Olive oil quality classification and measurement of its organoleptic attributes by untargeted GC-MS and multivariate statistical-based approach.

C. Sales¹, T. Portolés¹*, L.G. Johnsen², M. Danielsen², J. Beltran¹

¹Research Institute for Pesticides and Water (IUPA), University Jaume I, Avda. Sos Baynat, E-12071 Castellón, Spain.

²MS-Omics, Birkehegnnet 40, Álsgårde, Denmark

*Corresponding author: tportole@uji.es
ABSTRACT

The capabilities of dynamic headspace entrainment followed by thermal desorption in combination with gas chromatography (GC) coupled to single quadrupole mass spectrometry (MS) have been tested for the determination of volatile components of olive oil. This technique has shown a great potential for olive oil quality classification by using an untargeted approach. The data processing strategy consisted of three different steps: component detection from GC-MS data using novel data treatment software PARAFAC2, a multivariate analysis using EZ-Info, and the creation of the statistical models. The great amount of compounds determined enabled not only the development of a quality classification method as a complementary tool to the official established method “PANEL TEST” but also a correlation between these compounds and different types of defect. Classification method was finally validated using blind samples. An accuracy of 85% in oil classification was obtained, with 100% of extra virgin samples correctly classified.

Keywords

Dynamic headspace; Olive oil; GC-MS; PARAFAC2; Foodomics

1. INTRODUCTION

Olive oil quality is a matter of concern for consumers and producers. It establishes the differences between the products with poor attributes and the products with outstanding features, as well as it contributes to set oil prizes. For this reason and to avoid fraud, many times linked to this specific product (Jabeur, Zribi, & Bouaziz, 2016; Kalogiouri, Aalizadeh, & Thomaidis, 2017; Kalogiouri, Alygizakis, Aalizadeh, & Thomaidis, 2016; Portarena, Gavrichkova, Lauteri, & Brugnoli, 2014), guarantee of the genuine quality is a critical step
especially from an economical point of view. Thus, the total characterization of olive oil is an important aim where analytical chemists can be of great support.

Apart from physicochemical parameters that can determine the quality (as the acidity and turbidity of a sample), olive oil classification, as established by Spanish legislation (COI/T.20/Doc. No 15/Rev. 9 2017) and European Legislation (EEC No 2568/91), is performed by testers who establish if an olive oil must be labelled as extra virgin, virgin or lampante (not recommended for consumption). This strategy is known as “PANEL TEST”, which classifies the oils according to two main properties: defects (negative factors) and positive attributes (positive factors). The major defects are rancid, fusty/muddy sediment, musty/humid/earthy, acetone, burnt/heated, frozen/wet wood and winey/vinegary, while the positive attributes can be fruity (specifying green attribute), bitter and spicy. An extra virgin oil must have positive attributes and no defects, while the presence and amount of defects determines if an olive oil must be classified as Virgin or Lampante. According to the literature (Kalua et al., 2007; Luna, Morales, & Aparicio, 2006), and based on our previous work (Sales et al., 2017), the organic compounds responsible of these flavours are predominantly volatiles. This includes esters, ketones, aldehydes, alcohols, terpenes, phenols and their derivatives, with different concentrations and odour thresholds. To this extent, qualitative and quantitative analysis of volatile organic compounds (VOCs) has been an important issue of scientific interest for the organoleptic characterization of olive oil. Although PANEL TESTs are quite well trained in distinguishing these differences with an impressive precision, such methodology is rather expensive and remarkably time-consuming. In this scenario, a more objective methodology, based on instrumental responses, could be presented as cheaper and faster alternative approach to PANEL TESTs and could also be
useful as a complementary tool to prevent fraud due to sample adulteration by means of quality mislabeling.

Dynamic headspace with sorbent trapping (DHS) together with gas chromatography (GC) coupled to mass spectrometry (MS) in full scan mode is a well-known technique that has been used in our laboratory for the determination of VOCs in different food commodities (Beltran et al., 2006; Fredes et al., 2016), including olive oils (Sales et al., 2017), at low to trace levels. It allows to greatly concentrate most of the volatile compounds present in the sample with a good efficiency and significantly low cost. When coupled to thermal desorption, results improve considerably. This volatile-focused extraction technique makes use of no solvents, which helps to cut analysis costs and time while it enhances the sensitivity due to its high pre-concentration factor. Additionally, MS-spectra obtained when applying this sampling technique has been demonstrated to be cleaner than those obtained by traditional sampling methods, as the lack of solvents reduces column bleeding and overloading issues. (Marquez, Serratosa, Merida, Zea, & Moyano, 2014).

Other automatable alternatives to this approach rely on headspace (HS) coupled to MS or GC-MS, with high detection limits and no pre-concentration factor (Arrebola, González-Rodríguez, Garrido Frenich, Marín-Juan, & Martínez Vidal, 2005; Garrido-Delgado, Mercader-Trejo, Arce, & Valcárcel, 2011) or headspace-solid phase micro-extraction (HS-SPME) coupled to GC-MS, which has shown good performance in extraction of volatiles and has even been used for the determination of defect related compounds in olive oils (Benelli et al., 2015; Dierkes, Bongartz, Guth, & Hayen, 2012; Zhu, Wang, & Shoemaker, 2016). Most studies carried out on oil characterization are based on a targeted approach which can produce biased classification models that could lead to important misclassification of the
samples if the compounds responsible for a specific type of defect have not been considered in advance. Alternatively to target analyses for the determination of the chemical fingerprint of food samples, in the last years and together with the advance of data treatment technology, novel non-targeted methodologies have started to gain importance. Despite its great potential, only few application are found in olive oil analysis field (Gerhardt, Birkenmeier, Sanders, Rohn, & Weller, 2017; Gil-Solsona et al., 2016; Sales et al., 2017).

Metabolomics, defined as "the unbiased, global screening approach to classify samples based on metabolite patterns or fingerprints that change in response to disease, environmental or genetic perturbations with the ultimate goal to identify discriminating metabolites" (Cevallos-Cevallos, Reyes-De-Corcuera, Etxeberria, Danyluk, & Rodrick, 2009), has already demonstrated great capabilities to solve this problem. Application of a non-target approach based on analytical techniques to determine chemical fingerprint in food leads to a new field known as foodomics (Herrero, García-Cañas, Simo, & Cifuentes, 2010). Data processing together with data acquisition has to be carefully optimized through the use of specialized software to automatically obtain valuable markers (chromatographic peaks and masses) from raw data. As no compounds are selected in advance, chromatography must be robust and has to pursue the best peak resolution possible. Also, data acquisition has to be performed in full-scan in order to obtain the maximum information possible (Cevallos-Cevallos et al., 2009; Garcia & Barbas, 2011). After data acquisition, automatic deconvolution of spectra is needed, to deep scan relevant signals through the whole chromatogram (Meyer, Peters, & Maurer, 2010).

In literature, foodomics studies make use of different software to get this information, such as XCMS package of R (Díaz, Pozo, Sancho, & Hernández, 2014), MetAlign (Tikunov et
al., 2005) or MzMine 2.0 (Kind, Tolstikov, Fiehn, & Weiss, 2007). These software detect the relevant \( m/z \) values at a specific time and automatically integrate the signal (area or total intensity), in a procedure known as peak picking. Normally, it leads to different features detected in the same samples depending on their specifications and use (Li et al., 2018; Myers, Sumner, Li, Barnes, & Du, 2017). Recently, PARADiSe (Johnsen, Skou, Khakimov, & Bro, 2017), which makes use of the algorithm PARAFAC2 (Elcoroaristizabal, Bro, García, & Alonso, 2015; Johnsen et al., 2017; Lenhardt, Bro, Zeković, Dramićanin, & Dramićanin, 2015), has emerged as a really promising tool for GC-MS data treatment. This specific software presents a major difference compared with the others, which is the detection of compounds instead of singular ions. This reduces the data matrix and makes the statistical analyses easier and faster.

The aim of this work has been the development of a quality classification model for olive oil by the application of a novel untargeted methodology. For this purpose, the potential of GC-MS with DHS-TD has been exploited together with the use of the recently developed PARADiSe software for peak deconvolution purposes. As an additional aim, the correlation of detected compounds with the major defects reported by the PANEL test has been explored.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Internal standard toluene-d8 (tol-d8) \( \geq 99\% \) was purchased from Sigma Aldrich (Germany).

Tenax®TA glass desorption tubes 60/80 mesh, O.D. 6.00 mm x 4 mm I.D. x L 60 mm, used as traps were purchased from Gerstel (Mülheim an der Ruhr, Germany).
External standards of volatile compounds used for signal deviation correction (Z-3-hexenal, hexanal, E,E-2,4-hexadienal, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, E,E-2,4-heptadienal, R-limonene, 2-isobutylthiazole, guaiacol, E-2-octenal, linalool, 2-phenylethanol, methyl salicylate, α-terpineol, β-cyclocitral, Z-citral, E-citral, E,E-2,4-decadienal, diphenyl ether, geranylacetone, β-ionone, phenylacetaldehyde, benzaldehyde) were supplied by Supelco (Sigma–Aldrich and Fluka; Barcelona, Spain) as pure compounds (92–99.5%).

2.2. Olive oil samples

A total of 108 olive oil samples were provided by the Spanish Olive Oil Interprofessional Organization (INTERPRO, Spain), the Olive Oil Agency of the Ministry of Agriculture and Fisheries, Food and Environment (AAO, MAPAMA) and the official control services from the Council of Agriculture and Fisheries of Andalucia. Oil samples were obtained from several Spanish cultivar regions during the 2015 campaign. They were fully quality characterized by the official participating laboratories (Agricultural Laboratory from MAPAMA, Cordoba and Atarfe) using the official COI method (COI/T.20/Doc. No 15/Rev. 9 2017) by the corresponding panels accredited under EU REGULATION 2017/625. 87 samples were used for the training set of the models (18 extra virgin, 48 virgin and 19 lampante), and 21 were analyzed as blind samples (the quality was unknown during analysis and classification) and were used for validation of the created models. Samples were stored in freezer at −22 °C until their use. They were characterized by means of pH measurements and physicochemical and organoleptic properties (including
defects, positive attributes and quality classification). Each sample was analyzed once, due
to limited sample volume and due to the fact that, after desorption, the sample has to be re-
exttracted in case of a replicate is needed.

2.3. Sample treatment

Olive oil samples were allowed to defrost at room temperature before analysis. Then, they
were aliquoted in 4 different 10 mL vials. One aliquot was immediately used to perform the
extraction and the remaining ones were stored at 4 ºC.

3 g of oil were weighed on a precision balance directly into a 150 mL Erlenmeyer flask. The
general procedure was based on previous works (Fredes et al., 2016; Sales et al., 2017), but
improving the trapping and desorption steps. The trap consisted of a Tenax® TA TDU tube
(ID 4mm, 60mm length) previously conditioned by applying a desorption step (300 ºC during
8 minutes with a flow of high purity helium of 55 mL/min acting as carrier gas flowing
backflush). Prior to application of the extraction procedure, the trap was spiked with 10 µL
of a 50 ng/µL of toluene-d8 solution to correct extraction deviations.

For the extraction step, the sample was maintained at 40°C (by immersion of the flask in a
water bath) with magnetic agitation at 300 rpm and the headspace was purged with a flow of
100 mL/min of pure N2 for 1 hour into a Tenax tube trap. Figure S1 shows the experimental
set up used. After the extraction the traps were directly transferred to the GC/MS autosampler
to automatically carry out the thermal desorption on the TDU. In each sample extraction
batch, 6 samples (when possible, 2 extra virgin, 2 virgin and 2 lampante oils) were processed
simultaneously.

In order to avoid bias in the methodology, samples were analyzed in batches of 18 tubes,
randomly distributed. To ensure stability of the system and correct instrument deviation, replicate thermal desorptions of traps spiked with 10µL of a mixture of 50ng/µL of standards corresponding to volatile compounds present in vegetable matrices (and specific for tomato (Serrano, Beltrán, & Hernández, 2009)) were performed. These VOCs were used as they were already available in the laboratory and are coincident in many different vegetable matrices, including olive oil (Uriarte, Goicoechea, & Guillen, 2011). They are also used in volatile metabolomics studies (Gómez-Cortés, Brenna, & Sacks, 2012). These desorptions were planned at the beginning and at the end of each sequence batch, as well as every 6 samples.

2.4. GC-MS

The chromatographic analysis were performed using an Agilent 6890A gas chromatograph, equipped with a Gerstel MPS2 autosampler (Gerstel, Maryland, USA), coupled to a single quadrupole mass spectrometer, Agilent 5973N MSD (Agilent Technologies, California, USA), operating in EI mode. The GC separation was performed using a fused silica Supelcowax 10 capillary column with a length of 30m x 0.25mm ID and a film thickness of 0.25 µm (Sigma Aldrich, Germany). The oven program was set as follows: 40ºC (3 min); 5.00 ºC/min to 160ºC (1.00 min); 40.00 ºC/min to 260ºC (1.50 min). The injection system consisted of two units; the thermal desorption unit (TDU) and the programmable temperature vaporizing (PTV) – cooled injection system (CIS4) (Gerstel, Maryland, USA). The TDU parameters were set as follows; sample removal mode, splitless at an initial temperature of 40ºC (1 min equilibrium time); 60 ºC/min to 260ºC (4 min), transfer line temp 260ºC. The
CIS4 PTV was equipped with a Tenax® TA packed liner, CIS4 temperature program: 40°C (1 min equilibrium time); 12 °C/s to 260°C (4 min). A summary of the different temperature programs is graphically displayed in Figure S2.

2.5. Data processing

GC-MS data were converted to netCDF format using the Chemstation® (Agilent Technologies, California, USA,-Version G1701CA) export to .AIA function. Data mining was carried out using PARADiSe. After importing the netCDF data to the PARADiSe software, the regions of interest (ROIs), which are the time intervals where software applies the deconvolution, were selected manually along the full chromatogram. A total of 118 intervals were selected, paying attention to peak shape (when visible in the TIC) and leaving no empty spaces between intervals. Modelling options were set to a maximum of 8 compounds per interval and 50000 maximum iterations per interval. After the modelling step, models created for each interval were carefully optimized attending to: model fitting over 95%, model consistency over 95%, background removal, and avoiding model overfitting (in this order). Data matrix obtained after applying PARADiSe consisted in an .xls file which could be opened with Microsoft Excel for future data transformations. The areas provided by PARADiSe were divided by the area of tol-d8 to correct the differences between extraction batches and TDU tubes. The relative areas were corrected with the nearest external standard and then scaled applying mean-centering. Statistical analyses were performed using the EzInfo software (U-Metrics, Waters Corporation, Wilmslow, UK, Version 2.0.0.0).

3. RESULTS AND DISCUSSION
3.1. Extraction procedure optimization

Considering our previous experiences (Beltran et al., 2006; Fredes et al., 2016; Sales et al., 2017), efforts were devoted to optimize and apply static headspace-stir bar sorptive extraction-thermal desorption (SHS-SBSE-TD) and dynamic headspace entrainment followed by thermal desorption (DHS-TD). Though SPME has been extensively used for the analysis of olive oils (Arrebola et al., 2005; Benelli et al., 2015; Gómez-Cortés et al., 2012; Oliver-Pozo, Aparicio-Ruiz, Romero, & García-González, 2015; Uriarte et al., 2011; Zhu et al., 2016), it was discarded as no possibility for SPME automatically coupled to GC-MS was available at our laboratory.

The first step was to compare the performance of the two considered extraction methods in order to select only one of them for further development. Accordingly, a selected extra virgin olive oil sample was extracted by triplicate by HS-SBSE-TD and DHS-TD under the same conditions. Additionally, an aliquot of the same oil sample, spiked with the above mentioned mixture of VOCs (see experimental section), was extracted (n=3 for each method) with the same two methodologies. The analysis was performed in full-scan mode and then integrating the areas for the specified ions. Results obtained unequivocally demonstrated the higher performance of DHS-TD. On one hand, N$_2$ current (dynamic process) and the larger surface area of Tenax$^\text{®}$ TA tubes, enhanced the extraction from 4 to 10 times for most of components and up to $10^3$ times for the most volatile compounds when compared to Twister (SBSE) extraction. Furthermore, to test the reliability of the DHS-TD extraction procedure, 15 replicates of an extra virgin olive oil spiked with the IS mixture were extracted. This test gave RSD values below 15 % for most of the compounds, and permitted the detection of all the spiked compounds, together with a huge amount of additional VOCs present in the olive
oil sample. As an example, boxplots for a number of selected spiked compounds is shown in Figure S3. The plots show no outliers for the selected compounds, highlighting the repeatability of the methodology.

3.3. Data analysis optimization

Many different deconvolution software can be used for the automatic detection of chromatographic peaks in non-targeted approaches. There is plenty of literature regarding the use of xcms package of R (Fernández-Varela, Tomasi, & Christensen, 2015; Gil-Solsona et al., 2016) and MzMine (Hung, Lee, Yang, & Lee, 2014; Sales et al., 2017). More recently PARADiSe, integrating the algorithm PARAFAC2, has emerged as an efficient alternative (Elcoroaristizabal et al., 2015; Khakimov et al., 2016; Lenhardt et al., 2015; Vegge et al., 2016). From our previous knowledge on the application of these deconvolution tools to GC-MS data, and specifically related to the analyses of VOCs in olive oil, PARADiSe has been recently revealed as a potential tool in this field. It provides more robust integrations while removing a huge amount of interferent and ghost peaks. Additionally, it gives an additional benefit due to its easiness of use and peak visualization. PARAFAC2 algorithm (Harshman, 1972) performs peak deconvolution attending to the intensity and the spectra of the signals, so it is extremely powerful when resolving co-eluting peaks, even with unit mass resolution MS.

During the optimization of PARADiSe models for peak deconvolution, 118 individual intervals were obtained from the entire chromatogram, cropping the last 3 minutes to avoid ghost peaks from column bleeding at elevated temperatures. This step reduced data complexity and weight before the model validation. PARADiSe model validation was
performed as previously described elsewhere (Khakimov et al., 2016), testing the model fitting for each interval with one to eight components. Each model was carefully evaluated to find the optimal number of components, looking for a good model fitting (over 95%), noise removal and low residuals, with a core consistency over 95%. Also model overfitting was avoided while obtaining well resolved peaks. As an example, the capabilities of PARADISe for compound detection and noise reduction are displayed in Figure 1. In Figure 1(A), the total ion chromatogram shows a very complex interval, with three presumable compounds. The residuals in this case were up to $10^6$. After selecting 5 different components (Figure 1(B)), residuals were lowered by two orders of magnitude, and the algorithm detected four different signals and noise (the red component). The model fit increases from a 60% for one component to 100% with the selected compounds. At the same time, consistency is kept higher than 95%, ensuring the goodness of the selected model. Among the components selected, the green component was identified by using NIST08 as 3-(methylthio)-Propanal, and confirmed with the injection of its standard. Figure S4 highlights the potential of PARADISe for spectra deconvolution, as it is able to distinguish the signals coming from two different co-eluting components and column bleeding. This capability results in a higher number of components detected (green and blue in Figure 1B) with cleaner spectra, which results in better tentative identifications when using NIST. With the final selected model for each interval, all the samples were processed. Data exported from PARADISe lead to a .xls file containing a total of 230 different compounds, a number significantly lower than those obtained by other peak picking software commonly used, often close to ten thousand different features (Li et al., 2018). This step is determining to reduce the data matrix, which simplifies the statistical analysis. All these compounds were processed by dividing each compound peak
area by the area of the internal standard (tol-D₈) in each sample to correct instrument
development. Then they were corrected by nearest external standard and finally mean centered
to enhance the difference between groups. The whole dataset was divided in two groups, one
for method training, containing 87 samples (20 lampante, 48 virgen and 19 extra) and a
smaller subset of 21 blind samples for model validation. **Figure 2** shows the evolution of the
different principal component analyses (PCA) applied depending on different data
corrections applied. As it can be appreciated, the use of surrogate tol-D₈ for data correction
helps to minimize deviation in groups when compared to the raw data. Furthermore, the use
of the response of the nearest external standard for data correction enhances the differences
between groups, and consequently, was selected for further method development.

### 3.4. Classification Model Validation

At this point, the development of a quality classification model of olive oils by DHS-TD was
studied. Subsequently, after aforementioned data transformations and having checked the
PCA for goodness of data, a partial least squares discriminant analysis (PLS-DA) was
constructed according to the quality of each group (extra virgin, virgin and lampante groups
(see **Figure 3**). The PLS-DA showed a clear distinction between lampante and extra samples,
while the virgin samples, with both positive attributes and defects, were in the middle. In
order to verify the accuracy of the model, it was validated with the analysis of blind samples,
i.e with a priori unknown quality. **Figure S5** shows a confusion matrix presenting the results
for the training and the validation set of samples classified by PLS-DA. One of the greatest
outputs was the capability of the developed methodology to correctly classify 100% of extra
virgin olive oil samples. Another output was the great differentiation achieved between extra
and lampante samples which avoided any misclassification between these two extreme
groups. Finally, in order to determine the compounds responsible for extra and lampante qualities, two different orthogonal partial least squares-discriminant analysis (O-PLSDA) models were created. Firstly, the flawless extra virgin samples were faced to the virgin and lampante ones; and secondly, lampante samples were faced to the rest. From them, two S-PLOT graphs were obtained and inspected for endpoints. Table 1 lists the main compounds responsible of the positive attributes of extra samples and also those found as responsible of the lampante quality.

3.5. Defect-related compounds identification

As a final step, PARADISe automatically compares deconvoluted spectra with NIST library (in this case NIST08 (NIST, Maryland, USA)), giving the best fitted candidate for each peak. In order to add more confidence to identification, retention index for each compound was calculated using a C7-C30 alkane mixture which was injected along with the rest of the sequence.

Although all the deconvoluted features were used in the creation of the statistical model, only the compounds with a match over 850 and a RI match ± 20 (Chemspider) were considered as tentatively identified compounds. Compounds given as completely identified were confirmed by the injection of its corresponding standard.

In a previous work (Sales et al., 2017), it was demonstrated that the distinction between flawless extra samples and samples with a specific defect was larger than the difference between the three quality classes. Continuing with that work, our efforts were devoted towards the complete identification of the compounds responsible of each kind of defect or negative attribute. To that extent, a PLS-DA was constructed according to the main defect of
each sample (or the absence of it). **Figure 4** shows the results for the PLS-DA grouping the samples by quality and colored by: considering the predominant (main) defect (A), defect intensity (B) and main fruitiness intensity (C). From the first PLS-DA, distinguishing by the type of defect, several O-PLSDAs were performed facing samples with one defect against the flawless extra, one defect at a time. The next step was to obtain the corresponding S-PLOT graph for each case and to inspect them looking for endpoints, especially in the part of the defect, to see which compounds were highly related to each negative attribute. Applying this methodology to each defect, a group of compounds were considered as responsible of the bad quality of virgin/lampante olive oils, which are summarized in **Table 2**. The results show the great potential of this technique for the identification of defect-related compounds, as well as for the discrimination of samples according to their defect. These results correlate well with previous works in the field of defect identification using targeted approaches. Especially interesting are E-2-decenal and Heptenal, with odour thresholds in the low ppb level, which have been reported by many authors in different olive oils to be related with distinct major defects (Morales, Luna, & Aparicio, 2005; Zhu et al., 2016). Our approach, additionally, shows that their presence has stronger impact than other compounds when labelling an olive oil with rancid or fusty defects and frozen, respectively, and that their presence correlates normally with the label virgin rather than lampante. In a similar way, octanal, which in our results is indicative of fusty defect, and octane, with a stronger presence in defected oils (**Table S1**) are also reported as present in defected oils in previous literature (Morales et al., 2005; Oliver-Pozo et al., 2015). As complementary information, an overview of the signals (relative areas) for all the detected and unequivocally identified compounds in olive oils and their relation to quality and defects are also shown in **Tables S1 and S2**. Data shown in these tables allow to highlight the potential of the combination of DHS-TD together
with PARADISe for the detection of high number of relevant compounds in untargeted analysis. Apart from this, the use of this state-of-the art workflow for the determination of VOCs using EI source together with NIST library matching, allowed to tentatively identify several compounds detected in a previous work with the novel atmospheric pressure chemical ionization source (APCI) (Sales et al., 2017). Also, RI from the previous used non-polar column in GC-APCI-QTOF MS system, and RI from the polar Suplecowax 10 used in this work were compared and compounds were tentatively correlated when considering molecular ion and the molecular fragments found by both methodologies. Table S3 summarizes the results. Special attention must be paid to 4-ethyl phenol and 5-ethyl- 2(5H)-Furanone, which have been detected by both methodologies and have been found to be responsible of *fusty* defect and extra quality, respectively. It is also notable that *rancid* and *brine* defects are poorly characterized by volatiles, as only one compound has been linked to each defect.

### 4. CONCLUSIONS

A methodology coupling an advanced sample treatment technique for VOCs analyses, with a promising powerful deconvolution software for non-targeted analyses, has been developed for the quality classification of Spanish olive oils. This classification has been faced from an untargeted point of view, a novel contribution in a field where normally target approaches are applied. Also, this approach has allowed determining a wide number of compounds related to main defects found in olive oils.

The high pre concentration factor obtained by DHS-TD has allowed the detection of a huge
number of volatile compounds in olive oil at trace levels. PARADISe has demonstrated huge capabilities for robust peak detection. Thanks to its special algorithm (PARAFAC2), extremely clean mass spectra has been provided. This has been very useful for tentative identification of unknown compounds when matching their spectra with NIST libraries and also for resolving coeluting peaks.

The developed methodology has permitted to obtain an enhanced quality classification model, with a 100% discrimination of extra samples, and an overall 86% accuracy for the three different classes, which reveals it as a very important complement to the PANEL TEST.

As a final remark, the method has allowed also to putatively identify and completely identify (when standards were available) the main compounds responsible of each type of organoleptic defect in virgin olive oils. This work presents an affordable solution for olive oil classification thanks to the use of state-of-the-art sample treatment and data treatment methodologies for untargeted foodomics. It contributes to postulate DHS-TD methodology as a very powerful technique for the identification and quantitation of volatiles. Also it is feasible for the classification of samples trough untargeted analysis not only in oils, but in any complex sample with an important volatile composition.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors acknowledge the financial support of Generalitat Valenciana, as research group of excellence (PROMETEO II/2014/023), Collaborative Research on Environment and Food-Safety (ISIC/2012/016) and University Jaume I (UJI-B2016-10) C. Sales
acknowledges the financial support of Universitat Jaume I for his pre-doctoral grant. Tania Portolés acknowledges Juan de la Cierva Incorporation Program from Ministry of Economy and Competitiveness (IJC1-2014-20588) for funding her research. The authors are very grateful to prof. Jan H. Christensen for his support. The investigation has been performed within the frame of scientific collaboration between the “Ministerio de Agricultura, Alimentación y Medio Ambiente”, the “Consejería de Agricultura, Pesca y Desarrollo Rural de la Junta de Andalucía” and the “Interprofesional del Aceite de Oliva Español”.

FIGURE CAPTIONS

Figure 1: Evolution of PARADiSe model at interval 57 (17.53 – 17.87 min) for 1 component (A) and 5 components (B) finally selected.

Figure 2: Evolution of PCA plots after each data treatment step (mean centered in all cases): A Raw data, B corrected dividing by TOL-D8 area, C corrected by the nearest standard.

Figure 3: PLS-DA plot for the training set used in the construction of the classification model.

Figure 4: PLS-DA plots focused on defects: A colored by type of defect, B colored by intensity of the defect, C colored by fruity intensity.

BIBLIOGRAPHY

Arrebola, F. J., González-Rodríguez, M. J., Garrido Frenich, A., Marín-Juan, A., & Martínez Vidal, J. L. (2005). Determination of halogenated solvents content in olive oil by two completely automated headspace techniques coupled to gas
chromatography-mass spectrometry. *Analytica Chimica Acta*, 552(1–2), 60–66.

Beltran, J., Serrano, E., López, F. J., Peruga, A., Valcarcel, M., & Rosello, S. (2006). Comparison of two quantitative GC-MS methods for analysis of tomato aroma based on purge-and-trap and on solid-phase microextraction. *Analytical and Bioanalytical Chemistry*, 385(7), 1255–1264.

Benelli, G., Caruso, G., Giunti, G., Cuzzola, A., Saba, A., Raffaelli, A., & Gucci, R. (2015). Changes in olive oil volatile organic compounds induced by water status and light environment in canopies of *Olea europaea* L. trees. *Journal of the Science of Food and Agriculture*, 95(12), 2473–2481.

Cevallos-Cevallos, J. M., Reyes-De-Corcuera, J. I., Etxeberria, E., Danyluk, M. D., & Rodrick, G. E. (2009). Metabolomic analysis in food science: a review. *Trends in Food Science & Technology*, 20(11–12), 557–566.

Díaz, R., Pozo, O. J., Sancho, J. V, & Hernández, F. (2014). Metabolomic approaches for orange origin discrimination by ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *Food Chemistry*, 157, 84–93.

Dierkes, G., Bongartz, A., Guth, H., & Hayen, H. (2012). Quality evaluation of olive oil by statistical analysis of multicomponent stable isotope dilution assay data of aroma active compounds. *Journal of Agricultural and Food Chemistry*, 60(1), 394-401.

Elcoroaristizabal, S., Bro, R., García, J. A., & Alonso, L. (2015). PARAFAC models of fluorescence data with scattering: A comparative study. *Chemometrics and Intelligent Laboratory Systems*, 142, 124–130.

Fernández-Varela, R., Tomasi, G., & Christensen, J. H. (2015). An untargeted gas
chromatography mass spectrometry metabolomics platform for marine polychaetes.

*Journal of Chromatography A, 1384, 133–141.*

Fredes, A., Sales, C., Barreda, M., Valcárcel, M., Roselló, S., & Beltrán, J. (2016). Quantification of prominent volatile compounds responsible for muskmelon and watermelon aroma by purge and trap extraction followed by gas chromatography-mass spectrometry determination. *Food Chemistry, 190, 689-700.*

Garcia, A., & Barbas, C. (2011). Gas Chromatography-Mass Spectrometry (GC-MS)-Based Metabolomics. *Metabolic Profiling: Methods and Protocols* (pp. 191–204).

Garrido-Delgado, R., Mercader-Trejo, F., Arce, L., & Valcárcel, M. (2011). Enhancing sensitivity and selectivity in the determination of aldehydes in olive oil by use of a Tenax TA trap coupled to a UV-ion mobility spectrometer. *Journal of Chromatography A, 1218(42), 7543–7549.*

Gerhardt, N., Birkenmeier, M., Sanders, D., Rohn, S., & Weller, P. (2017). Resolution-optimized headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) for non-targeted olive oil profiling. *Analytical and Bioanalytical Chemistry, 409(16), 3933–3942.*

Gil-Solsona, R., Raro, M., Sales, C., Lacalle, L., Díaz, R., Ibáñez, M., Beltran, J., Sancho, J.V., Hernández, F. J. (2016). Metabolomic approach for Extra virgin olive oil origin discrimination making use of ultra-high performance liquid chromatography - Quadrupole time-of-flight mass spectrometry. *Food Control, 70, 350-359.*

Gómez-Cortés, P., Brenna, J. T., & Sacks, G. L. (2012). Production of isotopically labeled standards from a uniformly labeled precursor for quantitative volatile metabolomic
studies. *Analytical Chemistry*, 84(12), 5400–5406.

Harshman, R. A. (1972). PARAFAC2: Mathematical and technical notes. *UCLA Working Papers in Phonetics*, 22, 30-44.

Herrero, M., García-Cañas, V., Simo, C., & Cifuentes, A. (2010). Recent advances in the application of capillary electromigration methods for food analysis and Foodomics. *Electrophoresis*, 31(1), 205–228.

Hung, C.-H., Lee, C.-Y., Yang, C.-L., & Lee, M.-R. (2014). Classification and differentiation of agarwoods by using non-targeted HS-SPME-GC/MS and multivariate analysis. *Anal. Methods*, 6(18), 7449–7456.

Jabeur, H., Zribi, A., & Bouaziz, M. (2016). Extra-Virgin Olive Oil and Cheap Vegetable Oils: Distinction and Detection of Adulteration as Determined by GC and Chemometrics. *Food Analytical Methods*, 9(3), 712–723.

Johnsen, L. G., Skou, P. B., Khakimov, B., & Bro, R. (2017). Gas chromatography - mass spectrometry data processing made easy. *Journal of Chromatography. A*, 1503, 57–64.

Kalogiouri, N. P., Aalizadeh, R., & Thomaidis, N. S. (2017). Investigating the organic and conventional production type of olive oil with target and suspect screening by LC-QTOF-MS, a novel semi-quantification method using chemical similarity and advanced chemometrics. *Analytical and Bioanalytical Chemistry*, 409(23), 5413–5426.

Kalogiouri, N. P., Alygizakis, N. A., Aalizadeh, R., & Thomaidis, N. S. (2016). Olive oil authenticity studies by target and nontarget LC–QTOF-MS combined with advanced chemometric techniques. *Analytical and Bioanalytical Chemistry*, 408(28), 7955–
Kalua, C. M., Allen, M. S., Jr, D. R. B., Bishop, A. G., Prenzler, P. D., & Robards, K. (2007). Food Chemistry Olive oil volatile compounds, flavour development and quality: A critical review, *100*, 273–286.

Khakimov, B., Mongi, R. J., Sørensen, K. M., Ndabikunze, B. K., Chove, B. E., & Engelsen, S. B. (2016). A comprehensive and comparative GC–MS metabolomics study of non-volatiles in Tanzanian grown mango, pineapple, jackfruit, baobab and tamarind fruits. *Food Chemistry*, *213*, 691–699.

Kind, T., Tolstikov, V., Fiehn, O., & Weiss, R. H. (2007). A comprehensive urinary metabolomic approach for identifying kidney cancer. *Analytical Biochemistry*, *363*(2), 185–95.

Lenhardt, L., Bro, R., Zeković, I., Dramićanin, T., & Dramićanin, M. D. (2015). Fluorescence spectroscopy coupled with PARAFAC and PLS DA for characterization and classification of honey. *Food Chemistry*, *175*, 284–291.

Li, Z., Lu, Y., Guo, Y., Cao, H., Wang, Q., & Shui, W. (2018). Analytica Chimica Acta Comprehensive evaluation of untargeted metabolomics data processing software in feature detection, quantification and discriminating marker selection. *Analytica Chimica Acta*, *1029*, 50–57.

Luna, G., Morales, M. T., & Aparicio, R. (2006). Characterisation of 39 varietal virgin olive oils by their volatile compositions. *Food Chemistry*, *98*(2), 243–252.

Marquez, A., Serratosa, M. P., Merida, J., Zea, L., & Moyano, L. (2014). Optimization and validation of an automated DHS-TD-GC-MS method for the determination of
aromatic esters in sweet wines. *Talanta, 123*, 32–38.

Meyer, M. R., Peters, F. T., & Maurer, H. H. (2010). Automated mass spectral deconvolution and identification system for GC-MS screening for drugs, poisons, and metabolites in urine. *Clinical Chemistry, 56*(4), 575–584.

Morales, M. T., Luna, G., & Aparicio, R. (2005). Comparative study of virgin olive oil sensory defects. *Food Chemistry, 91*(2), 293–301.

Myers, O. D., Sumner, S. J., Li, S., Barnes, S., & Du, X. (2017). Detailed Investigation and Comparison of the XCMS and MZmine 2 Chromatogram Construction and Chromatographic Peak Detection Methods for Preprocessing Mass Spectrometry Metabolomics Data. *Analytical Chemistry, 89*(17), 8689–8695.

Oliver-Pozo, C., Aparicio-Ruiz, R., Romero, I., & García-González, D. L. (2015). Analysis of Volatile Markers for Virgin Olive Oil Aroma Defects by SPME-GC/FID: Possible Sources of Incorrect Data. *Journal of Agricultural and Food Chemistry, 63*(48), 10477–10483.

Portaarena, 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.

Sales, C., Cervera, M. I., Gil, R., Portolés, T., Pitarch, E., & Beltran, J. (2017). Quality classification of Spanish olive oils by untargeted gas chromatography coupled to hybrid quadrupole-time of flight mass spectrometry with atmospheric pressure chemical ionization and metabolomics-based statistical approach. *Food Chemistry, 216*, 365-373.
Serrano, E., Beltrán, J., & Hernández, F. (2009). Application of multiple headspace-solid-phase microextraction followed by gas chromatography-mass spectrometry to quantitative analysis of tomato aroma components. *Journal of Chromatography. A, 1216*(1), 127–33.

Tikunov, Y., Lommen, A., Vos, C. H. R. De, Verhoeven, H. A., Bino, R. J., Hall, R. D., & Bovy, A. G. (2005). A Novel Approach for Nontargeted Data Analysis for Metabolomics. Large-Scale Profiling of Tomato Fruit Volatiles. *Plant Physiology, 139*, 1125–1137.

Uriarte, P. S., Goicoechea, E., & Guillen, M. D. (2011). Volatile components of several virgin and refined oils differing in their botanical origin. *Journal of the Science of Food and Agriculture, 91*(10), 1871–1884.

Vegge, C. S., Jansen van Rensburg, M. J., Rasmussen, J. J., Maiden, M. C. J., Johnsen, L. G., Danielsen, M., … Kelly, D. J. (2016). Glucose Metabolism via the Entner-Doudoroff Pathway in Campylobacter: A Rare Trait that Enhances Survival and Promotes Biofilm Formation in Some Isolates. *Frontiers in Microbiology, 7*, 1–16.

Zhu, H., Wang, S. C., & Shoemaker, C. F. (2016). Volatile constituents in sensory defective virgin olive oils. *Flavour and Fragrance Journal, 31*(1), 22–30.