces an olive oil which is markedly influenced by pedoclimatic factors, in accordance with its geographical position. It is therefore coherent that the VOO produced on this island presents a characteristic chemical composition that allows one to distinguish it from all other VOO coming from different geographical regions.

3.2.3 Virgin olive oils from the main producing countries: Spain, Italy and Greece

The adulteration of VOOs from a certain country with VOOs produced in another country at a lower cost, or the false labeling of the VOOs as coming from a certain country when they

were actually produced in another, are actual events that the antifraud authorities have to deal with regularly. The need for chemical approaches to detect these fraudulent activities is evermore apparent.

The ^{1}H-NMR data of the VOOs from the main olive oil producing countries, i.e. Spain, Italy and Greece, were analyzed by multivariate techniques with the purpose of creating classification models to distinguish the geographical origin of VOOs from these three countries (Rosa M. Alonso-Salces et al., 2010b). PLS-DA, using LOO cross-validation, was applied to the autoscaled data to provide binary classification models (country *vs.* non-country), which were also evaluated by external validation (Table 4). The model 'Greece *vs.* non-Greece' distinguishes Greek VOOs from all the rest of the VOOs; it classified properly more than 97% of the samples of both categories, Greece and non-Greece, and predicted correctly more than 90% of the samples in the test set of the cross-validation, as well as in the external validation. The binary models for Italy and Spain presented classification abilities of 89% for the Italian oils and the Spanish oils, 84% for the non-Italy category and 85% for the non-Spain category. The prediction abilities in the cross-validation for the model for Spain were ca. 80% of hits for both classes; whereas the predictions in the external validation were considerably different, for the Spanish VOOs it was overoptimistic (92%), and for the non-Spanish VOOs it was considerably low (67%). In the model for Spain, the variability of the non-Spain category was under-represented in the training-test sets. As a result, this model did not provide good predictions for this category in the external set. The model for Italy provided prediction abilities in the cross-validation of ca. 76% for both classes; and in external validation, close to this value. So, these predictions were substantially lower than the recognition ability of the model, indicating that the model was dependent on the samples included in the training set.

Origin	**Cross-validation**			**Model**	**External Validation**		
Binary model[b]	***N***	***prior prob***	**% Prediction**	**% Classification**	***N***	***prior prob***	**% Prediction**
Italy	*72*	*0.35*	75.0	88.9	*568*	*0.78*	75.7
Non-Italy	*135*	*0.65*	77.0	84.4	*160*	*0.22*	71.9
Spain	*71*	*0.34*	78.9	88.7	*70*	*0.10*	92.9
Non-Spain	*136*	*0.66*	80.9	85.3	*658*	*0.90*	67.2
Greece	*64*	*0.31*	92.2	98.4	*31*	*0.04*	96.8
Non-Greece	*143*	*0.69*	93.7	97.9	*697*	*0.96*	90.0

[a] See abbreviations: Table 2; Models obtained by PLS-DA using autoscaling, LOO and The Unscrambler; Class codes: "Country", 1; "non-Country, 0.

[b] Binary models: Italy *vs* non-Italy: 4 PLS components, boundary at 0.4020; Spain *vs* non-Spain: 3 PLS components, boundary at 0.3563; Greece *vs* non-Greece: 5 PLS components, boundary at 0.4725.

Table 4. Classification results obtained by supervised pattern recognition techniques for the authentication of VOO from the main producing countries, i.e. Italy, Spain and Greece, using ^{1}H-NMR spectral data.[a]

The most influential variables, i.e. those with the highest weighted regression coefficients, on the binary PLS-DA models obtained for each country are due to signals in the phenolic regions at 6.45-6.47 ppm and 6.83-6.85 ppm, which were important for the three models (Rosa M. Alonso-Salces et al., 2010b). In contrast, the model for Spain was particularly influenced by the methylic proton of the C18-steroid group of β-sitosterol (0.67 ppm), the β-methylene protons of the acyl group (1.59 ppm, 1.67 ppm), the allylic protons of the acyl group (1.99-2.07 ppm), the diallylic protons of the acyl group of linoleic (2.73-2.77 ppm) and linolenic (2.77-2.81 ppm), the glycerol proton of *sn*-1,2-diglycerides (3.71 ppm), *sn*-1,3-diglycerides (4.05-4.07 ppm) and triglycerides (5.25 ppm, 5.29 ppm), the olefinic protons of the acyl groups (5.37 ppm), the signals in the phenolic region at 6.37 ppm, 6.61 ppm and 6.71 ppm, and the signals at 0.53 ppm, 1.75-1.77 ppm, 2.35 ppm. Among the most important variables, those who only affected the model for Greece were the methylic proton of the linolenic acyl group (0.97 ppm), and the terpene signal at 4.55-4.57 ppm, and the signals at 0.77 ppm and 3.81 ppm. The resonances of cycloartenol (0.31-0.33 ppm and 0.55 ppm) and phenolic compounds at 6.23 ppm and 6.27 ppm were important for the models of Italy and Greece.

These results show that ^{1}H-NMR fingerprinting of VOOs can be a useful tool to assure authenticity and traceability of VOOs at the national level. From this study, a stable model was achieved to distinguish Greek VOOs from oils from other countries. However, for Italian and Spanish VOOs further studies should be performed with a larger balanced data set, in which all categories will be well represented, to obtain robust models. In the present data set, Spain was clearly under-represented, being the main producer (50% of EU production of olive oil); and Italy, even though it was quite well-represented, the number of samples were very unbalanced regarding the other countries, and so few Italian samples were used in the modeling step. The classification results might therefore be very dependent on the samples in the training-test set.

3.3 Stability of virgin olive oil

Regarding the importance of oil stability on its quality and nutritional properties, the stability of VOO was studied over a period of 43 months in the dark at room temperature by ^{1}H-NMR. The high stability of VOO is mainly due to its relatively low degree of fatty acid unsaturation and to the antioxidant activity of some of the unsaponifiable components. For instance, the oxidative susceptibility of olive oil is related to the antioxidant activity of α-tocopherol, which also showed a synergistic effect in association with some phenolic compounds with significant activity (Deiana et al., 2002).

In contrast with previous studies on the oxidative stability of edible oils, which were performed at high temperatures (Frankel, 2010); we studied VOO stability at r.t. (Alonso-Salces et al., 2011a). The ^{1}H-NMR spectra of 40 VOO samples in the spectral region 0-10 ppm (492 buckets) were analyzed by PCA. The samples represented in the PCA score plot of the first two principal components (Fig. 2) are distributed in the direction of the first principal component (PC1) in clusters, partially overlapped, according to the length of time they had been at r.t. So, PC1, accounting for 15% TSV, contains information related to the change and evolution of the chemical composition of VOO during storage in the dark at r.t., and hence, about the degradation of olive oil under these conditions.

The most influential features, i.e. buckets of ^{1}H-NMR spectra, on PC1 are those with the highest loadings in absolute value, and are shown in Table 5. Some of these chemical shifts correspond to ^{1}H-NMR signals of compounds involved in the hydrolytic and oxidative degradation of VOO. During the oxidation process, hydroperoxides (primary oxidation compounds) are produced (Guillen & Ruiz, 2001, 2006) , which may degrade into secondary oxidation products such as aldehydes, ketones, lactones, alcohols, acids, etc. The oxidation of edible oils is a matter of major concern also from a safety point of view because some oxidation products such as aldehydes are toxic (Guillen & Ruiz, 2001, 2006). Furthermore, several saturated and unsaturated aldehydes have been found to be responsible for rancid sensory defect in VOO (Morales et al., 2005), as well as for off-odours (Kalua et al., 2007), altering its organoleptic properties.

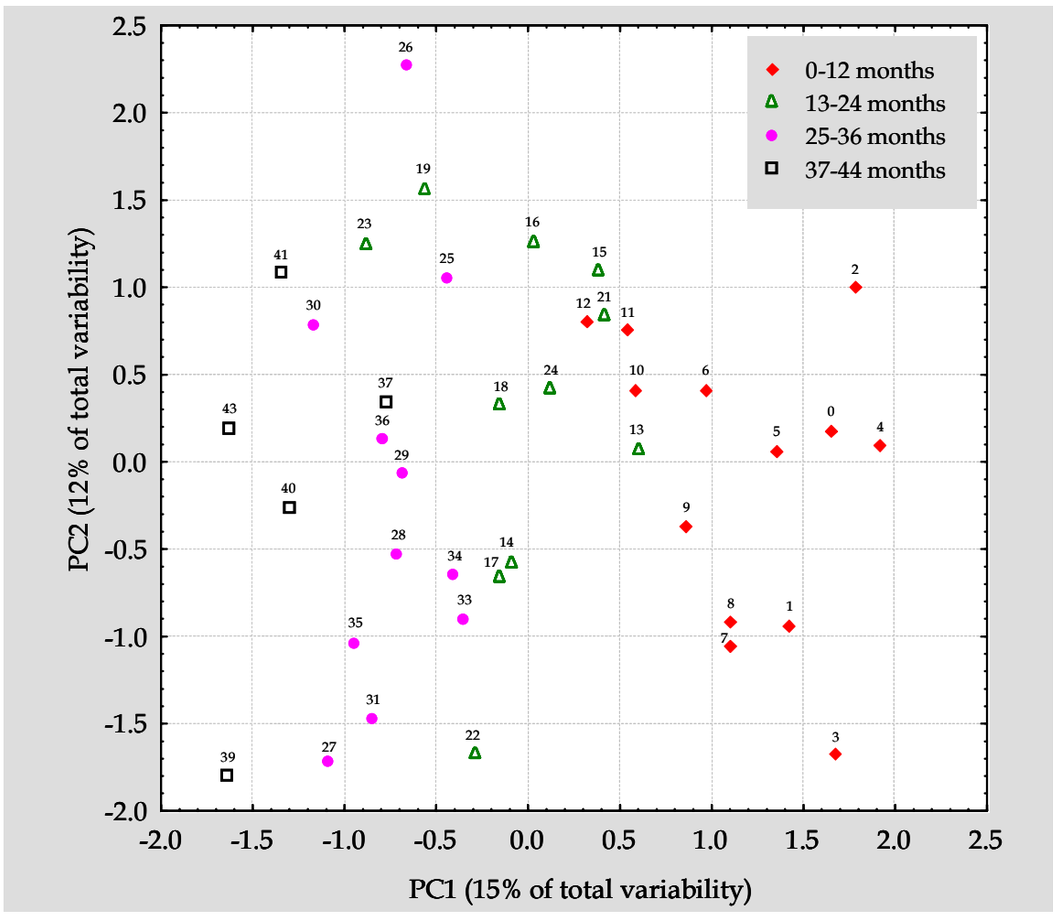

Fig. 2. PCA score plot of the samples used to study the stability of VOO on the space defined by the two first principal components. Samples are numbered according to the time (months) that they had been at r.t. in the dark before analysis.

Bucket (ppm)	Loadings	Multiplicity[a]	Functional group	Attribution
8.19 8.17 8.15 8.13 8.11 8.09	-0.807 -0.860 -0.823 -0.820 -0.749 -0.716	broad signal	-OO*H* (hydroperoxide group)	hydroperoxides
6.97	0.766	s	-Ph-*H* (phenolic ring)	phenolic compounds
6.75	0.811	d	-Ph-*H* (phenolic ring)	phenolic compounds
6.57 6.55 6.53 6.51	-0.792 -0.838 -0.834 -0.727	t	-C*H*=C*H*-C*H*=C*H*- (*cis, trans* diene system)conjugated	hydroperoxides
6.01 5.99 5.97 5.95	-0.833 -0.854 -0.860 -0.822	t	-C*H*=C*H*-C*H*=C*H*- (*cis, trans* conjugated diene system)	hydroperoxides
5.57 5.55	-0.824 -0.776	m	-C*H*=C*H*-C*H*=C*H*- (*cis, trans* conjugated diene system)	hydroperoxides
5.25	0.880	m	>C*H*OCOR (glyceryl group)	triglycerides
4.45	0.726	m	-CH_2OCOR (^{13}C satellite of signal at 4.09-4.32 ppm, glyceryl group)	triglycerides
4.37 4.35	-0.782 -0.786		>C*H*-OOH (methine proton of hydroperoxide group)	hydroperoxides
4.27	0.770	m	-CH_2OCOR (glyceryl group)	triglycerides
4.09 4.07 4.05	-0.795 -0.931 -0.875	q	>C*H*-OH (glyceryl group)	*sn* 1,3-diglycerides
3.89 3.87 3.85	-0.745 -0.802 -0.715	broad signal		saturated alcohols
3.59	-0.708	broad signal		saturated alcohols
2.79	0.924	t	=CH-CH_2-CH= (acyl group)	linolenic
2.77	0.839		=CH-CH_2-CH= (acyl group)	linolenic and linoleic
2.75	0.870	t	=CH-CH_2-CH= (acyl group)	linoleic
2.21 2.19 2.17 2.15	-0.740 -0.885 -0.913 -0.885	m	-OCO-CH_2- (^{13}C satellite of signal at 2.26-2.32 ppm, acyl group)	
2.03	0.792		-CH_2-CH=CH- (acyl group)	linoleic and linolenic
1.29 1.27	0.819 0.852		-(CH_2)$_n$- (acyl group)	linoleic and linolenic
1.25	0.784		-(CH_2)$_n$- (acyl group)	oleic

[a] Signal multiplicity: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet.

Table 5. Stability of VOO: Loadings of the most influential variables on the first principal component, and chemical shift assignments of the ^{1}H-NMR signals.

The presence of hydroperoxides in the samples which had been stored at r.t. and protected from light for more than one year was confirmed by the ^{1}H-NMR signals at 8.09-8.19 ppm due to hydroperoxide protons; 6.51-6.57 ppm, 5.95-6.01 ppm and 5.55-5.57 ppm due to protons of the conjugated diene systems; and 4.35-4.37 ppm due to the methine proton of the hydroperoxide group, as observed by other authors (Guillen & Ruiz, 2001). All these signals presented very small intensities in comparison with characteristic VOO signals, indicating that the oxidative degradation was taking place at a very low rate and yield. This was also supported by the fact that characteristic resonances of aldehydes (9.3-9.9 ppm), the main secondary oxidation products, were not detected in the VOO over the 3 and half years of storage at r.t., so the secondary oxidation process had not yet occurred. These facts agree with the recognized high oxidative stability of VOO. Some minor signals at 6.97 ppm and 6.75 ppm were assigned to phenolic compounds (Owen et al., 2003). The decrease or disappearance, respectively, of these signals during storage at r.t. was in agreement with the role that these substances play as antioxidants during the oxidative degradation process of VOO.

During hydrolytic degradation of olive oil, triglycerides hydrolyze thereby increasing the content of free fatty acids and consequently, the acidity of the oil, which means deterioration in the oil quality. Several resonances indicated the occurrence of hydrolytic degradation. In this sense, slight changes in the intensity of the tryglyceride signals at 5.25 ppm, 4.45 ppm and 4.27 ppm and the α-methylene protons of the acyl group (^{13}C satellite of the signal at 2.26-2.32 ppm) at 2.15-2.21 ppm were observed. Moreover, a slight decrease in the intensity of the signals at 2.75-2.79 ppm of the diallylic protons and at 2.03 ppm of the allylic protons of linoleic and linolenic acyl groups, and at 1.25-1.29 ppm of the methylene proton signal of oleic, linoleic and linolenic acyl groups, during storage at r.t., revealed that tryglycerides were degrading. The increase in the intensity of the signal at 4.05-4.09 ppm, assigned to the proton of the glyceryl group of *sn*-1,3-diglycerides, was indicative of the loss of quality and freshness of the VOO (Guillen & Ruiz, 2001). Young, good quality olive oils contain mainly native *sn*-1,2-diacylglyceride and only small amounts of *sn*-1,3-diacylglyceride. The increase in the latter was observed after one year of storage at r.t., which seems to be caused by intermolecular transposition and/or lipolytic phenomena (Sacchi et al., 1996). Moreover, in the samples stored for longer than 18 months, a broad signal also appeared in the region of saturated alcohols at 3.85-3.89 ppm, which can arise from lipolysis (Sacchi et al., 1996).

4. Conclusion

^{1}H-NMR fingerprinting of olive oil is a valuable analytical tool for the traceability of VOOs from different points of view, i.e. food authentication and food quality.

For authentication purposes, ^{1}H-NMR fingerprints of VOOs analyzed by supervised pattern recognition techniques allow the determination of their geographical origin at the national, regional and/or PDO level. PLS-DA afforded the best model to distinguish the PDO *Riviera Ligure* VOOs: 88% of the Ligurian and 86% of non-Ligurian oils were correctly predicted in the external validation. At the regional level, a stable and robust PLS-DA model was obtained to authenticate VOOs from Sicily, predicting well the origin of more than 85% of the samples in the external sample set. At the national level, Greek and non-Greek VOOs were properly classified by PLS DA: >90% of the oils were correctly predicted in the crossvalidation and external validation.

Regarding quality control, ^{1}H-NMR fingerprinting enables us to control the stability of VOO since this technique can detect its compositional changes due to oxidative and hydrolytic degradation. Under normal VOO storage conditions, i.e. at room temperature and protected from light, none of the signals present in the ^{1}H-NMR spectra of VOO at time zero disappeared or experienced significant decreases or increases over a period of more than 3 and half years. Only small changes in the signals and the appearance of some low intensity signals indicate that some oxidative and hydrolytic degradation of the VOO started after one year. These results confirm the high oxidative stability of VOO at r.t., and supports the best-before date for VOO that is normally between one and one and a half years, depending on the type of container and the olive variety used. Moreover, they show that VOO during this time period does not experience any significant changes which could render its consumption hazardous. In addition, aliquots (even small aliquots of 40 mL) can be preserved at r.t. in the dark (amber glass) until analysis for at least one year, which is of great interest to control laboratories of VOO with regard to storage space and expense. Furthermore, this research is a proof-of-concept that ^{1}H-NMR is a useful tool to study and evaluate the oxidative stability of edible oils in a quality control context at any temperature, since any toxic substances that may be generated during the degradation process can be detected and even quantified. Further studies would be needed to validate quantitative methods for this purpose.

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6. Abbreviations used

VOO, virgin and extra virgin olive oils; PDO, Protected Designation of Origin; NMR, nuclear magnetic resonance; ANOVA, analysis of variance; PCA, principal component analysis; PC, principal component; LDA, linear discriminant analysis; PLS-DA, partial least squares discriminant analysis; TSV, total system variability; CV, cross-validation; LOO, leave-one-out cross-validation; r.t., room temperature.

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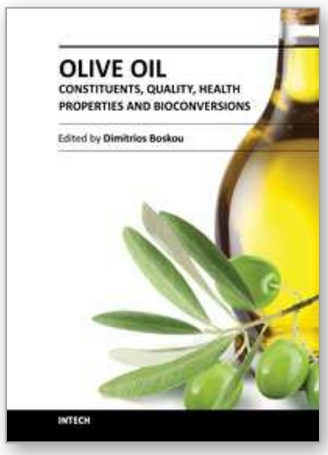

Olive Oil - Constituents, Quality, Health Properties and Bioconversions

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The health-promoting effects attributed to olive oil, and the development of the olive oil industry have intensified the quest for new information, stimulating wide areas of research. This book is a source of recently accumulated information. It covers a broad range of topics from chemistry, technology, and quality assessment, to bioavailability and function of important molecules, recovery of bioactive compounds, preparation of olive oil-based functional products, and identification of novel pharmacological targets for the prevention and treatment of certain diseases.

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