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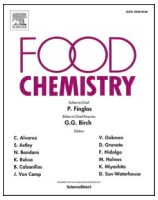

Contribution of specific volatile markers to green and ripe fruity attributes in extra virgin olive oils studied with three analytical methods

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ABSTRACT

An objective sensory evaluation of extra virgin olive oil (EVOO), involving the chemical characterization of positive attributes, is of interest. These attributes are objectively divided, according to fruitiness, into "green" and "ripe" fruity. This work studied the differentiation in the volatile profile of EVOOs into these two classes, obtained by three analytical methods, including different extraction techniques and detectors and two data processing strategies, and their relation with sensory results. According to the results, each method allowed the characterization of the two classes, providing information on different volatile compounds, which increased in number through PARADISe software (14 more than the conventional processing). Moreover, some volatile compounds showed significant differences between the two classes, 16 highlighted by the variables with importance in projection (VIP) for green fruity (e.g. (*Z*)-3-hexen-1-ol, methyl ether) and 23 for ripe fruity EVOOs (e.g. (Z)-2-hexen-1-ol), which could be considered as useful markers to complement quality assessment.

1. Introduction

Virgin olive oil production in recent years has been marked by the growing importance of the extra virgin olive oil (EVOO) category and the consumers' demand for optimized production procedures and better quality. In fact, quality is today identified as a competitive element (Conte et al., 2020). This context has led to a greater interest in understanding the sensory quality of virgin olive oil and the positive attributes to reposition the product in a competitive market in which other oils are also promoting positive aspects. In the search of a better quality, European Union regulations protect EVOOs of differentiated quality related with defined geographical indications (EC, 2006). Some private virgin olive oil brands have also opted to define higher quality standards within the extra virgin category to increase their competitiveness (for example, the SIQEV seal of QvExtra! International).

EVOOs is defined by the current regulation as that oil presenting an absence of negative attributes (median of defects = 0) and a fruitiness media above zero (>0) certified by the standard method of organoleptic assessment (EU, 1991; IOC, 2018). Beyond this basic definition, high value EVOOs known as premium oils are differentiated from other EVOOs by numerous positive sensory notes (Bongartz & Oberg, 2011; Casadei et al., 2021; Morales et al., 2013a). Thus, the differentiation of the enormous variety of sensory profiles within the EVOO category requires the study of descriptors other than those included in the standard method of organoleptic assessment (fruitiness, bitterness and pungency) that explain this quality diversity.

With the aim of understanding virgin olive oil quality, many studies have been conducted to explain the attributes from a sensory and chemical perspective (Morales et al., 2005; Kalua et al., 2007; Morales et al., 2013a; Morales et al., 2013b; Morales et al., 2013c; Cecchi et al., 2021; Genovese et al., 2021). However, many of these studies have been focused on the negative attributes of virgin olive oils (sensory defects). The study of positive attributes, mainly found in EVOO, requires specific strategies that are different from those used so far to detect lower quality oils (virgin olive oil and lampante olive oil) (Aparicio et al., 1996; García-González et al., 2011; Morales et al., 2013a; Morales et al., 2013b; Morales et al., 2013c; Cecchi et al., 2021). Furthermore, the study of positive attributes also requires working with a harmonized terminology. Some of the positive attributes perceived in virgin olive oil are listed in the International Olive Council (IOC) method for "organoleptic assessment of extra virgin olive oil applying to use a designation of origin" (IOC, 2005). This document presents a list of attributes, some of them being difficult to be detected by panelists due to the lack of definition and training and to the subjective component in their evaluation.

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However, in a first approach, these positive attributes could be divided into two subsets, "green fruity" and "ripe fruity". Each of these two qualifiers includes a high number of sensory notes that are difficult to be evaluated individually with enough accuracy. However, the panelists are able to qualify the oils with the general terms of "green" or "ripe" or a mixture of both with certain accuracy (Bongartz & Oberg, 2011). On the other hand, the virgin olive oil quality competitions that are commonly organized to award the best EVOOs typically differentiate these two categories. Thus, it is reasonable to propose that a study of the positive attributes and the volatile compounds responsible requires a specific study on differentiating the aromas of "green fruity" and "ripe fruity" EVOOs.

The positive attributes "green fruity" and "ripe fruity" in EVOOs are explained by the presence of a wide variety of volatile compounds (Aparicio et al., 1996; Cecchi et al., 2021; Neugebauer et al., 2020; Zhou et al., 2019; Morales et al., 1995). However, the knowledge of the individual contribution of volatile compounds to the greener or riper notes of the EVOOs is still scarce. Given the complexity of positive attributes found in EVOO associated to different volatile profiles rather than single volatile markers, the study of different isolation/extraction techniques and methods are needed to obtain the maximum information of the volatile profile. The perfection and validation of some methods based on headspace-solid-phase micro-extraction (HS-SPME) and gas chromatography (GC) with flame ionization (FID) or mass spectrometry (MS) detectors (Aparicio-Ruiz et al., 2018; Aparicio et al., 2012; Benelli et al., 2015; Casadei et al., 2021; Cecchi et al., 2019; Morales et al., 2005; Oliver-Pozo et al., 2015; Segura-Borrego et al., 2020) also provide the opportunity to determine volatile compounds with a minimized analytical error. The influence of the specificity of the SPME polymer in the volatile adsorption (Oliver-Pozo et al., 2015) could be avoided by using other extraction techniques such as the thermal extraction in micro-vials (ATEX) by Thermal Desorption Unit (TDU) coupled to GC–MS with a previous cryo-concentration with a Cooling Injector System (CIS) that applies liquid nitrogen (Francesca et al., 2015). Furthermore, the comprehensive analysis of volatile compounds of virgin olive oil headspace can be benefited by the PARAFAC2 based Deconvolution and Identification System (known as PARADISe software) (Johnsen et al., 2017), in which minor volatile compounds related with fruity notes compounds can be identified.

In this context, the aim of this work was the differentiation of the volatile profiles associated to EVOOs under the categorization of "green fruity" and "ripe fruity", looking for specific volatile markers that contribute to each class. For this purpose, the potential of two different extraction methods (HS-SPME and CIS-TDU) and two different detectors (GC–MS and GC-FID) have been exploited together with the use of the PARADISe software for peak deconvolution. In addition, the relationship between the detected compounds and the positive attributes reported by panelists was investigated.

2. Materials and methods

2.1. Sample collection

A total of 24 extra virgin olive oils (EVOOs) from different geographical origins and varieties were selected for this study from a first set of 105 collected EVOOs (Table 1). This set of selected samples was composed by high quality EVOOs from different regions of Spain, Italy, Portugal, Turkey, Slovenia, and Croatia, and of different varieties such as Arbequina, Picual, Hojiblanca, Frantoio, Istrska belica, Leccino, Oblica, Arbosana and Coratina. All the samples were verified regarding the fulfil of the legal limits for extra virgin olive oil classification (free acidity lower than 0.8 %, peroxide value lower than 20 mEq O_2/kg, extinction coefficients at 232 nm lower than 0.22 and at 268 nm lower than 2.50) (EC, 1991).

2.2. Sensory assessment of extra virgin olive oil samples

The primary selection of the 24 samples out of 105 oils was made by an open tasting procedure made twice by 4 trained panelists who tentatively selected those samples with distinctive and undoubtful sensory profiles of "green fruity" or "ripe fruity", discarding those samples with unclear positive attributes or a mixture of both sensory profiles.

Table 1
Samples under study.

Class*	Code	Geographical origin of the olives	Olive variety/varieties, PDO, PGI	Characteristics
Green fruity	**EVOO1**	Spain (Jaén)	Picual	Early harvest 2020/2021.
	EVOO2	Spain	–	–
	EVOO3	Spain	Hojiblanca	–
	EVOO4	Croatia (Dalmatia region)	Oblica	Healthy green olive fruits, processing within 24 h.
	EVOO5	Spain (Jaén)	–	
	EVOO6	Spain (Almería)	Coupage (Picual, Hojiblanca, Arbequina)	Organic, early harvest 2020/2021 or green olives in veraison harvested at the beginning of the season.
	EVOO7	Spain (Ciudad Real)	Arbequina	Early harvest.
	EVOO8	Spain (Jaén)	Picual	Early harvest 2020/2021.
	EVOO9	Italy (Ravenna)	Nostrana di Brisighella, PDO Brisighella	–
	EVOO10	Slovenia	Istrska belica, PDO Slovenska Istra	–
	EVOO11	Croatia (Istra)	Istrska belica, Leccino, Buža from Croatia	–
	EVOO12	Spain (Jaén)	Hojiblanca	Early harvest 2020/2021.
	EVOO13	Spain	Arbequina	–
	EVOO14	Spain (Seville)	Arbequina	Organic, early harvest 2019/2020.
	EVOO15	Italy (Apulia)	Coratina	–
	EVOO16	Italy (Tuscany)	Leccino/Frantoio/Pendolino	–
	EVOO17	Portugal (Beja)	Arbequina	–
	EVOO18	Spain (Ciudad Real)	Frantoio	–
Ripe fruity	**EVOO19**	Italy (Tuscany)	Coupage main cv.: Leccino; Other: Frantoio, Moraiolo	–
	EVOO20	Turkey (Akhisar-Manisa Egean)	Ayvalik, Domat blend	–
	EVOO21	Portugal (Algés)	–	Early harvest 2020/2021.
	EVOO22	Spain (Jaén)	Arbosana	Early harvest 2020/2021.
	EVOO23	Spain (Osuna)	Hojiblanca-Picual	–
	EVOO24	Spain (Jaén)	–	

* Note: this category has been attributed from the sensory analysis by the panelists.

Thus, the panelists selected 12 samples categorized as "green fruity" and 12 samples categorized as "ripe fruity" for further sensory analysis. Thus, after this tentative characterization, the panel test analyzed the samples and confirmed this characterization applying the official procedure described by the International Olive Council (IOC, 2018) in which a group of eight trained panelists detected and quantified (into a scale from 0 to 10) the intensity of different attributes. In the sensory evaluation, olfactory and gustatory, the panelists confirmed that the samples did not have any sensory defect and they scored the positive attributes included in the IOC regulation. Additionally, the positive sensory attributes described in the IOC method COI/T.20/Doc. n° 22, 2005 for the organoleptic assessment of extra virgin olive oil were evaluated (IOC, 2005). Thus, these attributes were: green fruit, ripe fruit, bitter, pungent, green almond, apple, artichoke, chamomile, citric fruits, eucalypt, exotic fruits, fig leaf, flowers, grass, green pepper, spices, olive leaves, pear, pine kernels, soft fruits, sweet pepper, green tomato, ripe tomato, vanilla, walnut, aromatic herbs. In addition to them, other attributes were also detected by the panelists in some of the samples, such as ripe raisins, dry wood, ripe banana, fruit mash, cooked vegetables, nuts, yogurt, cinnamon, dairy product, and aniseed.

2.3. Reagents and chemicals

The standards of volatile compounds were purchased from Sigma-Aldrich (St. Louis, Missouri, USA) for identification purposes, and 4-methyl-2-pentanol (CAS number 123-51-3, purity ≥95 %) was employed as internal standard (IS). A mixture of *n*-alkanes from 8 to 20 carbon atoms (~40 mg/L each, in *n*-hexane) purchased by Fluka (Madrid, Spain) was employed for calculating the Linear Retention Index (LRI).

2.4. Analysis of volatile compounds

The volatile compounds of the samples were analyzed by three methods: Headspace-solid phase microextraction (HS-SPME) coupled to gas chromatographic analysis with two detectors, mass spectrometry or with flame ionized detector (henceforth HS-SPME-FID or HS-SPME-MS, respectively); and thermal extraction in micro-vials (ATEX) by Cooled Injection System (CIS)-Thermal Desorption Unit (TDU) coupled to gas chromatographic analysis with mass spectrometry (henceforth TDU-GC–MS). Once the samples were collected, they were stored in a freezer at −18 °C using different vials for each analysis, in order to minimize the headspace volume and to only thaw them one time. Before each analysis, they were thawed until no solid phase was observable, at room temperature and shaken carefully. All the analyses were carried out in <2 months to avoid uncontrolled sensory changes. The samples were analyzed in duplicate for each methodology.

2.4.1. HS-SPME-GC-FID and HS-SPME-GC–MS analyses

The sample preparation and extraction of volatile compounds were carried out according to Casadei et al. (2021) in the case of HS-SPME-GC-FID and Aparicio-Ruiz et al. (2022) in the case of HS-SPME-GC–MS. Hence, 1.9 g of sample and 0.1 g of 4-methyl-2-pentanol standard solution at ≈2.5 mg/kg (added as internal standard-IS) were weighed in a 20 mL glass vial and hermetically closed with a polytetrafluoroethylene septum. The sample was heated for 10 min at 40 °C under agitation (250 rpm). After that, the volatile extraction was carried out by exposing the solid phase microextraction (SPME) fiber (21 mm of fiber depth) to the sample headspace, during 40 min at 40 °C. For desorption of volatile compounds, the fiber was inserted into the injector port of the gas chromatograph (GC) coupled to the MS detector of FID detector. The SPME fiber, which was previously conditioned by following the instructions of the supplier, was purchased from Supelco (Merck KGaA, Darmstadt, Germany), of length 1 cm, 50/30 μm film thickness and endowed with the Stable Flex stationary phase of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS).

Gas chromatography analysis was performed following the validated methods (Casadei et al., 2021; Romero et al., 2015), in the same conditions for both analysis and also for the extraction method described in the next section. The gas chromatograph was a 7820A (Agilent Technologies, Santa Clara, CA) with an autosampler MPS (Gerstel, Mülheim an der Ruhr, Germany) and coupled to a quadrupole mass spectrometer Series MSD 5975 (Agilent Technologies, Santa Clara, CA), with a capillary column DB-WAX (Agilent J&W, Santa Clara, CA. 60 m; I.D. 0.25 mm; film thickness 0.25 μm). The oven program consists of holding the oven at 40 °C for 10 min and then raised by 3 °C/min to a final temperature of 200 °C, that conforms a running time of 63.33 min. The carrier gas was hydrogen, at a flow rate of 1.5 mL/min in the case of FID and of 0.9 mL/min in the case of MS. The temperature of the FID was set at 260 °C. 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).

2.4.2. TDU-GC–MS analyses

Sample preparation was performed by adding 75 μL of virgin olive oil, with internal standard added (see section 2.5) into a disposable micro-vial insert for microvials ATEX (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) used for cryotrapping with a Cooled Injection System (CIS) and thermal desorption with a Thermal Desorption Unit (TDU2, Gerstel). The inserts are placed in the empty desorption liners and transferred to the TDU. The standby temperature was 40 °C. When the tube is heated in the TDU2, volatile analytes are extracted from the sample and transferred to the CIS where they are concentrated prior to be transferred to the GC, being the non-volatile matrix residue left behind in the disposable micro-vial. A temperature program was applied in the TDU: a rate of 25 °C/min until an end temperature of 90 °C and a hold time of 15 min. During this time, CIS was maintained at −150 °C (cryo timeout) to preconcentrate the volatile compounds. Then, a transfer temp of 325 °C was set. A flow-rate 0.9 mL/min of carrier gas (H_2) was set to inject extracted volatile compounds into the GC column. The temperature was adjusted with a computer-controlled valve supplying liquid nitrogen pulsed flow. All the variables for sample preparation and injection into GC column was controlled with Gerstel Maestro v1.4 software (Gerstel) adapted to Agilent MSD ChemStation software E.02.02.1431 (Agilent Technologies, Santa Clara, CA, USA). The GC–MS analysis was performed in the same conditions above described (Section 2.5), by using the same GC and MS detector, the same column, the same oven program and the same MS conditions.

2.4.3. Identification of volatile compounds and peak integration

All data were recorded using an MSD ChemStation software (Agilent technologies Inc.) which was used for a first conventional preprocessing performed for the data obtained from the three analytical methods (HS-SPME-GC-FID, HS-SPME-GC–MS and TDU-GC–MS).

Furthermore, the GC–MS data (HS-SPME-GC and TDU-GC–MS) was converted to netCDF format in order to use PARADISe tool for data mining. This tool allows peak deconvolution by PARAFAC2 and identification by using the deconvoluted mass spectra and the NIST MS library, generating an identification report (Johnsen et al., 2017). It is based on the so-called PARAllel FACtor analysis2 (PARAFAC2) modelling, which allows extraction of the pure spectra of co-eluting compounds as well as it simultaneously computes their peak areas. Before the deconvolution, a total of 117 and 113 intervals were selected along the full HS-SPME-GC–MS and TDU-GC–MS chromatograms, respectively. Modeling options were set to a maximum of 8 components per interval and non-negativity constrain was applied. For selecting the correct number of components for each model, the fit and the core consistency were carefully optimized. FID identification was performed by comparison with MS identification and standards.

The areas provided by PARADISe for each compound, (i.e., obtained using the entire pure spectrum and retention time region corresponding

to a specific peak) as well as the areas obtained by conventional pre-processing (total ion current), were used to obtain the quantitative results in mg/kg in relation to the internal standard.

2.5. Statistical analysis

An analysis of variance (ANOVA), followed by a post hoc comparison test (Tukey's test), was performed with the concentrations obtained by each method for each compound, grouping the samples into the two selected positive attributes ("ripe fruity" and "green fruity") using the INFOSTAT software 2016 (FCA, Universidad Nacional de Córdoba, Argentina). In addition, different principal component analyses (PCA) and partial least squares-discriminant analyses (PLS-DA) were carried out by using PLS_Toolbox 7.9.5 (Eigenvector Research Inc., Wenatchee, WA) working under MATLAB environment (R2016a, The MathWorks, Inc. USA). Prior to modeling, data was autoscaled.

3. Results and discussion

3.1. Sensory characterization of extra virgin olive oil samples and determination of volatile compounds with the three analytical methods

The extra virgin olive oil (EVOO) samples were collected under the premise that they were in the high rank of quality within the EVOO category and therefore with a clearly distinguishable fruity attribute. The attributes of "green fruity" and "ripe fruity" are typically present at different degrees in the global aroma of the product in these oils. However, for characterizing individually each of these attributes, it was necessary to select those samples in which one of the two attributes was clearly dominant over another. In the case of the "ripe fruity" attribute, those samples that clearly resembled aged EVOO in which the green attributes lowered (Lobo-Prieto et al., 2020) were also discarded. Each one of the two studied attributes was composed of different sensory notes that were also evaluated by the sensory panel. Fig. 1-**A** shows a spider chart in which the medians of the scores obtained for each of the samples are plotted. The results show the high variability in the sensory profiles within the selected EVOOs. In fact, the relative standard deviation of the assessed sensory notes ranged from 36 % (pungent values) to 490 % (eucalypt attribute). It should be noted that the perceived sensations and the intensity ranges found, are, in general, in agreement with those reported in the literature for other EVOOs (Rodrigues et al., 2022; Marx et al., 2021; Aparicio et al., 1996).

On the other hand, Fig. 1-**B** shows the medians of these sensory notes for the samples grouped in two classes, "green fruity" and "ripe fruity". This figure shows that the samples categorized by the panel as "green fruity" also presented higher bitterness and pungency, as well as higher scores of green tomato, olive leaves and grass attributes compared with "ripe fruity" EVOOs. Hence, all the "green fruity" samples presented medians of intensity for grass notes between 1.8 and 4.4, while it was only detected in 6 out of the 12 "ripe fruity" samples (medians between 0 and 3.1). In contrast, "ripe fruity" EVOOs showed higher median values for ripe tomato, flowers and forest fruits attributes. Similar results were recently obtained by Rodrigues et al., (2022), which results showed that oils with high olfactory and gustatory intensities of greenly fruity sensations, had also intense notes of tomato leaves, in addition to high bitter and pungent sensations, while there was another group of oils distinguished from the previous ones mainly due to the higher olfactory–gustatory intensity of banana, and the perceived gustatory fruit notes. Thus, in this study, ripe banana was a descriptor detected by all the panelists in more than a half of the samples categorized as "ripe fruity".

In order to study the volatile compounds responsible of these sensory differences perceived between the two general classes selected, "green fruity" and "ripe fruity", three analytical methods, including two different extraction techniques and two different detectors (HS-SPME-GC-FID, HS-SPME-GC–MS and TDU-GC–MS), were used and compared for the analysis of the samples. Unlike the study of volatile makers in

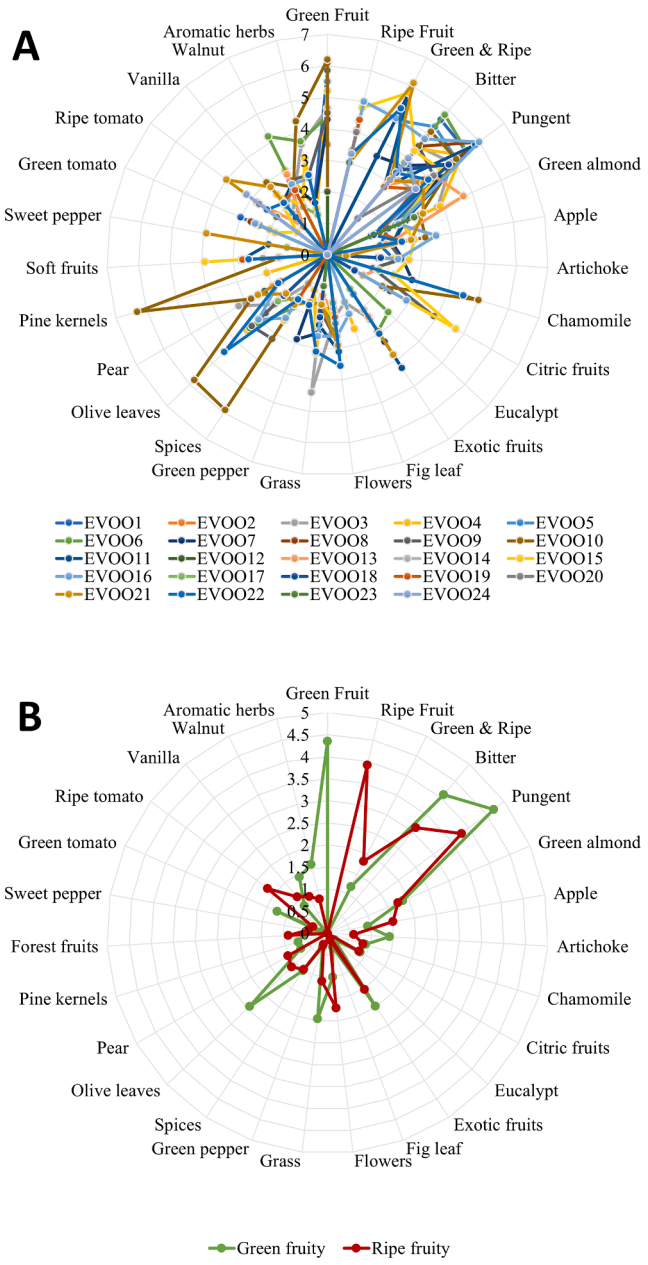

Fig. 1. Spider chart of the sensory attributes evaluated by the sensory panel in the selected extra virgin olive oil samples (A) and the results grouped by "green fruity" and "ripe fruity" classes (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sensory defects, the positive sensory attributes are due to a complex mixture of numerous compounds, mainly C6 and C5 compounds coming from the lipoxygenase pathway (Morales et al., 2013a; Morales et al., 2013b; Morales et al., 2013c; Cecchi et al., 2021), whose different combinations (qualitative and quantitative) lead to different sensory profiles ranging from green to ripe fruity. Therefore, the extraction of the complete volatile fraction and the consideration of different analytical procedures were included in this study to obtain a comprehensive approach to the aroma. Moreover, to deeper study the differences in the volatile profile of each sample and category, a targeted analysis was first performed (i.e., by conventional integration of compounds performed by the instrument software) and then, an untargeted analysis (i.e., deconvolution of compounds by PARADISe software) was also carried out for each dataset to search for unidentified volatile

compounds that may also have a sensory relevance.

When the information obtained from the three methods applying conventional integration were compiled and those compounds with doubtful identification were discarded, a total of 77 volatile compounds were considered as the initial set of identified compounds. In particular, 56 of them were identified by both HS-SPME-GC-FID and HS-SPME-GC–MS, and 52 were identified by TDU-GC–MS. These compounds included the chemical series of alcohols, aldehydes, hydrocarbons, ketones, acids, esters and ethers among others. With the aim of comparing the chromatographic capacity according to the three analytical methods, the sum of the concentrations as well as the number of compounds of each chemical series were studied and represented in Fig. 2. This figure shows that TDU extraction method was able to extract significantly higher ($p < 0.05$) concentrations of aldehydes compared to HS, either with MS or FID. In both extraction techniques, TDU and HS, the number of aldehydes were similar (11 for TDU and 10 for HS). TDU also extracted significantly higher concentrations for the alcohol series although the number of alcohols was lower than by HS (i.e., 9 by TDU and 15 by HS). In contrast, HS extracted a greater number of acids (3) and with higher concentration, which was observed with the two detectors used (MS and FID).

Comparing the results obtained with the two detectors used with HS, it could be seen that FID showed higher relative concentration for acids, alcohols and ketones than MS, although the number of compounds identified was the same in all the chemical series.

Once the differences between the chemical series were studied, the next step was to identify differences between methods regarding individual compounds. **Table S1-Supplementary material** shows the identified compounds by each of the analytical techniques with the mean and standard deviation of the concentrations obtained with conventional integration for EVOOs with “ripe fruity” and “green fruity” characteristics. Among the total volatile compounds identified by the three analytical methods, many of them have 5 or 6 carbon atoms and some of the later are produced by the lipoxygenase (LOX) pathway (Angerosa et al., 2000; Aparicio & Luna, 2002).

As it was observed in the case of chemical series, the differences were explained by the application of different extraction techniques (TDU vs HS) rather than detector (FID vs MS) (**Table S1-Supplementary material**). Thus, TDU-GC–MS allowed the detection and identification of 20 volatile compounds not detected by HS-GC-FID/MS: (*Z*)-3-hexene, (*Z*)-2-penten-1-ol, cyclopentanone, 2-octenal, octanal, 1-hydroxy-2-propanone, (*E,E*)-2,4-heptadienal, benzaldehyde, pentadecane, methyl benzoate, butyrolactone, hexadecane, (*E*)-2-decenal, acetophenone, heptadecane, α-muurolene, 2(5H)-furanone (sotolon), α-farnesene, octadecane, eicosane.

On the contrary, HS-SPME-GC-FID/MS enabled the determination of 25 volatile compounds not extracted by TDU-GC–MS: pentane, 1-pentene, 2-pentene, hexane, 1,3-pentadiene, (*E*)-1,3-pentadiene, heptane, propanal, 3-methyl-butanal, 3-ethyl-octane, 3-pentanol, (*Z*)-3-hexenal, 1R-α-pinene, 1-penten-3-ol, 2-nonenal, 3-methyl-1-butanol, 1-pentanol, 1-nonanol, (*E*)-3-hexen-1-ol acetate, (*E*)-2-penten-1-ol, 4-hexen-1-ol acetate, (*E*)-3-hexen-1-ol, pentanoic acid, 5-ethyldihydro-2(3H)-furanone, and hexanoic acid.

Thirty volatile compounds were identified by the three analytical methods, although 9 of them showed significantly different concentrations between methods. Thus, significantly higher concentrations were obtained by TDU-GC–MS for heptanal, nonanal, (*Z*)-2-penten-1-ol, toluene, (*Z*)-3-hexen-1-ol acetate, α-cubebene compared with the other two methods (HS-SPME-GC-FID/MS) (**Table S1-Supplementary material**). On the contrary, HS-SPME-GC-FID/MS yielded significant differences of concentrations for 3-pentanone, 2-butanone, hexyl acetate.

Although all the samples were within EVOO category and thereby no sensory defect was identified by the panelists, a specific study focused on the presence of volatile markers responsible for off-flavors were carried out (Casadei et al., 2021; Lobo-Prieto et al., 2020). Although all the oils were of EVOO quality, these volatile compounds may be present at such low amounts that did not contribute to the flavor of the oil or the off-flavor is masked by the intense fruity attribute. **Table S2-Supplementary material** shows the ranges of concentrations for these compounds considering the three analytical methods studied and the percentage of samples in which the compounds were identified. From these compounds, octane and ethanol, related to fermentative defects, and hexanal, related to rancidity at high concentrations or to green at lower concentrations (García-González et al., 2011), were present in 100 % of EVOOs by the three methods, but with higher concentrations for TDU than for HS. Acetic acid, attributed to winey-vinegary notes, was also identified in all the samples except in one sample analyzed with TDU-GC–MS, but in this case higher concentrations were found for HS. These results pointed out that the detection and identification of these volatile compounds related to sensory defects depended on the extraction technique used, TDU and HS-SPME. The effect of the extraction technique is even more evident in other compounds. Thus, 3-methyl-1-butanol was identified only by HS-SPME in almost all the samples, while (*E*)-2-decenal was identified only by TDU in the 100 % of EVOOs (**Table S2-Supplementary material**). Moreover, in those volatile compounds that were identified by the three methods, some of them

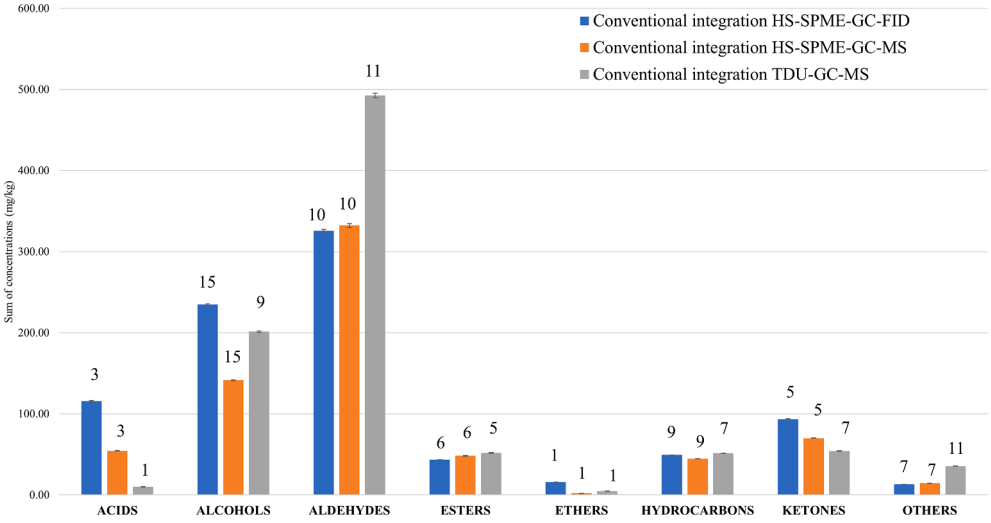

Fig. 2. Bar diagram of sum of concentrations (mg/kg) for each chemical series by each analytical method (HS-SPME-GC-FID, HS-SPME-GC–MS and TDU-GC–MS). The error bars and the number of compounds identified are indicated for each method and chemical series.

were more easily identified (i.e., higher chromatographic areas and/or found in more EVOOs) by one of the techniques, such as 6-methyl-5-hepten-2-one and nonanal by TDU, or ethyl acetate by HS-SPME. The difference in the working principle of the extraction procedures could explain the differences in the concentration since the detector (mass spectrometer) was the same in the methods HS-GC–MS and TDU-GC–MS in this study. Therefore, the absorption of volatile compounds in the SPME polymer and the associated competition phenomena (Oliver-Pozo et al., 2015) in contrast to the cryo-focusing trapping of TDU could lead to different extraction capacities. **Table S2-Supplementary material** also shows that the compounds associated to sensory defects propanoic acid, (*E*)-2-heptenal, 1-octen-3-ol, ethyl propanoate, and (*E,E*)-2,4-hexadienal were never identified in the EVOOs.

3.2. Differences between extraction methods and integration modes

In addition to the conventional integration carried out with the instrument software, the potential of HS-SPME and TDU extraction methods combined with GC–MS for the volatile characterization of the EVOOs was deeply studied with the use of the relatively recent PARADISe software for peak deconvolution purposes (Johnsen et al., 2017). This software tool provides the advantage to allow the extraction of the pure spectra of co-eluting compounds, at the same time that it computes their peak areas in a robust manner, correcting the base line and noise, and generates a possible identification using NIST database, which makes the statistical analyses easier and faster. These advantages mean an opportunity to identify minor compounds in complex volatile profiles such as those of EVOOs with high intensity of fruity attributes.

An illustrative example of the ability of this tool to improve identification could be seen in **Figure S2-Supplementary material.** This figure shows the morphological plot of a peak that was conventionally identified and integrated as 3-pentanone (**Figure S2-A Supplementary material**). However, when PARADISe was applied, the resolved elution profiles (**Figure S2-B Supplementary material**) showed the presence of three overlapped compounds, corresponding to 2-pentanone (clear blue peak), pentanal (blue peak) and 3-pentanone (green peak).

In this study, the total number of identified compounds and the total sum of concentrations was compared between methods (HS-SPME-GC–MS and TDU-GC–MS) and between integration modes (conventional vs PARADISe). Fig. 3 shows that PARADISe enables to obtain a significantly higher sum of concentrations and a higher total number of compounds for both methodologies compared with conventional integration. Thus, in both methods, the total concentration and the number of volatile compounds identified was multiplied by a factor of ≈1.20 (Fig. 3). The results obtained when comparing the sum of the concentrations between chemical series and analytical methods were consistent with those described above for the conventional integration (Fig. 2), except for ketones, which showed significantly larger concentrations with TDU-GC–MS than with HS-SPME-GC–MS, just the opposite to the results found with conventional integration (**Figure S1-Supplementary material**). This observation could be related with an effect of the different absorption capacity of the SPME fiber for different chemical series (Oliver-Pozo et al., 2015), while the TDU extraction method does not use any adsorbent for trapping volatile compounds.

Furthermore, regarding individual compounds, Table 2 shows the identified compounds by each of the analytical methods with the mean and standard deviation of the concentrations in this case obtained by PARADISe, for the "green fruity" and "ripe fruity" EVOOs. For comparative purposes, the conventional integration results of HS-SPME-GC-FID were also included in this table, even though FID data could not be processed with PARADISe. This table shows 14 new volatile compounds which were identified through PARADISe (i.e., those marked with a ^{p} in Table 2), which were unidentified with the conventional procedure. From these 14 compounds, 2-pentanone, pentanal and 3-methyl-4-penten-1-ol were extracted by both methods (HS-GC–MS and TDU-GC–MS), six were only detected by TDU-GC–MS (2-hexanone, ethylbenzene, 2-butenal, 3-methyl-, 4-penten-1-ol, (*Z*)-9-hexadecenal and 8-heptadecene) and five were only detected by HS-SPME-GC–MS (1-pentanol, (*Z*)-3-penten-1-ol, (*E*)-2-hexen-1-ol, 2-ethyl-1,3-butadiene and 4-hexen-1-ol).

Considering data from PARADISe, the total number of identified compounds was 91. From these compounds, like in conventional processing (**Table S1-Supplementary material**), also 30 volatile compounds were extracted by the three analytical methods (Table 2), and among them, 10 showed significant differences between the three methods. However, the volatile compounds with significant differences were not the same as in conventional processing. For example, with PARADISe, the concentrations of hexane, acetone, 2-methyl-butanal, (*E*)-3-hexen-1-ol acetate were significantly larger for TDU-GC–MS compared with HS-SPME-GC–MS, while the concentrations of these compounds did not show significant differences with conventional processing. These results could be explained by the fact that the processing of the signals by PARADISe could increase the ability to identify a higher number of compounds but also having a quantitative influence in the integrated chromatographic areas compared with the

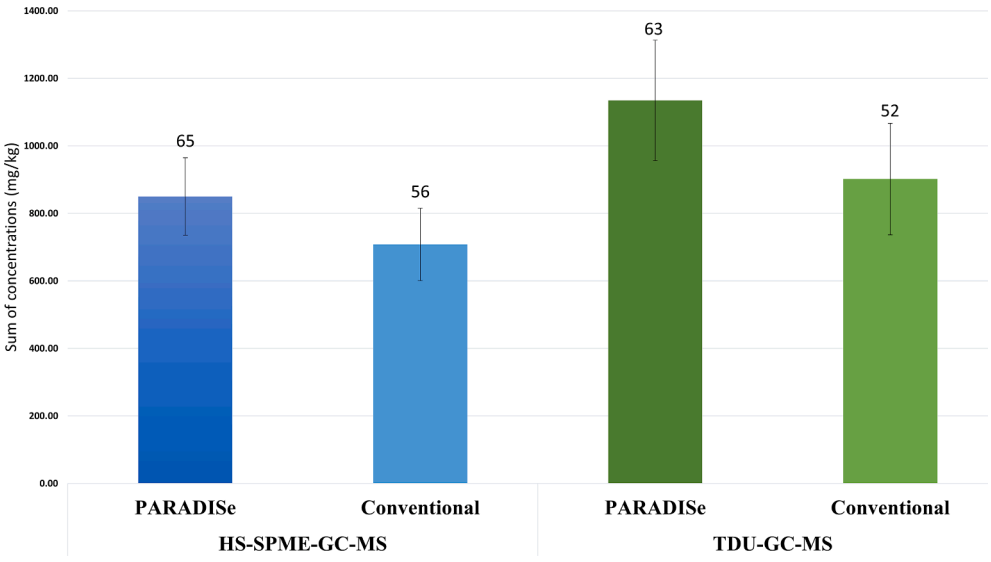

Fig. 3. Bar diagram of the comparative of the concentrations between PARADISe and conventional integration results. The error bars and the total number of compounds identified are indicated.

Table 2
Volatile compounds obtained by PARADISe processing of the data from HS-SPME-GC–MS and TDU-GC–MS, and by conventional processing of HS-SPME-GC-FID data. Mean and standard deviation of the concentrations (mg/kg) obtained for "green fruity" and "ripe fruity" EVOOs, and results of Tukey test.

LRI HS-SPME-GC-FID	LRI HS-SPME-GC–MS	LRI TDU-GC–MS	ID	Compounds	Chem serie	Odour description	HS-GC-FID						HS-SPME-GC–MS						TDU-GC–MS					
							Green fruity (N = 24)			Ripe fruity (N = 24)			Green fruity (N = 24)			Ripe fruity (N = 24)			Green fruity (N = 24)			Ripe fruity (N = 24)		
							Cm	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T
500	500	500	ST	Pentane	HC	–	0.28	0.10		0.34	0.13		0.25	0.13	a	0.36	0.20	b	0.33	0.31		0.33	0.38	
503	536	–	DB	1-Pentene	HC	–	0.13	0.08		0.17	0.12		1.26	0.56		1.46	0.74		nd	nd		nd	nd	
–	–	660	DB	(*Z*)-3-Hexene	HC	–	nd	nd		nd	nd		nd	nd		nd	nd		0.48	0.27		0.61	0.32	
506	580	–	DB	2-Pentene	HC	–	0.23	0.11	b	0.13	0.10	a	0.26	0.11	b	0.21	0.12	a	nd	nd		nd	nd	
600	600	600	ST	Hexane	HC	–	0.12	0.23		0.13	0.12		0.07	0.06		0.09	0.05		0.30	0.29		0.28	0.32	
714	667	–	MS	1,3-Pentadiene	HC	–	0.01	0.01		0.01	0.01		0.08	0.05		0.07	0.06		nd	nd		nd	nd	
757	706	–	MS	(*E*)-1,3-Pentadiene	HC	–	0.08	0.04		0.08	0.06		0.07	0.04		0.06	0.05		nd	nd		nd	nd	
700	700	–	ST	Heptane	HC	–	0.02	0.01		0.02	0.02		0.02	0.01		0.02	0.01		nd	nd		nd	nd	
763	789	–	DB	Propanal	ALD	ethereal, musty	0.01	0.01	a	0.02	0.02	b	0.02	0.02		0.02	0.02		nd	nd		nd	nd	
836	842	844	DB	Acetone	KET	pungent	0.07	0.05	a	0.11	0.09	b	0.07	0.04		0.07	0.03		0.40	0.15		0.46	0.18	
869	879	876	ST	Methyl acetate	EST	ethereal (solvent-like, fruity)	0.05	0.03		0.05	0.03		0.11	0.07	a	0.28	0.35	b	0.08	0.03	a	0.20	0.22	b
800	800	800	ST	Octane	HC	solvent	0.06	0.04	a	0.15	0.17	b	0.08	0.06	a	0.14	0.11	b	0.26	0.11	a	0.31	0.18	b
841	879	849	DB	Ethyl Acetate	EST	ethereal (fruity, sweet, aromatic, green)	0.17	0.14	a	0.37	0.48	b	0.24	0.20	a	0.57	0.72	b	0.09	0.09	a	0.29	0.40	b
853	901	866	DB	2-Butanone	KET	ethereal (fruity, camphoreus nuance)	0.40	0.33		0.32	0.15		0.04	0.06		0.03	0.04		0.09	0.07		0.08	0.04	
865	932	880	DB	2-Methylbutanal	ALD	musty, fusel, chocolate	0.03	0.01	a	0.05	0.03	b	0.04	0.02	a	0.07	0.03	b	0.11	0.13		0.11	0.06	
880	941	–	DB	3-Methylbutanal	ALD	–	0.02	0.01		0.02	0.01		0.02	0.01		0.02	0.01		nd	nd		nd	nd	
899	908	920	DB	Ethanol	ALC	alcoholic (ethereal, medical)	1.28	1.29		1.65	1.88		0.55	0.58		0.70	0.76		0.95	1.24		1.62	2.18	
913	933	951	DB	2-Ethylfuran	OTH	chemical (burnt, earthy, malty)	0.10	0.07	b	0.04	0.04	a	0.06	0.03	b	0.04	0.04	a	0.06	0.04		0.05	0.03	
927	934	960	DB	1-Methoxyhexane	OTH	herbal, ethereal (sweet, herbal, fruity)	0.02	0.01		0.03	0.06		0.13	0.12	b	0.07	0.09	a	0.13	0.13	b	0.08	0.10	a
–	957	991	DB	2-Pentanone	KET	fruity (sweet, ethereal, wine, banana, woody)	nd	nd		nd	nd		0.05	0.03		0.12	0.21		0.06	0.03		0.10	0.14	
956	959	992	DB	3-Pentanone	KET	ethereal (sweet, fruity)	1.51	0.39	b	1.22	0.55	a	1.03	0.33		0.84	0.48		0.25	0.11		0.39	0.30	
–	960	993	DB	Pentanal	ALD	fermented (almond, malt, pungent, woody, bitter, oily)	nd	nd		nd	nd		0.24	0.13		0.22	0.09		0.22	0.12		0.24	0.09	
910	1025	–	MS	3-Ethyloctane	HC	–	0.05	0.01	b	0.04	0.01	a	0.04	0.01		0.03	0.02		nd	nd		nd	nd	
–	–	1053	DB	2-Hexanone	KET	fruity	nd	nd		nd	nd		nd	nd		nd	nd		0.22	0.11		0.49	0.68	
934	1000	995	DB	(*Z*)-3-Hexen-1-ol, methyl ether	ETH	green (fruity, pear, green apple-like)	0.44	0.23	b	0.22	0.18	a	0.16	0.15	b	0.04	0.07	a	0.38	0.31	b	0.18	0.12	a
964	1038	976	DB	1-Penten-3-one	KET	spicy (pungent, mustard)	0.13	0.06		0.12	0.07		0.38	0.22	b	0.16	0.14	a	0.72	0.22	b	0.42	0.26	a
1000	1086	1015	DB	Toluene	HC	–	0.06	0.07		0.06	0.06		0.10	0.11		0.12	0.13		0.20	0.15		0.22	0.12	
1045	1079	1030	DB	Hexanal	ALD	green (fresh, apple, grass, leafy, fruity)	1.58	0.82	a	2.20	1.11	b	1.82	1.09	a	2.56	1.33	b	1.61	0.86	a	2.35	0.94	b
1070	1126	–	DB	3-Pentanol	ALC	herbal (sweet, oily, nutty)	0.20	0.06	b	0.15	0.06	a	0.13	0.04	b	0.09	0.04	a	nd	nd		nd	nd	
–	–	1078	DB	Ethylbenzene	HC	–	nd	nd		nd	nd		nd	nd		nd	nd		0.05	0.03		0.05	0.02	
1102	1119	1098	DB	(*E*)-3-Hexenal	ALD	green (fruity apple)	1.04	1.43	b	0.44	0.76	a	0.73	1.21	b	0.21	0.38	a	2.01	1.68	b	1.04	1.33	a
1180	1129	1106	MS	2,4-Hexadien-1-ol	ALC	green (musty, sweet, herbal, almond, nutty)	1.04	1.43		0.44	0.76		0.26	0.29		0.19	0.33		0.54	0.31		0.47	0.24	

(*continued on next page*)

Table 2 (*continued*)

LRI HS-SPME-GC-FID	LRI HS-SPME-GC–MS	LRI TDU-GC–MS	ID	Compounds	Chem serie	Odour description	HS-GC-FID						HS-SPME-GC–MS						TDU-GC–MS					
							Green fruity (N = 24)			Ripe fruity (N = 24)			Green fruity (N = 24)			Ripe fruity (N = 24)			Green fruity (N = 24)			Ripe fruity (N = 24)		
							Cm	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T
1160	1130	–	DB	(*Z*)-3-Hexenal	ALD	green (fruity, apple)	0.07	0.05	b	0.04	0.03	a	0.08	0.10	b	0.03	0.04	a	nd	nd		nd	nd	
1199	1135	–		1α-Pinene	OTH	herbal (minty)	0.03	0.03		0.02	0.02		0.01	0.02		0.02	0.03		nd	nd		nd	nd	
1123	1153	–	DB	1-Penten-3-ol	ALC	green (vegetable, horseradish-like)	0.26	0.13		0.21	0.10		0.13	0.08		0.09	0.06		nd	nd		nd	nd	
–	–	1154	DB	(*Z*)-2-Penten-1-ol	ALC	green (phenolic, ethereal, medicinal, cherry)	nd	nd		nd	nd		nd	nd		nd	nd		0.60	0.21		0.58	0.22	
–	–	1185	DB	Cyclopentanone	KET	–	nd	nd		nd	nd		nd	nd		nd	nd		0.11	0.11		0.08	0.02	
–	–	1216	DB	3-Methyl-2-butenal	ALD	green (fresh, fruity, pulpy, almond)	nd	nd		nd	nd		nd	nd		nd	nd		0.05	0.02	b	0.04	0.02	a
–	–	1222	DB	2-Octenal	ALD	fatty (fresh, cucumber, green, herbal, banana, waxy, green leaf)	nd	nd		nd	nd		nd	nd		nd	nd		0.32	0.30		0.18	0.22	
1168	1197	1189	DB	Heptanal	ALD	green (fresh, herbal)	0.02	0.00	a	0.02	0.01	b	0.02	0.01		0.02	0.01		0.03	0.02	a	0.08	0.11	b
21.23	21.87	–	DB	2-Nonenal	ALD	fatty (green, cucumber, aldehydic, citrus)	0.14	0.14		0.13	0.09		0.13	0.15		0.12	0.10		nd	nd		nd	nd	
–	1248	1180	DB	2-Methyl-1-butanol	ALC	roasted (winey, onion, fruity, fusel)	nd	nd		nd	nd		0.01	0.01	a	0.03	0.01	b	0.01	0.01	a	0.05	0.06	b
1165	1248	–	DB	3-Methyl-1-butanol	ALC	fermented (fusel, oil, alcoholic, whiskey, fruity, banana)	0.01	0.01	a	0.03	0.04	b	0.01	0.01	a	0.04	0.03	b	nd	nd		nd	nd	
–	1250	–	DB	1-Pentanol	ALC	fermented, fusel	nd	nd		nd	nd		0.02	0.01	a	0.04	0.03	b	nd	nd		nd	nd	
–	1257	1160	MS	3-Methyl-4-penten-1-ol	ALC	–	nd	nd		nd	nd		0.36	0.27	a	0.75	0.66	b	1.94	1.27	a	3.06	1.48	b
1187	1258	1190	DB	(*E*)-2-Hexenal	ALD	green (bitter almonds, leafy, green-fruity)	2.89	3.61	a	4.79	5.35	b	3.07	4.19	a	5.22	6.25	b	3.22	3.92	a	5.73	5.45	b
1266	1300	–	DB	1-Pentanol	ALC	fermented, fusel	0.10	0.03		0.10	0.04		0.06	0.02		0.06	0.03		nd	nd		nd	nd	
1318	1283	1278	DB	Hexyl acetate	EST	fruity (green, banana, apple)	0.20	0.13		0.17	0.19		0.21	0.15		0.21	0.22		0.06	0.04		0.07	0.08	
1349	1298	–	MS	1-Nonanol	ALC	floral (fresh, clean, fatty, rose)	0.02	0.01		0.02	0.01		0.02	0.02		0.02	0.03		nd	nd		nd	nd	
–	–	1306	DB	Octanal	ALD	aldehydic (citrus, orange, green, fatty)	nd	nd		nd	nd		nd	nd		nd	nd		0.26	0.10		0.27	0.08	
–	–	1272	DB	1-Hydroxy-2-propanone	OTH	caramellic (pungent, sweet, ethereal)	nd	nd		nd	nd		nd	nd		nd	nd		0.06	0.07		0.04	0.04	
–	1300	–	DB	(*Z*)-3-Penten-1-ol	ALC	–	nd	nd		nd	nd		0.02	0.01		0.02	0.01		0.73	0.65		0.67	1.00	
1331	1340	–	DB	(*Z*)-3-Hexen-1-ol, acetate	EST	green (fresh, sweet, fruity, banana apple)	0.25	0.14		0.25	0.27		0.05	0.03		0.05	0.06		nd	nd		nd	nd	
1337	1350	–	DB	(*Z*)-2-Penten-1-ol	ALC	green (medicinal, phenolic)	0.07	0.15		0.07	0.15		0.05	0.02		0.04	0.02		nd	nd		nd	nd	
1346	1367	1267	MS	(*E*)-3-Hexen-1-ol, acetate	EST	green (leaves, fruity, banana)	0.15	0.08		0.13	0.08		0.28	0.19		0.32	0.41		0.73	0.65		0.67	1.00	
1302	–	1276	DB	4-Penten-1-ol	ALC	–	0.02	0.01		0.02	0.02		nd	nd		nd	nd		0.22	0.14		0.21	0.26	
–	1370	1292	DB	(*E*)-2-Penten-1-ol	ALC	green (ethereal, medicinal, aldehydic, cherry)	nd	nd		nd	nd		0.12	0.08		0.10	0.07		0.23	0.08		0.22	0.10	
1340	1302	1306	DB	6-Methyl-5-hepten-2-one	KET		0.01	0.00	a	0.01	0.00	b	0.01	0.02		0.04	0.05		0.02	0.03	a	0.07	0.13	b

(*continued on next page*)

Table 2 (*continued*)

LRI HS-SPME-GC-FID	LRI HS-SPME-GC–MS	LRI TDU-GC–MS	ID	Compounds	Chem serie	Odour description	HS-GC-FID						HS-SPME-GC–MS						TDU-GC–MS					
							Green fruity (N = 24)			Ripe fruity (N = 24)			Green fruity (N = 24)			Ripe fruity (N = 24)			Green fruity (N = 24)			Ripe fruity (N = 24)		
							Cm	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T
						green (fruity, vegetative, grass, green bean-like)																		
1360	1317	–	MS	4-Hexen-1-ol, acetate	EST	–	0.01	0.00	a	0.01	0.00	b	0.02	0.01		0.02	0.02		nd	nd		nd	nd	
1386	1343	1346	DB	1-Hexanol	ALC	herbal, green (fruity tropical, apple, soft, oily)	0.73	0.29	a	1.25	0.86	b	0.55	0.19	a	1.05	0.67	b	0.19	0.09	a	0.49	0.40	b
1410	1361	–	DB	(*E*)-3-Hexen-1-ol	ALC	green, leafy	0.11	0.07		0.17	0.17		0.09	0.06		0.16	0.17		1.13	0.74		1.78	2.62	
1434	1402	1403	DB	(*Z*)-3-Hexen-1-ol	ALC	green (cut grass, foliage, vegetable, herbal, oily)	0.52	0.42		0.78	1.06		0.45	0.40		0.78	1.19		1.13	0.74		1.78	2.62	
–	1363	–	DB	(*E*)-2-Hexen-1-ol	ALC	fruity (fresh, leafy, banana)	nd	nd		nd	nd		0.05	0.05	a	0.21	0.29	b	nd	nd		nd	nd	
–	1374	–	MS	2-Ethyl-1,3-butadiene	HC	–	nd	nd		nd	nd		0.04	0.03		0.08	0.09		nd	nd		nd	nd	
1444	1424	1342	DB	Nonanal	ALD	aldehydic (waxy, citrus)	0.03	0.02		0.03	0.02		0.01	0.01		0.02	0.02		0.70	0.26		0.88	0.41	
1418	–	1358	DB	(*Z*)-2-Hexen-1-ol	ALC	fruity (fresh, leafy, banana)	0.10	0.07	a	0.35	0.43	b	nd	nd		nd	nd		0.05	0.05	a	0.32	0.44	b
1461	1457	1350	MS	(*E*)-4-Hexen-1-ol	ALC	green (vegetable, oily)	0.03	0.02		0.04	0.05		0.06	0.02	a	0.10	0.06	b	0.11	0.08	a	0.41	0.36	b
–	1486	–	MS	(*Z*)-4-Hexen-1-ol	ALC	green (vegetable, oily)	0.03	0.02		0.04	0.05		0.02	0.02		0.04	0.05		nd	nd		nd	nd	
1496	1414	1425	DB	Acetic acid	*ACI*	acidic (sour)	0.67	0.44		0.99	0.88		0.72	0.57		1.30	1.36		0.11	0.10		0.15	0.20	
–	–	1463	DB	(*E,E*)-2,4-Heptadienal	ALD	fatty (green, oily, aldehydic with a vegetative nuance)	nd	nd		nd	nd		nd	nd		nd	nd		0.06	0.04		0.05	0.04	
1498	1474	1480	DB	α-Cubebene	OTH	woody/spicy (fruity, mango)	0.09	0.06	b	0.05	0.03	a	0.10	0.11		0.07	0.07		0.20	0.22		0.17	0.19	
–	1565	1564	DB	Benzaldehyde	ALD/FRU	fruity (oily, almond, cherry)	nd	nd		nd	nd		0.01	0.00		0.01	0.00		0.04	0.02	a	0.06	0.05	b
1625	1655	–	MS	Pentanoic acid	*ACI*	acidic and sharp, cheese-like	0.01	0.00		0.01	0.00		0.05	0.04	b	0.02	0.02	a	nd	nd		nd	nd	
–	–	1500	ST	Pentadecane	HC	–	nd	nd		nd	nd		nd	nd		nd	nd		0.04	0.03		0.04	0.02	
–	–	1663	DB	Methyl benzoate	EST	phenolic (cherry pit)	nd	nd		nd	nd		nd	nd		nd	nd		0.01	0.01		0.02	0.03	
–	–	1678	DB	Butyrolactone	OTH	creamy (fruity peach-like)	nd	nd		nd	nd		nd	nd		nd	nd		0.03	0.03	b	0.02	0.01	a
–	–	1600	ST	Hexadecane	HC	–	nd	nd		nd	nd		nd	nd		nd	nd		0.04	0.03		0.04	0.02	
–	–	1622	DB	(*E*)-2-Decenal	ALD	waxy	nd	nd		nd	nd		nd	nd		nd	nd		0.07	0.08		0.05	0.02	
–	–	1633	DB	Acetophenone	KET	floral (sweet, cherry pit, marzipan and coumarinic)	nd	nd		nd	nd		nd	nd		nd	nd		0.02	0.02		0.02	0.02	
–	–	1700	ST	Heptadecane	HC	–	nd	nd		nd	nd		nd	nd		nd	nd		0.04	0.04		0.03	0.03	
–	–	1722	MS	α-Muurolene	OTH	–	nd	nd		nd	nd		nd	nd		nd	nd		0.03	0.02		0.02	0.02	
–	–	1744	DB	2(5H)-furanone	OTH	buttery	nd	nd		nd	nd		nd	nd		nd	nd		0.05	0.05		0.03	0.03	
–	–	1749	MS	(*Z*)-9-Hexadecenal	ALD	–	nd	nd		nd	nd		nd	nd		nd	nd		0.06	0.09		0.04	0.03	
–	–	1751	DB	α-Farnesene	OTH	woody, green (citrus, herbal, lavender, bergamot)	nd	nd		nd	nd		nd	nd		nd	nd		0.04	0.03		0.21	0.43	
1798	1726	–	MS		OTH		0.03	0.02	b	0.02	0.01	a	0.04	0.02	b	0.03	0.02	a	nd	nd		nd	nd	

(*continued on next page*)

Table 2 (*continued*)

LRI HS-SPME-GC-FID	LRI HS-SPME-GC–MS	LRI TDU-GC–MS	ID	Compounds	Chem serie	Odour description	HS-GC-FID				HS-SPME-GC–MS						TDU-GC–MS					
							Green fruity (N = 24)			Ripe fruity (N = 24)			Green fruity (N = 24)			Ripe fruity (N = 24)			Green fruity (N = 24)			Ripe fruity (N = 24)		
							Cm	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T	*Cm*	SD	T
				5-Ethyldihydro-2 (3H)-furanone		herbal (coumarin, tobacco)																	
1831	1739	1756	DB	5-Ethyl-2(5H)-furanone	OTH	–	0.01	0.00		0.01	0.00		0.02	0.02	b	0.01	0.01	a	0.02	0.02	b	0.01	0.01	a
–	–	1780	MS	8-Heptadecene	HC	–	nd	nd		nd	nd		nd	nd		nd	nd		0.04	0.04		0.03	0.03	
1897	1814	–	DB	Hexanoic acid	*ACI*	fatty (cheesy)	0.08	0.06		0.06	0.04		0.03	0.04	a	0.09	0.12	b	nd	nd		nd	nd	
–	–	1800	ST	Octadecane	HC	–	nd	nd		nd	nd		nd	nd		nd	nd		0.04	0.03		0.03	0.02	
1990	1952	1977	DB	Phenylethyl Alcohol	ALC	floral (fresh, bready, rosey, honey)	0.01	0.00		0.01	0.00		0.01	0.00	a	0.01	0.01	b	0.01	0.00	a	0.02	0.02	b
–	–	2000	DB	Eicosane	OTH	–	nd	nd		nd	nd		nd	nd		nd	nd		0.03	0.02		0.03	0.02	

Notes: LRI: Linear Retention Index; ID (identification): reliability of identification: ST, mass spectrum and LRI agreed with standards (own or literature); DB, mass spectrum and LRI agreed with mass spectral data base and/or literature. MS, mass spectrum agreed with mass spectral data base. Chem. Serie: Chemical serie; *Cm*: Mean of concentrations (mg/kg); SD: Standard deviation; T: Tukey test results. Different letters in different columns indicate significant differences according to Tukey's test ($P < 0.05$). From letter 'a' to letter 'b' indicates increasing concentrations; nd: peak not detected; HC: hydrocarbon; ALD: aldehyde; KET: ketone; EST: ester; ETH: ether; *ACI*: acid; ALC: alcohol; OTH: other.

conventional processing.

According to the advantages presented by PARADISe in identifying more volatile compounds than with conventional identification, the study of the differences between the two classes of EVOOs "ripe fruity" and "green fruity" was addressed with PARADISe results.

3.3. Characterization of the volatile compounds associated to "ripe fruity" and "green fruity" EVOOs

The data presented in Table 2 were studied with ANOVA to highlight the significant differences in concentrations between EVOOs of the two studied classes, "green fruity" and "ripe fruity". A total of 39 volatile compounds showed significant differences between the two classes, 16 with significantly higher concentrations for "green fruity" samples, and 23 with significantly higher concentrations for "ripe fruity" samples (Table 2). **Table S3-Supplementary material** summarizes the ANOVA results, and it shows these volatile compounds and the methodologies that allowed their identification. The group of volatile compounds with significantly higher concentrations in the "ripe fruity" samples included more aldehydes (6), alcohols (8) and esters (3) compared with "green fruity" samples (3, 1, and none, respectively). Moreover, the volatile compounds with significantly higher concentrations in "green fruity" samples were sensory characterized with herbal (e.g. 3-pentanol, 1-methoxyhexane), and fruity aroma, such as pear (e.g. (*Z*)-3-hexen-1-ol, methyl ether), green apple (e.g. (*E*)-3-hexenal and (*Z*)-3-hexenal), or mango (e.g. α-cubebene); while the sensory descriptors for the compounds with higher concentrations in "ripe fruity" samples were ethereal notes (e.g. propanal, methyl and ethyl acetate), as well as green notes but in this case with vegetable (e.g. (*E*)-4-hexen-1-ol) and floral (e.g. phenethyl alcohol) descriptors, or fruity notes mainly related to banana (e.g. (*Z*)-2-hexen-1-ol, (*E*)-2-hexen-1-ol or 3-methyl-1-butanol) (Table 2).

These results agreed with those previously obtained in the literature. Thus, another study (Procida et al., 2016) also showed that there were a specific number of volatile compounds which were correlated with positive odor properties and could be classified into two main groups of sensory notes, "green substances" and "sweet substances". In this study, "green substances" (e.g. (Z)-3-hexenal) were associated to bitter and pungent attributes, as could occur with EVOOs categorized as "green fruity". Moreover, Procida et al. (2016) also reported a relationship between the content of acetone, ethyl acetate and 3-methyl-1-butanol with the sweet sensorial sensation, that could be considered as the "ripe fruity" sensory category.

On the one hand, among the significantly different volatile compounds between the two classes summarized in **Table S3-Supplementary material**, 12 were only determined by HS-SPME-GC-FID/MS. Among them, 6 volatile compounds showed significantly higher concentrations for "ripe fruity" EVOOs: propanal, 3-methyl-1-butanol, 4-hexen-1-ol acetate and hexanoic acid (detected by FID and MS) and 1-pentanol and (*E*)-2-hexen-1-ol, detected only by MS. Regarding those related to "ripe fruity" samples, propanal is one volatile compound identified in oxidized olive oil samples by different authors (Morales et al., 2013a; Morales et al., 2013b; Morales et al., 2013c), although it is a compound that has been identified in olive fruits (Rosati et al., 2014). Moreover, it has also been recently considered as one of the important aroma compounds in extra virgin olive oils (Neugebauer et al., 2020) with fresh, malty and fruity nuances. On the other hand, hexanoic acid is related to fatty notes and with fat oxidation (Dierkes et al., 2012; García-González et al., 2011; Oliver-Pozo et al., 2015), although it can be identified in EVOOs at low concentration (Dorota et al., 2021; García-González et al., 2011).

From the 12 compounds only identified by HS-SPME-GC–MS/FID, 6 compounds showed significantly higher concentration in "green fruity" EVOOs: 2-pentene, 3-ethyl-octane, 3-pentanol, (*Z*)-3-hexenal, pentanoic acid and 5-ethyldihydro-2(3H)-furanone. (*Z*)-3-hexenal has been described with cut grass odor note (Aparicio & Morales, 1998), which is a sensory attribute remarked by the panel test for these samples (Fig. 1).

Furthermore, the contribution of this compound to green notes of EVOOs has been also reported by Cecchi et al. (2021).

On the other hand, two volatile compounds were only determined by TDU-GC–MS, which showed significantly larger concentrations in "green fruity" samples: butyrolactone and 3-methyl-2-butenal. Butyrolactone has been described in the literature as aroma-active compounds contributing to the fruity odor of virgin olive oils (Cecchi et al., 2021) with a peach-like odor, while 3-methyl-2-butenal has not been yet reported on olive oils but it is related to green notes (fresh, fruity, green, pulpy, almond).

The remaining 25 compounds out of 40 with significant difference in concentrations between the two classes were extracted by the three analytical methods (**Table S3-Supplementary material**), 8 with significantly higher concentrations in "green fruity" samples and 17 in "ripe fruity" samples. Regarding those related to "green fruity" EVOOs, (*Z*)-3-hexen-1-ol methyl ether and (*E*)-3-hexenal could be highlighted as showing significant differences by the three analytical methods. The first one has been described with the sensory attributes of fruity, mainly pear (The Good Scents Company, 2022), and (*E*)-3-hexenal has been described as one of the characteristic volatile compounds associated to artichoke, green and flowers notes in virgin olive oil (Aparicio & Morales, 1998). The higher presence of these two compounds in "green fruity" samples was consistent with the sensory evaluation, which showed higher median scores for pear and artichoke attributes (Fig. 1). Regarding those related to "ripe fruity" samples, (*E*)-2-hexenal, octane, ethyl acetate, hexanal, and 1-hexanol could be highlighted for having significant differences in concentrations by the three analytical methods. (*E*)-2-hexenal has been identified as the most abundant component of the C6 aldehydes formed from the LOX pathway in high-quality virgin olive oils and related with "green" "fruity" and "floral" sensory notes (Aparicio & Morales, 1998; Casadei et al., 2021; Dabbou et al., 2011; Ríos-Reina et al., 2021). The "floral" odor descriptor was also highlighted by the panelists when they evaluated the "ripe fruity" samples (Fig. 1).

As it was mentioned before, the presence of octane and ethyl acetate has been related to fusty/muddy and winey-vinegary defects, respectively (Aparicio et al., 2012; Casadei et al., 2021; Cecchi et al., 2021; Morales et al., 2005; Oliver-Pozo et al., 2015). However, they are produced in the advanced stage of spoilage and have relatively high odor threshold, which indicates that they could not have sensory impact in the samples under study (Aparicio et al., 2012; Morales et al., 2005; Oliver-Pozo et al., 2015). Finally, hexanal and 1-hexanol are two compounds coming from LOX pathway contributing with green aroma and they are characteristic compounds in EVOOs (García-González et al., 2011; Lobo-Prieto et al., 2020). Hexanal is also originated by fatty acid oxidation (Morales et al., 2013a; Morales et al., 2013b; Morales et al., 2013c). However, given that they were EVOOs, it is expected that most of the hexanal content were originated from the LOX pathway.

In order to easily explore and visualize the capacity of the different methodologies and volatile profiles obtained in the differentiation between samples analyzed, principal component analyses (PCA) were carried out with the data of the three analytical methods. In a first step, all the dataset was submitted to PCA (data not shown), and later, only those compounds that showed significant differences between the classes "green fruity" and "ripe fruity" for each of the analytical methods (i.e., 24 volatile compounds for HS-SPME-GC-FID, 29 for HS-SPME-GC–MS, and 21 for TDU-GC–MS from the 40 volatile compounds summarized in **Table S3-Supplementary material**), were included in the PCAs, in order to move towards a more targeted approach. From the three PCA models developed with HS-SPME-GC-FID data set (conventionally processed), HS-SPME-GC–MS and TDU-GC–MS data sets (processed by PARADISe), 3 significant principal components (PCs) were chosen on the basis of Kaiser's criterion (eigenvalues higher than 1.0 are chosen) accounting for 70.80 %, 70.18 % and 60.53 % of total variability, respectively. Fig. 4 shows the scores **(A)** and loadings **(B)** plots for the first two PCs of the three PCA models.

Firstly, regarding the scores plots in Fig. 4**-A**, which included the samples in duplicate, the fact that each pair of duplicate samples are clustered together indicated that the sampling method, the GC–MS/FID analysis and the integration procedures were robust enough. Secondly, considering the two different sensory classes ("green fruity" and "ripe fruity"), PC1, which accounted 30.07 %, 27.95 % and 27.94 % for HS-SPME-GC-FID, HS-SPME-GC–MS and TDU-GC–MS, respectively, seemed to be the responsible for the grouping (Fig. 4**-A**). Although there was some degree of overlapping between groups, this overlapping is not observed when the other PCs were considered. Furthermore, the PCA scores plots of the three methods showed that the "ripe fruity" samples were less homogeneous than the group of "green fruity" samples. These results can be explained by the fact that that oils with "ripe fruity" sensory profile may come from a cultivar characteristic aroma and also from a more advanced ripening stage of olives. The loadings plots (Fig. 4**-B**) confirmed that the compounds listed in **Table S3-Supplementary material** could be the markers that could be used to differentiated EVOOs in each of the classes, "green fruity" and "ripe fruity".

Finally, PLS-DA models were developed with the aforementioned dataset in order to go deeper in the exploration of data. Classification rates are shown in **Table S4-Supplementary material**. In fact, these models were not developed for a classification approach because of the relatively low number of samples, but to study the variables with importance in prediction (VIP). VIP is scaled in such a way that all of the predictors having a VIP > 1 are considered to be relevant (Mehmood et al., 2012). Thus, VIP values could be useful for identifying the volatile compounds, selected from those with significant differences (**Table S3-Supplementary material**), that are more relevant and effective in the differentiation between the two sensory classes. **Figure S3-Supplementary material** showed the VIP scores for the volatile compounds for each analytical method obtained by the PLS-DA models developed. Thus, the volatile compounds that showed VIP values higher than 1 and could be considered as markers of "green fruity" samples were 7: (*Z*)-3-hexen-1-ol methyl ether (for the three analytical methods), 1-penten-3-one (for HS-SPME-GC–MS and TDU-GC–MS) and 3-pentanol, 2-pentene, 2-ethylfuran, 3-ethyloctane (for HS-SPME-GC-FID), and 5-ethyl-2(5H)-furanone (for TDU-GC–MS); while those VIPs with more relation to "ripe fruity" samples were 11: ethyl acetate, 2-methylbutanal (for HS-SPME-GC–MS and FID), 1-hexanol, (*E*)-4-hexen-1-ol and phenethyl alcohol (for HS-SPME-GC–MS and TDU-GC–MS), 2-methyl-1-butanol, 3-methyl-1-butanol, 1-pentanol (for HS-SPME-GC–MS), and (Z)-2-hexen-1-ol, 3-methyl-4-penten-1-ol and butyrolactone (for TDU-GC–MS). These compounds were present in the oils at different concentrations. Thus, for example, the low concentration of (*E*)-4-hexen-1-ol or phenethyl alcohols, among others, may lead to a more difficult analytical determination. On the contrary, some compounds as 1-penten-3-one, 1-hexanol, 3-methyl-1-butanol and 1-pentanol are easily identified in virgin olive oils.

Hence, these compounds could be considered as the volatile markers of "green fruity" and "ripe fruity" EVOOs. Moreover, all these results also confirmed that although the three analytical methods led to differentiate the two classes of EVOOs with successfully results, they provided different information about the volatile compounds more relevant for it, so the use of different extraction techniques and methodological approaches could be complementary.

4. Conclusions

The data obtained in this study have shown that, with the volatile analyses of the EVOO samples obtained by three analytical gas chromatographic methods (HS-SPME-GC-FID, HS-SPME-GC–MS and TDU-GC–MS), it has been possible to differentiate EVOO samples into two sensory classes, "green fruity" and "ripe fruity" by means of their volatile profile. Each analytical method allowed the characterization of the two classes enabling the identification of different volatile compounds and a better definition of their aroma profile. Hence, TDU extraction method seemed to have more potential for extracting volatile compounds of the

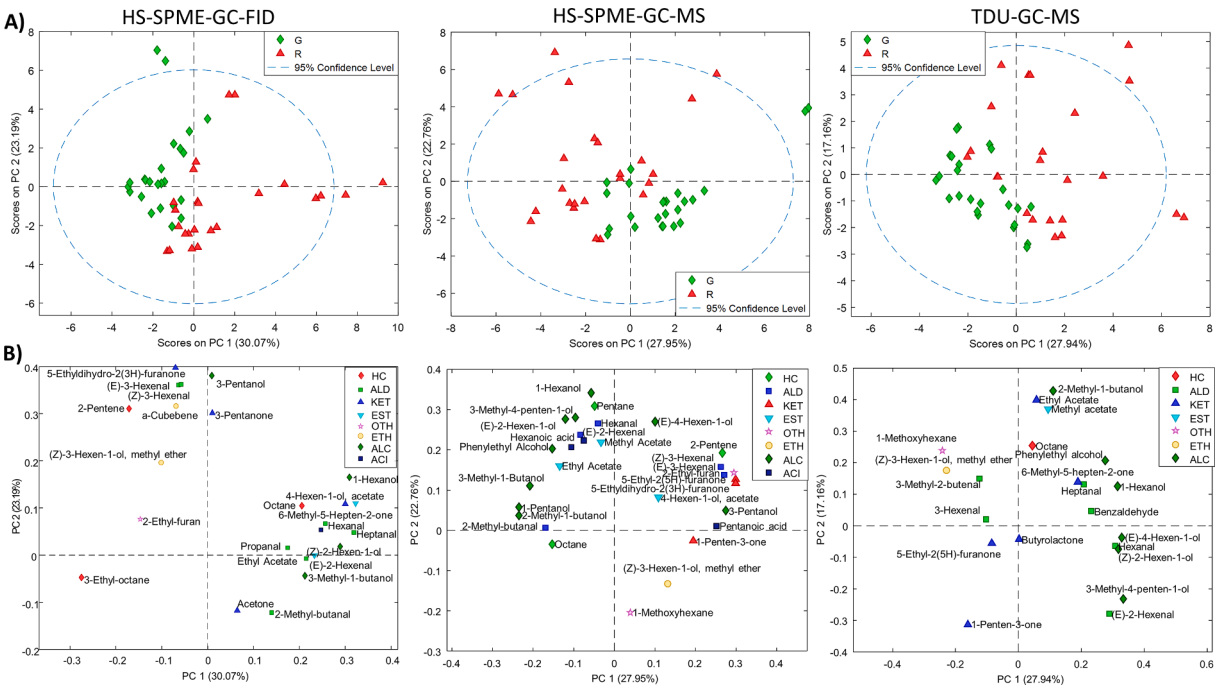

Fig. 4. (A) PCA scores plot and (B) loadings plots obtained with the concentrations of the volatile compounds with significant differences between "green fruity" and "ripe fruity", for each analytical method and obtained by PARADISe integration of HS-SPME-GC–MS and TDU-GC–MS data, and the conventional integration of HS-SPME-GC-FID data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chemical series of aldehydes and alcohols compared to HS-SPME, either with MS or FID, while HS-SPME extraction (either with MS or FID) seemed to be more powerful for extracting acids.

Moreover, the application of a deconvolution software such as PARADISe has been useful for the detection and identification of more compounds than with the conventional integration, due to its ability for resolving co-eluting peaks, for robust peak detection and for its ability of extracting extremely clean mass spectra. This tool is particularly interesting for the study of aroma profiles with complex differences as is the case of the differentiation between "green fruity" and "ripe fruity" attributes.

In order to improve the applicability of the proposed methodologies, and given the complexity of the problem, it would be convenient to verify the results with more samples of each EVOO class (e.g., different cultivars and protected designations of origin). This would permit gaining knowledge about other positive attributes of EVOO, thus contributing to a better definition of these attributes in their sensory evaluation. Thus, in this work, the studied samples presented only one of the two considered attributes, "green" or "ripe" fruity attributes since the objective was to gain knowledge about which volatile compounds were associated to each one of these two classes. Although natural virgin olive oils typically present a mixture of both attributes at different degree, it is important to note that these two attributes are due to different sensory profiles. Their chemical and sensory understanding of these two attributes is relevant because they are often indicated by panelists in the sensory assessment and it is the first classification when evaluating extra virgin olive oils (e.g. in the sensory assessment in quality awards). Next steps in research is the study of other sensory notes within "green" and "ripe" fruity attributes and the study of samples with a complex mixture of these attributes. The establishment of volatile profiles associated to particular positive attributes could contribute to a better understanding of sensory quality of virgin olive oil and their change over time.

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CRediT authorship contribution statement

Rocío Ríos-Reina: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing, Software, Data curation. **Ramón Aparicio-Ruiz:** Conceptualization, Investigation, Methodology, Software, Data curation, Writing – review & editing. **María T. Morales:** Conceptualization, Methodology, Validation, Data curation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Diego L. García-González:** Conceptualization, Methodology, Validation, Data curation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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