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A metabolic fingerprinting approach based on selected ion flow tube mass spectrometry (SIFT-MS) and chemometrics: A reliable tool for Mediterranean origin-labeled olive oils authentication

Aadil Bajoub[a], Santiago Medina-Rodríguez[a], El Amine Ajal[b], Luis Cuadros-Rodríguez[a], Romina Paula Monasterio[c], Joeri Vercammen[d], Alberto Fernández-Gutiérrez[a], Alegría Carrasco-Pancorbo[a, *]

[a] *Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Ave. Fuentenueva s/n, 18071 Granada, Spain*
[b] *Provincial Department of Agriculture of Azilal, PO Box 13, 22000 Azilal, Morocco*
[c] *Instituto de Biología Agrícola de Mendoza (IBAM), CONICET, Alt. Brown 500, Chacras de Coria, Mendoza, Argentina*
[d] *Interscience, Avenue J.E. Lenoir 2, 1348 Louvain-la-Neuve, Belgium*

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ABSTRACT

Ion flow tube mass spectrometry (SIFT-MS) in combination with chemometrics was used to authenticate the geographical origin of Mediterranean virgin olive oils (VOOs) produced under geographical origin labels. In particular, 130 oil samples from six different Mediterranean regions (Kalamata (Greece); Toscana (Italy); Meknès and Tyout (Morocco); and Priego de Cordoba and Baena (Spain)) were considered. The headspace volatile fingerprints were measured by SIFT-MS in full scan with H_3O^+, NO^+ and O_2^+ as precursor ions and the results were subjected to chemometric treatments. Principal Component Analysis (PCA) was used for preliminary multivariate data analysis and Partial Least Squares-Discriminant Analysis (PLS-DA) was applied to build different models (considering the three reagent ions) to classify samples according to the country of origin and regions (within the same country). The multi-class PLS-DA models showed very good performance in terms of fitting accuracy (98.90–100%) and prediction accuracy (96.70–100% accuracy for cross validation and 97.30–100% accuracy for external validation (test set)). Considering the two-class PLS-DA models, the one for the Spanish samples showed 100% sensitivity, specificity and accuracy in calibration, cross validation and external validation; the model for Moroccan oils also showed very satisfactory results (with perfect scores for almost every parameter in all the cases).

1. Introduction

The fact that VOO trade is becoming increasingly internationalized and very competitive is causing that various olive producing regions use different instruments to protect and promote specific qualities and attributes of their olive oils. They basically want to increase their economic profitability and gain competitive advantages in the global market (Parras-Rosa, 2013). Among these tools, there are geographical indications labels such as protected designations of origin (PDOs), protected geographical indications (PGIs) and traditional specialties guaranteed (TSGs). The emergence and further proliferation of these geographical indications labeling systems as a differentiating marketing strategy was mainly stimulated by the recent trend in consumers´ purchasing habits, that are increasingly placing value on products that they can associate with a certain place and/or special means of production (Barham, 2003; Lenglet, 2014; Verbeke & Roosen, 2009).

Under these labeling systems, two main types of olive oil attributes are enhanced: tangible characteristics related to olive oil distinctive quality and composition profile; and intangible features, linked to the cultural heritage from the protected region, local know-how, ancestral traditions, and legacy of olive tree (*Olea europaea* L.) cultivation and oil production. For all these characteristics (which constitute distinctive peculiarities of origin-labeled olive oils), this kind of products are usually commercialized at higher prices in the market. As a consequence, frauds and adulterations of these products with cheaper oils (with the obvious aim of obtaining a greater production) have become a raising problem over the last decades (López-Feria, Cárdenas, García-Mesa, &

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[*] Corresponding author.
Email address: alegriac@ugr.es (A. Carrasco-Pancorbo)

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Valcárcel, 2008). Therefore, the assessment of the traceability and authenticity of this type of products is a relevant concern which is attracting a lot of interest internationally at the moment.

Indeed, fraudulent practices have become more sophisticated, even if the use of production and origin labels of olive oil is strictly controlled by regulations, directives and laws that define a set of rules for its production, management, conservation and packaging (as well as internal and external control measures) (European Commission Regulation (EEC), 2012). For this reason, there is a need for reliable and effective methodological approaches to verify the authenticity of origin-labeled olive oils (Galtier et al., 2007; Giacalone, Giuliano, Gulotta, Monfreda, & Presti, 2015; Korifi, Le Dréau, Molinet, Artaud, & Dupuy, 2011).

In the described context, metabolomic approaches could offer (and are already doing it) positive perspectives for the assessment of olive oil geographical origin authenticity. Indeed, metabolomic strategies based on obtaining qualitative and/or quantitative information from major and/or minor olive oil compounds have been proposed as powerful methods to trace the geographical origin of this matrix. To date, various approaches, coming from the classical ones (based on the analysis of specific marker compounds) to the most advanced (more holistic approaches based on the use of olive oil fingerprints, considering the contribution of a wider number of compounds of this matrix, which can make adulteration detection more efficient) have been developed and successfully applied to olive oil geographical origin traceability monitoring (Beltrán, Sánchez-Astudillo, Aparicio, & García-González, 2015; Ben-Ayed, Kamoun-Grati, & Rebai, 2013; Nescatelli et al., 2014; Petrakis, Agiomyrgianaki, Christophoridou, Spyros, & Dais, 2008; Pizarro, Rodríguez-Tecedor, Pérez-Del-Notario, Esteban-Díez, & González-Sáiz, 2013; Portarena, Gavrichkova, Lauteri, & Brugnoli, 2014). In general, these approaches take advantage of the high sensitivity, selectivity and robustness of advanced analytical platforms (such as high performance liquid chromatography, gas chromatography, mass spectrometry, spectroscopic techniques and nuclear magnetic resonance) and the power of chemometric tools (such as PCA, Linear Discriminant Analysis (LDA), and PLS-DA, among others). At this point, it is important to bear in mind that, as it happens with other foodstuffs, the quality of olive oil and the concentration of its chemical components exhibit seasonal variations; moreover, this vegetable oil can also suffer some qualitative and quantitative changes on its composition along its shelf-life (Beltrán, Del Rio, Sánchez, & Martínez, 2004; Criado, Romero, Casanovas, & Motilva, 2008; Romero, Tovar, Ramo, & Motilva, 2003; Salvador, Aranda, Gómez-Alonso, & Fregapane, 2003). This means that the mathematical models classifying or characterizing samples of a particular geographical origin have to be updated or enriched over the time to keep being useful; pluri-annual projects as well as studies including a very wide number of samples are absolutely advisable in order to get robust, reliable and powerful models.

Among the minor components of olive oil, volatile compounds are of great interest within this context. The crucial role of aroma compounds to define sensorial attributes of olive oil is very well-known (Kesen, Kelebek, Sen, Ulas, & Selli, 2013; Morales et al., 1995). Moreover, they are among the most widely investigated components of this oily matrix for their ability to authenticate the geographical origin of this product (Ben Temime, Campeol, Cioni, Daoud, & Zarrouk, 2006; Cajka et al., 2010; Pizarro, Rodríguez-Tecedor, Pérez-del-Notario, & González-Sáiz, 2011). In most of the studies focused on olive oil aroma, the analysis of the volatile fraction is carry out by headspace plus chromatographic techniques and selective MS detection, especially GC–MS. The usefulness of this coupling has been, beyond a doubt, demonstrated; however (but not questioning the suitability of GC–MS), these methodologies are often time-consuming, require skilled operators and are relatively expensive.

SIFT-MS partially overcomes the just listed limitations. It is a direct injection-mass spectrometric technique which allows real-time monitoring of most volatile compounds. It has been characterized by very low detection limits, typically low part-per-billion (ppb) levels (Gibson, Dekker, & Ross, 2015). Furthermore, SIFT-MS allows collecting the overall MS fingerprints of the samples which can be further processed with chemometric techniques trying to achieve a successful discrimination and classification. Even though SIFT-MS has been previously used to investigate the volatile compounds present in olive oil headspace (Davis & McEwan, 2007; Davis, Senthilmohan, & McEwan, 2011; Davis, Senthilmohan, Wilson, & McEwan, 2005), this is the first report about the use of the same technology applied to the geographical assessment of origin-labeled olive oils. Thus, the aim of the present study was to develop a fast and reliable method for the discrimination of geographical indication labeled-olive oils by means of SIFT-MS fingerprinting measurements in combination with subsequent statistical interpretation of the obtained MS data.

2. Material and methods

2.1. Sample collection

A total of 130 samples belonging to six different Mediterranean origin-labeled regions were purchased from local stores or supplied by Agro-pôle Olivier National School of Agriculture's partners (*i.e.* organisms located in Meknès (Morocco)). The number of EVOO samples from each region was as follows: PDO Kalamata, Greece ($n = 15$); PDO Priego de Córdoba, Spain ($n = 25$); PDO Baena, Spain ($n = 20$); PDO Tyout-Chiadma, Morocco ($n = 25$); PGI Toscana, Italy ($n = 20$); and Meknès, Morocco ($n = 25$). A homogeneous bulk sample was prepared by mixing the same quantity of all bottles from the same region, creating 6 samples-mixtures which could be considered as overall representations of each geographical indication. Those batch materials were considered as quality control samples and were used during the preliminary study and, later on, for the method optimization and validation. Sufficient quantity of each sample (both individual olive oils and mixtures) was stored in dark bottles at − 20 °C under nitrogen atmosphere until instrumental analysis.

2.2. Samples preparation and SIFT-MS experiments

After preliminary studies, for each investigated sample, 10 g were exactly weighed in a glass bottle of 100 mL (Schott Duran bottles, Germany), which was then sealed with screw cap with two closed ports. Each one of them was equipped with a tube connection. Immediately after, glass bottles were placed in a water bath heated at 40 ± 1 °C for 10 min. A temperature sensor (± 0.1 °C) was used to ensure a homogeneous temperature distribution in the hot water bath. In order to allow the equilibrium of the analytes between the liquid phase and the headspace, the sample was continuously agitated with a magnetic stirring bar. After the heating time finished, the samples were removed from the bath for headspace analysis by mean of a Voice 200Ultra SIFT-MS (Syft Technologies, Christchurch, New Zealand).

Thus, one of two tubes connection was coupled to a nitrogen gas bag and the other was connected to the inlet needle of the SIFT-MS instrument. So, to sweep volatile compounds present in the headspace of the glass bottle, the screw cap ports were opened, nitrogen gas was added by passing an adjusted air stream through the headspace of the glass bottle at 0.35 bar using a needle valve, and volatile compounds were, then, sampled *via* a needle into the SIFT-MS flow tube through a calibrated and heated stainless steel flow limiter at a rate of 1.69 ± 0.1 Torr L s^{-1} (133 ± 8 mL/min under standard conditions).

The reagent ions (H_3O^+, NO^+ and O_2^+) were generated by a microwave air discharge at 0.5 Torr, selected by using a quadrupole mass filter and injected into a stream of carrier gas in the flow tube. Afterwards, the sample headspace was introduced into the carrier gas at a known rate, and product ions started to be formed (Fig. 1-supplementary information). All the ion products of the chemical ionization reaction and un-reacted reagent ions were monitored by a quadrupole mass spectrometer in the full scan mode in the mass-to-charge ratio (m/z) range of 10–200 over 60 s. The full scan data (ion counts per second) averaged over the sampling time for each m/z value was used for the statistical analyses.

The sample analysis order was randomized, at the beginning of each sequence and after every 10 analyses a blank and a test or control analysis was conducted.

Sample analyses were carried out in triplicate. For each replicate, and for each ion precursor, four full mass scans were recorded, but the first and last cycles were discarded for further calculations. The second and third scans were averaged yielding a mean mass spectrum/replicate. Then, the three averaged mass spectrums of the three replicates of each sample were put together to compute another average, obtaining a mean mass spectrum/sample. LabSyft software package (version 1.4.4, Syft Technologies) was used for the mass spectrum acquisition and data exportation as comma-separated values (CSV) files. In total, three data sets were obtained: H_3O^+ data matrix [130 (samples) × 187 (m/z) values]; NO^+ data set [130 × 187 (m/z) values] and O_2^+ data matrix [130 × 187 (m/z) values].

2.3. Chemometrics

The usefulness of the headspace fingerprints generated using SIFT-MS to authenticate the origin of geographical labeled Mediterranean VOOs was examined. For this purpose, the three built data sets mentioned above (H3O$^+$, NO^+ and O2$^+$) were imported into MATLAB (version 7.9) to create the data matrices and split data into training and testing sets. The Classification toolbox for MATLAB (version 7.9) and Solo - Eigenvector Research environment (version 8.5.1) were used for chemometric treatment of data.

Prior to the chemometrics analysis, individual data matrices from H_3O^+, NO^+ and O_2^+ analyses were preprocessed using an 'auto-scaling' method (*i.e.*, it standardizes the columns of the processed data matrix to have zero mean and unit variance).

As a first step, an exploratory data analysis based on PCA was applied as unsupervised multivariate technique just to reduce the dimensionality of the three data sets, detect the presence of outliers, explore samples and variables correlations and visualize general trends within the data. In a second step, multi-class and two-class PLS-DA models were built for each SIFT-MS data set obtained (H_3O^+, NO^+ and O_2^+) with the aim of evaluating the ability of the different proposed analytical approaches to authenticate the studied oils according to their geographical origin. In multi-class models, all the countries were included within analysis, whereas the two-class models were built to discriminate between samples from the same country, but coming from different regions. This latter approach was especially interesting to study samples from Spain and Morocco. In other words, three PLS-DA models were constructed for each data set (H_3O^+, NO^+ and O_2^+) after removing outliers:

Model 1: a multi-class model built from the entire sample set (128 samples) to classify the samples according to their country of origin; Model 2: a two-class model constructed with the subset of Spanish oils (44 samples), and Model 3: a two-class model built from the subset of Moroccan oils (49 samples). For each of the mentioned data sets, samples were randomly divided into training and test sets, containing 70% and 30% of the total number of considered samples, respectively (see Table 1 for details). In each case, the training set was used to calibrate the PLS-DA classification models, whereas test samples were only used in the final stage to evaluate the true predictive ability of the calibrated models.

An important issue when calibrating a PLS-DA model is the selection of the optimal number of Latent Variables (LVs), which is usually carried out by cross validation procedures. Cross validation is usually performed by dividing the training samples in several cross validation groups. Each cross validation group is then removed from the original training set, one at a time. Each time the model is calibrated on the remaining training samples and, then, used to predict samples of the cross validation group (Ballabio & Consonni, 2014). In this work, a venetian blind cross-validation comprising 10 data splits was used to determine the classification error average of cross-validation. The number of latent variables (LVs) for each PLS-DA model was estimated using the plots of the classification error average obtained in calibration and cross-validation. The optimal number of LVs was chosen to minimize the classification error average obtained by cross-validation (these results will be illustrated in Results and Discussion section).

Once the optimal number of latent variables for each model was chosen, the training sets were used to build the PLS-DA models. Calculation of the final PLS-DA models was performed selecting 7 latent variables (for model 1) and 2 (for models 2 and 3) and 10 cross validation groups for internal validation. After model calibration, the optimal models were validated on the external test sets. In PLS-DA, the n-di-

Table 1
Olive oil dataset for the three PLS-DA models studied: oil classes included in each model, number of samples per class, as well as sample partition in training and test sets for the three considered SIFT-MS data sets (H_3O^+, NO^+, O_2^+) after elimination of outliers.

PLS-DA model 1 (6 classes)

	Baena	Kalamata	Meknès	Priego de Córdoba	Toscana	Tyout	
	(Class 1)	(Class 2)	(Class 3)	(Class 4)	(Class 5)	(Class 6)	Total
Training set	14	11	17	17	14	18	91
Test set	6	4	7	7	6	7	37
Total	20	15	24	24	20	25	128

PLS-DA model 2 (2 Spanish classes)				PLS-DA model 3 (2 Moroccan classes)		
	Baena	Priego de Córdoba		Meknès	Tyout	
	(Class 3)	(Class 4)	Total	(Class 3)	(Class 6)	Total
Training set	14	17	31	17	18	35
Test set	6	7	13	7	7	14
Total	20	24	44	24	25	49

mensional class vector (containing the membership of samples to the *M* classes) is transformed into a binary two-dimensional matrix Y, with *n* rows (samples) and *M* columns (the class information). Each element y_{im} of Y represents the membership of the *i-th* sample to the *m-th* class expressed with a binary code (1 = in class or 0 = not in class). For each *i-th* sample and for each *m-th* class, PLS-DA returns estimated values class $y_{im}{}^{est}$ (raw predictions) which may have values between zero and one. A value closer to zero indicates the new sample is not in the modeled class, while a value closer to one is evidence that the sample is in the modeled class. In practice, two major classification rules are used to make the class assignment of the samples: 'strict class predictions' and 'most probable predictions'. Strict class predictions are based on the rule that each sample belongs to a class if the probability is greater than a specified threshold probability value (typically > 0.5) for one and only one class. If no class has a probability greater than the threshold, or if more than one class has a probability exceeding it, then the sample is assigned to class zero (0) indicating no class could be assigned. On the other hand, most probable predictions are based on choosing the class that has the highest probability regardless of the magnitude of that probability. Unlike to strict class predictions, under this approach, samples are always classified in one of the classes. In this work, this latter approach was used as class prediction rule for all PLS-DA models built.

The classification performance of PLS-DA models for each class was assessed from the confusion matrices obtained in fitting, cross validation and test. Confusion matrix is a table of results showing True Positive (TR), False Positive (FP), True Negative (TN) and False Negative (FN) rates as a matrix for each class modeled with a given classification model. These classification rates are defined as: TPR: proportion of positive cases that were correctly identified (Sensitivity), = TP / (TP + FN); FPR: proportion of negatives cases that were incorrectly classified as positive, = FP / (FP + TN); TNR: proportion of negatives cases that were classified correctly (Specificity), = TN / (TN + FP); FNR: proportion of positive cases that were incorrectly classified as negative, = FN / (FN + TP). In this work, different classification parameters, such as the class specificity and sensitivity, non-error rate (NER or accuracy), error rate (ER), precision and F1-score were derived from the confusion matrices in order to better evaluate the classification performance. The last three classification parameters were obtained as: ER or misclassification error: proportion of samples which were incorrectly classified, = 1 – accuracy = (FP + FN) / (TP + TN + FP + FN); precision: proportion of correctly assigned positive samples, = TP / (TP + FP); and F1-score: is the harmonic mean of the precision and sensitivity, = 2 ∗ TP/(2 ∗ TP + FP + FN). It should be noted that, TP/TN/FP/FN refer to the counts rather than the rates for these quantities. All parameters were reported as percentages, with the exception of the F1-score parameter (range of values between 0 and 1).

3. Results and discussion

3.1. SIFT-MS full scan headspace analysis

Fig. 1 shows the MS spectrum of headspace of a "control sample" using H_3O^+ (Fig. 1a), NO^+ (Fig. 1b) and $O_2{}^+$ (Fig. 1c) as precursor ions. The possibility of using three different precursor ions is one of the advantages of SIFT-MS, since it can provide complementary information which could be very useful for the identification of the sample compounds. For example, E-2-hexenal ($C_6H_{10}O$) reported as one of the major olive oil volatile compounds gives rise to the following reactions: with the H_3O^+, in a primary reaction a proton is transferred to the molecule producing $C_6H_{11}O^+$ with an m/z of 99. There is also the possibility to form clusters with the atmospheric water which is introduced alone with the sample. This secondary reaction can create two ion products $C_6H_{11}O^+\cdot 2H_2O$ (m/z = 135) and $C_6H_{11}O^+\cdot H_2O$ (m/z = 117). With NO^+, however, a hydride abstraction reaction can take place leading to the formation of $C_6H_9O^+$ (m/z = 97). Finally, reactions ratios with O_2 remain very small (between 0.2 and 0.3), which indicates the low reactivity of the mentioned compounds with this precursor ion.

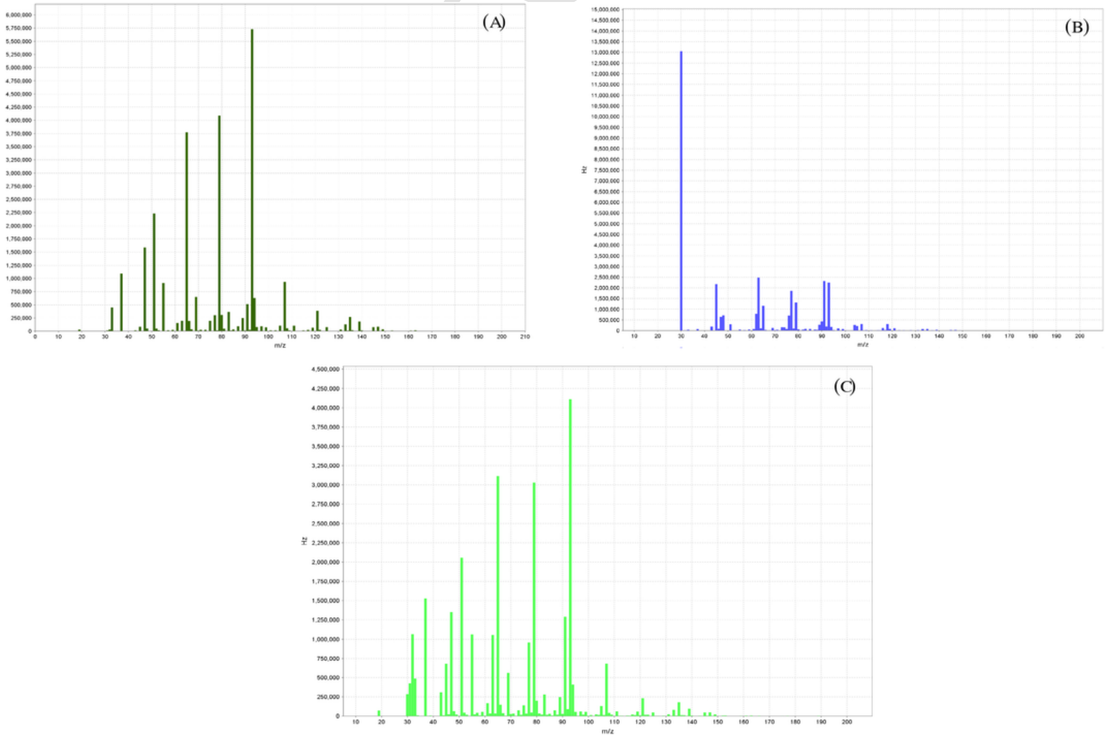

Fig. 1. Example of the fingerprint mass spectra of a quality control sample-headspace volatile fraction: (a) precursor ion H_3O^+; (b) precursor ion NO^+ and (c) precursor ion $O_2{}^+$.

3.2. Geographical classification of the studied oils

The data matrices containing the whole SIFT-MS spectrum (187 m/z values and their intensities) for samples coming from the six studied regions were submitted to PCA without any prior class label (in an unsupervised manner) to identify outliers and visualize the underlying trends. The projection of the samples onto the first four principal components allowed the observation of the distribution of the samples and gave us the chance to have a look at their grouping. Results are given in Fig. 2; there is a clear discrimination of Tyout-Chiadma (PC1 *vs*. PC2) and Toscana (PC2 *vs*. PC3, and PC3 *vs*. PC4) from the oils coming from the remaining regions (for which a significant overlapping is shown). Two samples (from Meknès and Priego de Córdoba) were identified as outliers.

Subsequently, the attention was driven towards the application of a supervised statistical technique (PLS-DA) to classify the olive oil samples on the basis of their geographical origins. As stated above, before building the PLS-DA models, the optimal number of LVs was chosen to minimize the classification error average obtained by cross-validation (see Fig. 2-supplementary material).

Fig. 3 shows the PLS-DA score plots of LV1, LV2 and LV3 obtained with the model 1 using the three calibration SIFT-MS data sets (H_3O^+, NO^+, O_2^+) after elimination of outliers. Similarly, Fig. 4 shows the PLS-DA score plots of LV1 and LV2 obtained with the models 2 and 3 for the three calibration SIFT-MS data sets. On the other hand, the achieved classification results in fitting, cross validation and test for

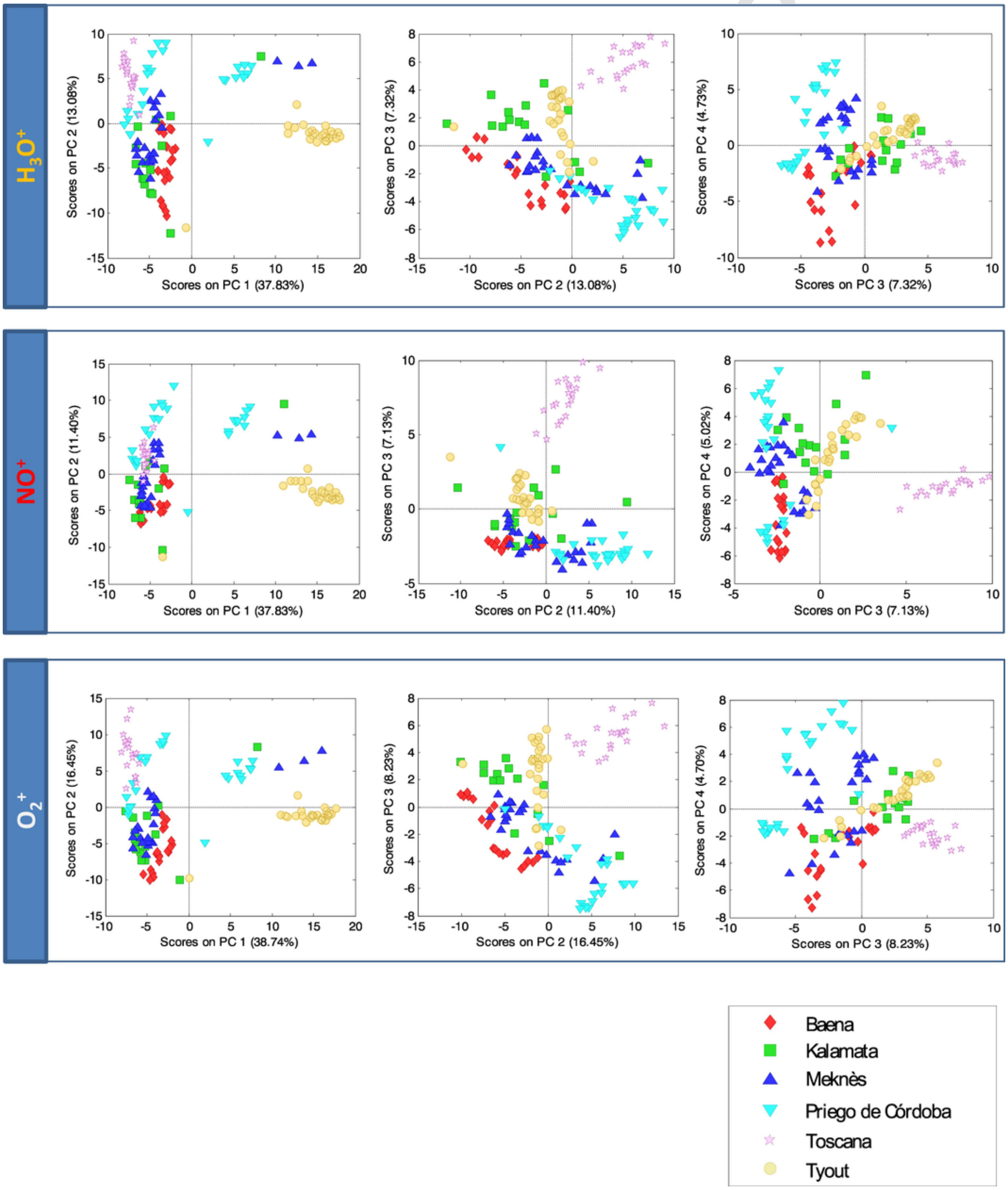

Fig. 2. PCA score plots of the studied olive oil samples on the first four principal components using the H_3O^+, NO^+ and O_2^+ data sets.*Samples' colour/shape codes are indicated and will be the same in illustrations 3 and 4 of the manuscript.

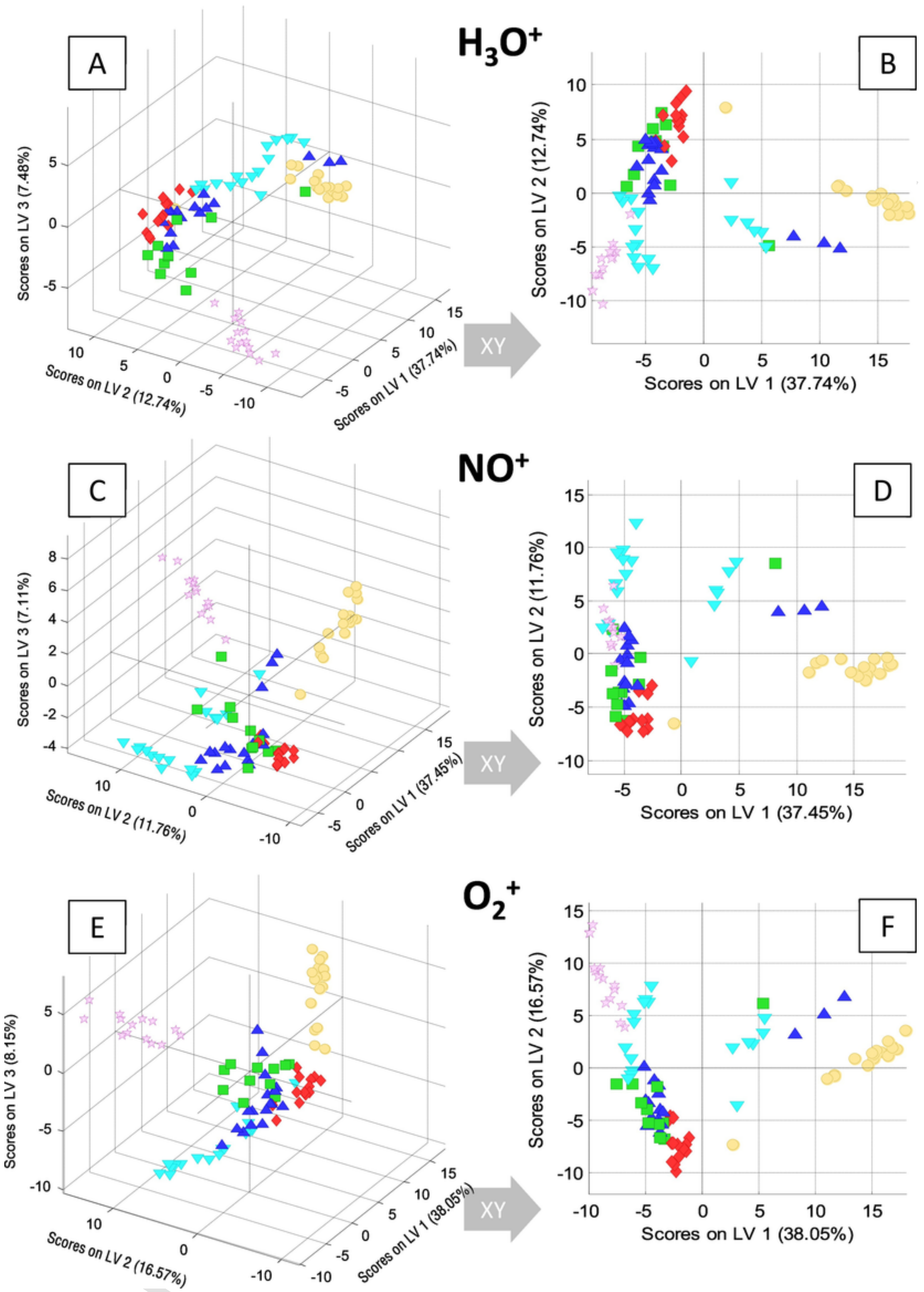

Fig. 3. Score plots (LV1 *vs* LV2 *vs* LV3) of PLS-DA model 1 for the three calibration SIFT-MS data sets (H_3O^+, NO^+, O_2^+) after elimination of outliers.

PLS-DA models 1, 2 and 3 are summarized in Tables 2 and 3, respectively. In addition, three of the most representative classification parameters (sensitivity, specificity and accuracy) are also represented graphically in Fig. 5 to facilitate comparison of data.

When considering the multi-class PLS-DA model (Table 2), it is possible to say that, regardless of the data set used, the multi-class PLS-DA models provided the possibility to discriminate among the six studied groups. They ensured almost excellent specificity and sensitivity in discrimination; the models were slightly worse for Kalamata oils. Besides, the similarity between the parameters obtained for the training and test sets indicate that overfitting did not occur, which assured the robustness and reliability of the PLS-DA models developed. Furthermore, very good classification and prediction rates were obtained regardless of the precursor ion employed. Thus, in calibration PLS-DA models showed an accuracy of 100% for all the studied categories, except for Priego de Córdoba and Tyout-Chiadma class, for which a value of

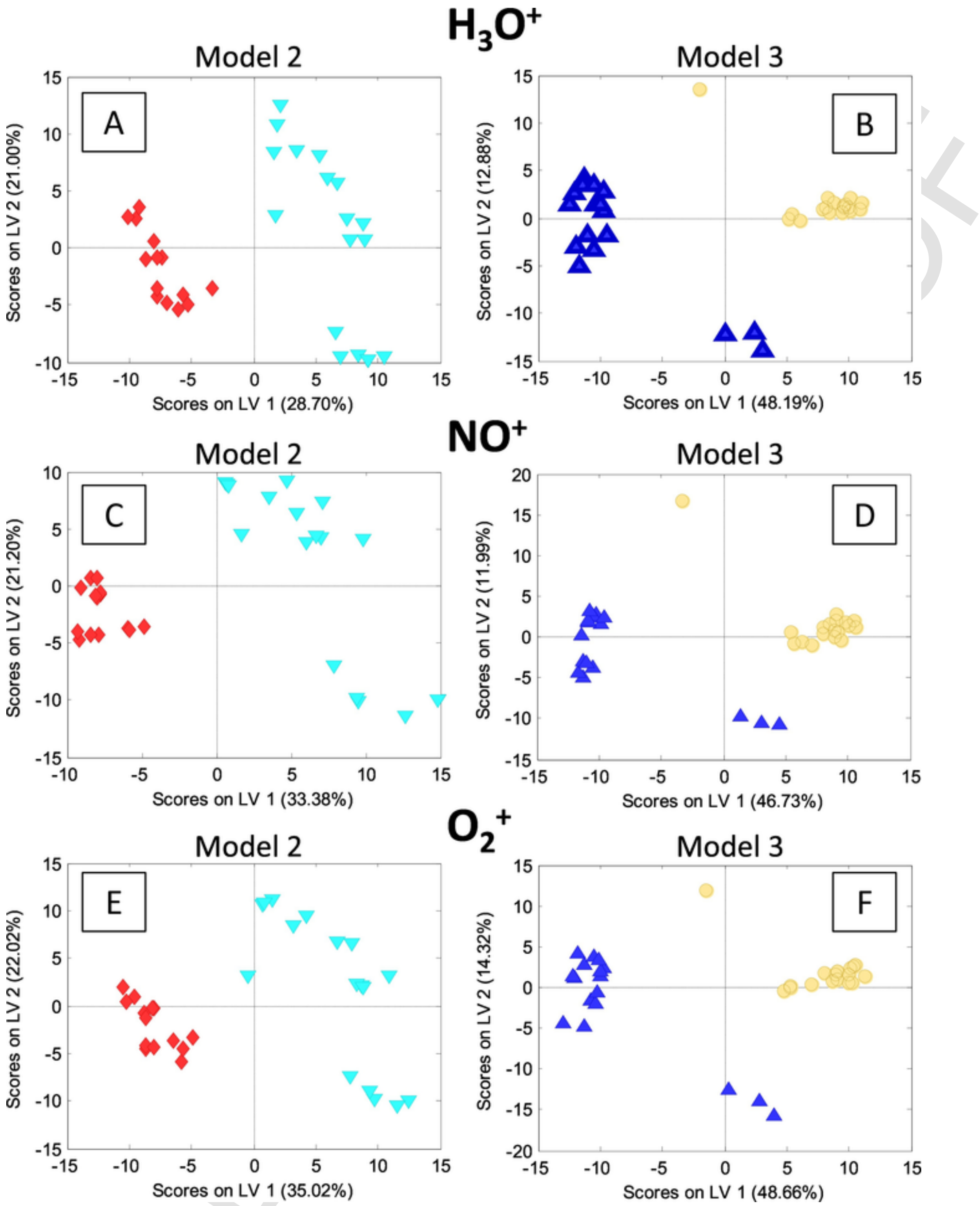

Fig. 4. Score plots (LV1 *vs* LV2) of PLS-DA models 2 and 3 for the three calibration SIFT-MS data sets (H_3O^+, NO^+, O_2^+) after elimination of outliers.

98.90% was obtained. In terms of prediction, accuracy values (in cross validation) were between 96.70% and 100%; whereas in external validation (test set), calculated rates were between 97.30% and 100%. The applied PLS-DA models gave the best results (100%, in both fitting and test set) for samples from Toscana (see Table 2 and Fig. 5A).

Considering the two-class PLS-DA models (Table 3), it is possible to stand out that regardless of the data sets used, model 2 showed 100% sensitivity, specificity and accuracy in calibration, cross validation and external validation (Fig. 5B). This result, from our point of view, proves the ability of the proposed methodology to correctly identify oils of the two selected Spanish geographical labeled regions (Priego de Cordoba and Baena). Likewise, model 3 -computed to discriminate between oils from the two considered Moroccan regions- showed very satisfactory results (see Table 3 and Fig. 5B). Sensitivity, specificity and accuracy values were 100% in all the cases (fitting, cross validation and test set), just finding few exceptions (when PLS-DA was applied to H_3O^+ and NO^+ data sets in cross validation, reporting values of sensitivity and specificity between 94.44% and 100%, and accuracy values between 97.14% and 100%). This outcome allowed us to verify the suitability of SIFT-MS, in combination with PLS-DA chemometric treatment, to efficiently differentiate the oils from the studied Moroccan regions.

Volatile compounds analysis had been already reported to be suitable for tracing the geographical origin of olive oil. However, to the best of our knowledge, the methodology proposed herewith seems to be advantageous (in comparison, for instance, with GC–MS), as it is less time-consuming (only 21 min in total are required for sample preparation and analysis) and provides higher recognition and prediction abilities.

4. Conclusions

The developed chemometric models, based on the SIFT-MS data of the volatile fingerprints of 130 VOO samples from six of the most important Mediterranean regions, provided a strong discrimination. The use of the entire headspace volatile fingerprinting matrices, of the

Table 2

Classification parameters obtained by PLS-DA multi-class models (model 1) in fitting, cross validation (with 10 groups split venetian blinds) and on the external test set for the three SIFT-MS data sets (H_3O^+, NO^+, O_2^+).

	PLS-DA model 1[a]														
Parameters[b]	H_3O^+						NO^+						O_2^+		
Fitting	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)
Sensitivity (%)	100.00	100.00	100.00	100.00	100.00	94.44	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Specificity (%)	100.00	100.00	100.00	98.65	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Accuracy (%)	100.00	100.00	100.00	98.90	100.00	98.90	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
ER (%)	0.00	0.00	0.00	1.10	0.00	1.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Precision (%)	100.00	100.00	100.00	94.44	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
F1-score	1.000	1.000	1.000	0.971	1.000	0.971	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Cross validation	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)
Sensitivity (%)	100.00	100.00	100.00	100.00	100.00	94.44	100.00	90.91	94.12	94.12	100.00	94.44	100.00	100.00	100.00
Specificity (%)	100.00	98.75	100.00	100.00	100.00	100.00	100.00	100.00	100.00	97.30	100.00	97.26	100.00	100.00	100.00
Accuracy (%)	100.00	98.90	100.00	100.00	100.00	98.90	100.00	98.90	98.90	96.70	100.00	96.70	100.00	100.00	100.00
ER (%)	0.00	1.10	0.00	0.00	0.00	1.10	0.00	1.10	1.10	3.30	0.00	3.30	0.00	0.00	0.00
Precision (%)	100.00	91.67	100.00	100.00	100.00	100.00	100.00	100.00	100.00	88.89	100.00	89.47	100.00	100.00	100.00
F1-score	1.000	0.957	1.000	1.000	1.000	0.971	1.000	0.952	0.970	0.914	1.000	0.919	1.000	1.000	1.000
Test set	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)
Sensitivity (%)	100.00	75.00	100.00	100.00	100.00	100.00	100.00	75.00	100.00	100.00	100.00	100.00	100.00	75.00	100.00
Specificity (%)	96.77	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	96.67	100.00	100.00	100.00
Accuracy (%)	97.30	97.30	100.00	100.00	100.00	100.00	100.00	97.30	100.00	100.00	100.00	97.30	100.00	97.30	100.00
ER (%)	2.70	2.70	0.00	0.00	0.00	0.00	0.00	2.70	0.00	0.00	0.00	2.70	0.00	2.70	0.00
Precision (%)	85.71	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	87.50	100.00	100.00	100.00
F1-score	0.923	0.857	1.000	1.000	1.000	1.000	1.000	0.857	1.000	1.000	1.000	0.933	1.000	0.857	1.000

Sample codes: (1) Baena; (2) Kalamata; (3) Meknès; (4) Priego de Córdoba; (5) Toscana; (6) Tyout.

[a] 7 LVs were chosen to build the PLS-DA models for all data sets. PLS-DA classification rule was "Class Pred Most Probable". This rule makes predictions based on choosing the class that has the highest probability (regardless of the magnitude of that probability). Note this differs from "Strict class predictions" because if more than one class has > 0.50 probability, the highest probability will "win" the sample. Likewise, if all probabilities are below 0.50, the largest probability still "wins".

[b] Parameters: Sensitivity (%): proportion of positive cases that were correctly identified, $= 100 \times TP/(TP + FN)$ (in percentage); Specificity (%): proportion of negatives cases that were classified correctly, $= 100 \times TN / (TN + FP)$ (in percentage); Accuracy (%): proportion of samples which were correctly classified, = 1-ER (in percentage); ER or Misclassification error (%): proportion of samples which were incorrectly classified (*i.e.*, for a class A, ER represents: 1) samples of class A which were incorrectly classified as not class A, and 2) samples not of class A which were incorrectly classified as being class A), $=(FP + FN) / (TP + TN + FP + FN)$ (in percentage); Precision (%): proportion of correctly assigned positive samples, $= TP / (TP + FP)$ (in percentage); F1-score: is the harmonic mean of the precision and sensitivity, $= 2 * TP/(2 * TP + FP + FN)$ (range of values between 0 and 1).

Table 3

Classification parameters obtained by PLS-DA two-class models (models 2 and 3) in fitting, cross validation (with 10 groups split venetian blinds) and on the external test set for the three SIFT-MS data sets (H_3O^+, NO^+, O_2^+).

	PLS-DA model 2[a]						PLS-DA model 3[a]					
Parameters[b]	H_3O^+		NO^+		O_2^+		H_3O^+		NO^+		O_2^+	
Fitting	(1)	(4)	(1)	(4)	(1)	(4)	(3)	(6)	(3)	(6)	(3)	(6)
Sensitivity (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Specificity (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Accuracy (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
ER (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Precision (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
F1-score	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Cross validation	(1)	(4)	(1)	(4)	(1)	(4)	(3)	(6)	(3)	(6)	(3)	(6)
Sensitivity (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	94.44	100.00	94.44	100.00	100.00
Specificity (%)	100.00	100.00	100.00	100.00	100.00	100.00	94.44	100.00	94.44	100.00	100.00	100.00
Accuracy (%)	100.00	100.00	100.00	100.00	100.00	100.00	97.14	97.14	97.14	97.14	100.00	100.00
ER (%)	0.00	0.00	0.00	0.00	0.00	0.00	2.86	2.86	2.86	2.86	0.00	0.00
Precision (%)	100.00	100.00	100.00	100.00	100.00	100.00	94.44	100.00	94.44	100.00	100.00	100.00
F1-score	1.000	1.000	1.000	1.000	1.000	1.000	0.971	0.971	0.971	0.971	1.000	1.000
Test set	(1)	(4)	(1)	(4)	(1)	(4)	(3)	(6)	(3)	(6)	(3)	(6)
Sensitivity (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Specificity (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Accuracy (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
ER (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Precision (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
F1-score	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Model 2: Discrimination between Spanish olive oils: (1) Baena and (4) Priego de Córdoba; Model 3: Discrimination between Moroccan olive oils: (3) Meknès and (6) Tyout.

[a] 2 LVs were chosen to build the PLS-DA models for all data sets.

[b] The description of the classification parameters can be found in Table 2.

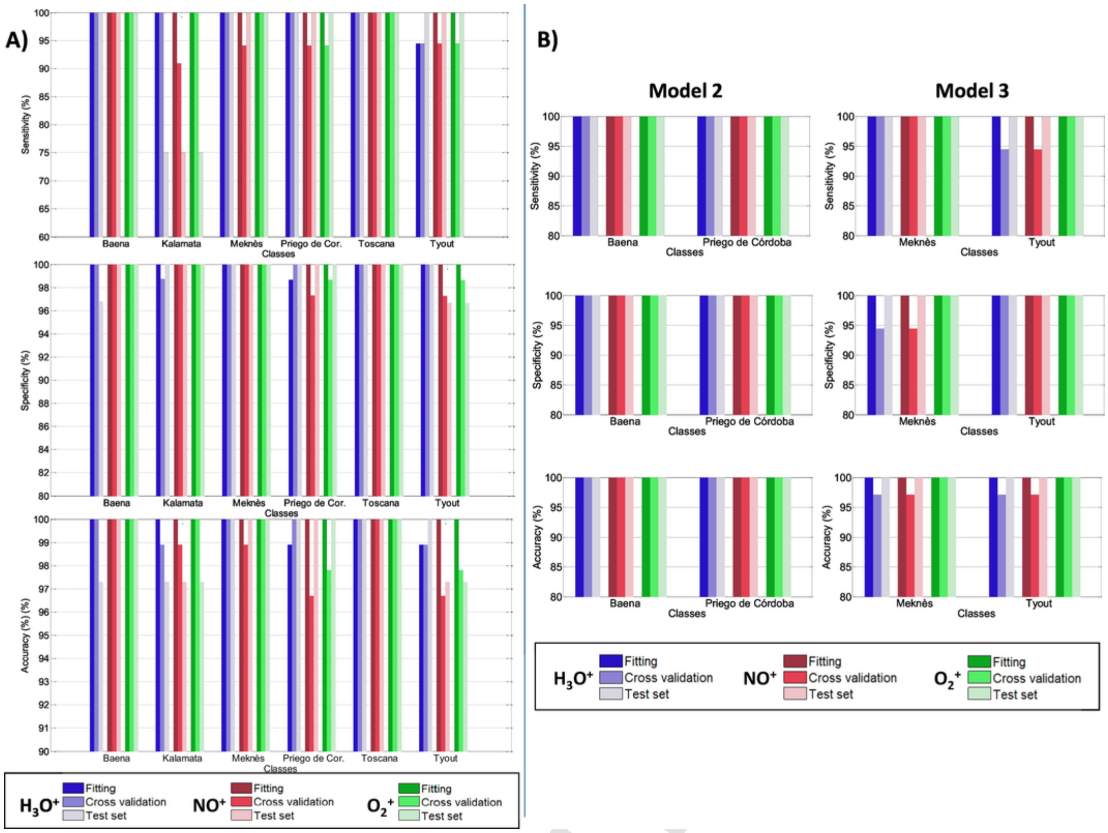

Fig. 5. A) Graphical representation of classification parameters (sensitivity, specificity and accuracy) obtained by PLS-DA multi-class models (model 1) in fitting, cross validation (with 10 groups split venetian blinds) and on the external test set for the three SIFT-MS data sets (H_3O^+, NO^+, O_2^+). B) Idem for PLS-DA two-class models (models 2 and 3).

three precursor ions, provided a holistic approach and the possibility of deducing the best possible combination to achieve our discrimination purpose for each studied region. This study indicates that the described methodology can be considered as a non-time consuming strategy suitable for routine analysis of origin-labeled olive oils.

To the best of our knowledge, the present work is the first report highlighting the use and potential of SIFT-MS fingerprinting for geographical origin authentication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2017.12.027.

References

Ballabio, D., Consonni, V., 2014. Classification tools in chemistry. Part 1: Linear models PLS-DA. Analytical Methods 5, 3790–3798.

Barham, E., 2003. Translating terroir: The global challenge of French AOC labeling. Journal of Rural Studies 19, 127–138.

Beltrán, G., Del Rio, C., Sánchez, S., Martínez, L., 2004. Influence of harvest date and crop yield on the fatty acid composition of virgin olive oils from cv. Picual. Journal of Agricultural and Food Chemistry 52 (11), 3434–3440.

Beltrán, M., Sánchez-Astudillo, M., Aparicio, R., García-González, D.L., 2015. Geographical traceability of virgin olive oils from south-western Spain by their multi-elemental composition. Food Chemistry 169, 350–357.

Ben Temime, S., Campeol, E., Cioni, P.L., Daoud, D., Zarrouk, M., 2006. Volatile compounds from Chétoui olive oil and variations induced by growing area. Food Chemistry 99 (2), 315–325.

Ben-Ayed, R., Kamoun-Grati, N., Rebai, A., 2013. An overview of the authentication of olive tree and oil. Comprehensive Reviews in Food Science and Food Safety 12 (2), 218–227.

Cajka, T., Riddellova, K., Klimankova, E., Cerna, M., Pudil, F., Hajslova, J., 2010. Traceability of olive oil based on volatiles pattern and multivariate analysis. Food Chemistry 121 (1), 282–289.

Criado, M.-N., Romero, M.-P., Casanovas, M., Motilva, M.-J., 2008. Pigment profile and colour of monovarietal virgin olive oils from Arbequina cultivar obtained during two consecutive crop seasons. Food Chemistry 110 (4), 873–880.

Davis, B.M., McEwan, M.J., 2007. Determination of olive oil oxidative status by selected ion flow tube mass spectrometry. Journal of Agricultural and Food Chemistry 55 (9), 3334–3338.

Davis, B.M., Senthilmohan, S.T., McEwan, M.J., 2011. Direct determination of antioxidants in whole olive oil using the SIFT-MS-TOSC assay. Journal of the American Oil Chemists' Society 88 (6), 785–792.

Davis, B.M., Senthilmohan, S.T., Wilson, P.F., McEwan, M.J., 2005. Major volatile compounds in head-space above olive oil analysed by selected ion flow tube mass spectrometry. Rapid Communications in Mass Spectrometry 19 (16), 2272–2278.

European Commission Regulation (EEC), 2012. Regulation (EU) No 29/2012 of 13 January 2012 on marketing standards for olive oil. Official Journal of the European Union L12 (14), 14–21.

Galtier, O., Dupuy, N., Le Dréau, Y., Ollivier, D., Pinatel, C., Kister, J., Artaud, J., 2007. Geographic origins and compositions of virgin olive oils determinated by chemometric analysis of NIR spectra. Analytica Chimica Acta 595 (1–2), 136–144.

Giacalone, R., Giuliano, S., Gulotta, E., Monfreda, M., Presti, G., 2015. Origin assessment of EV olive oils by esterified sterols analysis. Food Chemistry 188, 279–285.

Gibson, A., Malek, L., Dekker, R.F.H., Ross, B., 2015. Detecting volatile compounds from Kraft lignin degradation in the headspace of microbial cultures by selected ion flow tube mass spectrometry (SIFT-MS). Journal of Microbiological Methods 112, 40–45.

Kesen, S., Kelebek, H., Sen, K., Ulas, M., Selli, S., 2013. GC–MS–olfactometric characterization of the key aroma compounds in Turkish olive oils by application of the aroma extract dilution analysis. Food Research International 54 (2), 1987–1994.

Korifi, R., Le Dréau, Y., Molinet, J., Artaud, J., Dupuy, N., 2011. Composition and authentication of virgin olive oil from French PDO regions by chemometric treatment of Raman spectra. Journal of Raman Spectroscopy 42 (7), 1540–1547.

Lenglet, F., 2014. Influence of terroir products meaning on consumer's expectations and likings. Food Quality and Preference 32, 264–270, (PA).

López-Feria, S., Cárdenas, S., García-Mesa, J.A., Valcárcel, M., 2008. Classification of extra virgin olive oils according to the protected designation of origin, olive variety and geographical origin. Talanta 75 (4), 937–943.

Morales, M.T., Alonso, M.V., Rios, J.J., Aparicio, R., De, I., Csic, G., Tejero, A.P.G., 1995. Virgin olive oil aroma : Relationship between volatile compounds and sensory attributes by chemometrics. Journal of Agricultural and Food Chemistry 43, 2925–2931.

Nescatelli, R., Bonanni, R.C., Bucci, R., Magrì, A.L., Magrì, A.D., Marini, F., 2014. Geographical traceability of extra virgin olive oils from Sabina PDO by chromatographic fingerprinting of the phenolic fraction coupled to chemometrics. Chemometrics and Intelligent Laboratory Systems 139, 175–180.

Parras-Rosa, M., 2013. New global and local marketing strategies: Creation of added value through differentiation and high quality products. Options Méditerranéennes, Séries A: Mediterranean Seminars 106, 87–95.

Petrakis, P.V., Agiomyrgianaki, A., Christophoridou, S., Spyros, A., Dais, P., 2008. Geographical characterization of Greek virgin olive oils (cv. Koroneiki) using ^1H and ^{31}P

NMR fingerprinting with canonical discriminant analysis and classification binary trees. Journal of Agricultural and Food Chemistry 56 (9), 3200–3207.

Pizarro, C., Rodríguez-Tecedor, S., Pérez-Del-Notario, N., Esteban-Díez, I., González-Sáiz, J.M., 2013. Classification of Spanish extra virgin olive oils by data fusion of visible spectroscopic fingerprints and chemical descriptors. Food Chemistry 138 (2–3), 915–922.

Pizarro, C., Rodríguez-Tecedor, S., Pérez-del-Notario, N., González-Sáiz, J.M., 2011. Recognition of volatile compounds as markers in geographical discrimination of Spanish extra virgin olive oils by chemometric analysis of non-specific chromatography volatile profiles. Journal of Chromatography A 1218 (3), 518–523.

Portarena, S., Gavrichkova, O., Lauteri, M., Brugnoli, E., 2014. Authentication and traceability of Italian extra-virgin olive oils by means of stable isotopes techniques. Food Chemistry 164, 12–16.

Romero, M.P., Tovar, M.J., Ramo, T., Motilva, M.J., 2003. Effect of crop season on the composition of virgin olive oil with protected designation of origin "Les garrigues.". Journal of the American Oil Chemists' Society 80 (5), 423–430.

Salvador, M.D., Aranda, F., Gómez-Alonso, S., Fregapane, G., 2003. Influence of extraction system, production year and area on Cornicabra virgin olive oil: A study of five crop seasons. Food Chemistry 80, 359–366.

Verbeke, W., Roosen, J., 2009. Market differentiation potential of country-of-origin, quality and traceability labeling. The Estey Centre Journal of International Law and Trade Policy 10 (1), 20–35.