Comparison of the Amounts of Volatile Compounds in French Protected Designation of Origin Virgin Olive Oils

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Headspace solid-phase microextraction (HS-SPME) −gas chromatography using flame ionization detection and multivariate analysis were applied to the study of the specificity of protected designation of origin (PDO) virgin olive oils produced in a southern French region (Alpes-Maritimes) based on their volatile compounds. A total of 35 PDO olive oils from Nice, 6 commercial oils, and 12 other French PDO olive oils were analyzed. Recorded data were subjected to principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA). The method developed here was able to perfectly distinguish different qualities of olive oils. Representative samples from each class obtained by chemometric treatment were analyzed by HS-SPME and GC-MS. PCA and SIMCA of chromatographic data were related to sensory analysis and led to a better understanding of the chemical features and observed sensory effects of olive oils.

KEYWORDS: Headspace solid-phase microextraction; GC-FID; GC-MS; principal component analysis; soft independent modeling of class analogy; classification; olive oil; PDO

INTRODUCTION

Oleiculture has great economic and social importance in the countries of the Mediterranean region. While France is a relatively small producer country, it is ranked sixth in terms of olive oil consumption. In the South of France, many producers are engaged in the production of high quality virgin olive oils, which are more expensive than olive oils usually traded on the olive oil market and which are evaluated for various quality criteria. At the European level, two regulatory measures were adopted in 1992 (*1*, *2*), reflecting the need to adapt to changing attitudes on the part of both producers and consumers. For this reason, virgin olive oils from Nice received the protected designation of origin (PDO) "Olive de Nice" in 2001 in recognition of their specific geographical environment of production and the use of established processes. Oils receiving this designation are subjected to strict specifications that ensure the quality and thus increase the commercial value of the product (*3*).

Establishing vegetable oil authenticity, particularly in the case of virgin olive oils, is of prime importance in the food industry because high quality extra virgin olive oils are more expensive than commercial oils sold in supermarkets (extra virgin, virgin, or simply "olive oil") (*4*). The need for effective analytical techniques for evaluating the quality and authenticity of virgin olive oils is obvious, to protect both the economic interests of producers and the rights of consumers who prefer to know exactly what the olive oil contains (*5*). Various analytical methods have been used to identify and quantify characteristic compounds of vegetable oil: fatty acids, triglycerides, waxes, sterols, tocopherols, hydrocarbons, alcohols (*6*), and volatile compounds such as the products of the lipoxygenase pathway or terpenoid hydrocarbons (*7*).

Today, establishing faster alternative methods for determining the origin or quality of olive oil represents a considerable challenge. All such methods have the same principle in common: multivariate statistical analysis of analytical data. Various analytical methods, such as NMR (^{1}H and ^{13}C) (*8*, *9*), IRTF (*10*, *11*), synchronous scanning fluorescence spectroscopy (*12*), and excitation−emission fluorescence spectroscopy (*13*), have been used. An alternative to these methods is the use of electronic noses to characterize the entire oil aroma. Fingerprints obtained with this technique can be exploited by statistical analysis and allow the classification of oils (*14*).

Volatile compounds can be used as an alternative way to evaluate oil authenticity. The identification and quantification of volatile compounds involve enrichment techniques that can be applied to low concentration compounds (*15*). Dynamic headspace, also called the purge and trap technique when used with liquid samples, is a commonly used technique for aroma analysis of virgin olive oils (*16*). This enrichment process allows the detection of low concentrations of analytes, which are thought to contribute significantly to the flavor of samples (*17*,

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18). This method, however, tends to be time-consuming and requires inert gas purge and thermal desorption systems.

Solid-phase microextraction (SPME) is a solvent-free sample preparation technique for the extraction of volatile and low volatile compounds. This method, developed by Arthur and Pawliszyn (*19*) in 1990, is used in many applications including the analysis of pollutants in water, headspace analysis of aromatic and medicinal plants (*20*), and food flavor analysis, particularly with vegetable oils (*21*, *22*). In just a few years, SPME has considerably extended its range of applications and is now used in many fields. Recently, we demonstrated that SPME may be an appropriate technique for routine quality control analysis of olive oils, thanks to its operational simplicity, repeatability, and low cost (*23*). This method has also been successfully applied to the identification of quality-freshness markers in French and Spanish virgin olive oils (*24*).

The use of multivariate methods such as PCA and SIMCA allows both the identification of the most important directions of variability in a multivariate data matrix and the multivariate classification of the analyzed samples. Chemometrics in conjunction with SPME-GC data has been applied to differentiation studies of various food matrices, such as coffee (*25*), honey (*26*), soft fruit purées (*27*), and vegetable oils (*28*).

This method has also been used to characterize virgin olive oils produced in two geographical areas of northern Italy (*29*), to analyze olive oils from southern Italian regions (*30*), and to study the impact of the variety, harvest date, malaxation time, and temperature on the volatile profile of Australian olive oils (*31*). To our knowledge, however, this approach has never been used to analyze PDO virgin olive oils from southern France and to distinguish between their qualities.

The aim of this study was to assess the performance of headspace solid-phase microextraction (HS-SPME)−gas chromatography using flame ionization detection (GC-FID) and multivariate analysis (MVA) in evaluating the quality of French olive oils and in demonstrating the typicality of PDO olive oils from Nice. We also wanted to examine the relationships between results obtained by chemometric treatment of GC-FID data and results of sensory analysis in order to identify markers of olive oil quality.

MATERIALS AND METHODS

Materials. PDO virgin olive oils (*cailletier* variety) from Nice (35 samples) were supplied by the "Syndicat Interprofessionnel de l'Olive de Nice" (SION) in 2002 and 2003. The other PDOs from Southern France were supplied by the "Association Française Interprofessionnel de l'Olive" (AFIDOL) in 2003. Four different PDOs (four samples per variety) were supplied: Nyons PDO (*Tanche* variety, samples 67−70), Haute Provence PDO (*Aglandau* variety, samples 63−66), and Aix en Provence PDO (mixture of *Solenenque*, *Aglandau*, and *Cayanne* varieties, samples 59−61). Commercially available virgin olive oils (six samples) were purchased at a local supermarket in early 2002; these oils are mixtures of different Spanish oils (*picudo*, *hojiblanca*, and *picual* varieties). All these oils have physical data (peroxide value and acidity) corresponding to virgin olive oils (according to the latest EU classification system that entered into force on 1 November 2003). All samples were hermetically sealed (purged with argon) and stored at −20 °C until used for chemical analysis.

Headspace Solid-Phase Microextraction. A manual SPME device including the fiber was obtained from Supelco (Bellefonte, PA). The fiber used for the extraction of the volatile components was divinyl-benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm.

Before use, the fiber was conditioned as recommended by the manufacturer. The olive oil (20 g) was placed in a 40 mL amber vial closed by a PTFE/silicone septum (Supelco). Before extraction, stabilization of the headspace in the vial was accomplished by

equilibration for 60 min at 25 °C. The extraction was carried out at room temperature.

To determine the optimal adsorption time of the fiber to the sample headspace, the fiber was exposed for various time periods: 10, 30, 60, 90, and 120 min. A sampling time of 90 min was chosen for the analysis (*23*).

After exposure, the fiber was thermally desorbed into the GC injector and left in the injection port (equipped with a 0.75 mm i.d. inlet liner) for 4 min. The injector temperature was set at 250 °C, and the injector was operated in splitless mode for 4 min unless otherwise stated. Before sampling, the fiber was reconditioned for 5 min in the GC injection port at 250 °C. Blank runs were carried out periodically during the study.

Chemical Analysis. *Analytical GC.* GC analysis was carried out using a Hewlett-Packard 5890 Series II gas chromatograph equipped with an FID and with HP-1 fused-silica capillary columns (polydimethylsiloxane, 50 m × 0.2 mm i.d., film thickness: 0.33 μm). The carrier gas for GC-FID was nitrogen; the column head pressure was 25 psi; the oven temperature was programmed to increase from 60 to 250 °C by 2 °C/min and was then held isothermally for 20 min. The FID temperature was set at 250 °C.

Retention indices were determined using C5 to C26 alkane standards as references (SPME extraction time of the alkane standard solution: 20 s at 50 °C). Relative amounts of individual compounds were based on the peak areas obtained without FID response factor correction. Three replicates were performed for each sample. The average of these values and the standard deviation were determined for each component identified.

GC-MS Analysis. Each oil was analyzed by GC-MS using a Hewlett-Packard 5890/5970A system with HP-1 fused-silica capillary columns (50 m × 0.20 mm; film thickness, 0.5 μm). Oven conditions were the same as above for GC, under the following operating conditions: carrier gas, helium; injector temperature, 250 °C; ion source and transfer line temperatures, 170 °C and 280 °C, respectively; splitless mode. Retention indices were determined with C5 to C26 alkane standards as references. The mass spectra were performed at 70 eV over a mass range of 35−350 amu.

The constituents were identified by comparing their retention times with those of authentic samples on using a computer matching against commercial libraries (NIST 1998, Wiley6N, MassFinder 2.1 Library 2001) and our own laboratory-made spectral library built from pure substances and MS literature data (*32−34*); the results were then confirmed by comparing the retention indices with published index data (*35*, *36*).

Chemicals. The standard compounds used were as follows: absolute ethanol (VWR, EURLEMR134001), propan-2-one (Riedel de Haën, 24201), acetic acid (Aldrich, 242853), ethyl acetate (Riedel de Haën, 27227), 2-methyl propan-1-ol (Aldrich, 320048), 3-methylbutanal (Aldrich, M330048), 2-methylbutanal (Aldrich, 146455), pentan-3-one (Aldrich, 127604), heptane (Aldrich, A154873), toluene (Riedel-de Haën, 24529), octane, (Aldrich, 412236), (*E*)-2-hexenal (Aldrich, 132659), hexanal (Aldrich, 115606), hexanol (Aldrich, H13303), ethyl 2-methylbutanoate (Aldrich, 306886), (*Z*)-3-hexen-1-ol (Aldrich, 12900), (*E*)-2-hexen-1-ol (Aldrich, 132667), heptan-2-one (Aldrich, 537683), heptanal (Aldrich, H2120), *o*-xylene (Aldrich, 294780), *p*-xylene (Aldrich, 134449), α-pinene (Aldrich, 147524), β-pinene (Aldrich, 420166), δ-3-carene (Aldrich, 115576), *p*-cymene (Aldrich, C121452), limonene (Fluka, 89188), (*E*)-β-ocimene (Fluka, 74730), γ-terpinene (Acros, 207501000), and nonanal (Acros, 357571000).

(*Z*)-3-hexenyl acetate, (*E*)-3-hexenyl acetate, and hexyl acetate were obtained by reaction of acetyl chloride (Aldrich, 114189) and (Z)-3-hexen-1-ol, (*E*)-3-hexen-1-ol, and hexanol (*37*, *38*). Products were characterized by ^{1}H and ^{13}C NMR and by GC/MS (Electronic Impact, 70 eV).

Chemometrics. *Statistical Software.* Data were computed with Pirouette 3.11 (Infometrix Inc., WA, U.S.A.) (*32*), an easy-to-use multivariate analysis program designed to facilitate the integration and automation of chemometrics in chemical data treatment. We used this software to carry out an exploratory analysis of our data and to build and test a suitable classification model.

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Data Matrices, Preprocessing, and Chemometrics. All data matrices used in this work for SPME analysis had the following form:

$$C_{ij} = (S_i, RI_j)$$

with S_i the *i*th element of the C_{ij} matrix, that is the *i*th sample, and RI_j the *j*th element of C_{ij}, that is the area measured under the *j*th peak of the SPME chromatogram, with the *j*th retention index obtained by comparison with an alkane series (see analytical conditions below). Each value in the matrix represents the average of the measured peak areas of three replicates of the considered sample.

For the comparison between PDO Nice and commercial olive oils (denoted "Com"), 41 (35 PDO Nice and 6 Com) samples were analyzed, leading to 72 X vars. For the comparison between PDO Nice and other PDO olive oils, there were 46 (34 PDO Nice, 12 others), also resulting in 72 X vars.

Before applying chemometric treatments, the data matrix was normalized to 100. For gas chromatography data, we used the retention index of each chemical compound as a variable, and the integrated area under each peak as a value in the matrix. Therefore, our data preprocessing involved mean-centering. The mean was computed for each variable (in our case, variables labeled with a retention index contained the area measured under the peak located at a given retention index) and then subtracted from each data value to produce a mean-centered matrix.

Two chemometric procedures were used to compute data: PCA and SIMCA. Principal Component Analysis (PCA) is usually used in exploratory analysis. It gives graphical representations of intersample and intervariable relationships. In addition, it reduces the complexity of the data and transforms the original variables into new axes, called principal components (PCs). These PCs are orthogonal in such a way that the data presented in the axes are uncorrelated with each other; PCA expresses as much as possible the total variation of the data in just a few principal components and in decreasing order with respect to the amount of the variation. Score plots represent the projections of the objects (samples) in the planes defined by the PCs, whereas loading plots represent the projections of the original variables in the same planes. The score and loading plots can be represented separately or in the same drawing. Objects that are projected close to each other in the score plots have similar characteristics, and, for instance, samples to the right in the score plot have high values for variables placed to the right in the loading plot. The same holds for samples located in other regions of the graph. The farther a variable is from the axis origin, the more its contribution can be considered a major contribution in the statistical model generated by the principal component analysis.

SIMCA (Soft Independent Modeling of Class Analogy), introduced by Svante Wold in 1974 (*39*), involves carrying out a PCA on each class in the data set and the retention of a sufficient number of principal components (PCs) to account for most of the variation within each class. Hence, a principal component model is used to represent each class in the data set. The number of PCs retained for each class is usually different. A cross-validation procedure ensures that the model size can be determined directly from the data. The variance that is explained by the class model is called the modeled variance, which describes the signal, whereas the noise in the data is described by the residual variance or the variance not accounted for by the model. By comparing the residual variance of an unknown sample to the average residual variance of the samples that make up a class, it is possible to obtain a direct measurement of the degree of similarity between the unknown sample and the class. This comparison is also a measure of the goodness of fit of the sample to a particular principal component model. An attractive feature of SIMCA is that an unknown sample can only be assigned to a class to which it has a high probability of belonging. If the residual variance of a sample exceeds the upper limit for every modeled class in the data set, then the sample would not be assigned to any of the classes, either because it is an outlier or because it comes from a class that is not represented in the data set.

The technique implemented in Pirouette software also provides a rich set of diagnostic tools addressing other interesting aspects of classification, such as the modeling power and the discriminating power. Modeling power, or MP, describes how well a variable helps the principal components model the variation (it typically ranges from 0 to 1). Discriminating power, or DP, (always ≥ 0) describes how well the variable helps the principal components classify the samples in the data set. Variables with low MPs and low DPs are usually deleted from the data because they only contribute noise to the principal component models. To use discriminating power values, the user has to define a minimum limit in order to determine which variables have an acceptable DP. The choice is linked to the maximum value computed for the DP scale for the given data matrix, as well as to the classification performance for a given SIMCA model built with a specific variables. In our study, we kept variables having a DP > 30.

RESULTS AND DISCUSSION

Olive Oil Analysis. In previous studies (*23,24*), we described the optimization of HS-SPME sampling conditions and evaluated its reproducibility for monitoring the quality of virgin olive oils. The relative performances of four fibers (PDMS, CAR/PDMS, CW/DVB, and DVB/CAR/PDMS) were compared using a single olive oil sample (Sabine variety). This study showed that the signal obtained with the DVB/CAR/PDMS fiber was the most suitable for the analysis of olive oil volatiles, and it was superior to fibers with PDMS, CAR/PDMS, or CW/DVB coatings.

Different sampling temperatures (25, 60, and 75 °C) were tested, and the observed standard deviations led us to use room temperature as the operating temperature. HS−SPMEs of all olive oil samples were analyzed in triplicate. When deviations were observed in the results, the SPME fiber was changed. The relative standard deviations were between 5 and 10% on average, although they sometimes reached as high as 30% for the most volatile compounds present at low concentrations. Blank samples were carried out to ensure the specific use of signals coming from the samples. The signal area averages obtained were used to perform chemometrics. Each sample was also analyzed by HS-SPME and GC-MS. A total of 45 compounds were thus characterized by combining the GC-MS and retention indices (GC-RI, see Materials and Methods) (**Table 1**). The majority of these compounds were previously reported in the literature as being present within olive oil volatile fractions (*22−24*). (*E*)-2-hexenal was the main compound extracted by SPME and was characteristic of the olive oil headspace for the great majority of PDO virgin olive oils. In the 41 French Cailletier olive oils, the isolated and identified compounds were mainly aldehydes, representing from 56.6% to 91.7% of the total peak area percentage for 40 samples, although only 0.2% for one atypical sample (no. 356). The more abundant compounds were aldehydes, such as (*E*)-2-hexenal (56.0−92.2%, 0.0% for the atypical sample), hexanal (0.0−8.8%), or nonanal (0.0−0.3%); alcohols (0.2−29.6%), such as (*E*)-2-hexen-1-ol (0.0−7.0%), hexanol (0.0−5.0%, 17.8% for the atypical sample), or (*Z*)-3-hexen-1-ol (0.0−2.5%); monoterpenes (β-pinene, limonene, (*E*)-β-ocimene); and sesquiterpene hydrocarbon (α-ylangene). Four isomeric unsaturated hydrocarbons (3,4-diethyl 1,5-hexadiene, 3-ethyl 1,5-octadiene, known as pentene dimers) were identified in the volatile fraction of the different virgin olive oils studied (**Table 1**). Concerning the chemical composition of commercial olive oil presented in **Table 1**, 25 compounds (78.9% of the total GC area percentage) were identified. This relatively low percentage can be explained by the high number of unidentified compounds in this oil, in particular low concentration (<0.1%) compounds that were difficult to identify.

For the next phase of the study, the use of retention indices was preferred over the use of retention times. Indeed, when studying a large number of complex mixtures over a period of

Table 1. Compounds Identified by HS-SPME and GC-MS

| | | HS-SMPE of virgin olive oils (%) | | | | | |
compound[a]	RI[b]	PDO Nice 1[c]	PDO Nice 2[d]	commercial	PDO 2[e]	PDO 3[f]	identification methods[g]
ethanol	<500	tr	–	4.2 ± 0.5	tr	tr	MS, Std
propan-2-one	<500	tr	–	2.8 ± 0.3	tr	tr	MS, Std
(E)-1,3-pentadiene[h]	519	0.3[i] ± 0.1[j]	–	–	0.7	1.2 ± 0.1	MS, RI
unknown[k]	528	tr	–	2.6	0.3	1.6	–
acetic acid	558	–	–	25.3 ± 1.7	–	–	MS, RI, Std
ethyl acetate	594	–	0.2	14.6	5.7 ± 0.1	3.0	MS, RI, Std
2-methylpropan-1-ol	612	–	–	1.5	–	–	MS, RI, Std
unknown	627	–	–	0.9	0.1	–	–
3-methylbutanal	640	–	–	0.7 ± 0.2	0.1	0.3	MS, RI, Std
2-methylbutanal	647	0.1	–	–	–	0.6 ± 0.1	MS, RI, Std
1-penten-3-one[h]	660		–	–	–	3.2 ± 0.1	MS, RI
pentan-2-one[h]	662	–	8.3 ± 1.0	–	5.7 ± 0.3	11.0 ± 0.3	MS, RI
pentan-3-one	675	–	5.3 ± 1.8	2.1 ± 0.1	2.1 ± 0.1	–	MS, RI, Std
n-heptane	697	0.4	0.6 ± 0.2	0.8 ± 0.2	0.7 ± 0.3	1.4 ± 0.1	MS, RI, Std
3-methylbutan-1-ol[h]	719	–	–	–		tr	MS, RI
2-methylbutan-1-ol[h]	714	–	0.8 ± 0.2	–	–	tr	MS, RI
(Z)-2-pentenal[h]	727	–	–	1.6 ± 0.3	–	–	MS, RI
toluene	753	0.6	1.1 ± 0.1	0.4 ± 0.1	0.2	1.6 ± 0.1	MS, RI, Std
hexanal	775	0.8 ± 0.1	–	4.9 ± 0.3	7.6 ± 0.4	–	MS, RI, Std
unknown	784	–	–	0.2	–	tr	–
1-octene[h]	786	–	0.3 ± 0.1	1.6 ± 0.3	–	–	MS, RI
n-octane	798	2.0 ± 0.1	7.7 ± 0.6	–	0.5	0.5 ± 0.1	MS, RI, Std
(Z)-2-octene[h]	809	–	–	0.7 ± 0.1	0.2	1.3	MS, RI
(E)-2-hexenal	825	86.9 ± 2.2	–	5.8 ± 0.1	55.7 ± 0.2	28.3 ± 0.4	MS, RI, Std
ethyl 2-methylbutanoate	830	–	–	1.6 ± 0.4	–	–	MS, Std
(Z)-3-hexen-1-ol	837	–	–	1.6 ± 0.6	–	–	MS, RI, Std
(E)-2-hexen-1-ol	845	0.1	10.2 ± 0.9	–	0.1	–	MS, RI, Std
hexanol	848	–	16.5 ± 1.2	–	–	–	MS, RI, Std
p-xylene	853	0.6	12.5 ± 2.2	–	0.4 ± 0.1	0.6	MS, RI, Std
heptan-2-one	859	–	1.7 ± 0.3	–	–	–	MS, RI, Std
o-xylene	876	–	1.0 ± 0.7	–	0.1	0.1	MS, RI, Std
unknown	879	tr	–	0.4	0.1	0.1	–
3,4-diethyl 1,5-hexadiene[h,l]	893	0.2	0.2	0.2	0.1	0.7	MS, RI
3,4-diethyl 1,5-hexadiene[h,l]	898	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1	0.7	MS, RI
α-pinene	923	–	22.4 ± 1.7	–	0.2	0.2	MS, RI, Std
α-thujene	929	–	0.7 ± 0.1	–	–	–	MS, RI, Std
unknown	930	0.1	–	1.1	tr	–	–
3-ethyl 1,5-octadiene[h,l]	932	1.0	–	0.7	0.7 ± 0.1	2.5 ± 0.1	MS, RI
3-ethyl 1,5-octadiene[h,l]	939	0.8	0.8 ± 0.2	0.6	0.4	1.9 ± 0.1	MS, RI
β-pinene	970	–	0.3	–	–	–	MS, RI, Std
(Z)-3-hexenyl acetate	985	–	–	3.5 ± 0.5	–	4.6 ± 0.3	MS, RI, Std
(E)-3-hexenyl acetate	987	1.8 ± 0.1	–	–	1.3	3.7 ± 0.3	MS, RI, Std
hexyl acetate	997	–	0.2	2.0 ± 0.2	1.3	2.5 ± 0.3	MS, RI, Std
δ-3-carene	1005	–	6.8 ± 0.6	–	–	–	MS, RI, Std
p-cymene	1011	–	–	–	–	0.4 ± 0.1	MS, RI, Std
limonene	1020	–	0.2 ± 0.1	–	7.7 ± 0.8	8.2 ± 0.9	MS, RI, Std
(E)-β-ocimene	1036	–	1.2 ± 0.1	0.7 ± 0.1	–	tr	MS, RI, Std
γ-terpinene	1050	–	0.1	–	0.6	0.6	MS, RI, Std
nonanal	1081	–	0.2	–	–	–	MS, RI, Std
unknown	1206	–	0.2 ± 0.1	4.3 ± 0.6	0.1	1.3	–
α-ylangene[h]	1376	0.1	0.2	0.4 ± 0.1	1.0 ± 0.1	3.6	MS, RI
Total of identified compound		95.8 ± 0.2	99.6 ± 0.5	78.9 ± 0.3	92.9 0.1	87.1 ± 0.7	

a Order of elution and percentage are given on apolar column (HP-1). *b* Retention indices as determined on HP-1 column using the homologous series of *n*-alkanes. *c* PDO Nice 2: Sample N*o*. 356, characterized by a strubble-mildewed aroma. *d* PDO Nice 1: Sample No. 353, characterized by a green and herbaceous aroma. *e* PDO 2: Nyons PDO. *f* PDO 3: Haute Provence PDO. *g* Method of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI: by comparison of RI with those from the literature; Std: by injection of an authentic sample. *h* Compound tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (RI). *i* Peak area percent (percent normalized areas) determined by HS-SPME GC-FID analysis (mean values of three replicates). *j* Standard deviation. *k* Unknown compounds present with a percentage of <0.1% are not presented in the table. *l* Correct isomer not characterized.

several months, deviations in retention times are frequently observed. This raises many difficulties, particularly with respect to compounds present at trace levels. The use of retention indices can, however, overcome this limitation. To build the data matrices, all peaks were used, including both identified and unidentified compounds. However, in order to work exclusively with volatile compounds that were characteristic of the samples, some unidentified, low concentration compounds were suppressed. This work allowed us to select 72 chromatographic peaks for the construction of the matrices.

Comparison between PDO "Olive de Nice" and Commercial Olive Oils. A total of 35 PDO "Olive de Nice" oils and 6 commercial olive oils bought in French supermarkets were analyzed by HS-SPME and GC-FID. The commercial olive oils used were all "extra virgin olive oils" in order to compare the best qualities of oils. The application of the PCA algorithm to the data revealed strong differences between the PDO Nice samples and the commercial samples, leading to the score plot shown in **Figure 1**. The first two PCs accounted for 92.1% of the total variance. The PDO Nice samples were all located on

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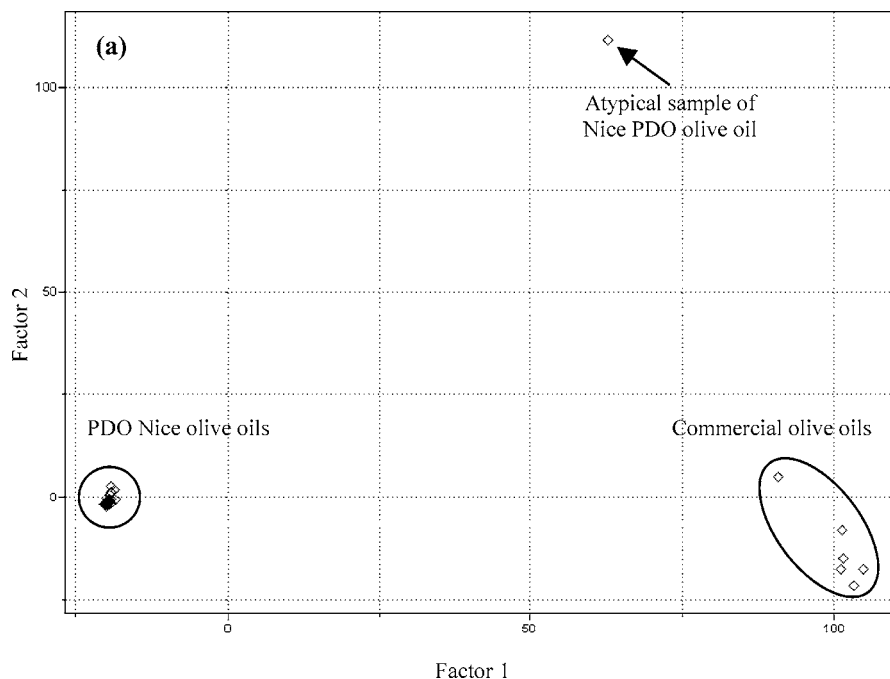
Figure 1. PCA score plot of PDO Nice and commercial olive oils.

the left-bottom area of the score plot, whereas the other commercial olive oil samples were distributed symmetrically. This result indicates the existence of substantial differences in the chemical compositions of the headspace of the samples. We also note the presence of a single sample located at the top center of the score plot. This observation was not surprising, because this sample was provided to us as an atypical sample of Nice PDO virgin olive oil. The loading plot (not shown) provides some explanation of this relative distribution of samples. Indeed, the PDO Nice samples were strongly correlated with a compound having a retention index of 825, identified as (*E*)-2-hexenal by GC-MS, and the commercial olive oils were strongly correlated with compounds having retention indices of 594 (ethyl acetate) and 558 (acetic acid); the atypical olive oil sample seemed to be correlated with compounds having retention indices of 929 (α-thujene), 848 (hexanol), and 853 (*p*-xylene). These observations fit very well with data in the literature; it is well-known, for instance, that (*E*)-2-hexenal is characteristic of the "green" flavor found in PDO olive oils from the Nice region. This product comprises C_6 compounds that are enzymatically produced from polyunsaturated fatty acids through the lipoxygenase (LOX) pathway, and its concentration depends on the level and the activity of each enzyme involved in the reaction (*30, 40, 41*).

Commercial virgin olive oils are generally preserved for a long time, contrary to PDO olive oils which are sold quickly and little preserved. Levels of (*E*)-2-hexenal decrease during olive oil storage (aldehyde oxidation) (*24*), explaining the low concentrations observed in the commercial virgin olive oil samples. Our ability to easily discriminate between the two groups of oils is thus perfectly logical.

More interesting is the sample known as "stubble-mildewed" olive oil (located at the top center of the score plot). Three compounds known to be produced during the fermentation of olive oils mainly characterize this sample. Pentan-2-one and pentan-3-one are not produced by the lipoxygenase pathway by enzymatic action, but rather by homolytic cleavage of 13-hydroperoxides that are formed during the first stage of the LOX pathway (*42*). That is why this sample appears different from the others.

Table 2. Main Molecular Markers Extracted and Analyzed from Olive Oil Headspace and Allowing Discrimination between Olive Oil Samples[a]

compound	retention indices	discrimination between all olive oil samples	discrimination between Nice PDO/ other French PDO
(*E*)-pent-1,3-diene	519		X
unknown	528		X
acetic acid	558	X	
ethyl acetate	594		
pentan-2-one	662	X	X
pentan-3-one	675	X	X
2-methylbutanol	714	X	
hexanal	775		X
(*Z*)-2-octene	809	X	
(*E*)-2-hexenal	825	X	X
(*Z*)-3-hexenol	837		X
(*E*)-3-hexenol	845		X
p-xylene	853		X
α-pinene	923	X	
(*E*)-3-hexenyl acetate	987		X
δ-3-carene	1005	X	
limonene	1020		X
(*E*)-β-ocimene	1036	X	
γ-terpinene	1050	X	X
unknown	1206	X	

[a] Discriminating power > 30.

The application of the SIMCA method gave us an efficient classification model that was able to distinguish between these two olive oil classes. As expected, the atypical sample was plotted away from (did not match) the PDO Nice group due to its particular chemical properties. This sample was thus removed from the matrix in order to make a valid classification model. **Table 2** presents those compounds that had a discriminating power (DP) sufficient to differentiate among all the categories of olive oil samples, as well as those compounds with a DP sufficient to discriminate more specifically between the Nice samples and other French olive oil samples. The contribution of these compounds to the class separation process was significant compared to the others.
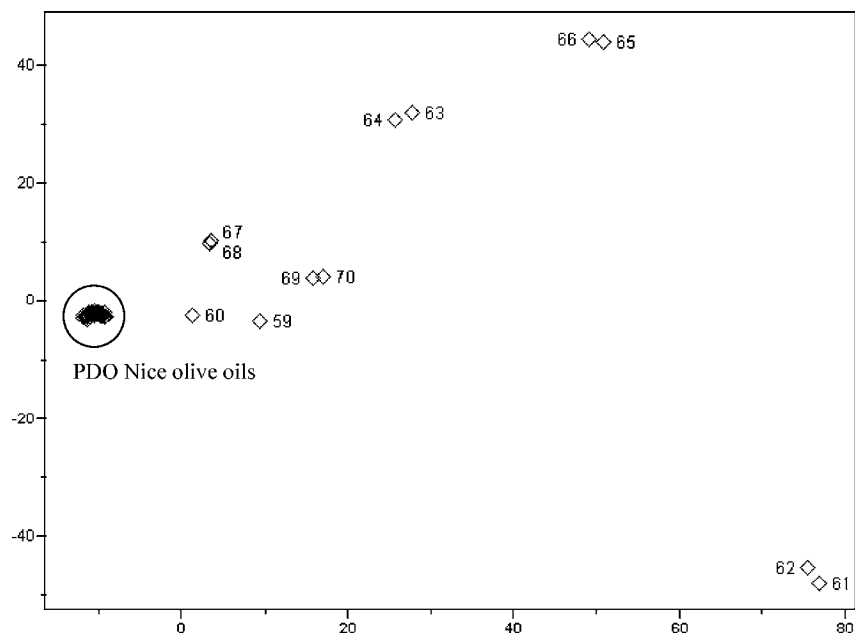
Figure 2. PCA score plot of PDO Nice and other French PDO olive oils. 59–62: Aix en Provence PDO. 63–66: Haute Provence PDO. 67–70: Nyons PDO.
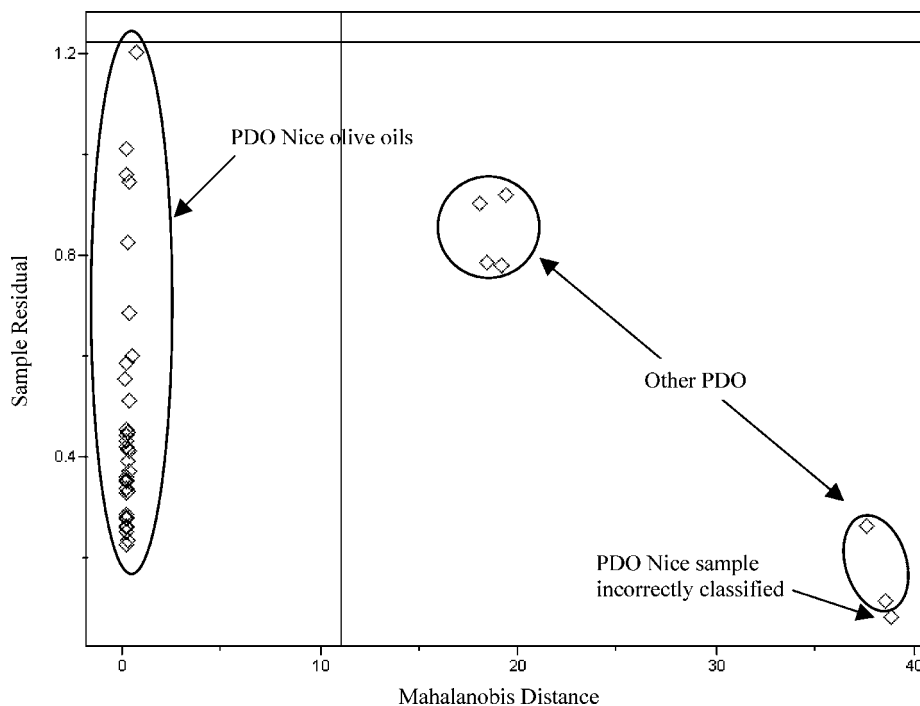
Figure 3. Classification model obtained in the study between PDO Nice and other French PDO olive oils.

Comparison between PDO Nice and Other PDO Olive Oils. The second question we wished to answer was whether the chemical specificity of the PDO Nice samples was sufficient to allow them to be distinguished from other PDO olive oil samples coming from other southern French regions. The data matrix used contained 34 PDO Nice samples (all the oils used in the first part of the study except for the atypical sample 356) and 12 other French PDO samples from different regions and olive varieties. **Figure 2** presents the scores obtained from a PCA procedure applied to this matrix. It is immediately evident that the PDO Nice class is compactly located at the left-center region of the score plot, whereas the other class of samples is relatively spread out over the rest of the graph, with both classes following the two principal components. This indicates that the other PDO samples were more heterogeneous than the PDO

Nice samples. The first two PCs were responsible for 84.5% of the total variance, with the third accounting for about 7.7%. Therefore, the majority of the variance extracted from the original data matrix can be represented by only three principal components.

The main interest of the loading plot is to show the positive correlation between the PDO Nice samples and the compound with a retention index of 825 ((*E*)-hex-2-enal). The situation with the other PDO samples on the right side of the loading plot is more complex, due to the broad distribution of samples. Nevertheless, it is possible to highlight a correlation between the samples located at the top-right of the score plot (samples 63−66) and the compounds located in the same region on the loading plot (compounds with RI values of 594, 662, 675, 987, and 1020), indicating that the anaerobic degradation of olives
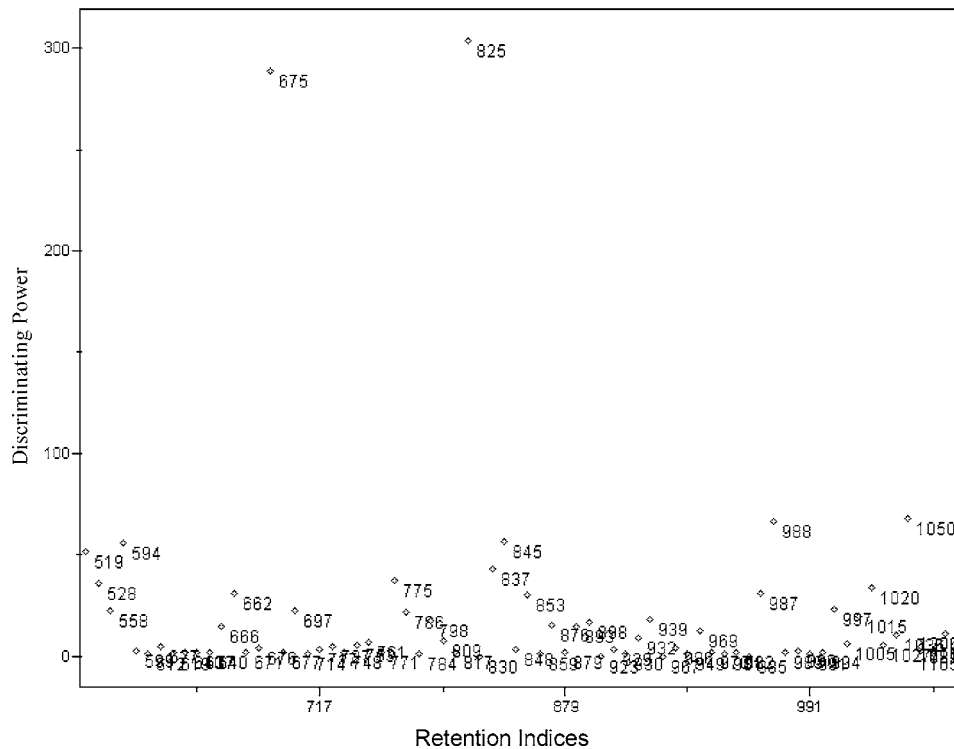
Figure 4. Discriminating power graph data matrix: PDO Nice olive oils and other French PDOs.
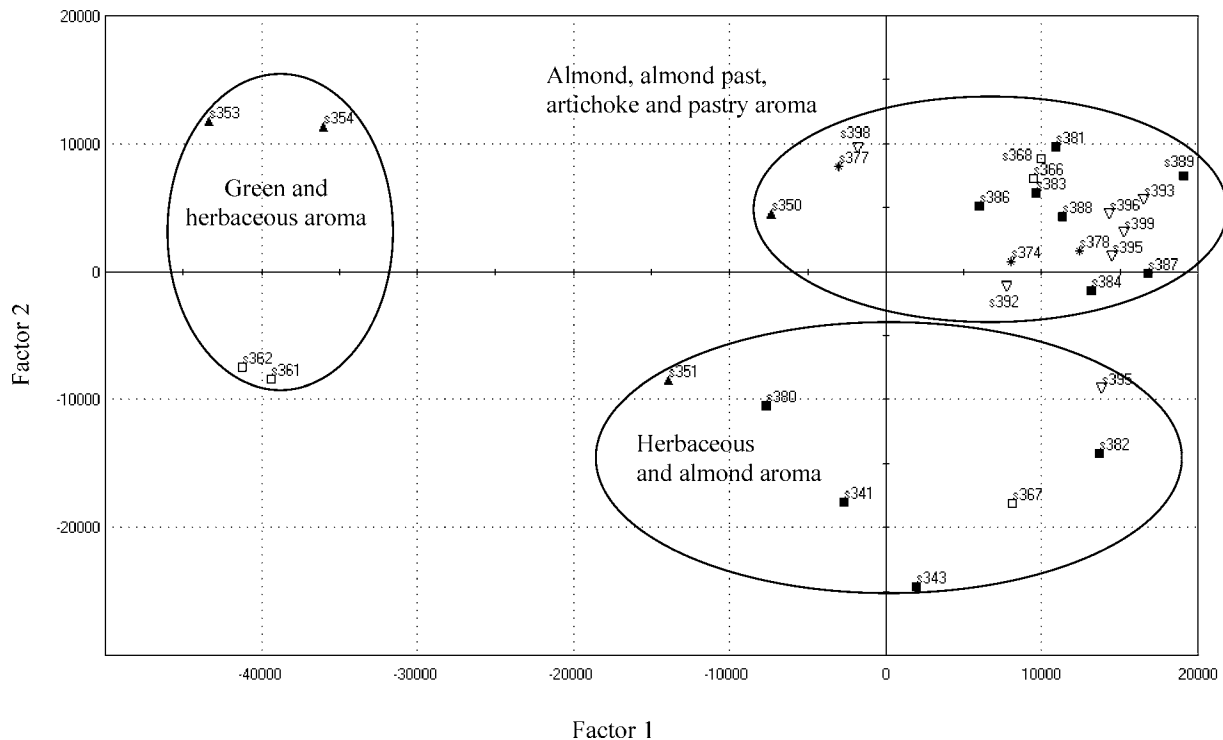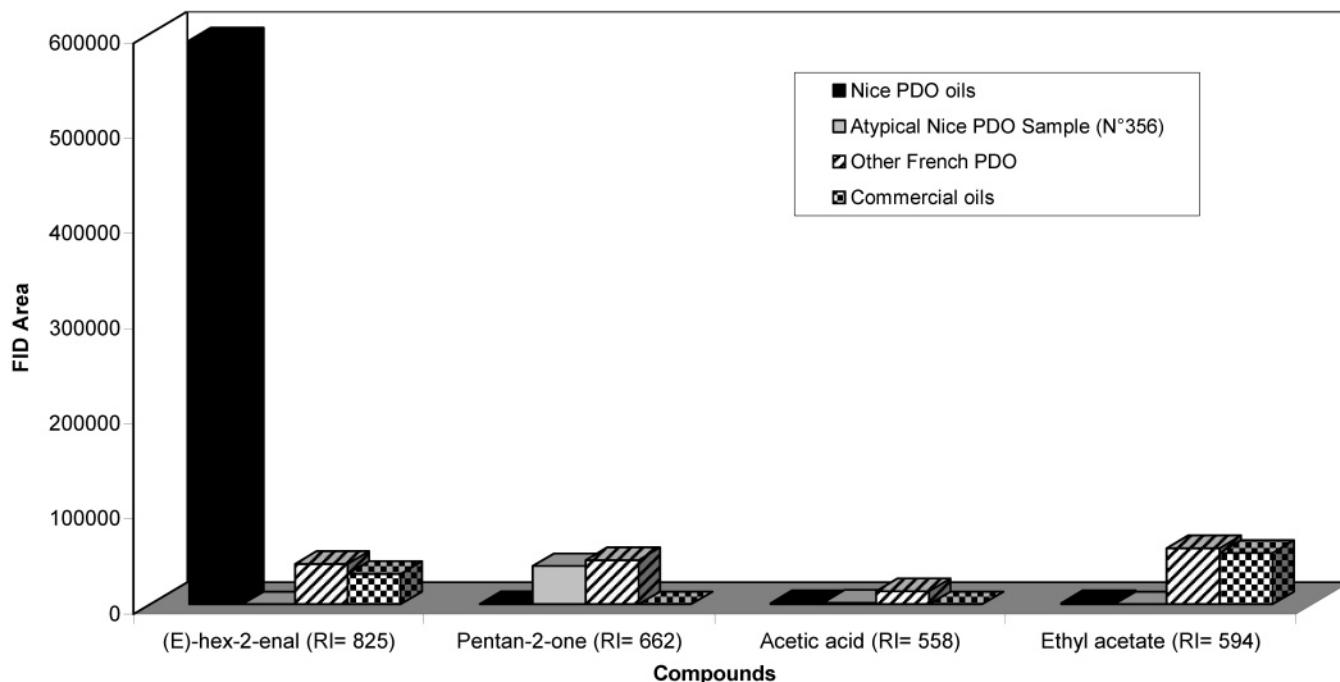

Figure 5. PCA score plot of PDO Nice olive oils; correlation with evaluations of olfactive attributes.

probably started in storage prior to oil extraction (*43*). In the same way, samples located at the bottom-right of the score plot (*61*, *62*) are correlated with compounds having RIs of 837 ((*Z*)-3-hexen-1-ol), 845 ((*E*)-2-hexen-1-ol), and 675 (pentan-3-one). Hexan-1-ol is obtained from the corresponding aldehyde by enzymatic reduction with alcohol dehydrogenase (ADH) (*40*, *41*). However, the other PDO olive oils seem to be characterized by other compounds, and these compounds are present in lesser quantities in PDO Nice samples. The classification model (**Figure 3**) obtained leads to only one misclassification.

The discriminating power graph (**Figure 4**) provides a good illustration of the relative abilities of the compounds present in the headspace to differentiate between PDO Nice oils and other French PDO oils (*44*). It is readily apparent that the compound with the RI of 825 ((*E*)-2-hexenal) shows the highest discriminating power, followed by the compound with the RI of 675 (pentan-3-one). By removing the compound with an RI of 825 from the model, it is possible to obtain a new discriminating power graph showing the next highest discriminating compounds (not shown here).

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Figure 6. Variation of (*E*)-2-hexenal, pentan-2-one, acetic acid, and ethyl acetate extracted by HS-SPME in the different olive oils studied.

Study of PDO Nice and Comparison with Sensorial Analysis. Many studies have been carried out on the classification of olive oils, particularly for verifying their quality (olive oil, virgin, or extra virgin, PDO) or origins (*7, 29−31*). However, correlations between analytical data and sensorial evaluations have received little attention.

All PDO Nice virgin olive oil samples were submitted to sensorial analysis. The panel was composed of fully trained assessors, each with more than 5 years of experience in evaluating and characterizing virgin olive oils. The perceptions were mainly odor and taste (combination of olfactory and gustatory sensations, but also tactile sensations).

The third question we wanted to answer was whether the method used correlates with results obtained by sensorial analysis. The data matrix used contains 30 samples. **Figure 5** presents the PCA score plot resulting from this matrix that allowed us to characterize the PDO oils from Nice.

PDO Nice olive oils feature a green herbaceous aroma with slight differences. The correlation analysis between the PCA scores and the olfactory properties revealed that these differences correspond to three different groups: a first group is characterized by a very green and herbaceous aroma; the two others present closer characteristics, with one defined by an herbaceous and almond aroma and the second presenting an almond, artichoke and pastry odor. This method highlights the significant correlations that exist between the chemical compositions of the volatile compounds present in PDO Nice olive oils and their sensory evaluations. Comparisons with taste properties are more difficult due to the role of combinations of sensorial sensations in determining such properties.

Discussion about the Applied Method. The applied method appears to be very efficient at identifying volatile compounds responsible for the typicity of French PDO virgin olive oils. The variations in the identified markers for the different virgin olive oils are presented in **Figure 6**.

PDOs from Nice are characterized by a high level of (*E*)-2-hexenal that distinguishes them from other oils, in particular commercial virgin oils. The latter are usually homogeneous mixtures of various olive oils and are characterized by their ethyl acetate and acetic acid contents.

Our method is well suited for distinguishing Nice PDOs from other French PDO olive oils and is capable of detecting a possible defect in a sample described as atypical (no. 356, with a stubble-mildewed odor). The use of the SIMCA procedure is very efficient, even more efficient than the widely used PCA. In comparison with electronic noses, particularly those coupled with SPME-enhanced headspace enrichment, the method we used is time-consuming and costly (*14*). Indeed, electronic noses have the advantage of supplying results rapidly and with ever increasing efficiency. However, once results are supplied by electronic noses, it is difficult to identify the chemical components responsible for any differences observed.

The use of HS−SPME−GC followed by chemometrics to study virgin olive oil quality suffers from some drawbacks. These difficulties mainly originate from the extraction method employed. Indeed, the state of the fiber has to be frequently monitored by running blank samples and by visual fiber examination. In this study, extractions were manually performed. While the results obtained were good, the manipulations were very time-consuming, particularly for routine analysis. The use of a specific autosampler such as CombiPAL (CTC Analytics, Switzerland) would facilitate the automation of the procedure and reduce the risk of fiber damage during sample preparation.

However, the main drawback encountered comes from the heterogeneity of the fiber lots. Indeed, the results obtained can vary substantially depending on the batches used. In this study, we used the same batch of fibers to ensure the level of repeatability required for chemometrics. But this issue could become a major hurdle for time-series studies, for example to measure the impact of storage on product quality. However, the ongoing progress that is being made with SPME technology, especially in the field of stationary phases, should reduce the impact of these limitations.

In conclusion, the use of HS-SPME−gas chromatography−multivariate analysis constitutes a useful technique for studying the typicity of protected designation of origin virgin oils. A total of 35 PDO olive oils of Nice, 6 commercial oils, and 12 other

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PDO oils were analyzed. Recorded data were submitted to principal component analysis and soft independent modeling of class analogy analysis. The method developed here is well suited for perfectly distinguishing among different qualities of olive oils and highlights the specificity of Nice PDOs. These oils are easily distinguishable from commercial virgin olive oils and from other PDO oils. Combining multivariate classification with the results of sensorial analysis, particularly olfactive properties, allowed the identification of three subgroups of Nice PDOs, in agreement with the sensorial analysis.

Complementary GC-MS analyses led to the identification of the volatile compounds responsible for the sample typicity. The method could be further improved by examining the effects of automation on the analytical results.

ACKNOWLEDGMENT

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