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EVOO or not EVOO? A new precise and simple analytical tool to discriminate extra virgin olive oils

C. Taiti ^{1(*)}, E. Marone ²

¹ *Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università degli Studi di Firenze, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy.*

² *Bioscienze e Tecnologie Agro-Alimentari e Ambientali, Università di Teramo, Via R. Balzarini, 1, 64100 Teramo, Italy.*

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(*) **Corresponding author:**
cosimo.taiti@unifi.it

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

Abstract: International Olive Oil Council (IOOC) states chemical and organoleptic parameters to classify the commercial grade of olive oil. Finding tools or analytical procedures able to support the organoleptic evaluation would be helpful to streamline and facilitate the commercial classification. The aim of the present study was to evaluate a new tool and validate a procedure that allows a fast and non-invasive volatile compounds detection system, able to assign each sample to its right trade category. Moreover, we tried to test the capability of PTR-ToF-MS in grading olive oils according to their *fruity* intensity levels. A total of 273 olive oil samples collected from Argentina (21), Chile (10), Italy (191), Morocco (17), Tunisia (4) and EU (30) were analyzed and classified through: (1) Panel Test and (2) PTR-ToF-MS analysis. On the whole PTR-ToF-MS data EVOO and Not EVOO as resulted by Panel Test were clustered by PCA in two main groups and correctly classified by PLS-DA model, confirming the high confidence level (95%) in utilizing analytical spectral data for helping Panel Test and able to easy monitoring the quality formation in the oils, by a fast and cheap control from harvest until the store. The eight protonated masses detected as VIP by the model may be linked to negative olfactory notes. Finally, PCA applied on the volatile profile of 122 classified EVOO highlighted a shift of the samples distribution following the trend of the *fruity* intensity as assessed by the panelists. In conclusion, this trial confirmed the availability of a new, precise and simple analytical tool as the PTR-ToF-MS, which coupled with an appropriate multivariate data analysis, allows to classify EVOO according to their trade category and *fruity* intensity.

1. Introduction

The virgin olive oil is the only vegetable oil consumed without any refinement and characterized by a peculiar synthesis between taste and aroma. The importance of extra virgin olive oil (EVOO) is due to its high content of oleic acid and phenolic compounds, which act as natural

antioxidants (Bendini *et al.*, 2007). Its composition makes it not only a food and dressing, but also a product able to protect the human organism from some dysfunctions and pathologies (Marone and Fiorino, 2012). As reported by Aued-Pimentel *et al.* (2013), EVOO has unique characteristics compared to other vegetable oils, such as exceptional sensory and nutritional attributes, therefore worldwide the olive oils are the most valuable ones with a price (normally 3-5 times) higher than other edible oils (Zou *et al.*, 2009). As a consequence, in the last years, some adulterations of EVOO with olive oils of lower quality, or with oils of different botanical origin (Catharino *et al.*, 2005; Vlachos *et al.*, 2006) have been found. As defined by the International Olive Oil Council (IOOC), olive oil is split in trade categories of different quality and commercial value. Because the high commercial value, and the relatively low availability against a high consumption, some traders and bottlers are prompted to sell as EVOO inferior olive oils that does not reflect the parameters established by the IOOC. According with the IOOC rules, the trade class attribution depends not only on chemically defined parameters (i.e. free acidity and peroxides index) but also on a sensory evaluation (SE) that assesses off-flavors and *fruity* presence and intensity. Therefore the EVOO is the only traditional food that must be tested through a Panel Test. Taste and aroma are determined by the presence and the amount of peculiar volatile organic compounds (VOCs), giving to the product unique appealing proprieties. On average the olive oil contains more than 100 volatile compounds belonging to different chemical categories (Guadarrama *et al.*, 2000). VOCs emission by the olive fruit and/or olive oil is mostly related to oxidative reactions (i.e. due to injuries during the fruits crushing and malaxation processes). VOCs develop according to distinctive biosynthetic pathways and, among these, the “LipOxygenase (LOX) cascade” determines the enzymatic splitting of polyunsaturated fatty acids (linoleic and linolenic) with the “controlled” production of aldehydes, ketones, alcohols, carboxylic acids, esters and other VOCs (Angerosa *et al.*, 2004; Kalua *et al.*, 2007).

The importance of the SE for the olive oils, is due both to its ability to identify the positive attributes and also to evaluate the defects (Peri and Rastelli, 1994). Indeed, the volatile compounds can be used: (a) to discriminate EVOO and virgin olive oil (VOO); and (b) as quality parameters, being the VOCs responsible especially for the green notes and *fruity* of high-quality EVOO oils (Gomez-Rico *et al.*, 2006).

While the chemical parameters are easily evaluated through chemical analyses, the flavor and off-flavors are assessed with more subjectivity through the sensory analyses. The SE by the Panel Test is based on strict and laborious rules, and needs trained peoples; therefore, as currently planned, the Panel Test is time consuming and very expensive. Thus, while the chemical analyses guarantees objectivity, repeatability and speediness, the sensory analysis does not allow this result. Indeed, as reported by Marone *et al.* (2017) the SE presents some disadvantages such as: (1) subjectivity of the analysis which could influence the overall evaluation; (2) the need of a large number of trained panelist (8-12) to allow the statistical validation of the results; (3) a limited number of samples evaluable by each panelist a day. Moreover, the results are difficult to generalize, because a lack of a common standard shared in the world, neither easy exploitable in any situations, nor to apply at any step of chain of olive oil making before sale (i.e. processing, storage). Consequently, there is no doubt that the detection of each type of olive oil manipulation needs to be addressed to ensure a correct trade classification, quality and consumer price.

Currently, the most common used analytical techniques to detect VOCs emitted by olive oil are both chromatographic and spectrophotometric methods, as the dynamic headspace gas chromatograph (DHS-GC) (Procida *et al.*, 2016), electronic nose and electronic tongue (Aparicio *et al.*, 2000; Cosio *et al.*, 2007) and the Proton Transfer Reaction-Time of Flight-Mass Spectrometer (PTR-ToF-MS) (Aprea *et al.*, 2006; Marone *et al.*, 2017).

The PTR-ToF-MS shows a high resolution coupled to a rapid screening power of samples, it is easy to handle and does not need any sample manipulation (Blake *et al.*, 2009; Taiti *et al.*, 2017). Moreover, this tool is applicable to any step of the olive oil production, from the processing to the market, including the product storage (Marone *et al.*, 2017). Furthermore, as the PTR-ToF-MS could work at temperature near those of the tasting, it should give as output a bulk of VOCs at least similar to those perceived by panelists or consumers. A first attempt to directly link spectral data from PTR-ToF-MS as protonated masses to the olfactory sensations perceived by the panelists, to distinguish EVOO from Not EVOO, and consequently correctly classify the virgin olive oils in their trade category, was recently carried out by Marone *et al.* (2017). In this cited work, although employing a low number of samples, it was possible to build up, in a

statistically meaningful way, a color codified card highlighting some specific VOCs that seem to characterize the off flavors as perceived by the panelist. Starting from this result, the aim of the current work was to develop and to test a fast analytical method that combines efficiency, accuracy and reliability for a rapid screening and quantification of volatile compounds in olive oil samples. This analysis method should be helpful to: (1) detect the main defective odors and distinguish the olive oils trade categories; (2) understand if there is an accurate and precise correlation between the judgment provided by the Panel Test and the analysis of the volatile component by PTR-ToF-MS; (3) evaluate different quality and types of EVOO using the positive attributes (i.e. *fruity* and *green* notes).

2. Materials and Methods

Oil sampling

Analyses were carried out during 3 years of surveys (from 2015 to 2017) on the whole spectra of 273 olive oil samples, produced from 2012 to 2017 (Table 1). The olive oils came from three different continent (Africa, South America, and Europe); most samples were obtained from producers or supermarkets, both blend or monocultivar stocks; in this last case, the most of the olive oil samples were obtained at the olive mill. To enhance and enlarge the samples set variability, EVOO from supermarkets labeled as origin were acquired together with “aged” samples (certainly processed two or more years before to be analyzed). For each sample, two filled dark bottles of 250 ml were collected and quickly sent to the storage refrigerated room (17°C) until the organoleptic and VOCs analyses were carried out. Finally, for some samples, the VOCs and SE analyses were repeated during the three years of analysis.

Panel test

After the spectrometric determinations all samples were submitted to a Panel Test. All panels were organized according to the official E.U. olive oil sensory analysis Regulation (n. 2568/91 and its successive modifications) (Table 1). Each taster on the panel shall enter the intensity of the negative and positive attributes on the 10-cm scale in the profile sheet. The oil is graded by the Panel Leader in line with the median of the defects and the median for the *fruity* attribute. According to the reference ranges, an olive oil is graded as extra virgin if the median of the

defects is 0 and the median of the *fruity* attribute is above 0. In the present work, all the samples that did not result EVOO were classified as Not EVOO, without any further distinction.

Volatile compounds detection

Measurements were performed with a chemical ionization mass spectrometer (PTR-MS) equipped with a Time-of-Flight (ToF) analyzer (PTR-ToF 8000 model, Ionicon Analytik, Innsbruck, Austria) in its standard mode and using H_3O^+ as ions for the chemical ionization. PTR-ToF-MS has some advantages compared to the other traditional electron ionization such as: reduced fragmentation which eases compound identification and guarantees high sensitivity with a very high time resolution and no need of sample treatments (Taiti *et al.*, 2017). Previously Blake *et al.* (2009) provided a complete and detailed description of the PTR-MS technology. All the instrumental parameters used during the measurement were set as follow: a constant drift voltage of 600 V and a pressure of 2.20 ± 0.02 mbar were maintained in the reaction chamber and the instrument operated at a standard E/N value (electric field strength/gas number density) of 138 Td ($1 \text{ Td} = 10^{-17} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$). Each sample was prepared on the basis of the following protocol: 15 g of oil (T 25°C) were introduced in apposite glass jar (750 ml), afterwards were fluxed with clean air (Zero air generator, Peak scientific) for 120 seconds and subsequently were hermetically sealed and incubated for 60 seconds at 25°C inside an incubator. Each jar was provided with inlet and outlet Teflon pipes, which connect the glass jar to the PTR-ToF-MS system and to the zero-air generator, respectively. Two replicates of each sample were analyzed and the order of samples was randomized. Besides, at the beginning of the experiment and always after three oils sample an identical empty jar was analyzed for background subtraction. Headspace concentrations of each oil sample were subsequently averaged over the two replicates and used for further statistical analysis. The range of mass spectra was recorded in the range of 20-210 m/z at 1 spectrum per 1 second, and the mass calibration was based on $m/z = 21.022$ (H_3O^+), $m/z = 59.049$ ($\text{C}_3\text{H}_7\text{O}^+$) and $m/z = 137.132$ ($\text{C}_{10}\text{H}_{17}^+$); the calibration was made before starting each files and, subsequently, all files were recalibrated off-line. Data were recorded with the TofDaq software (Tofwerk AG, Switzerland) and all spectra were acquired and analyzed using a procedure previously reported by Taiti *et al.*, 2017. Data were expressed in ppbv following a procedure

Table 1 - Description of 273 olive oil samples in relation to provenience zone, cultivar, acquisition from producer or supermarkets, processing campaign or getting year, PTR-ToF-MS analysis year, and Panel Test judgement (EVOO/Not EVOO)

Provenience zone	Cultivar/blend	Number of samples	Get from: producer (1), supermarkets (2)	Processing campaign (A), or acquisition (B) year	PTR-ToF-MS analysis year	EVOO (0)/Not EVOO (1)
Argentina	Arbosana	4	1	2017 A	2017	1 (4)
Argentina	Blend	4	2	2012 B	2016	1 (4)
Argentina	Blend	4	1	2013 A	2016	1 (4)
Argentina	Blend	4	1	2014 A	2016	1 (4)
Argentina	Coratina	2	1	2015 A	2016	1 (2)
Argentina	Coratina	1	1	2017 A	2017	0 (1)
Argentina	Koroneiki	2	1	2017 A	2017	0 (2)
Chile	Arbequina	2	1	2012 A	2017	1 (2)
Chile	Arbosana	4	1	2012 A	2017	1 (4)
Chile	Arbosana	1	1	2013 A	2017	1 (1)
Chile	Koroneiki	1	1	2012 A	2017	1 (1)
Chile	Koroneiki	2	1	2013 A	2017	1 (2)
Italy	Arbequina	4	1	2012/13 A	2016	1 (4)
Italy	Arbequina	3	1	2013/14 A	2016	1 (3)
Italy	Arbequina	7	1	2015/16 A	2016	0 (7)
Italy	Arbequina	5	1	2016/17 A	2017	0 (5)
Italy	Arbosana	3	1	2015/16 A	2016	0 (3)
Italy	Arbosana	2	1	2016/17 A	2017	1 (2)
Italy	Blend	2	1	2012/13 A	2017	1 (2)
Italy	Blend	11	1	2015/16 A	2015	0(11)
Italy	Blend	12	1	2015/16 A	2016	0(12)
Italy	Blend	14	1	2016/17 A	2017	0(10)
Italy	Blend	17	2	2017 B	2017	1(4) 0(13)
Italy	Carolea	5	1	2013/14 A	2015	1(5)
Italy	Frantoio	8	1	2013/14 A	2015	1(8)
Italy	Frantoio	2	1	2015/16 A	2015	0(2)
Italy	Gentile di Chieti	9	1	2015/16 A	2015	0(9)
Italy	Gentile di Chieti	4	1	2015/16 A	2016	0(4)
Italy	Intosso	7	1	2015/16 A	2015	0(7)
Italy	Intosso	4	1	2015/16 A	2016	0(4)
Italy	Itrana	7	1	2015/16 A	2015	0(7)
Italy	Itrana	4	1	2015/16 A	2016	0(4)
Italy	Koroneiki	3	1	2015/16 A	2016	0(3)
Italy	Koroneiki	4	1	2016/17 A	2017	0(4)
Italy	Leccino	5	1	2013/14 A	2015	1(5)
Italy	Maurino sel. Vittoria	3	1	2015/16 A	2016	0(3)
Italy	Maurino sel. Vittoria	4	1	2016/17 A	2017	0(4)
Italy	Oliana	5	1	2016/17 A	2017	1(5)
Italy	Olivastra seggianese	18	1	2015/16 A	2016	0(3) 1(15)
Italy	Peranzana	5	1	2015/16 A	2015	0(5)
Italy	Peranzana	4	1	2015/16 A	2016	0(4)
Italy	Sikitita	5	1	2015/16 A	2016	0(5)
Italy	Sikitita	5	1	2016/16 A	2017	1(5)
Morocco	Arbequina	2	1	2012/13 A	2017	1(2)
Morocco	Picholine maroccaïne	3	1	2014/15 A	2016	1(3)
Morocco	Picholine maroccaïne	3	1	2014/15 A	2017	1(3)
Morocco	Picholine maroccaïne	3	1	2015/16 A	2016	1(3)
Morocco	Picholine maroccaïne	6	1	2015/16 A	2017	1(6)
Tunisia	Koroneiki	4	1	2012/13 A	2017	1(4)
U.E.	Blend	5	2	2016 B	2016	1(5)
U.E.	Blend	25	2	2017 B	2017	1(25)

described by Lindinger and Jordan (1998). Then, the data obtained were filtered by eliminating peaks that were lower than a threshold of 0.50 ppbv and elimi-

nating all signals relative to ions hard to quantify precisely. After filtration, data have been sent to the statistical analysis (Fig. 1).

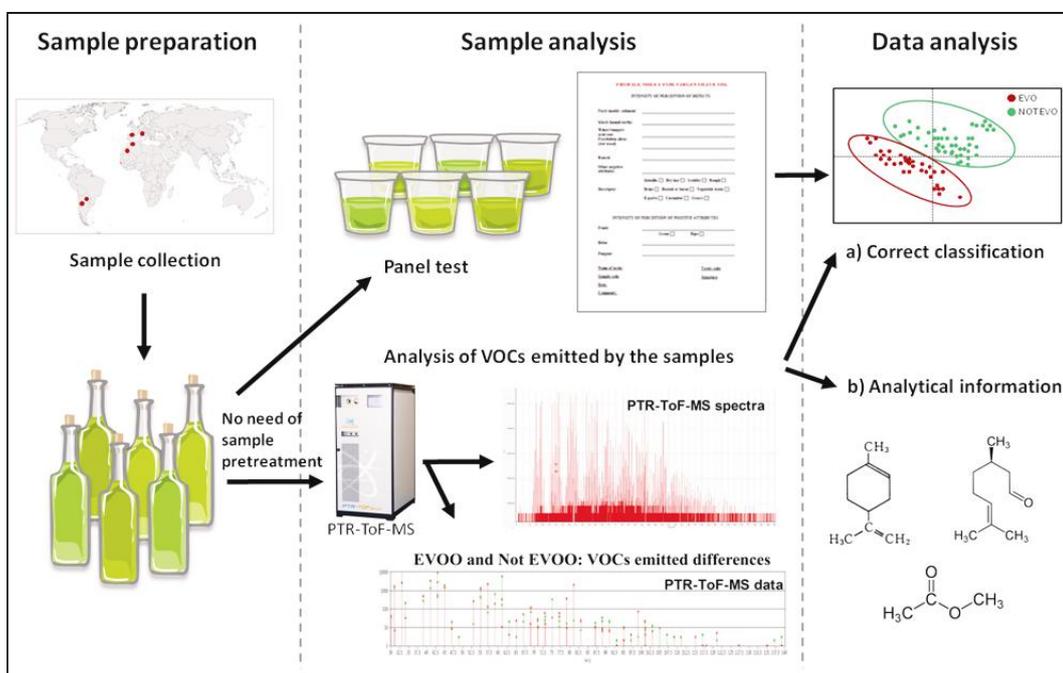


Fig. 1 - Schematic representation of oil samples analyses and classification using the PTR-ToF-MS. This technique allows rapid and non-destructive VOCs detection throughout the entire food-to-fork chain (e.g. oils) without any sample pretreatment. All data acquired by PTR-ToF-MS were used to obtain analytical information regarding the quality of product and for trade categories, varieties and geographical origin classification applying different multivariate analyses.

Multivariate data analysis

A principal component analysis (PCA, unsupervised method) was applied to the spectral data of 273 olive oil samples, submitted to a logarithmic transformation and mean centering as pre-processing. Computations were performed by PLS-Toolbox v. 8.0.2 (Eigenvector Research Inc., West Eaglerock Drive, Wenatchee, WA) for MATLAB® R2015b (Mathworks Inc., Natick, MA, USA). A multivariate partial least squares-discriminant analysis (PLS-DA, supervised method) was applied on the spectra of the 273 olive oil samples, to develop a model for differentiating EVOO from Not EVOO. As pre-processing data, they were submitted to a logarithmic transformation and auto-scaling. The training set (85% of the samples) allowed to select the optimal number of latent variables (LVs) throughout the calibration and cross validation phases. The training and validation subsets were obtained by the Euclidean distances based on the algorithm of Kennard and Stone (1968). The test set (prediction) consisted of 15% of the samples previously removed from the dataset. As cross validation procedure, Venetian blind with 10 splits and 1 sample per split was chosen. The performances of the model were evaluated by the number of correct assignments and the root-mean-squared error of cross-validation (RMSECV), and prediction (RMSEP). The optimal number of LVs resulted associated to the minimum error and misclassification rate of the cali-

bration dataset. The reliability of the model was tested by confusion matrices. The threshold to assign a sample to a class was chosen based on the Bayes theorem, minimizing the number of false positives and false negatives. Variable Importance in Projection (VIP) scores ($p=0.01$) were also calculated. A random permutation of the class labels (permutation test) was also performed (500 iterations), so to generate nonsense datasets for comparison with the true model, to evaluate the probability that the model is significantly different from one casually built up under the same conditions. PLS-DA analysis was performed by PLS-Toolbox v. 8.0.2 (Eigenvector Research Inc., West Eaglerock Drive, Wenatchee, WA) for MATLAB® R2015b (Mathworks Inc., Natick, MA, USA). A PCA was then applied to the spectral (PTR-ToF-MS) data of the 122 samples resulting EVOO based on the Panel Test, previously submitted to a logarithmic transformation and auto-scaling.

3. Results and Discussion

EVOO or Not EVOO

VOCs emission by olive oil is characterized by the presence of different compounds belonging mainly to alcohols, esters, aldehydes, ketones, terpenes and hydrocarbons. C6 molecules are the main volatile compounds derived from polyunsaturated fatty acids

through the LipOxygenase pathway (Cecchi and Alfei, 2013), generally characterized by low molecular weight. These compounds easily come in contact with the olfactory cells and help to create flavor and sometimes off-flavor. According to Marone *et al.* (2017), there is the possibility to directly relate the volatile profile obtained by PTR-ToF-MS to distinguish EVOO from Not EVOO, and, as a consequence, to correctly classify the virgin olive oils in their trade category. To confirm the preliminary results obtained by Marone *et al.* (2017), and validate the new procedure and methodology, we used a huge number of samples that were collected and analyzed in different years. In the present work, to define their trade category, 273 olive oil samples were submitted to the SE, that classified 151 samples as Not EVOO, and 122 as EVOO. By analyzing each oil sample (Table 1), 63 volatile compounds were detected within a mass range of $m/z = 20-210$ (data not shown). PCA applied to the whole dataset (ppbv) allowed to get a first general overview of the data distribution. Two main groups of EVOO and Not EVOO were clearly highlighted (Fig. 2) in the bidimensional space of the first two components, despite the great variability present in the original data set, due to the great diversification in the olive oil samples. This variability is also evidenced by the need to consider the first 7 components to justify 90.17% of the total variance (respectively: 60.26%, 12.56%, 5.17%, 3.81%, 3.39%, 2.78, and 2.20%). The data ordination clearly highlights that the VOCs spectra provided by Not EVOO samples were well distinguishable from those of the EVOO, with a few partially overlapping zones in the upper right and bottom left quadrants. This behaviour indicates a different spectral distribution between flavors and off-flavors, confirming the same result

obtained by the SE. Subsequently, a partial least squares discriminant analysis (PLS-DA) approach was applied to determine the trade category of the olive oil samples. A seven-component PLS-DA model, evaluated by its performances indicators (Table 2), resulted robust to discriminate the Not EVOO from the EVOO samples in the model/validation data set, and in the independent test set. The optimal number of latent variables (LVs), associated to the minimum error rate and the minimum number of not assigned samples, resulted in 7 (Table 2). The permutation test

Table 2 - PLS-DA statistics for each Y-Block (class 1 = Not EVOO; class 0 = EVOO) related to 273 olive oil samples. Sensitivity (SE); Specificity (SP); Class error, RMSEC, RMSECV, and RMSEP for Calibration (Cal), Cross Validation (CV), and Prediction (Pred), respectively. Confusion matrices for Calibration, Cross Validation, and Prediction

Statistics	LVs	SE	SP	SE	SP	Class.	Class.	Class.	RMSEC	RMSECV	RMSEP
		(Cal)	(Cal)	(CV)	(CV)	error (Cal)	error (CV)	error (Pred)			
Not EVOO	7	0.972	0.968	0.954	0.960	0.029	0.043	0.042	0.206	0.261	0.226
EVOO		0.968	0.972	0.960	0.954						

		Classes		Matthew's correlation coefficient	
		1-Not EVOO	0-EVOO		
Calibration results	Predicted as	1-Not EVOO	105	4	0.940
		0-EVOO	3	121	
Cross validation results	Predicted as	1-Not EVOO	103	5	0.914
		0-EVOO	5	120	
Prediction results	Predicted as	1-Not EVOO	16	2	0.903
		0-EVOO	0	22	

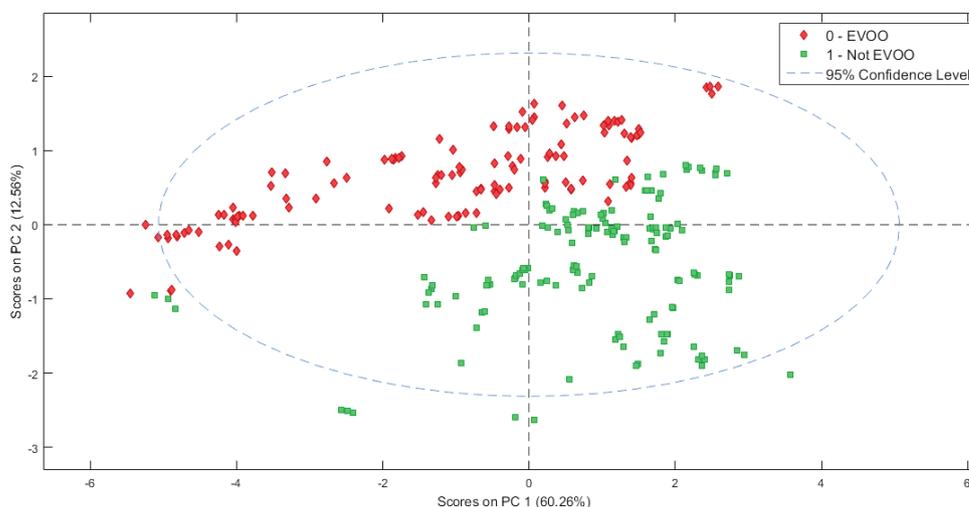


Fig. 2 - PCA ordination of 273 olive oil samples. Green = Not EVOO, red = EVOO.

indicated that the model is significant at 95% confidence level. In fact, the probability of model insignificance vs. permuted samples resulted 0.0 based on the Wilcoxon and Sign Test, both in Self-Prediction and Cross-Validated, and 0.005 by the Rand t-test. The model successfully classified 96.9% of samples into their trade category based on the Panel Test results in fitting, 95.5% in cross validation (internal validation) and 95% in prediction (external validation). That is, as reported in the confusion matrices (Table 2), in the calibration, on a total of 233 samples, 226 were correctly classified, while 3 resulted false positive (predicted as EVOO from the Panel Test, but classified as Not EVOO by the spectrometer) and 4 false negative (predicted as Not EVOO from the Panel Test, but classified as EVOO by the spectrometer). In the cross validation, 223 samples were correctly classified, while 5 resulted false positive, and 5 false negative. In the prediction results, on 40 samples, 38 were correctly assigned to their right class, while only 2 resulted false positive. The occurring of false positive (3 samples in prediction, judged as EVOO by the Panel Test, and classified as Not EVOO by the spectrometer), can be related to the fact that all compounds (including off-flavors) are only perceived by the human olfactory when they exceed their specific threshold values (Morales *et al.*, 2013). Thus, we can assume that, below this threshold values, the presence of a given compound linked to a defect is not perceived by the human olfactory, but is inexorably detected by the spectrometer. On the other hand, only a few borderline olive oils judged as Not EVOO by the panelists but classified as EVOO by the tool (false negative) were detected. A scores plot of the first two components of the PLS-DA model for all oil samples is shown in figure 3. The

PLS-DA model also allowed to evidence the significant (>1.5) VIP scores, indicating the role of the selected protonated masses to differentiate the two classes (Fig. 3). VIP scores reported in figure 3 confirm the results of our preliminary work (Marone *et al.*, 2017). In particular, the masses $m/z = 47.050$ (Tentatively identified (TI) as: ethanol), $m/z = 61.030$ (TI: acetic acid), $m/z = 75.040$ (TI: propanoic acid) and $m/z = 89.060$ (TI: butanoic acid) resulted as factors able to distinguish EVOO from Not EVOO. Indeed, ethanol and acetic acid are generally considered as compounds deriving from microbial alterations due to a long time of olive storage before processing (Morales *et al.*, 2000) and therefore represent a known defect. Likewise propanoic acid and butanoic acid are both considered defective compounds, that can be linked to fermentation processes in olive fruits as a long time of storage (Angerosa *et al.*, 1996) or related to the sugar fermentation (Morales *et al.*, 2013).

Overall, for all the samples evaluated, it is interesting to note that the procedure applied in this study to discriminate EVOO from Not EVOO is not affected by factors such as: year under analysis, harvesting year, variety and geographical origin. In fact the model resulted significant at 95% confidence level only considering as classification (variability) factor the EVOO/Not EVOO distinction.

Classification of different EVOO fruity intensity

EVOO are currently also labeled according to the *fruity* intensity perceptions (*robust, medium, delicate*), based on the IOOC regulation (COI/T.20/Doc. No 15/Rev. 8, 2015). Thus, we tried to evaluate the different EVOO *fruity* intensity using the dataset provided only by the VOCs profile of the 122 EVOO samples (according to the Panel Test) (Table 1) through a

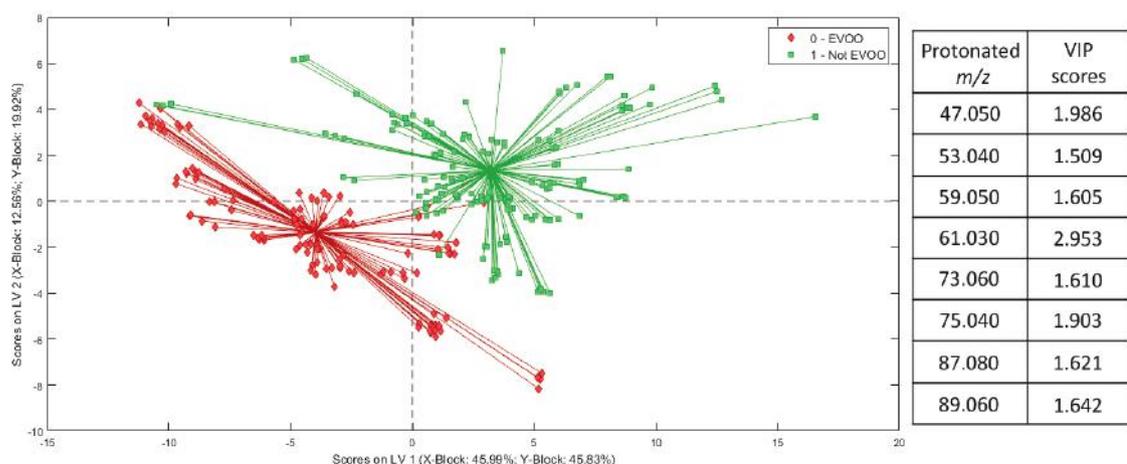


Fig. 3 - Score plot (LV1, LV2) of the PLS-DA model. Green = Not EVOO, red = EVOO; VIP scores > 1.5.

PCA analysis. The first two components explained about 63% of the total variability, and the derived scatterplot (Fig. 4) showed three groups of samples that are rather well separated. It is interesting to note that linking the *fruity* score assigned by the Panel Test to each sample, the three groups distributed in the chart according to their *fruity* intensity (Fig. 4), even if the samples grouped by the tool at the bottom of the figure (Fig. 4) show VOCs profiles relatively close, while the *fruity* intensity scores attributed to the same samples by the panelists ranged from 4 to 7. According to this chemometric approach, the subjectivity of the Panel Test becomes evident at intermediate values of *fruity* intensity. PCA analysis also underlines two outliers group. In the first one, labeled with "M", the three samples belonging to cv. Maurino sel. Vittoria harvested in year 2016 are found; this can be linked to peculiar flavor notes characterizing this Tuscan clone, that showed the highest amount of terpene compounds compared to all other samples (data not shown). The second one, represented by a few samples separated from the central bulk and shifting to the right part, labeled with "S" (Fig. 4), is formed by samples with particular flavor notes (data not shown). These samples, belonging to the cv. Sikitita, as reported by García-Gonzalez *et al.* (2010), are in fact characterized by typical aromas.

Associating the *fruity* score assigned by the Panel Test to each sample, it is remarkable as the entire

aromatic profile detected by the PTR-ToF-MS seems to be linked to changes in the amount of masses within the spectra rather than to the presence of specific compounds in the human olfactory perception of the *fruity* intensity.

4. Conclusions

The chemometric classification model proposed in this trial and based on the VOCs fingerprint acquired by the PTR-ToF-MS allows to distinguishing olive oil samples of different trade category. In particular, it was demonstrated that: (1) the entire volatile profile can be useful to classify oils belonging to different commercial categories (as the Panel Test), (2) the different qualities and types of EVOO can be split by using the *fruity* intensity. The accuracy of classification proposed is very high and it is more efficient than that obtained by other authors using different tools. Indeed, this tool does not require any sample pre-treatment and allows identifying compounds with low molecular weight (i.e. methanol, ethanol, etc.) compared to other ones.

Given our results and the emerging need of the olive oil sector that requires the developmental analytical tools to support or integrate the Panel Test, this work opens the way for the use of PTR-ToF-MS coupled with an appropriate multivariate analysis, as a quick and cheap tool with high confidence level and

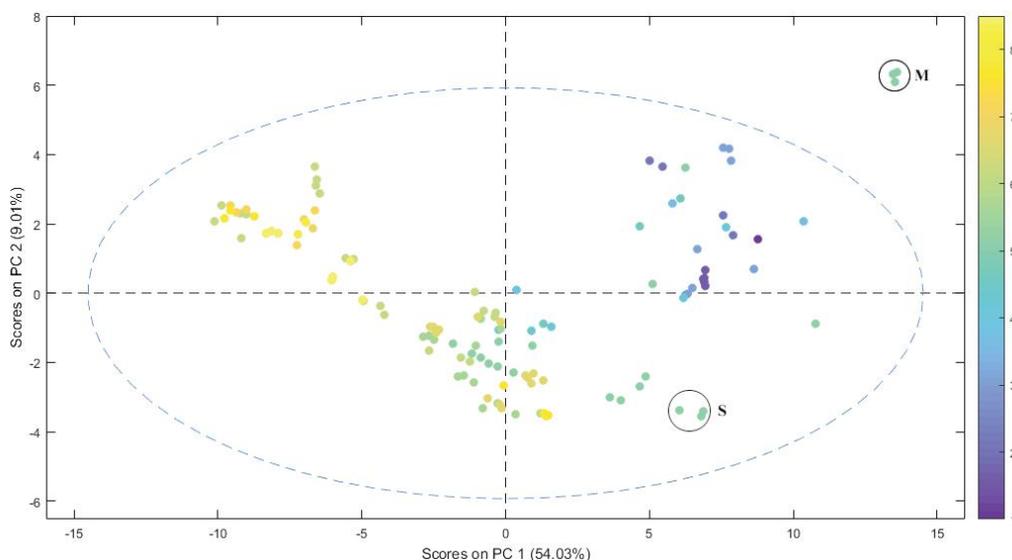


Fig. 4 - PCA ordination of 122 extra virgin olive oil samples. The objects key color indicates *fruity* intensity scores as evaluated by the Panel test increasing from blue (*fruity* = 1) to yellow (*fruity* = 8.5). Black circled samples indicate: Maurino sel. Vittoria (M) and Sikitita (S).

comparable to the Panel Test, for the olive oil quality identification.

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