Talanta 134 (2015) 394–401

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# Validation of SPME–GCMS method for the analysis of virgin olive oil volatiles responsible for sensory defects

I. Romero [a], D.L. García-González [a,*], R. Aparicio-Ruiz [a], M.T. Morales [b]

[a] Instituto de la Grasa (CSIC), Universidad Pablo de Olavide, Edificio 46. Ctra. de Utrera, km. 1, 41013 Sevilla, Spain
[b] Department of Analytical Chemistry, University of Seville, c/Prof. García González 2, 41012 Seville, Spain

ARTICLE INFO

*Article history:*
Received 7 August 2014
Received in revised form
6 November 2014
Accepted 17 November 2014
Available online 25 November 2014

*Keywords:*
Virgin olive oil
Volatiles
Sensory defects
Analytical validation
SPME–GCMS

ABSTRACT

Volatile compounds are responsible for the aroma of virgin olive oil and also for its quality. The high number and different nature of volatile compounds drive to the need of a reliable analytical method that allows their proper quantification to explain the standard method of panel test. Although there are some analytical solutions available, they have not been validated and the regulatory bodies are reluctant to adopt them since they can be subjected to unknown errors. In this regards, the European Union has encouraged the validation of these analytical tools through the research program Horizon2020, which involves gaining knowledge from the analytical properties of the chemical methods for sensory assessment. This work is focused on the analytical validation of the methodology used to determine the actual concentration of volatiles in virgin olive oils when applying SPME–GCMS. The validation process includes the calibration curves for 29 volatile compounds responsible for the most common sensory perceptions in virgin olive oils, the determination of their working ranges with linear response, the detection and quantification limits, the sensitivity, the accuracy estimated as trueness and precision and the selectivity. Sixty-seven percent of the compounds presented a relative standard deviation in repeatability lower than 10%, and this percentage rises to 95% in lampante virgin olive oils. The accuracy was established in 97% of the studied volatile compounds. Finally, an empirical example of the ability of the method to discriminate virgin olive oils of different categories (extra virgin, virgin, ordinary and lampante) by the quantification of their volatiles is provided.



## 1. Introduction

Virgin olive oil (VOO) is extracted from the fruit of the olive tree, *Olea europaea* L., by mechanical processes only. No further treatment is required before its consumption, so a considerable concentration of minor compounds is retained in the oil. VOOs characteristic aroma depends on volatile compounds, many of which derived from the degradation of polyunsaturated fatty acids through the lipoxygenase pathway, which occurs during the oil extraction process [1]. However, several processes can alter the initial profile of the volatiles, originating unpleasant sensory notes, which are known as sensory defects [2]. When VOOs reach high intensities of sensory defects they are classified as lampante virgin olive oils (LVOO) and must undergo refining before being consumed, which explains the economic importance of detecting the presence of volatile compounds responsible for sensory defects in VOOs [2–4]. Volatile compounds are then crucial to determine VOO quality from an objective viewpoint.

The standard method for analyzing VOO sensory quality is, however, the sensory assessment by trained panelists [5]. The method determines the category of a VOO (extra-virgin, virgin, ordinary and lampante) [6] according to the detection and intensity of sensory defects. Nevertheless, the sensory assessment methodology (henceforth panel test) is not simple, and a permanent staff of trained panelists is required. Moreover, panel test is a costly and slow procedure that is not always at the disposal of small and medium-sized enterprises and cooperative societies. In some instances, the subjective opinion of the panelists influences the final overall evaluation too, and some flaws have been pointed out [7]. For this reason, analytical methods based on identification and quantification of volatiles [2,4,8,9] are needed to achieve the correct classification of VOOs in a rapid and efficient way.

In this context, solid phase microextraction (SPME) is the most used system in the isolation and preconcentration of volatiles, prior to gas-chromatographic analysis [1], among the proposed for evaluating VOO [10,11], and also for characterizing several monovarietal VOOs [11–13]. However, none of the authors had published a complete validation of the applied methodologies so far, which results in confusion about the concentrations of volatiles quantified in VOOs when comparing results described in the

* Corresponding author. Tel.: +34 954 61 15 50; fax: +34 954 61 67 90.
*E-mail address:* dluisg@cica.es (D.L. García-González).

http://dx.doi.org/10.1016/j.talanta.2014.11.032
0039-9140/

bibliography [3], although a partial validation was carried out with a SPME–GC/Ion Trap Mass Spectrometric method in both EI and CI ionization modes [9] As a consequence, none of these methods based on SPME–GC can be considered fully established since the quantification of volatiles may be subjected to significant errors that hinder the sensory interpretation from the chemical data. That is one of the reasons that today no regulatory body has adopted any of proposed methods as alternative to sensory assessment by panel test [1,14]. This analytical problem arises in the context of a series of criticisms to the sensory quality of European extra virgin olive oil as well as to the standard method of sensory assessment [15]. These criticisms have resulted in a strong demand by producers, consumers and regulatory bodies for alternative methods based on the quantification of the volatile compounds. On the other hand, the European Union, as the main producer and consumer of olive oil [16], has reacted by funding new activities in the validation of analytical methods for substituting/complementing the organoleptic assessment of virgin olive oil through the research program for 2014–2020 Horizon2020 [17]. Since there are analytical alternatives for aroma analysis that have not been validated, the European Union has centered the attention into the validation of these techniques to promote the establishment of those within the industrial and regulatory realms.

The aim of this work is the validation of a SPME–GCMS analytical method for identification and quantification of VOO volatile compounds to demonstrate "suitability for its intended purpose" since the objective of analytical measurements is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal, and the results from the method validation can be used to judge quality, reliability and consistency of analytical results, which are part of the integrated quality assurance for analytical measurement. In consequence, the validation is useful in order to enable a continuous control of VOO sensory defects, which is one of the current challenges in olive oil authentication and quality [14,17].

## 2. Materials and methods

### 2.1. Reagents

Octane, pentanal, hexanal, heptanal, octanal, nonanal, *E*-2-hexenal, *E*-2-heptenal, ethanol, butan-1-ol, butan-2-ol, 2-methyl-butan-1-ol, 3-methyl-butan-1-ol, hexan-1-ol, *E*-3-hexen-1-ol, heptan-2-ol, 1-octen-3-ol, pentan-3-one, heptan-2-one, 1-penten-3-one, 6-methyl-5-hepten-2-one, octan-3-one, 1-octen-3-one, acetic acid, propanoic acid, butanoic acid, pentanoic acid, ethyl acetate and ethyl butanoate were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2. Samples

A refined olive oil carefully deodorized (Aceites del Sur, S.L.), and checked for absence of volatiles, was used to prepare the dilutions of the standard volatile compounds. Additionally, an extra virgin olive oil (EVOO) *var.* Hojiblanca from Sierra de Yeguas (Málaga, Spain) (Fig. 1) and a LVOO qualified with the rancid sensory defect (5.5 intensity) (Fig. 2) – supplied by the International Olive Council (IOC) – were used to validate the analytical method.

A total of 22 LVOO samples, from different cultivars and collected from different oil mills of Oleoestepa SCA (Estepa, Spain) were analyzed using the validated method. These samples were qualified with main sensory defects (rancid, winey-vinegary, mustiness-humidity, hay-wood and fusty) by Oleoestepa sensory panel (Table 1), which is a recognized official panel test by IOC [6].

### 2.3. Sample preparation

The oil sample (2 g) was placed in a 20 mL glass vial, tightly capped with polytetrafluoroethylene (PTFE) septum, and left for 10 min at 40 °C to allow for the equilibration of the volatiles in the headspace. After the equilibration time, the septum covering each vial was pierced with a SPME needle and the fiber was exposed to the headspace for 40 min. The SPME fiber (1 cm length and 50/30 μm film thickness) was purchased from Supelco (Bellefonte, PA), and it was endowed with the Stable Flex stationary phase of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The fiber was previously conditioned following the instructions of the supplier.

### 2.4. Gas chromatography–mass spectrometry

The volatiles adsorbed by the fiber were thermally desorbed in the hot injection port of a gas chromatograph 7820 coupled to a quadrupole mass spectrometer Series MSD 5975 (Agilent Technologies, Santa Clara, CA) for 5 min at 300 °C (splitless mode). ATR-WAX capillary column (60 m × 0.25 mm i.d., 0.25 μm coating) (Teknokroma, Spain) was used. The carrier gas was hydrogen, at a flow rate of 0.9 mL/min. The oven temperature was held at 40 °C for 10 min and then programmed to rise a final temperature of 200 °C at 3 °C/min. The GC–MS interface was heated at 280 °C with the actual temperature reaching 180 °C in MS source and 150 °C in MS-quadrupole. The electron impact energy was set at 70 eV, and data were collected in the range of 40–300 atomic mass units (amu). Compounds identification was based on mass spectra by comparison with MS spectra database Wiley 7 (John Wiley & Sons Limited, NJ) and checked with standards. Each sample was analyzed in duplicate. The integrations were performed with Enhanced ChemStation software E.02.02.1431 (Agilent Technologies, Palo Alto, CA).

### 2.5. Statistical analysis

Data were imported to Statistica 8.0 (Statsoft, Tulsa, OK), which was the package used to perform the statistical analyses.

Calibration curves were created for the quantification of 29 volatile compounds and checking the linearity of the analytical method. Simple linear regression procedure was used for modelling the calibration curves.

The statistical procedure for the multivariate analysis of the volatile profiles of the samples was principal component analysis (PCA) because it is an unsupervised procedure oriented toward modelling the variance/covariance data matrix [18].

## 3. Results and discussion

### 3.1. Method validation

The validation of analytical methods is crucial for the quality of results, which is relevant because many important decisions are based on the test results of chemical analyses. The analytical validation was focused on the evaluation of the linearity with the building of the calibration curves, the limits of detection and quantification (LOD and LOQ respectively), the working ranges, the accuracy estimated as trueness and precision (repeatability and intermediate precision), and the sensitivity and the selectivity of the method.

#### 3.1.1. Linearity

Linearity is the ability of a method of analysis to provide an instrumental response or result proportional to the quantity of analyte to be detected in a laboratory sample [19]. For any quantitative method,

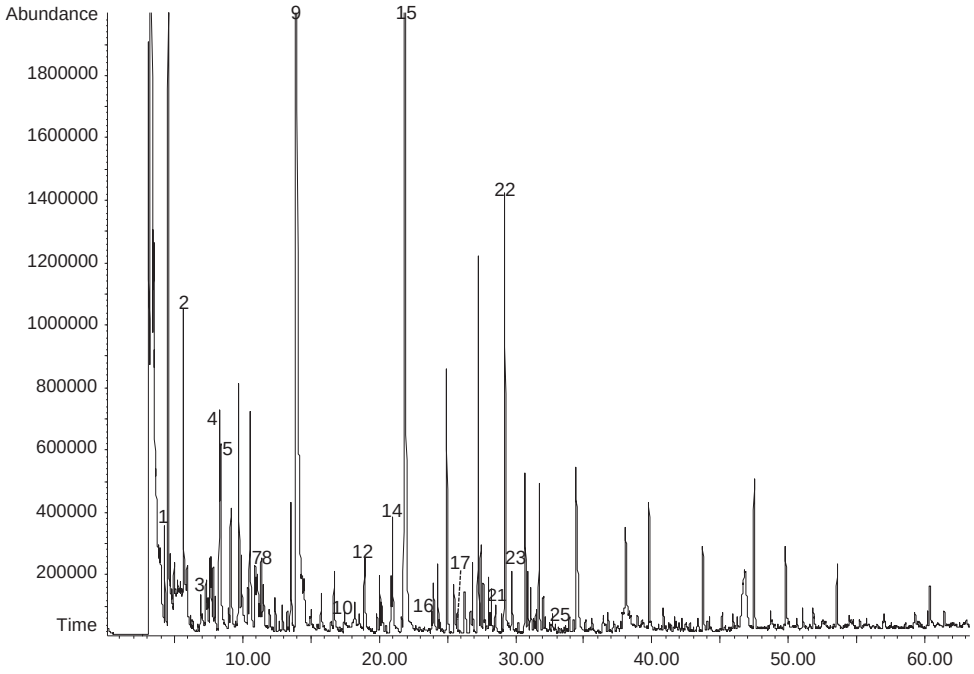


**Fig. 1.** SPME–GCMS chromatogram of EVOO sample. Codes are described in Table 2.

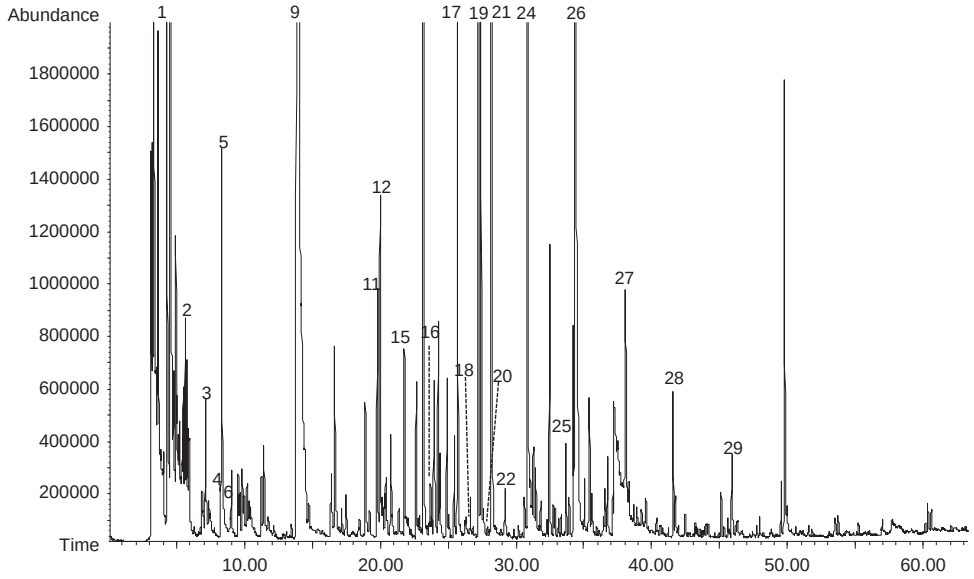


**Fig. 2.** SPME–GCMS chromatogram of LVOO sample. Codes are described in Table 2.

it is necessary to determine the range of analyte concentrations or property values over which the method may be applied [20,21]. The lower end of the working range is limited by the values of the limits of detection and/or quantitation. At the upper end of the concentration range, however, limitations are imposed by various effects depending on the instrument response system. Thus, the working range of the method should be in line with that qualified by a linear response, which means the method gives results that are proportional to the analyte concentration. Table 2 shows the correlation coefficients (from 0.96 to 0.99) for the calibration curves obtained within a concentration level ranging from 0.1 to 15.0 mg/kg for 29 volatiles. All the calibration showed squared adjusted regression coefficients ($R^2_{adj}$) higher than 0.90. As in the assessment of linearity, the correlation coefficient does not always guarantee the linearity of the calibration curve [22], the information on the correlation coefficients was supplemented by the relative standard deviation ($RSD_b$), which should be lower than 5% to confirm the linearity. Additionally, the plot of the residual values was considered to check lack of linearity. Aldehydes showed $RSD_b$ values lower than other volatiles studied (Table 2). The mean $RSD_b$ value (%) of aldehydes, except nonanal, was 1.56. Alcohols mean $RSD_b$ value was 3.1% and ketones 3.5, both higher than aldehydes.

According to the $RSD_b$ criterion, heptanal and 6-methyl-5-hepten-2-one show a linear response for the whole concentration range (0.1–15.0 mg/kg). However, most of the volatiles show the best linearity at lower range of concentration (0.1–3.0 mg/kg), with the exception of ethyl acetate, nonanal and 2-methyl-1-butanol, which does not show a linear response in the lower range of concentration either. The worst case of linearity corresponds to pentanoic acid since its residual values shows a bias, its correlation coefficient is low ( $< 0.990$) and its $RSD_b$ value is upper than 5%.

**Table 1**
Cultivar, quality category and sensory properties of VOO samples.

| Code | Variety | Oil category | Main sensory defect | Secondary sensory defect |
|---|---|---|---|---|
| 1 | Hojiblanca | VOO[a] | Slighly rancid | – |
| 2 | Arbequina | VOO | Slighly winey | – |
| 3 | Hojiblanca | VOO | Slighly rancid | – |
| 4 | Arbequina | EVOO[b] | – | – |
| 5 | Arbequina-Manzanilla | EVOO | – | – |
| 6 | Hojiblanca | LVOO[c] | Fusty[d] | Musty[f] |
| 7 | Hojiblanca | LVOO | Fusty[d] | Musty[f] |
| 8 | Picual | LVOO | Fusty[d] | Musty[f] |
| 9 | Picual | LVOO | Fusty[d] | Musty[f] |
| 10 | Picual | LVOO | Fusty[d] | Musty[f] |
| 11 | Hojiblanca | LVOO | Winey[e] | Musty[f] |
| 12 | Hojiblanca | LVOO | Winey[e] | Musty[f] |
| 13 | Picual | LVOO | Winey[e] | Rancid |
| 14 | Cornicabra | LVOO | Hay-wood | Fusty[d], Musty[f] |
| 15 | Cornicabra | LVOO | Hay-wood | Winey[e], Musty[f] |
| 16 | Picual | LVOO | Hay-wood | Winey[e], Musty[f] |
| 17 | Hojiblanca | LVOO | Musty[f] | Winey[e] |
| 18 | Picual | LVOO | Musty[f] | Fusty[d], Rancid |
| 19 | Picual | LVOO | Musty[f] | Fusty[d] |
| 20 | Hojiblanca | LVOO | Musty[f] | Winey[e], Fusty[d] |
| 21 | Picual | LVOO | Frozen[g] | Rancid |
| 22 | Picual | LVOO | Frozen[g] | Musty[f], Winey[e] |

Note
[a] Virgin olive oil.
[b] Extra-virgin olive oil.
[c] Lampante virgin olive oil.
[d] Fusty-muddy sediment.
[e] Winey-vinegary.
[f] Musty-humid-earthy.
[g] Frostbitten olives.

### 3.1.2. Limits of detection and quantification

Limits of detection (LOD) and quantification (LOQ) were determined from the data of the calibration curves [23]. LOD is defined as the minimum amount or concentration of substance that can be reliably detected by a given analytical method [24]. LOQ is, however, a performance characteristic that denotes the ability of a chemical measurement process to adequately quantify an analyte, and it is defined as the lowest amount or concentration of the analyte that can be determined with an acceptable level of precision and accuracy [25]. LOD and LOQ were calculated as three and ten times the value of the relationship between the standard deviation of the regression and the slope of the calibration curve [26]. Table 2 shows the values for both parameters. Although the values are quite diverse, it is important to note that the regular concentrations in virgin olive oils are quite diverse as well [1]. LOD and LOQ obtained for aldehydes were amongst the lowest with the exception of nonanal and *E*-2-heptenal. In the application of quality assessment, the correct determination of aldehydes is important to detect incipient rancidity [2]. On the contrary, pentanoic acid and octane had the highest values of LOQ. The lack of linearity of pentanoic acid can be due to the difficulty for a correct determination of this compound. This compound is absent in good quality oils, and its concentration rises in the case of fusty and rancid oils [2]. On the other hand, octane has no sensory significance in virgin olive oil [1], and its analytical determination is not relevant.

### 3.1.3. Working range

A good knowledge and definition of the working range is essential for conducting a proper investigation of the method linearity [27]. The working range of volatiles is determined by the minimum value of LOQ and the highest concentration tested with good linearity [22]. Table 2 shows that volatile compounds with low molecular mass and high polarity (as the compounds eluting at the beginning of the chromatography) showed higher LOQ values. Besides, volatile compounds with high molecular mass and low polarity (eluting at the end of the chromatography) showed higher LOQ values too. However, volatiles placed in the middle of chromatogram (medium polarity) showed, in general, lower LOQ values (Figs. 1 and 2 and Table 2). The lowest LOQ value corresponds to ethyl butanoate and the highest to pentanoic acid, which also has the narrowest working range; heptanal has the widest working range of all the volatiles.

### 3.1.4. Precision

The precision of the method [12,22,28,29] was studied in terms of intra-day precision (repeatability) and inter-day precision (intermediate precision) of 29 volatiles quantified in an EVOO (*var*. Hojiblanca). Seven replicates were analyzed in a unique session to determine the repeatability. Intermediate precision was determined by analyzing the sample in 8 non-consecutive analytical sessions. The results, shown in Table 3, were calculated as relative standard deviation (RSD%).

An analysis of the values found in the repeatability evaluation showed that 10 of the volatiles identified in EVOO sample (67%) showed a RSD% lower than 10%, 2 volatiles (13%) presented values of 10–15%, and only 3 volatiles (20%) had values higher than 15%. The highest value (20.75%) corresponds to 1-butanol, followed by 6-methyl-5-hepten-2-one and acetic acid. Intermediate precision showed RSD% slightly higher than repeatability.

Repeatability was also evaluated by analyzing seven replicates of a LVOO characterized by the rancid sensory defect (Table 3). The objective was to check the values of RSD% when the concentrations of some volatile compounds are higher [2], and therefore the precision may be different. Thus, RSD% values of LVOO were lower than EVOO. Thus, 94.7% of the volatiles identified in LVOO showed RSD% values lower than 10% (16 volatiles have values lower than 5%), and only *E*-2-heptenal has a value higher than 10% (12.4%). The results of the study focused on the intermediate precision were also better. These results point out the importance to take into account the differences in the oil matrix composition when this analytical procedure is employed to analyze VOO.

The precision of a method can be used to obtain the measurement uncertainty through the estimation of the so-called critical differences. If the difference between two analytical results is greater than the critical differences, then it may be assumed that the sample in question does not fulfill any statutory or contractual requirement. The calculation of critical differences can also be understood as a definition of the measurement uncertainty [19]. Thus, repeatability ($r=2.8s_r$; $s_r$ being the repeatability standard deviation) limits were calculated in this work. Two test results ($x1$ and $x2$) were performed for each sample analyzed in the precision study under repeatability conditions and checked if the difference $|x1-x2|$ was less than the repeatability limit r of the method. For all volatile compounds the absolute difference did not exceed r and the mean of these two results was quoted as the final result.

### 3.1.5. Accuracy

Accuracy is a performance characteristic that refers to the total error (systematic and random errors) and comprises two components: trueness and precision [30]. Accuracy is assessed with recovery studies for large concentration ranges, recovery being understood as the proportion of the amount of analyte, present or added to the analytical portion of test material, which is extracted and presented for measurement [31]. In this work accuracy has been assessed in terms of 'apparent accuracy' to express the ratio

**Table 2**
Summary of the method validation data (I).

| Peak code | Volatile compounds | $R_t$[a] | $I_i$[b] | $R^2$[c] | RSD$_b$[d] | Calibration curve | $W_R$[e] | LOD[f] | LOQ[f] | Accuracy | Sensitivity | Sensory properties |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Octane | 4.25 | 43/57/85 | 0.997 | 4.20 | $Y=1.478\times10^8+2.801\times10^8\times X$ | 5.44–6.00 | 1.63 | 5.44 | $t_{exp}\le t_{tab}$ | 2.80 | Solvent |
| 2 | Ethyl acetate | 5.62 | 43/70/88 | 0.987 | 11.84 | $Y=3.051\times10^7+2.716\times10^8\times X$ | 3.92–6.00 | 1.18 | 3.92 | $t_{exp}\le t_{tab}$ | 2.72 | Sweet, aromatic, ethereal |
| 3 | Ethanol | 6.85 | 45/46 | 0.998 | 3.50 | $Y=7.463\times10^{6.}+3.009\times10^7\times X$ | 1.15–3.00 | 0.57 | 1.15 | $t_{exp}\le t_{tab}$ | 0.30 | Apple, sweet |
| 4 | 3-Pentanone | 8.26 | 57/86 | 0.983 | 4.79 | $Y=7.190\times10^7+3.360\times10^8\times X$ | 1.59–3.00 | 0.48 | 1.59 | $t_{exp}\le t_{tab}$ | 3.35 | Sweet, fruity |
| 5 | Pentanal | 8.31 | 44/58/71 | 0.996 | 1.77 | $Y=-1.519\times10^6+2.920\times10^7\times X$ | 0.59–3.00 | 0.17 | 0.59 | $t_{exp}\le t_{tab}$ | 0.29 | Almond, malt, pungent |
| 6 | 1-Penten-3-one | 10.30 | 55/84 | 0.990 | 3.37 | $Y=3.520\times10^8+2.580\times10^6\times X$ | 1.11–3.00 | 0.33 | 1.11 | $t_{exp}\le t_{tab}$ | 0.03 | Pungent, mustard |
| 7 | 2-Butanol | 10.92 | 45/59/72 | 0.991 | 4.72 | $Y=2.005\times10^8+9.399\times10^7\times X$ | 0.34–1.00 | 0.10 | 0.34 | $t_{exp}\le t_{tab}$ | 0.94 | Medicine, fruity |
| 8 | Ethyl butanoate | 11.37 | 43/71/88 | 0.992 | 4.24 | $Y=6.118\times10^7+1.513\times10^8\times X$ | 0.30–1.00 | 0.09 | 0.30 | $t_{exp}\le t_{tab}$ | 1.51 | Apple, sweet |
| 9 | Hexanal | 13.82 | 44/56/72 | 0.998 | 2.26 | $Y=1.462\times.10^7+1.677\times10^8\times X$ | 0.74–3.00 | 0.22 | 0.74 | $t_{exp}\le t_{tab}$ | 1.67 | Green apple, grass |
| 10 | 1-Butanol | 17.99 | 31/41/56 | 0.972 | 3.03 | $Y=9.920\times10^7+9.569\times10^7\times X$ | 0.99–3.00 | 0.30 | 0.99 | $t_{exp}\le t_{tab}$ | 0.96 | Winey |
| 11 | 2-Heptanone | 19.81 | 43/58/71 | 0.998 | 2.31 | $Y=2.275\times10^7+2.270\times10^8\times X$ | 0.76–3.00 | 0.22 | 0.76 | $t_{exp}\le t_{tab}$ | 2.27 | Sweet, fruity, cinnamon |
| 12 | Heptanal | 19.99 | 44/55/70 | 0.996 | 0.81 | $Y=1.045\times10^7+7.466\times10^7\times X$ | 0.26–3.00 | 0.18 | 0.60 | $t_{exp}\le t_{tab}$ | 0.75 | Fatty, citrus, rancid |
| 13 | 2-Methyl-1-butanol | 21.67 | 41/57/70 | 0.973 | 6.89 | $Y=4.837\times10^7+2.006\times10^8\times X$ | 2.25–3.00 | 0.68 | 2.25 | $t_{exp}>t_{tab}$[g] | 2.00 | Winey, onion |
| 14 | 3-Methyl-1-butanol | 21.70 | 41/55/70 | 0.997 | 1.88 | $Y=1.016\times10^8+7.833\times10^7\times X$ | 0.63–3.00 | 0.19 | 0.63 | $t_{exp}\le t_{tab}$ | 0.78 | Whiskey, malt, burnt |
| 15 | *E*-2-Hexenal | 21.79 | 55/69/83 | 0.995 | 1.56 | $Y=-5.622\times10^6+6.163\times10^7\times X$ | 0.51–3.00 | 0.15 | 0.51 | $t_{exp}\le t_{tab}$ | 0.62 | Bitter almonds, green-fruity |
| 16 | 3-Octanone | 23.73 | 43/57/72/99 | 0.995 | 3.57 | $Y=7.291\times10^6+9.205\times10^7\times X$ | 1.17–3.00 | 0.35 | 1.17 | $t_{exp}\le t_{tab}$ | 0.92 | Nut |
| 17 | Octanal | 25.71 | 43/57/84 | 0.996 | 1.40 | $Y=7.776\times10^5+2.567\times10^7\times X$ | 0.44–3.00 | 0.13 | 0.44 | $t_{exp}\le t_{tab}$ | 0.26 | Fatty, soap, lemon, green |
| 18 | 1-Octen-3-one | 26.33 | 55/70/97 | 0.997 | 2.98 | $Y=2.716\times10^6+1.005\times10^8\times X$ | 0.98–3.00 | 0.30 | 0. 98 | $t_{exp}\le t_{tab}$ | 1.00 | Mushroom, metal |
| 19 | *E*-2-Heptenal | 27.38 | 41/55/83 | 0.997 | 4.93 | $Y=-2.336\times10^6+5.480\times10^6\times X$ | 3.88–10.00 | 1.16 | 3.88 | $t_{exp}\le t_{tab}$ | 0.05 | Soap, fat, almond |
| 20 | 2-Heptanol | 27.71 | 45/55/83 | 0.997 | 3.23 | $Y=3.758\times10^7+2.946\times10^8\times X$ | 1.06–3.00 | 0.32 | 1.06 | $t_{exp}\le t_{tab}$ | 2.95 | Mushroom, green, chemical |
| 21 | 6-Methyl-5-hepten-2-one | 28.21 | 43/55/69/108 | 0.995 | 4.38 | $Y=1.913\times10^6+7.914\times10^7\times X$ | 1.45–3.00 | 0.43 | 1.45 | $t_{exp}\le t_{tab}$ | 0.79 | Green-fruity, grass, pungent |
| 22 | 1-Hexanol | 29.22 | 43/56/69 | 0.998 | 1.48 | $Y=6.972\times10^6+1.209\times10^8\times X$ | 0.50–3.00 | 0.15 | 0.50 | $t_{exp}\le t_{tab}$ | 1.21 | Fruity, soft, aromatic, alcoholic |
| 23 | *E*-3-Hexen-1-ol | 29.75 | 41/67/82 | 0.985 | 5.60 | $Y=1.170\times10^7+1.830\times10^7\times X$ | 2.86–6.00 | 0.86 | 2.86 | $t_{exp}\le t_{tab}$ | 0.18 | Astringent, bitter |
| 24 | Nonanal | 30.87 | 43/57/70 | 0.987 | 6.35 | $Y=1.437\times10^5+3.711\times10^6\times X$ | 3.98–6.00 | 1.14 | 3.98 | $t_{exp}\le t_{tab}$ | 0.04 | Fat, citrus, green |
| 25 | 1-Octen-3-ol | 33.71 | 57/67/81 | 0.998 | 1.17 | $Y=-8.092\times10^6+8.838\times10^7\times X$ | 1.07–3.00 | 0.32 | 1.07 | $t_{exp}\le t_{tab}$ | 0.88 | Mushroom, moldy |
| 26 | Acetic acid | 34.25 | 43/45/60 | 0.997 | 5.23 | $Y=1.339\times10^7+4.634\times10^7\times X$ | 1.72–3.00 | 0.52 | 1.72 | $t_{exp}\le t_{tab}$ | 0.46 | Sour |
| 27 | Propanoic acid | 37.97 | 45/57/74 | 0.998 | 1.75 | $Y=-1.142\times10^7+5.605\times10^7\times X$ | 1.61–3.00 | 0.48 | 1.61 | $t_{exp}\le t_{tab}$ | 0.56 | Pungent, rancid, soy |
| 28 | Butanoic acid | 41.70 | 42/60/73 | 0.998 | 5.32 | $Y=1.593\times10^6+1.432\times10^7\times X$ | 1.75–3.00 | 0.53 | 1.75 | $t_{exp}\le t_{tab}$ | 0.14 | Rancid, pungent, soy |
| 29 | Pentanoic acid | 45.93 | 41/60/73 | 0.965 | 12.53 | $Y=-2.124\times10^7+1.554\times10^7\times X$ | 8.49–10.00 | 2.54 | 8.49 | $t_{exp}\le t_{tab}$ | 0.15 | Sweat |

[a] Retention time (min).
[b] Identification ions ($m/z$); 3.
[c] Squared adjusted regression coefficient.
[d] Relative standard deviation.
[e] Working range (mg/kg).
[f] Limits of detection and quantification (mg/kg).
[g] The accuracy of the method cannot be established.

of concentration found (extracted on SPME fiber, and quantified by GCMS and the calibration curves) versus the reference value instead of the term 'recovery' [32]; this kind of accuracy has been applied with success to a chromatographic method [33]. The mean apparent concentration ($C_{ap}$) and standard deviation (SD) were calculated from the replicate values, the actual concentration being 2 mg/kg. Accuracy was then assessed by statistically comparing the mean apparent concentration with the 100% [22,33]. This comparison was carried out using the following $t$ test:

$$t\text{exp} = \frac{|100 - \overline{C_{ap}}|}{\text{SD}/\sqrt{n}}$$

The calculated $t$-value ($t_{exp}$) was then compared with two-sided $t$ tabulated value, $t_{tab}$, for $\alpha=0.02$ and $n-1$ degrees of freedom. Table 2 shows that 29 of the volatile compounds (97% of the studied volatiles) showed $t_{exp}\le t_{tab}$, so the accuracy of the method was established. 2-Methyl-1-butanol did not show a good accuracy (it showed $t_{exp}>t_{tab}$). This compound has been associated to sensory defect winey-vinegary [2].

### 3.1.6. *Selectivity*

Determining selectivity is necessary to ensure that the signal produced in the measurement stage is only due to the analyte of interest, and not to the presence of interferences in the sample. Selectivity is particularly crucial when validating an analytical method by which tens of compounds can be quantified like in this case [1]. For this reason, the selectivity of the analytical method was studied by calculation of the resolution [22,34]. A resolution of 1.5 has been thought that represents a fully resolved peak without any baseline or space between the two peaks. A lower value indicates there is some overlapping or peaks are partially resolved [23]. The selectivity of the method, evaluated with an EVOO (*var.* Hojiblanca) sample, showed that all the selected peaks were completely resolved (resolution $>1.5$), except octanal, which was not completely resolved when it was identified at low concentration. 3-Methyl-1-butanol and *E*-2-hexenal could have some degree of overlapping in olive oil samples characterized by different sensory defective notes like musty-humid-earthy, fusty-muddy sediment and winey-vinegary. Overlap is due to the high concentrations of 3-methyl-1-butanol and the low concentrations

**Table 3**
Summary of the method validation data (II).

| Peak code | Volatile compounds | Repeatability | | Intermediate precision | |
|---|---|---|---|---|---|
| | | RSD%[a] EVOO | RSD%[a] LVOO | RSD%[a] EVOO | RSD%[a] LVOO |
| 1 | Octane | 9.38 | 2.05 | 9.97 | 1.86 |
| 2 | Ethyl acetate | 3.41 | 5.07 | 7.80 | 3.31 |
| 3 | Ethanol | 8.57 | 7.45 | 10.16 | 8.43 |
| 4 | 3-Pentanone | 7.92 | 3.64 | 8.69 | 3.74 |
| 5 | Pentanal | 6.22 | 2.60 | 6.47 | 2.58 |
| 6 | 1-Penten-3-one | nd | 9.29 | nd | 13.28 |
| 7 | 2-Butanol | 8.43 | nd | 10.43 | nd |
| 8 | Ethyl butanoate | 4.45 | nd | 8.61 | nd |
| 9 | Hexanal | 9.16 | 4.42 | 9.78 | 6.76 |
| 10 | 1-Butanol | 20.75 | nd | 32.15 | nd |
| 11 | 2-Heptanone | nd | 1.85 | nd | 1.60 |
| 12 | Heptanal | 6.07 | 2.26 | 6.55 | 2.60 |
| 13 | 2-Methyl-1-butanol | nd | nd | nd | nd |
| 14 | 3-Methyl-1-butanol | 5.29 | nd | 32.80 | nd |
| 15 | *E*-2-Hexenal | 4.50 | 3.56 | 6.30 | 1.64 |
| 16 | 3-Octanone | 12.81 | 4.58 | 14.55 | 6.68 |
| 17 | Octanal | 14.24 | 1.65 | 7.46 | 2.93 |
| 18 | 1-Octen-3-one | nd | 10.45 | nd | 8.00 |
| 19 | *E*-2-Heptenal | nd | 12.4 | nd | 15.43 |
| 20 | 2-Heptanol | nd | 8.54 | nd | 10.12 |
| 21 | 6-Methyl-5-hepten-2-one | 16.15 | 1.22 | 21.36 | 2.46 |
| 22 | 1-Hexanol | 9.11 | 3.04 | 10.27 | 10.5 |
| 23 | *E*-3-Hexen-1-ol | 11.85 | nd | 43.50 | nd |
| 24 | Nonanal | nd | 4.02 | nd | 6.97 |
| 25 | 1-Octen-3-ol | 6.13 | 4.35 | 6.52 | 8.00 |
| 26 | Acetic acid | 17.30 | 3.46 | 22.76 | 5.37 |
| 27 | Propanoic acid | nd | 1.99 | nd | 3.90 |
| 28 | Butanoic acid | nd | 3.20 | nd | 7.72 |
| 29 | Pentanoic acid | nd | 2.77 | nd | 5.02 |

Note

[a] Relative standard deviation; nd, not detected; EVOO, extra virgin olive oil; LVOO, lampante virgin olive oil.

of *E*-2-hexenal in contrast to the usual concentrations of these compounds in EVOOs [2].

### 3.1.7. Sensitivity

Sensitivity, which is defined as the change in the response of a measuring instrument divided by the corresponding change in the stimulus, is in fact the slope of the calibration straight-line [22]. Mass spectroscopy shows usually better sensitivity when working with lower concentrations than gas chromatography [35]. Sensitivity values were obtained within a range of values due to the diversity of volatile compounds studied with different structures and natures (Table 2). In general, acid compounds showed lower sensitivity values (average 0.33), although the lowest value corresponded to 1-penten-3-one (0.03). On the contrary, the highest value corresponded to 3-pentanone (3.35). Table 2 also shows that aldehydes (average 0.56) have lower sensitivity values than the ketones (average 1.39).

## 3.2. Analyzing extra virgin, virgin and lampante olive oil samples

The validated method was applied to the analysis of twenty two samples (Table 1); two being EVOOs; and hence without sensory defects, three VOOs with slight sensory defects; and the rest being LVOOs, which are characterized by various sensory defects according to the evaluation carried out by the sensory panel. The sensory qualification as LVOOs, however, is not usually caused by the presence of a single sensory defect but a combination of them as shown in Table 1. Thus, the secondary sensory defect is detected by assessors at less intensity of odor than the primary defect.

Table 4 shows the concentrations (mg/kg) of the volatiles though two of them (2-methyl-1-butanol and 1-octen-3-one), which were studied in the method validation, were not detected in these samples. The detection of sensory defects by the assessors is due to differences of the concentration of some volatiles, in comparison with their concentrations in EVOOs [2]. In general, LVOOs, which are qualified with sensory defects, are characterized by higher concentration of total volatiles (e.g. 38.75 mg/kg for winey-vinegary defect) than EVOOs (18.26 mg/kg).

In order to verify the classification ability of the method, the statistical procedure of principal component analysis (PCA) was applied to concentration values of volatiles determined in the whole set of samples. Fig. 3 shows the PCA plot, in which factor 1, with 30.9% of explained variance, allowed for the separation of EVOO and VOO samples from LVOO samples. EVOO samples were characterized by higher values of 3-pentanone, hexanal, *E*-2-hexenal, 1-hexanol and *E*-3-hexen-1-ol; C6 compounds are produced by the lipoxygenase pathway, and they are responsible for green and fruity sensory attributes, which characterize EVOOs [1]. It is also noticeable the high content of *E*-2-hexenal in the VOO samples.

Also LVOOs are apart from EVOO samples because of the concentrations of volatiles responsible for sensory defects, such as nonanal (with exception of fusty LVOO), acetic acid or ethanol (Table 4) although the latter does not contribute to aroma because of its high odor threshold. Thus, LVOO samples mainly qualified with musty-humid-earthy sensory defect have high concentrations of ethanol (10.75 mg/kg) while the frostbitten olives defect is characterized with high concentrations of nonanal (12.08 mg/kg), 6-methyl-5-hepten-2-one (0.31 kg/kg) and *E*-2-heptenal (4.76 mg/kg). LVOOs qualified with winey-vinegary are obviously characterized by a high concentration of acetic acid (14.55 mg/kg). The hay-wood sensory perception, which sometimes is detected together with frostbitten olives by panelists, is not characterized by the highest but high concentrations of volatiles like 1-penten-3-one, *E*-2-hexenal, and *E*-2-heptenal. The first two volatiles characterize EVOOs as well, which indicates the need of looking for a marker [1] and of harmonizing its perception as it has been relatively uncommon so far. Other volatile compounds also were determined at higher concentrations in LVOOs than in EVOOs, such as ethyl acetate and octanal. Thus, PCA plot (Fig. 3) shows a great dispersion of VOO and EVOOs because of the differences in their volatile profiles, which are inherent to selected cultivars (Arbequina, Hojiblanca, Manzanilla) [12,36].

The samples characterized by sensory defective perceptions were placed in the second and third quadrants of the PCA plot (Fig. 3), with the exception of one sample qualified as hay-wood (H), perhaps because the main qualifier is not decisive enough to distinguish the samples in a clear different group. Thus, samples characterized by the sensory note musty-humid-earthy were grouped in the central zone of Y axis very closed to those characterized by the notes hay-wood and winey-vinegary. Fusty-muddy sediment defect oils showed high concentrations of some volatile compounds (ethyl acetate, butanoic and pentanoic acids), so these samples were grouped and differentiated from the rest of LVOOs in the PCA plot.

These results show the utility of the validated method as a tool to discriminate between virgin olive oils of different quality. The classification of samples was attributed to the different concentration of some volatiles compounds, each one of them having different validation parameters in their determination. In this study, all the volatile compounds were included in the PCA. However, the reliability of the method can be adjusted leaving out those volatile compounds with less appropriate analytical

**Table 4**
Concentration (mean ± standard deviation) (mg/kg) of volatiles quantified in 17 LVOOs qualified by sensory defects (Table 1), 3 VOOs and 2 EVOOs.

| Peak code | Volatile | LVOO | | | | | EVOO[d] | VOO[e] | Odor threshold |
|---|---|---|---|---|---|---|---|---|---|
| | | Winey[a] | Fusty | Frozen[b] | Musty[c] | Hay-wood | | | |
| 1 | Octane | 0.49 ± 0.07 | 0.84 ± 0.12 | 0.85 ± 0.10 | 0.52 ± 0.03 | 0.37 ± 0.08 | 0.11 ± 0.05 | 0.26 ± 0.11 | 0.94 |
| 2 | Ethyl acetate | 0.53 ± 0.11 | 1.37 ± 0.21 | 0.31 ± 0.11 | 0.93 ± 0.09 | 0.67 ± 0.20 | 0.04 ± 0.02 | 0.42 ± 0.32 | 0.94 |
| 3 | Ethanol | 7.42 ± 1.40 | 6.91 ± 0.94 | 1.53 ± 0.17 | 10.75 ± 1.79 | 4.73 ± 2.08 | 0.17 ± 0.04 | 4.24 ± 1.75 | 30.00 |
| 4 | 3-Pentanone | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.002 | 0.04 ± 0.004 | 0.07 ± 0.01 | 0.77 ± 0.38 | 0.21 ± 0.09 | 70.00 |
| 5 | Pentanal | 0.72 ± 0.30 | 0.20 ± 0.09 | 1.22 ± 0.26 | 0.69 ± 0.18 | 1.28 ± 0.15 | 1.11 ± 1.00 | 0.19 ± 0.19 | 0.24 |
| 6 | 1-Penten-3-one | 0.36 ± 0.20 | nd | 0.04 ± 0.006 | 0.09 ± 0.04 | 0.08 ± 0.03 | 0.12 ± 0.04 | 0.02 ± 0.01 | $0.70 \times 10^{-3}$ |
| 7 | 2-Butanol | 0.48 ± 0.37 | 0.004 ± 0.002 | 1.08 ± 0.83 | 0.88 ± 0.07 | 2.05 ± 0.90 | 1.80 ± 0.01 | 5.03 ± 2.78 | 0.15 |
| 8 | Ehtyl butanoate | nd | 0.52 ± 0.01 | nd | nd | 0.06 ± 0.06 | 0.32 ± 0.02 | nd | 0.03 |
| 9 | Hexanal | 0.22 ± 0.02 | 0.23 ± 0.02 | 0.50 ± 0.02 | 0.24 ± 0.01 | 0.27 ± 0.05 | 0.95 ± 0.13 | 0.79 ± 0.17 | 0.80 |
| 10 | 1-Butanol | 0.05 ± 0.03 | 1.20 ± 0.27 | 0.02 ± 0.02 | 0.03 ± 0.005 | 0.01 ± 0.005 | nd | 0.04 ± 0.02 | 0.40 |
| 11 | 2-Heptanone | 0.05 ± 0.03 | 0.01 ± 0.003 | nd | 0.01 ± 0.002 | 0.002 ± 0.002 | 0.01 ± 0.005 | nd | 0.30 |
| 12 | Heptanal | 0.05 ± 0.02 | 0.04 ± 0.006 | 0.04 ± 0.01 | 0.04 ± 0.005 | 0.03 ± 0.003 | 0.07 ± 0.03 | 0.03 ± 0.002 | 0.50 |
| 14 | 3-Methyl-1-butanol | 0.95 ± 0.58 | 1.25 ± 0.19 | 0.88 ± 0.12 | 1.00 ± 0.15 | 0.49 ± 0.05 | 0.69 ± 0.26 | 0.06 ± 0.03 | 0.10 |
| 15 | *E*-2-Hexenal | 0.30 ± 0.30 | 0.05 ± 0.01 | 1.14 ± 0.30 | 0.78 ± 0.21 | 2.09 ± 0.85 | 0.94 ± 0.02 | 7.34 ± 5.35 | 0.42 |
| 16 | 3-Octanone | 0.41 ± 0.17 | 0.04 ± 0.01 | 0.40 ± 0.06 | 0.39 ± 0.14 | 0.12 ± 0.02 | 0.84 ± 0.38 | 0.07 ± 0.03 | – |
| 17 | Octanal | 0.39 ± 0.09 | 0.20 ± 0.02 | 0.31 ± 0.06 | 0.43 ± 0.02 | 0.24 ± 0.04 | 0.16 ± 0.01 | 0.12 ± 0.02 | 0.32 |
| 19 | *E*-2-Heptenal | 0.80 ± 0.20 | nd | 4.76 ± 0.33 | 0.72 ± 0.05 | 1.48 ± 0.14 | 0.23 ± 0.23 | 0.92 ± 0.50 | $5.00 \times 10^{-3}$ |
| 20 | 2-Heptanol | 0.01 ± 0.002 | 0.006 ± 0.002 | 0.004 ± 0.004 | 0.02 ± 0.004 | 0.01 ± 0.008 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.01 |
| 21 | 6-Methyl-5-hepten-2-one | 0.08 ± 0.02 | 0.07 ± 0.02 | 0.31 ± 0.02 | 0.16 ± 0.05 | 0.13 ± 0.02 | 0.02 ± 0.002 | 0.19 ± 0.16 | 1.00 |
| 22 | 1-Hexanol | 0.49 ± 0.08 | 0.86 ± 0.14 | 0.53 ± 0.05 | 0.42 ± 0.07 | 0.77 ± 0.25 | 3.69 ± 0.34 | 1.42 ± 0.36 | 0.40 |
| 23 | *E*-3-Hexen-1-ol | 0.26 ± 0.09 | 0.43 ± 0.04 | 0.20 ± 0.02 | 0.16 ± 0.03 | 0.30 ± 0.09 | 2.10 ± 0.40 | 0.47 ± 0.23 | 1.00 |
| 24 | Nonanal | 9.63 ± 1.45 | 2.71 ± 0.36 | 12.08 ± 2.62 | 6.02 ± 0.76 | 5.72 ± 1.27 | 3.60 ± 1.75 | 4.56 ± 2.28 | 0.15 |
| 25 | 1-Octen-3-ol | 0.01 ± 0.01 | nd | 0.07 ± 0.01 | 0.01 ± 0.004 | 0.02 ± 0.004 | nd | nd | 0.001 |
| 26 | Acetic acid | 14.55 ± 6.87 | 3.35 ± 0.46 | 8.12 ± 4.31 | 5.50 ± 1.45 | 8.97 ± 3.42 | 0.33 ± 0.09 | 3.03 ± 0.88 | 0.50 |
| 27 | Propanoic acid | 0.04 ± 0.02 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.03 ± 0.01 | 0.03 ± 0.003 | 0.08 ± 0.03 | 0.01 ± 0.005 | 0.72 |
| 28 | Butanoic acid | 0.40 ± 0.23 | 2.03 ± 0.38 | 0.19 ± 0.04 | 0.17 ± 0.07 | 0.07 ± 0.07 | 0.11 ± 0.02 | 0.13 ± 0.12 | 0.14 |
| 29 | Pentanoic acid | 0.05 ± 0.05 | 0.11 ± 0.03 | 0.04 ± 0.02 | nd | 0.04 ± 0.04 | nd | 0.04 ± 0.03 | 0.60 |
| | Total volatiles | 38.75 ± 0.47 | 22.43 ± 0.12 | 34.66 ± 0.68 | 30.01 ± 0.20 | 30.08 ± 0.70 | 18.26 ± 0.38 | 29.60 ± 1.11 | |

[a] Winey-vinegary.
[b] Frostbitten olives.
[c] Musty-humid-earthy.
[d] Extra-virgin olive oil.
[e] Virgin olive oil.

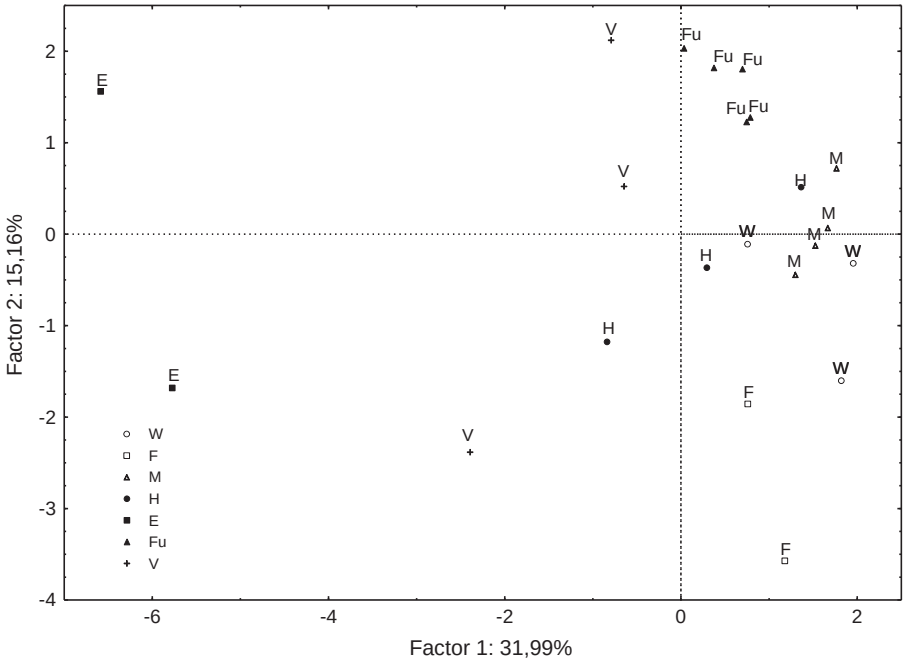


**Fig. 3.** Principal component analysis of extra virgin olive oils (E), virgin olive oils (V), and lampante virgin olive oil samples characterized by the sensory defects frostbitten olives (F), winey-vinegary (W), musty-humid-earthy (M), hay-wood (H) and fusty-muddy sediment (Fu).

characteristics (e.g. 2-methyl-1-butanol for non-having the accuracy established). There is a need in the olive oil sector to develop analytical tools to support the panel tests [17]. Although there are available analytical procedures for this purpose, the implementation of those must be supported by the validation of the method. Further work should address the validation of the application, not

only in quantitative but also in qualitative terms, including the statistical analysis to achieve the quality classification.

## Acknowledgment

This work was supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT-EU, Spanish and European Government), Spanish State Secretary for Research (Ramón y Cajal Program) and FEDER (CE) in the Project AGL 2011-30371-C01.

## References

[1] M.T. Morales, R. Aparicio-Ruiz, R. Aparicio, Chromatographic methodologies: compounds for olive oil odor issues, in: R. Aparicio, J. Harwood (Eds.), Handbook of Olive Oil. Analysis and Properties, second ed., Springer, New York, 2013, pp. 261–309.
[2] M.T. Morales, G. Luna, R. Aparicio, Food Chem. 91 (2005) 293–301.
[3] C.M. Kalua, M.S. Allen, D.R. Bedgood, A.G. Bishop, P.D. Prenzler, K. Robards, Food Chem. 100 (2007) 273–286.
[4] S. Vichi, J.M. Guadayol, J. Caixach, E. Lopez-Tamames, S. Buxaderas, Food Chem. 105 (2007) 1171–1178.
[5] Commission Regulation 640/2008/EC of 4 July 2008, Off. J. Eur. Union (2008) L178, 11–16.
[6] IOC, Sensory Analysis of Olive Oil. Method for the Organoleptic Assessment of Virgin Olive Oil. COI/T.20/Doc. no 15/Rev, 2011.
[7] R. Aparicio, S.M. Rocha, I. Delgadillo, M.T. Morales, J. Agric. Food Chem. 48 (2000) 853–860.
[8] D.L. García-González, N. Romero, R. Aparicio, J. Agric. Food Chem. 58 (2010) 12899–12905.
[9] C. Benincasa, A. De Nino, N. Lombardo, E. Perri, G. Sindona, A. Tagarelli, J. Agric. Food Chem. 51 (2003) 733–741.
[10] A. Jiménez, M.P. Aguilera, G. Beltrán, M. Uceda, J. Chromatogr. A 1121 (2006) 140–144.
[11] D.L. García-González, N. Tena, R Aparicio, J. Agric. Food Chem. 58 (2010) 8357–8364.
[12] G. Luna, M.T. Morales, R. Aparicio, Food Chem. 98 (2006) 243–252.
[13] C. Pizarro, S. Rodríguez-Tecedor, N. Pérez-del-Notario, J.M. González-Sáiz J. Chromatogr. A 1218 (2011) 518–522.
[14] D.L. García-González, R. Aparicio, J. Agric. Food Chem. 58 (2010) 12569–12577.
[15] E.N. Frankel, R.J. Mailer, C.F Shoemaker, Wang S.C., J.D. Flynn, Tests Indicate That Imported "Extra Virgin"Olive Oil Often Fails International and USDA Standards. Report UC Davis Olive Center (July, 2010). ⟨http://olivecenter.ucdavis.edu/research/files/oliveoilfinal071410updated.pdf⟩.
[16] J-L. Barjol, Introduction, in: R. Aparicio, J. Harwood (Eds.), Handbook of Olive Oil. Analysis and Properties, second ed., Springer, New York, 2013, pp. 1–17.
[17] EU, HORIZON 2020 Work Programme 2014–2015. Olive Oil Authentication, 2014. ⟨http://ec.europa.eu/research/participants/portal/desktop/en/opportunities/h2020/topics/2329-sfs-14a-2014.html⟩.
[18] I.T. Jolliffe, Principal Component Analysis, second ed., Springer, NewYork, 2002.
[19] R. Aparicio, L.S. Conte, H.J. Fiebig, Olive oil authentication, in: R. Aparicio, J. Harwood (Eds.), Handbook of Olive Oil. Analysis and Properties, second ed., Springer, New York, 2013, pp. 589–653.
[20] IUPAC, Pure Appl. Chem. 74 (2002) 835–855.
[21] L. Carrasco, E. Vassileva, Talanta 122 (2014) 106–114.
[22] R. Boqué, A. Maroto, J. Riu, F.X. Rius, Grasas Aceites 53 (2002) 128–143.
[23] N.J. Miller, J.C. Miller, Estadística y Quimiometría para Química Analítica, fourth ed., Pearson Educación, Madrid, 2002.
[24] IUPAC, International Union of Pure and Applied Chemistry, Compendium of Analytical Nomenclature, Definitive Rules, Blackwell Scientific Publications, Oxford, 1987.
[25] EURACHEM, The Fitness for Purpose of Analytical Methods, A Laboratory Guide to Method Validation and Related Topics, EURACHEM Secretariat, Teddington, Middlesex, 1998.
[26] J. Uhrovčík, Talanta 119 (2014) 178–180.
[27] A. Ribes, G. Carrera, E. Gallego, X. Roca, M.J. Berenguer, X. Guardino J. Chromatogr. A 1140 (2007) 44–55.
[28] R.M. Callejón, A.G. González, A.M. Troncoso, M.L. Morales, J. Chromatogr. A 1204 (2008) 93–103.
[29] E. Barros, N. Moreira, G. Pereira, S. Gomes, Ferreira Leite, C. Moraes Rezende, P. Guedes de Pinho, Talanta 101 (2012) 177–186.
[30] G. González, M.A. Herrador, A.G. Asuero, Talanta 82 (2010) 1995–1998.
[31] IUPAC, International Union of Pure and Applied Chemistry, Harmonized Guidelines for In-House Validation of Methods of Analysis (Technical reports), 1999.
[32] IUPAC, International Union of Pure and Applied Chemistry, Selectivity in Analytical Chemistry Technical Draft, 2001.
[33] G. Luna, Characterization of European Virgin Olive Oils: Implications of Sensory Assessment and Consumers' Attitudes, Doctoral Thesis, University of Seville, Spain, 2003.
[34] FDA Bioanalytical, Method Validation, Guidance for Industry, 2001.
[35] D.O. Sparkman, Z.E. Penton, F.G. Kitson, Gas Chromatography and Mass Spectrometry: A Practical Guide, second ed., Elsevier, Oxford, 2011.
[36] R. Aparicio, D.L. García-González, Olive oil characterization and traceability, in: R. Aparicio, J. Harwood (Eds.), Handbook of Olive Oil. Analysis and Properties, second ed.,Springer, New York, 2013, pp. 431–478.