Check for updates

Received: 18 April 2023 | Revised: 12 September 2023

DOI: 10.1002/ejlt.202300079

European Journal of Lipid Science and Technology

RESEARCH ARTICLE

Multianalyte analysis of volatile compounds in virgin olive oils using SPME-GC with FID or MS detection: results of an international interlaboratory validation

Diego L. García-González[1] | Enrico Casadei[2] | Ramón Aparicio-Ruiz[1,3] | Clemente Ortiz Romero[1] | Enrico Valli[2] | Paul Brereton[4] | Anastasios Koidis[4] | Martyna Korytkowska[4] | Maurizio Servili[5] | Roberto Selvaggini[5] | Florence Lacoste[6] | Julien Escobessa[6] | Stefania Vichi[7] | Beatriz Quintanilla-Casas[7] | Alba Tres[7] | Pierre-Alain Golay[8] | Paolo Lucci[9] | Erica Moret[10] | Alessandra Bendini[2] | Tullia Gallina Toschi[2]

[1]Instituto de la Grasa (CSIC), Sevilla, Spain

[2]Department of Agricultural and Food Sciences, Alma Mater Studiorum - Università di Bologna, Bologna and Cesena, Italy

[3]Department of Analytical Chemistry, Universidad de Sevilla, Sevilla, Spain

[4]Institute for Global Food Security, Queen's University Belfast, Belfast, UK

[5]Department of Agricultural, Food and Environmental Sciences, Università degli Studi di Perugia, Perugia, Italy

[6]ITERG (Institut des Corps Gras), Canejan, France

[7]Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Campus de l'Alimentació de Torribera, Universitat de Barcelona, Santa Coloma de Gramenet, Spain

[8]Nestlé Research Center, Lausanne, Switzerland

[9]Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy

[10]Department of Agri-Food, Animal and Environmental Science Università degli Studi di Udine, Udine, Italy

Correspondence
Prof. Enrico Valli, Piazza Goidanich, 60, 47521 Cesena (FC), Italy.
Email: enrico.valli4@unibo.it

Funding information
Horizon 2020 European Research; European Commission within the Horizon 2020 Programme (2014–2020), Grant/Award Number: 635690

Abstract

The organoleptic assessment (Panel test) is the only procedure within the official methods for determining the quality of virgin olive oils that involves an expert panel. There is an urgent need for analytical methodology that can reliably measure volatile compounds in virgin olive oils that is capable of supporting and anticipating the official Panel test. For this reason, a new method based on solid-phase microextraction–gas chromatography with the choice of two possible detectors (FID or MS) was subjected to a large international interlaboratory validation study. The study involved a two-stage process: first, a pretrial phase in which 7 participants were exposed to the method for the first time to identify any initial problems with the methodology; then,

Abbreviations: CAR, Carboxen adsorbent; DVB, divinylbenzene; EV, extra virgin olive oil; FID, flame ionization detector; IS, internal standard; L, lampante olive oil; LOD, limit of detection; LOQ, limit of quantification; LRI, linear retention indexes; PEG, polyethylene glycol; SMs, standard mixtures; SOP, Standard Operating Procedure; SPME, solid-phase microextraction; V, virgin olive oil; VOC, volatile organic compounds; VOO, virgin olive oil.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2023 The Authors. European Journal of Lipid Science and Technology published by Wiley-VCH GmbH.

a formal validation stage (trial proper), which involved 20 laboratories from Europe, USA, Japan and China. The performance of the different detectors was investigated. While both methods have advantages, the method using FID provided better results for 11 compounds, in terms of reproducibility, compared to MS. This information will allow to implement the method with accurate information of the method performance depending on the detector used.

Practical applications: This study provides information from an interlaboratory validation of a method for measuring volatile compounds in virgin olive oils conducted with laboratories (from industry and academia) working in the olive oil sector. The information on the expected analytical errors in the determination of each volatile compound is necessary to apply this method for supporting the official Panel test (sensory analysis). The SPME-GC-MS/FID methods proposed in this work can be used for the internal quality control of a company/distributor/quality control laboratory and could also be used in cases of difficult/contradictory organoleptic assessment, or to confirm results from sensory panels in cases of disputes/disagreement (Reg. EU 2022/2105).

KEYWORDS
collaborative trial validation, sensory analysis, SPME-GC-FID/MS, virgin olive oil, volatile compounds

1 | INTRODUCTION

The sensory quality of virgin olive oil (VOO) is one of the most distinctive properties of this oil compared to other edible oils, and for that reason quality characteristics are carefully controlled.[1,2] by international regulations and trade standards that facilitate international trade and attempt to ensure the quality of the oil from production to the consumer.[3–7] The trade standards are specified by the International Olive Council (IOC),[8] which establishes the description of the corresponding methods. In addition other national and international regulatory frameworks also establish specific provisions for VOO quality control (e.g., EU, USDA, CODEX).[3,9–11] Among the methods for controlling quality, the organoleptic assessment (sensory analysis) is one of the most crucial.[12] An oil will be formally attributed as VOO due to the Panel test identifying a sensory defect even though the rest of the chemical and physical–chemical parameters are within extra virgin (EVOO) category.[4,13,14] In this context, a recent report from the European Union (EU) highlighted that the marketing of VOO sold as EVOO is the most widespread type of non-compliance identified with respect to the organoleptic characteristics of olive oils that are declared as "extra virgin olive oil."[15] Furthermore, this report points out some key issues in the organization and performance of organoleptic assessment, which has led to the search for alternative analytical approaches to support the official Panel test. As a result, the EU H2020 OLEUM research project developed a new method for measuring volatiles based on solid-phase microextraction-gas chromatography (SPME-GC) with the choice of two alternative detectors: flame ionization detector (FID) or mass spectrometry (MS).[16–18] The method resulted from a previous investigation into sources of analytical errors that focused on reducing errors resulting from the quantification procedure.[16,17] This procedure consisted of developing calibration curves by using two mixtures of standards. The composition and concentration of the volatile compounds included in these mixtures were optimized to take into account separation/coelution and concentration of the compounds in VOO. The method underwent an initial peer study to obtain initial information about the performance parameters of the method, such as linearity, repeatability, reproducibility, recovery, and limits of detection (LOD) and quantification (LOQ).[16,17] The peer study revealed that the normalization of the chromatographic areas by the selected internal standard (IS) (4-methyl-2-pentanol) introduced in the calibration curves produced similar (e.g., 23.04% vs. 25.83% for hexanal determined by MS) or better reproducibility results (e.g., 19.18% vs. 30.57% for (*Z*)—3-hexenyl acetate) compared with the calibration of the chromatographic areas conducted without IS normalization.[16,17]

Following the peer study, the method was then subjected to a formal international validation (collaborative trial) with laboratories from industry or other areas working in olive oil sector, some with limited experience of conducting a multianalyte analysis of organic compounds (VOCs) as it is currently not a requirement in the EU regulation; that is, there no methods for the headspace analysis of VOO for assessing sensory quality,[8] and therefore this analytical technique is considered as new procedure for the laboratories in the study.[19] A key part of the method focuses on avoiding evaporation of VOCs or contamination with other volatile molecules present in the environment. In addition, the method includes a GC system adaptation: new injection configuration, new column, new procedure, and procedures for identifying the VOCs on the chromatograms, that are all relatively novel for analysts

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

less experienced in the analysis of VOCs.[20–22] The lack of experience may play a relevant role in introducing more errors so it is import to address this factor when designing the validation scheme while ensuring that an internationally recognized process is followed.[23] In this research work, the study and statistical processes were carried out in compliance with ISO 5725,[24–27] a well-established internationally agreed method validation procedure.[25] With this purpose, an international validation was launched in 2020 following the Protocol for the Design, Conduct and Interpretation of Method-Performance Studies, which is compliant with ISO 5725.[25] The study ran between June 2020 and January 2021. In total, 20 laboratories from Europe, the United States, Japan, and China took part in the study. The method was supplied to these labs in an internationally accepted form (ISO format).[28] A large proportion of participants were involved in olive oil analysis, as such the results of the validation study reflect the results from real end-users.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Pure standards of 18 VOCs analyzed were purchased from Merck (Darmstadt, Germany). These were used to prepare two standard mixtures and to prepare the calibration curves. The IS (4-methyl-2-pentanol, purity ≥98%) and the mixture of *n*-alkanes from 8 to 20 carbon atoms (~ 40 mg L^{-1} each, in *n*-hexane) for the calculation of the linear retention indexes (LRI) were purchased by each participating laboratory.

2.2 | Samples

A group of 10 "blind samples," comprising five paired samples randomly numbered, were used in the trial proper (see Section 2.6). These samples were selected to ensure 18 VOCs were included in the study and at relevant concentrations. This selection took place after analysis of a range of commercial filtered samples obtained from different producers in order to check for their volatile composition and their suitability for the validation study. In addition, the sensory analysis (Panel test) was carried out by the professional committee of the University of Bologna and all samples were classified according to the commercial category (extra virgin, virgin, and lampante). Those samples in which many VOCs were absent (not detected) were not selected as samples for the trial proper (Table 1).

2.3 | IS solution

The IS solution was prepared as described by Casadei et al.[16] The IS (4-methyl-2-pentanol) was diluted in freshly refined olive oil in order to have an approximate concentration of 50 mg kg^{-1}.

2.4 | Gas chromatographic analysis

2.4.1 | SPME-GC-FID analysis

SPME-GC-FID analysis was carried out according to Casadei et al.[16] An Excel file was sent to the participant labs (see Supplementary Information) in which the calculations were carried out automatically, just requiring the laboratories to input the exact weights and chromatographic areas (see Supplementary Information), thus reducing the likelihood of error in such calculations. The vial was closed with a septum (polytetrafluoroethylene) and was left for 10 min at 40°C under agitation to allow for equilibration of the VOCs in the headspace. The SPME fiber was exposed to the sample headspace for 40 min at 40°C. The volatiles adsorbed by the fiber were thermally desorbed in the hot injection port of a GC for 5 min at 250°C with the purge valve off (splitless mode) and injected into a capillary column of a gas chromatograph with FID detector. The analysis allowed the use of an auto sampler or manual injection.

The capillary column was of a polar phase based on polyethylene glycol (PEG), length 60 m, internal diameter 0.25 mm, and coating 0.25–0.50 μm. The transfer line temperature was set at 260°C. The carrier gas used was open to helium or hydrogen if the lab facility was configured for that. Although hydrogen is the most efficient and economical carrier gas for GC, carbon-carbon double bonds may be hydrogenated in the hot GC injector if introduced by SPME coated with divinylbenzene (DVB) polymer or Carboxen (CAR) porous particles. During this validation process, parallel analyzes were made (both with hydrogen and helium as carrier gas) and no artifacts were detected.[29,30]

The oven temperature was held at 40°C for 10 min and then programmed to increase by 3°C min^{-1} to a final temperature of 200°C. A cleaning step was added at the end of the oven programmed temperature by all participants (20°C min^{-1} to 250°C for 5 min) to ensure that the column was ready for the next analysis.[16]

2.4.2 | SPME-GC-MS analysis

SPME-GC-MS analysis was carried out according to Aparicio-Ruiz et al.[17] The calculation of the concentrations was also made with the same Excel file mentioned in the previous section (see Supplementary Information).

2.5 | Identification and quantification of VOCs

Linear Retention Index (LRI) and standards were used for identification[16] in addition to mass spectrometry.[17] The quantification procedure was based on the calibration curves of the 18 selected volatile compounds built as linear regression (intercept equal to 0) corrected by the chromatographic areas of the IS as described by Casadei et al.[16] and Aparicio-Ruiz et al.[17] Thus, the following equation was used: $A_{Analyte}/A_{IS} = m \cdot C_{Analyte}$, where $A_{Analyte}$ is the area

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 Information and sensory characteristics of the samples (VOO) considered for the selection in the pretrial and trial proper studies.

Characteristics	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Pretrial code	M4A007	M4A008	–	–	–		–	–	–
Trial proper code	TP3, TP11	–	TP2, TP4	–	TP10, TP12	TP6, TP7	–	TP1, TP8	–
Quality grade	Virgin olive oil[a]	Lampante olive oil	Extra virgin olive oil	Lampante olive oil	Lampante olive oil[b]	Virgin olive oil[a]	Virgin olive oil	Virgin olive oil	Lampante olive oil[c]
Sensory characteristics	Fusty/muddy sediment, fermentative defects and slightly rancid	Winey/vinegary, fusty/muddy sediment and brine	No sensory defect detected	Fusty/muddy sediment, winey/vinegary, musty/humid/earthy	Fusty/muddy sediment, winey/vinegary, musty/humid/earthy, slightly rancid	Rancid	Slight rancid	Winey/vinegary	Fusty/muddy sediment, winey/vinegary, musty/humid/earthy, rancid
No. of nondetected compounds	2	2	3	3	0	0	4	3	1

[a] Near lampante olive oil.
[b] Near virgin olive oil.
[c] Oil resulted from the mixture of 10 virgin olive oils from the three quality grades (extra virgin, virgin, and lampante).

corresponding to the analyte, A_{IS} is the area corresponding to the IS, and m is the slope of the calibration curve (built for the selected analyte).

The calibration curves were prepared using standard mixtures (SMs), as reported by Casadei et al.[16] The two mixtures, coded as SM-A and SM-B, were prepared to have a concentration of 10 000 mg kg^{-1} for each VOC and were supplied to participants for s subsequent dilutions, coded as SM1 (200 mg kg^{-1}), SM2 (20 mg kg^{-1}), and SM3 (2 mg kg^{-1}). Thus, the calibration started from the same materials avoiding need for each participant to purchase 18 standards of the volatile compounds. The calibration solutions were prepared by the participants to have 0.05, 0.10, 0.15, 0.20, 0.25, 0.5, 1.00, 1.50, 2.00, 2.50, 5.00, and 10.00 mg kg^{-1} for the compounds included in SM-A and from 0.20 to 10 ppm (adding three points at 15.00, 20.00, and 25.00 mg kg^{-1}) for the compounds included in SM-B. The concentration values were higher in the latter since some of the compounds in this mixture are present at higher amounts in VOO.

2.6 | Collaborative study design (pretrial and trial proper)

The design of the collaborative study involved two consecutive phases, namely, the initial ("pretrial") and the formal validation stage ("trial proper"). Figure S1 shows the general validation scheme used in the OLEUM project. The initial pretrial phase took place in 2019. The objective of the pretrial was a two-way knowledge transfer: (1) to allow the participants to familiarize with the protocol (called Standard Operating Procedure or SOP) as well as with the reporting procedure (Excel file shown in Supplementary Information) and to provide important information regarding deviations from the provided protocol by the participants and (2) to obtain end-user feedback to improve the written procedure (SOP). A key part of the pretrial process was the two-way hands-on knowledge transfer workshops where participants shared their experiences with the developers and provided feedback on the method so that any errors could be corrected, and changes could be made to make the method easier to understand and use. The resulting workshop in Bologna (Italy)[31] was well attended, with over 70 laboratories and stakeholders in the olive oil sector participating in the event. A timeframe of 3 months from the launch date of each phase was granted for submitting the results of the requested analyses.

The formal collaborative trial (trial proper) ran between June 2020 and January 2021. At the start of each phase of the collaborative trial, the participant laboratories were provided with Excel electronic spreadsheets for reporting the results (Supplementary Information), as well as with the appropriate SOP. Any deviation from the protocol had to be reported to the organizers of the study.

Twenty laboratories from 9 countries (Europe, the United Kingdom, the United States, China, and Japan) took part in the trial proper and received 10 test materials comprising 5 sets of individually numbered blind duplicates. Twelve laboratories (4 from Italy, 3 from Spain and 1 each from France, Slovenia, China and Japan) submitted valid (compliant) results for the MS procedure and 8 laboratories for FID

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(5 from Italy, 2 from Spain, and 1 from Slovenia). The collaborative study was designed according to the harmonized protocol for method validation of the International Union of Pure and Applied Chemistry (IUPAC).[25] The criteria that must be met for the protocol are as follows: (i) a minimum number of 8 laboratories that deliver valid results; (ii) use a minimum number of 5 test materials that are different in terms of analyte and/or matrix concentration; and (iii) finally undertake the statistical analysis to calculate method performance parameters after rejection of outliers.

2.7 | Data processing and statistical analysis

Prior to the statistical analysis of the results, the reported data and metadata were assessed for compliance, that is, whether the laboratories strictly followed the designated SOP. The assessment of noncompliance was based on (1) comments in the Excel reporting spreadsheet (Supplementary Information) and/or subsequent dialogue with participants and (2) clear gross errors in the analysis/noncompliance assessed.

After exclusion of noncompliant results, the data were processed according to the harmonized protocol, which includes stepwise assessment of statistical outliers using Cochrans and Grubbs tests respectively.[25] Method performance parameters were then calculated: (i) relative standard deviation for repeatability (RSD_r %), which is a primary estimate of precision when the method is applied within a laboratory; (ii) relative standard deviation for reproducibility (RSD_R %), which is an estimate of the precision when the method is applied between laboratories; and (iii) Horrat value (HoR), defined as the ratio between the reproducibility standard deviation and the Horwitz function (SR/H). Horrat values in the interval $0.5 < HoR < 2.0$ indicate that the precision is within expected values.[32] Additionally, the repeatability and reproducibility limits were calculated. The terms "repeatability limit" and "reproducibility limit" were applied specifically to a probability of 95% and they were taken as $2.8 \times S_r$ (S_r, standard deviation of the repeatability) and $2.8 \times S_R$ (S_R, standard deviation of the reproducibility), respectively as specified in the harmonized protocol.[24]

Data processing, calculations, and Student's *t*-test of paired samples ($p < 0.05$) were carried out with Microsoft spreadsheet program 2016 (Microsoft Corp., Redmond, WA). Analysis of variance ($p < 0.05$) was carried out with Statistica (StatSoft, Tulsa, OK).

3 | RESULTS AND DISCUSSION

3.1 | Sample selection

The previous evaluation of the SPME-GC-FID/MS[16,17] method showed that the selection of the validation samples is critical since these oils should contain all the relevant 18 VOCs as they are markers of different sensory defects or fruitiness, as positive attributes. Furthermore, a natural oil (real VOO sample) could not present all the sensory defects at the same time. For that reason, 9 samples were selected that represented either complex aromas or different sensory defects, and, therefore, they were likely to show many if not most of 18 VOCs. The VOCs were analyzed by SPME-GC-FID and the number of nondetected compounds were evaluated to select those 5 samples to be used in the pretrial and trial proper phases. Table 1 shows those 5 samples selected, which presented most of the volatile compounds studied. This selection was also made in order to balance the samples set not only in terms of commercial category (EV, V, L) but also in terms of sensory defects. Sample 9 only presented one nondetected VOC (3-methyl-1-butanol). This sample was a lampante olive oil resulting from the mixture of 10 VOOs from the three quality grades—extra virgin, virgin, and lampante—with different sensory defects. However, this sample was finally not selected because its aroma was considered nonrepresentative of a real VOO.

3.2 | Pretrial phase

The pretrial phase allowed for the identification of potential unforeseen problems. One of the critical issues was the possible alteration of the volatile fraction during transport and distribution to the labs, since external variables (e.g., light and temperature) may alter the volatile profile,[2,33] thereby introducing errors that are not associated with the method performance. In order to investigate stability during transportation a pilot exercise was carried out by shipping the two pretrial samples from Seville (Spain)—York (UK)—Seville (Spain) and analyzing the VOCs before and after the transportation to identify possible stability issues. No significant differences were found between the concentrations obtained before and after the shipping.

Table 2 shows the results of the pretrial, which were computed from 7 labs (2 with FID and 5 with MS). Since the number of labs using FID and MS detectors were low for a separate analysis for each detector the results of all the labs were studied together regardless of type of detector used. The analyses of outliers determined that one of the labs presented the half of the values (9 out of 18) as outliers and as a consequence it was removed from the data set. It was established that a technical problem had occurred in the sample analysis which explained the anomalous values. The other outliers removed from the data set corresponded to cases of wrong identification or a clear problem in the chromatogram integration that resulted in anomalously high/low chromatographic areas. No errors were identified in the calibration curves, provided by participants in their Excel data sheets. As a result of the pretrial, and the following knowledge transfer workshop, a revision of the SOP was made that provided a more detailed description of the method with an example chromatogram for each product category to more easily facilitate identification. Furthermore, a statement about the importance of applying the same integration procedure, in both calibration and sample chromatograms, was added to the written method. The resulting relative standard deviations (RSD%) were in accordance with those published regarding the peer study of the method carried out within OLEUM project[16,17] for some compounds: octane, ethyl acetate, ethanol,

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 Mean concentrations (mg kg^{-1}) and standard deviations (SD) resulted from the pretrial study carried out with two samples (coded as M4007 and M4008).

		M4007		M4008		Previous interlab study[1]	
Code	Volatile compounds	Mean ± SD	RSD_R%	Mean ± SD	RSD_R%	RSD_R% FID	RSD_R% MS
1	Octane	2.812 ± 0.313	25.9	2.874 ± 0.349	28.0	12.0	38.5
2	Ethyl acetate	2.568 ± 0.664	23.0	5.723 ± 1.600	25.9	18.2	28.2
3	Ethanol	0.730 ± 0.168	24.7	18.220 ± 4.715	25.4	35.7	32.3
4	Ethyl propanoate	9.590 ± 2.368	112.3	30.007 ± 7.607	49.7	122.0	39.0
5	Hexanal	0.012 ± 0.014	23.1	0.382 ± 0.190	33.7	28.0	23.0
6	3-Methyl-1-butanol	2.428 ± 0.560	51.6	1.236 ± 0.417	15.0	23.1	26.0
7	(*E*)−2-hexenal	0.199 ± 0.103	14.5	7.774 ± 1.169	128.0	30.1	19.6
8	(*Z*)-3-hexenyl acetate	8.855 ± 1.282	22.9	0.151 ± 0.194	64.8	32.8	19.2
9	(*E*)-2-Heptenal	1.094 ± 0.251	37.9	0.209 ± 0.136	48.7	26.0	24.9
10	6-methyl-5-hepten-2-one	0.423 ± 0.160	33.9	0.195 ± 0.095	24.2	47.8	43.2
11	1-hexanol	0.047 ± 0.016	65.6	0.239 ± 0.058	37.1	48.1	13.3
12	Nonanal	1.322 ± 0.867	16.3	2.341 ± 0.867	41.4	44.2	46.1
13	1-octen-3-ol	3.693 ± 0.602	15.8	8.272 ± 3.427	55.7	37.2	31.5
14	(*E*,*E*)−2,4-hexadienal	0.038 ± 0.006	44.7	0.188 ± 0.105	104.9	39.3	63.5
15	Acetic acid	0.051 ± 0.023	20.1	0.189 ± 0.198	21.7	44.8	17.5
16	Propanoic acid	3.815 ± 0.768	36.6	17.257 ± 3.735	34.2	21.4	26.7
17	(*E*)−2-decenal	0.126 ± 0.046	74.0	1.072 ± 0.367	55.8	57.8	36.7
18	Pentanoic acid	0.474 ± 0.350	30.0	1.391 ± 0.777	39.0	29.7	27.1

Note: The relative standard deviation for reproducibility (RSDR%) resulted from this study (regardless the detector) and from two previous studies carried out with labs with experience in VOC analysis (FID and MS separated) are also shown.

hexanal, 6-methyl-5-hepten-2-one, acetic acid, propanoic acid, and (*E*)−2-decenal. Other compounds showed higher RSD% although only in one of the two samples, highlighting a probable effect of the sample (their whole volatile profile) or concentration as was the case for 3-methyl-1-butanol, (*E*)−2-hexenal, (*Z*)−3-hexenyl acetate, 1-hexanol, 1-octen-3-ol, (*E*,*E*)−2,4-hexadienal, and (*E*)−2-decenal. The fact that the volatile profile of the sample and the concentration of volatile compounds apparently showed some effect highlights the importance of integration procedure, which may be affected by the signal around the analyte peak. Alternatively, the low number of participants (7) in the pretrial, and the fact that the data comprised results from both FID and MS detector systems could explain some of the variability in results. The differences observed with respect to the previous evaluation of the method[16,17] could be explained by incorrect identification and/or integration of the peaks, which supports the idea of introducing changes in the protocol to guide the identification procedure and to give importance to the chromatographic integrators and data systems.[34] It has been reported, that a manual integration carried out on the same chromatogram by 4 different analysts may lead to a maximum variation (RSD%) of 7% in the determined areas.[16]

According to the results and information acquired in the pretrial, there were two key sources of errors: (1) calibration and (2) identification/integration. One of them arose from the calibration procedure (from the sample analysis, integration, or the calibration curve). When calibration was the source of error for the concentration data of one VOC, atypically high or low values were observed but the magnitude in relation to other samples was similar to the results from other laboratories, for example, if one sample contained higher concentration than another, in that laboratory their results were still in a similar proportion to those of from other laboratories. The other type of error was due to failings in the identification/integration, not surprising given the complexity of VOO aroma.[21,22,35] In this case, the order of the samples with respect to the magnitude of the concentration was dissimilar to other laboratories with incorrect peaks being quantified. This error was expected to be more frequent when FID detector was used due to its lack of selectivity. However, some errors in identification were also found in some laboratories working with MS, although usually in compounds at low concentration, or in the cases of coeluting peaks. For example, some identification/integration problems were detected for octane with one laboratory reporting no value for this analyte. Similarly, 3-methyl-1-butanol was apparently problematic, probably due to the overlapping with (*E*)−2-hexenal, and also because it was observed that the order of elution of this compound could be altered depending on the brand of the capillary column used. Some laboratories did not report values for (*E*)−2-hexenal and (*Z*)−3-hexenyl acetate, suggesting a problem in their identification.

The conclusion of the pretrial phase and the resulting feedback from participants led to some improvements to the SOP and accompanying documentation that could be summarized as follows:

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

1. The Excel data sheet for reporting the results in the trial proper included fields to be completed with all the information regarding the weights for building the calibration curves (Supplementary Information). Thus, introducing all the weights and the chromatographic areas, Excel provided the output in mg kg^{-1}.
2. The information to be introduced in the Excel file allowed the traceability of the quantification method to identify the source of gross errors (e.g., anomalous chromatographic area).
3. The information about the injector liner or sleeve to be used was included in the section of the apparatus in the updated SOP. This information was identified as missing in the previous version used in the Pretrial and it was considered that it should be included.
4. The importance of equilibrating the samples to room temperature before their preparation was also highlighted. Samples must look homogeneous, and no waxes or solid particles should be observed. This new input in the SOP comes from a discussion about how to handle the samples with respect to the room temperature.
5. For the method using SPME-GC-FID, the detector temperature was set at 260°C and the desorption temperature in the injector was reduced from 260°C to 250°C.
6. The need for applying an adequate and uniform integration method for both calibration solutions and virgin olive oil samples was specified in the SOP since it was discussed that it was one of the error sources in the pretrial.

In addition to these improvements, it was decided to provide the labs with a table with the exact concentrations of each volatile compound present in the two standard mixtures (SM-A and SM-B) considering the exact measured weights during the preparation of these mixtures. Thus, using the Excel file, participants could build the calibration curves taking into account the exact concentrations of the specific mixtures that they received. Four lots of SM-A and SM-B (10 vials in each lot) were produced and each lab received one SM-A and SM-B of one of these lots. The actual concentrations are indicated in Table S1. Special attention was also paid to the reproducibility of the preparation of SM-A and SM-B to provide standard mixtures with the lowest variation as possible. The relative standard deviations (RSD%) of the concentrations were in the range of 0.69–8.50, and except for 5 compounds, the RSD% were always lower than 2.81% (Table S1). On the other hand, when the quantification was carried out with the exact concentrations (Table S1) and the generic concentration of 10 000 mg kg^{-1} for all the compounds (concentrations without considering the exact weights in the preparation), the two resulting concentrations differed in only 1.16% (mean value for the 18 volatile compounds). Thus, the error coming from the differences in the preparation of the standard mixtures was considered to be acceptable.

3.3 | Trial proper

The trial proper was informed by the results and experience acquired in the pretrial, not only in the method itself, but also in the sample selection, homogenization, and the materials (flasks and vials) used. For example, in the trial proper, the use of plastic bottles for refined olive oil (sent for building the calibration curves) was avoided since some peaks derived from plastic were observed during the analysis of refined olive oil in the pretrial. Table 3 shows the mean concentration values for the validation process of FID and MS methods, which were calculated from 20 participants (8 for FID and 12 for MS). The comparison of concentrations obtained between the FID and MS methods for the determination of the 18 VOCs in VOO revealed that, for 13 VOCs, no significant differences in concentration were found ($p \leq 0.05$, two tails by Student's *t*-test of paired samples) while 5 VOCs showed significant differences in concentration between the two method variants. The 5 compounds were 3-methyl-1-butanol, (*E*)−2-heptenal, 6-methyl-5-hepten-2-one, propanoic acid, and pentanoic acid, with the concentrations obtained with the FID method being higher than the MS method for these analytes (Table 3). These differences could be related to the different sensitivity of the detectors at low concentrations, for example, (*E*)−2-heptenal.

In terms of repeatability, Table 4 shows the RSD_r% values for the FID and MS methods from the trial proper. The RSD_r% values were lower than 10% in all cases except for ethyl propanoate, (*E*)−2-heptenal, 6-methyl-5-hepten-2-one, nonanal, (*E*,*E*)−2,4-hexadienal, (*E*)−2-decenal, pentanoic acid. These compounds presented RDS_r% lower than 20% except for ethyl propanoate, (*E*)−2-decenal, and pentanoic acid in two samples (Table 4). In general, the RDS_r% values were similar between FID and MS detectors, pointing out that the sensitivity of these detectors did not have a large impact on repeatability. No significant differences were found in the RSD_r% values ($p \leq 0.05$, two tails by Student's *t*-test of paired samples) for 17 VOCs (94.4% of the compounds) except for ethyl acetate. In this case, the RSD_r% value was slightly higher for FID compared to MS. The mean RSD_r% values for the 5 paired samples were 6.7% and 5.0% for FID and MS detectors, respectively.

Table 5 shows the reproducibility in terms of RSD_R% for FID and MS methods. In this case, there were differences in reproducibility for 50% of the VOCs between FID and MS methods. The reproducibility of these compounds (octane, ethyl acetate, ethanol, 6-methyl-5-hepten-2-one, acetic acid, and pentanoic acid) were lower for FID method compared to MS while 1-hexanol showed a higher reproducibility value for FID compared to MS. For FID, the compounds with the lowest RSD_R% (< 30%) were ethyl acetate, ethanol, acetic acid, and octane, while the compounds with high RSD_R (> 60%) were (*E*)−2-heptenal, pentanoic acid, (*E*)−2-decenal, (*E*,*E*)−2,4-hexadienal, ethyl propanoate, and 1-octen-3-ol, with the last three compounds having a RSD_R% > 100%. The results could be related to the low concentration of these compounds in the samples, the limits of quantification, and/or the low recovery for these compounds in FID.[16,17] The values of RSD_R% were not associated to the concentration value apparently: the highest RSD_R% was not necessarily found in those samples with lower concentration, and a regression analysis between RSD_R% values in each compound and the concentration values resulted in R^2 below 0.6 in all cases, except for (*Z*)-hexenyl acetate (0.70), 1-octen-3-ol (0.91), propanoic acid (0.63), and (*E*)−2-decenal (0.7) in the case of the method with FID detector. For MS detector, octane (0.98),

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 3 Mean concentrations and standard deviation (mg kg^{-1}) from the trial proper performance study of the method (SPME-GC-FID/MS) carried out with five paired samples.

Volatile compounds	Samples 1 and 8		Samples 2 and 4		Samples 3 and 11		Samples 6 and 7		Samples 10 and 12	
	FID	MS	FID	MS	FID	MS	FID	MS	FID	MS
Octane	1.541 ± 0.190	1.552 ± 0.068	0.162 ± 0.019	0.161 ± 0.012	0.244 ± 0.015	0.219 ± 0.018	0.450 ± 0.034	0.377 ± 0.015	1.249 ± 0.084	1.438 ± 0.040
Ethyl acetate	2.892 ± 0.191	3.421 ± 0.152	0.542 ± 0.032	0.678 ± 0.038	0.448 ± 0.019	0.593 ± 0.016	0.193 ± 0.019	0.232 ± 0.016	1.035 ± 0.068	1.299 ± 0.039
Ethanol	51.519 ± 5.745	45.892 ± 4.080	0.345 ± 0.154	0.460 ± 0.058	10.249 ± 0.518	13.182 ± 0.715	4.558 ± 0.215	7.102 ± 0.446	13.663 ± 0.752	17.138 ± 0.409
Ethyl propanoate	0.011 ± 0.004	0.009 ± 0.001	0.028 ± 0.008	0.031 ± 0.139	0.014 ± 0.003	0.004 ± 0.014	0.013 ± 0.002	0.005 ± 0.013	0.038 ± 0.008	0.020 ± 0.039
Hexanal	0.457 ± 0.049	0.584 ± 0.035	0.954 ± 0.024	0.584 ± 0.042	2.265 ± 0.091	1.379 ± 0.088	1.786 ± 0.092	1.494 ± 0.034	1.939 ± 0.107	1.784 ± 0.047
3-Methyl-1-butanol*	0.507 ± 0.016	0.425 ± 0.019	0.286 ± 0.014	0.215 ± 0.010	0.242 ± 0.010	0.150 ± 0.005	0.128 ± 0.010	0.076 ± 0.007	1.048 ± 0.041	0.877 ± 0.032
(*E*)−2-hexenal	0.111 ± 0.011	0.137 ± 0.007	2.619 ± 0.127	2.746 ± 0.150	12.092 ± 0.482	10.140 ± 0.424	3.222 ± 0.104	3.211 ± 0.137	3.875 ± 0.109	4.714 ± 0.109
(*Z*)−3-hexenyl acetate	0.645 ± 0.042	0.810 ± 0.078	1.753 ± 0.063	2.079 ± 0.179	0.641 ± 0.042	0.520 ± 0.038	0.598 ± 0.036	0.666 ± 0.038	0.722 ± 0.065	0.795 ± 0.025
(*E*)−2-heptenal*	0.231 ± 0.024	0.194 ± 0.015	0.103 ± 0.020	0.075 ± 0.008	0.296 ± 0.016	0.161 ± 0.021	0.442 ± 0.029	0.298 ± 0.018	0.577 ± 0.037	0.376 ± 0.011
6-methyl-5-hepten-2-one*	0.059 ± 0.006	0.051 ± 0.003	0.059 ± 0.010	0.048 ± 0.007	0.080 ± 0.008	0.068 ± 0.007	0.068 ± 0.007	0.058 ± 0.007	0.069 ± 0.006	0.047 ± 0.002
1-hexanol	0.514 ± 0.030	0.530 ± 0.010	1.727 ± 0.055	1.763 ± 0.094	1.403 ± 0.050	1.298 ± 0.058	0.579 ± 0.020	0.532 ± 0.032	1.121 ± 0.057	1.103 ± 0.022
Nonanal	2.533 ± 0.283	2.839 ± 0.405	0.546 ± 0.056	0.911 ± 0.063	1.064 ± 0.107	1.022 ± 0.177	0.606 ± 0.054	0.648 ± 0.046	2.886 ± 0.378	2.908 ± 0.138
1-octen-3-ol	0.029 ± 0.009	0.025 ± 0.003	0.017 ± 0.005	0.018 ± 0.001	0.026 ± 0.002	0.023 ± 0.004	0.112 ± 0.002	0.030 ± 0.004	0.040 ± 0.006	0.039 ± 0.003
(*E*,*E*)−2,4-hexadienal	0.202 ± 0.048	0.020 ± 0.007	1.039 ± 0.148	0.112 ± 0.012	1.883 ± 0.075	0.219 ± 0.028	0.395 ± 0.027	0.101 ± 0.012	0.500 ± 0.032	0.294 ± 0.055
Acetic acid	10.893 ± 1.049	10.402 ± 0.906	8.487 ± 0.179	7.935 ± 0.376	3.521 ± 0.179	3.141 ± 0.142	1.790 ± 0.074	1.515 ± 0.036	3.215 ± 0.095	3.755 ± 0.118
Propanoic acid*	0.366 ± 0.013	0.310 ± 0.019	0.229 ± 0.022	0.165 ± 0.023	0.162 ± 0.007	0.010 ± 0.006	0.221 ± 0.019	0.124 ± 0.005	0.537 ± 0.024	0.476 ± 0.017
(*E*)−2-decenal	0.651 ± 0.173	0.994 ± 0.390	0.292 ± 0.112	0.295 ± 0.092	0.664 ± 0.083	0.522 ± 0.072	0.929 ± 0.246	0.669 ± 0.071	0.829 ± 0.150	0.858 ± 0.088
Pentanoic acid*	0.084 ± 0.017	0.049 ± 0.012	0.071 ± 0.005	0.038 ± 0.002	0.051 ± 0.010	0.038 ± 0.008	0.073 ± 0.022	0.041 ± 0.003	0.080 ± 0.004	0.062 ± 0.003

*Significant differences between results from FID and MS at $p \leq 0.05$, two tails by Student's *t*-test.

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Politi Delle Marche-Ancona Cir Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 4 Results of relative standard deviation of repeatability (RSDr%) for the five paired samples used in the trial proper.

Volatile compounds	Mean RSDr% pairs		RSDr% 1 and 8		RSDr% 2 and 4		RSDr% 3 and 11		RSDr% 6 and 7		RSDr% 10 and 12	
	FID	MS	FID	MS	FID	MS	FID	MS	FID	MS	FID	MS
Octane	9.5	5.9	12.3	4.4	11.9	7.2	6.0	8.2	7.6	3.9	6.8	2.8
Ethyl acetate*	6.7	5.0	6.6	4.4	5.9	5.6	4.3	2.8	9.8	7.0	6.6	3.0
Ethanol	16.4	8.3	11.2	8.9	44.6	12.7	5.1	5.4	4.7	6.3	5.5	2.4
Ethyl propanoate	24.6	27.3	34.9	14.2	28.6	13.5	20.2	36.5	14.7	44.9	22.1	6.6
Hexanal	5.6	5.5	10.8	6.0	2.5	7.2	4.0	6.4	5.1	2.3	5.5	2.6
3-Methyl-1-butanol	5.1	5.4	3.1	4.6	5.0	4.7	4.3	3.0	8.1	9.3	4.0	3.7
(*E*)−2-hexenal	5.4	4.7	9.6	4.9	4.8	5.5	4.0	4.2	3.2	4.3	2.8	2.3
(*Z*)−3-hexenyl acetate	5.7	7.7	6.5	9.6	3.6	8.3	6.6	7.3	6.0	5.7	9.1	3.2
(*E*)−2-heptenal	10.6	9.3	10.6	7.8	19.8	10.7	5.5	12.8	6.6	6.0	6.5	3.0
6-methyl-5-hepten-2-one	11.8	10.4	10.6	6.6	17.0	13.5	10.0	10.2	9.6	11.4	8.0	5.2
1-hexanol	4.1	4.4	5.9	1.8	3.2	5.3	3.6	4.5	3.5	5.9	5.1	2.0
Nonanal	10.1	11.4	11.2	14.3	10.2	6.9	10.0	17.3	8.8	7.2	13.1	4.7
1-octen-3-ol	17.8	12.2	32.6	11.9	27.8	7.5	9.1	15.4	1.7	13.9	14.3	6.5
(*E*,*E*)−2,4-hexadienal	12.1	17.9	23.0	35.4	14.4	11.0	4.0	12.9	6.8	12.1	6.4	18.6
Acetic acid	5.2	5.1	9.6	8.7	2.1	4.7	5.1	4.5	4.1	2.4	3.0	3.1
Propanoic acid	6.5	7.4	3.7	6.0	9.4	13.6	4.4	6.2	8.6	3.7	4.4	3.6
(*E*)−2-decenal	26.1	23.7	26.6	39.2	38.5	31.1	12.6	13.8	26.5	10.6	18.1	10.3
Pentanoic acid	19.3	14.1	20.2	23.7	6.3	5.7	20.0	20.8	30.6	6.2	5.4	4.3

*Significant differences between results from FID and MS at $p \leq 0.05$, two tails by Student's *t*-test.

TABLE 5 Results of relative standard deviation of reproducibility (RSD_R%) for the five paired samples used in the trial proper.

Volatile compounds	Mean RSD_R% pairs		RSD_R% 1 and 8		RSD_R% 2 and 4		RSD_R% 3 and 11		RSD_R% 6 and 7		RSD_R% 10 and 12	
	FID	MS	FID	MS	FID	MS	FID	MS	FID	MS	FID	MS
Octane*	27.7	39.1	21.9	32.0	30.5	44.9	23.6	42.2	28.1	41.4	34.6	35.1
Ethyl acetate*	15.9	29.1	12.4	27.1	8.5	29.9	12.1	29.5	23.9	32.1	22.6	26.7
Ethanol*	23.8	45.4	27.1	45.2	53.5	53.3	8.0	41.0	13.0	46.2	17.2	41.5
Ethyl propanoate	111.3	107.2	150.3	82.7	117.8	158.4	98.8	121.8	92.1	102.7	97.4	70.3
Hexanal	39.7	28.0	20.3	17.3	54.5	33.9	56.7	27.4	35.6	30.1	31.6	31.1
3-Methyl-1-butanol	33.5	57.0	12.9	37.4	23.2	63.0	54.6	70.8	48.5	76.1	28.1	37.8
(*E*)−2-hexenal	38.7	37.6	61.9	64.7	31.6	31.2	21.5	30.3	29.9	31.3	48.7	30.7
(*Z*)−3-hexenyl acetate	44.7	38.9	49.9	42.9	35.3	43.4	43.5	32.7	52.0	39.5	42.7	36.1
(*E*)−2-heptenal	68.5	70.4	58.3	67.2	64.8	74.2	100.0	76.0	58.4	67.3	61.2	67.4
6-methyl-5-hepten-2-one*	34.0	74.2	27.1	78.2	29.8	66.2	36.3	82.7	30.9	74.4	45.9	69.3
1-hexanol*	54.7	43.1	53.1	44.7	39.6	38.0	59.9	44.8	68.2	47.4	52.6	40.8
Nonanal	57.2	59.4	49.6	66.9	61.5	68.1	55.5	60.0	55.5	55.9	63.8	46.0
1-octen-3-ol	114.2	52.4	108.9	43.0	87.4	45.8	79.9	61.6	208.6	56.1	86.0	55.7
(*E*,*E*)−2,4-hexadienal	108.7	103.4	113.2	142.4	126.6	93.3	125.7	79.3	99.0	80.2	79.0	121.6
Acetic acid*	25.1	40.0	26.4	43.5	20.3	39.2	23.0	35.7	23.4	35.4	32.6	46.0
Propanoic acid	39.4	74.5	25.5	76.8	38.3	68.4	61.1	80.4	43.6	70.3	28.3	76.8
(*E*)−2-decenal	88.9	92.3	66.8	94.6	145.2	100.1	78.8	105.5	76.6	71.5	77.3	90.0
Pentanoic acid*	74.7	86.5	80.9	82.8	70.3	91.1	77.5	87.1	76.8	94.6	68.0	77.0

*Significant differences between results from FID and MS at $p \leq 0.05$, two tails by Student's *t*-test.

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 6 Results of Horrat values (HoR) for the five paired samples used in the trial proper.

Volatile compounds	Mean HoR% pairs		HoR 1 and 8		HoR 2 and 4		HoR 3 and 11		HoR 6 and 7		HoR 10 and 12	
	FID	MS	FID	MS	FID	MS	FID	MS	FID	MS	FID	MS
Octane*	1.6	2.2	1.5	2.1	1.5	2.1	1.2	2.1	1.6	2.2	2.2	2.3
Ethyl acetate*	0.9	1.7	0.9	2.0	0.5	1.7	0.7	1.7	1.2	1.6	1.4	1.7
Ethanol*	1.8	3.9	3.1	5.0	2.8	3.0	0.7	3.8	1.0	3.9	1.6	4.0
Ethyl propanoate	3.8	3.4	4.8	2.5	4.3	5.9	3.3	3.3	3.0	2.9	3.7	2.4
Hexanal	2.6	2.0	1.1	1.9	3.4	2.0	4.0	1.8	2.4	2.0	2.2	2.1
3-Methyl-1-butanol*	1.7	2.8	0.7	2.1	1.2	3.1	2.8	3.3	2.2	3.2	1.8	2.3
(*E*)−2-hexenal	2.6	2.5	2.8	3.0	2.3	2.3	2.0	2.7	2.2	2.3	3.7	2.4
(*Z*)−3-hexenyl acetate	2.7	2.4	2.9	2.6	2.4	3.0	2.5	1.9	3.0	2.3	2.5	2.2
(*E*)−2-heptenal	3.5	3.4	2.9	3.3	2.9	3.1	5.2	3.6	3.2	3.5	3.5	3.6
6-methyl-5-hepten-2-one*	1.4	3.0	1.1	3.1	1.2	2.6	1.6	3.5	1.3	3.0	1.9	2.7
1-hexanol*	3.4	2.7	3.0	2.5	2.7	2.6	3.9	2.9	3.9	2.7	3.3	2.6
Nonanal	3.7	3.9	3.6	4.9	3.5	4.2	3.5	3.8	3.2	3.3	4.7	3.4
1-octen-3-ol	4.5	1.9	4.0	1.5	3.0	1.6	2.9	2.2	9.4	2.1	3.3	2.1
(*E*,*E*)−2,4-hexadienal	6.4	4.6	5.6	4.9	7.9	4.2	8.6	3.9	5.4	3.5	4.5	6.3
Acetic acid*	2.0	3.1	2.4	3.9	1.8	3.3	1.7	2.6	1.6	2.4	2.4	3.5
Propanoic acid*	2.0	3.7	1.4	4.0	1.9	3.3	2.9	3.6	2.2	3.2	1.6	4.3
(*E*)−2-decenal	5.1	5.4	3.9	5.9	7.5	5.2	4.6	6.0	4.7	4.2	4.7	5.5
Pentanoic acid	3.1	3.4	3.5	3.3	3.0	3.5	3.1	3.3	3.2	3.7	2.9	3.2

*Significant differences between results from FID and MS at $p \leq 0.05$, two tails by Student's *t*-test.

3-methyl-1-butanol (0.97), 6-methyl-5-hepten-3-one (0.87) showed R^2 higher than 0.6 denoting a certain relationship of higher RDS_R% with lower concentration.

The Horrat value was also calculated for each one of the VOCs. Table 6 shows the Horrat values for the FID and MS methods, which were computed from the 8 participants who used FID and the 12 participants using MS. From this table, it can be observed that 8 VOCs (44.4% of the compounds: octane, ethyl acetate, ethanol, 3-methyl-1-butanol, 6-methyl-5-hepten-2-one, 1-hexanol, acetic acid, and propanoic acid) have significant differences in Horrat values between the two detectors ($p \leq 0.05$, two tails by Student's *t*-test of paired samples), with the Horrat values for the FID method being lower than the MS for all of VOCs, except 1-hexanol. Furthermore, 7 out of 18 volatile compounds have acceptable Horrat mean values (Horrat ≤2) for the FID method. These VOCs were octane, ethyl acetate, ethanol, 3-methyl-1-butanol, 6-methyl-5-hepten-2-one, acetic acid, and propanoic acid.

4 | CONCLUSIONS

The VOC mean concentrations obtained with methods based on SPME-GC-FID and SPME-GC-MS assessed in this study were similar. However, in general terms, the method using FID detector provided better results in terms of reproducibility than the method using MS. The observation of a different reproducibility for the two detectors agrees with our previous experience. In this validation study, the RSD_R% values were lower for SPME-GC-FID in 11 compounds (octane, ethyl acetate, ethanol, 3-methyl-1-butanol, (*E*)−2-heptenal, 6-methyl-5-hepten-2-one, nonanal, acetic acid, propanoic acid, (*E*)−2-decenal, and pentanoic acid). However, that difference is not significant in some cases (e.g., pentanoic acid, nonanal, (*E*)−2-decenal, and (*E*)−2-heptenal), and it is important to keep both detectors for the validation, to consider their different prerogatives together with advantages and disadvantages. FID, which generally costs much less than a MS, and is more commonly used in routine laboratories is adequate for quantitative studies in which the concentration ranges of the analytes are wide, as the case of VOCs in VOO. FID can also be used as a dedicated detector to acquire a lot of data favoring the intercomparison between different labs. In fact, the GC standard methods approved by IOC and EU are mostly based on FID detector. Nonetheless, the method using the MS detector provides the clear advantage of a confirmed identification. Furthermore, most of MS detectors permit quantification with enough linear working range. Regarding the identification of VOCs, new instructions were included in the revised methods, after the pretrial experience to facilitate identification in the SPME-GC-FID method. These instructions seem to be enough since no problem or feedback on this issue were reported in the collected comments in the reporting sheet.

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

This investigation was carried out to study the performance of GC-FID and GC-MS methods for the detection of VOCs in VOO, also considering that the labs may have different configurations and availability of GC-MS or GC-FID only. The methods may be developed further to have different implementation strategies, for example, as an additional/confirmatory method for litigation where there is a disagreement in the sensory classification made by two different panels, as well as screening methods as appropriate. Thus, one of both detectors may show more advantages than the other in one specific implementation strategy. In laboratories where both detectors are available, an optimum approach is to use MS for accurate identification (or confirmations, e.g., in the case of overlapping of peaks) and carry out routine analyses using GC-FID. This strategy fits with the logic of analytical sustainability, that is, to carry out the maximum number of analyzes at a lower cost. Therefore, the analysts, considering their specific objective, the analytes of interest, and the available lab facilities and equipment may choose one of the two detectors, while always keeping in mind the performance characteristics of the methods for each compound.

AUTHOR CONTRIBUTIONS

Diego L. Garcia-Gonzalez: conceptualization; methodology; supervision; validation; writing original draft; writing review & editing. Enrico Casadei: conceptualization; data curation; formal analysis; methodology; writing—original draft; writing—review & editing. Clemente Ortiz Romero: data curation; formal analysis; investigation; methodology; software; validation; writing—review & editing. Enrico Valli: conceptualization; data curation; methodology; supervision; validation; writing—original draft; writing—review & editing. Paul Brereton: conceptualization; data curation; formal analysis; methodology; supervision; validation; visualization; writing—review & editing. Martyna Korytkowska: data curation; formal analysis; investigation; methodology; validation. Maurizio Servili: conceptualization; formal analysis; investigation; methodology; supervision; writing—review & editing. Florence Lacoste: conceptualization; data curation; investigation; methodology; supervision; validation; visualization. Julien Escobessa: data curation; formal analysis; investigation; methodology; validation. Stefania Vichi: data curation; formal analysis; methodology; validation; writing—review & editing. Beatriz Quintanilla-Casas: data curation; formal analysis; methodology; writing—review & editing. Alba Tres: data curation; formal analysis; methodology; validation; writing—review & editing. Pierre-Alain Golay: conceptualization; data curation; formal analysis; methodology; writing—review & editing. Paolo Lucci: conceptualization; data curation; methodology; validation; writing—review & editing. Erica Moret: data curation; formal analysis; methodology; writing—review & editing. Alessandra Bendini: conceptualization; funding acquisition; methodology; supervision; validation; writing—review & editing. Tullia Gallina Toschi: conceptualization; data curation; funding acquisition; investigation; methodology; resources; supervision; validation; writing—review & editing.

ACKNOWLEDGMENTS

The authors would like to thank Fera Science Ltd for preparing and distributing the samples used in the validation study. The authors are also grateful to the sensory panel leader and tasters of the UNIBO professional committee, to the labs that participated in the pretrial and trial proper stages and to the numerous olive oil producers that provided the commercial virgin olive oils for this work. The authors would like to express their gratitude to Prof. Lanfranco Conte for his contribution in terms of discussion and ideas on the herein presented method and validation process. This work was supported by the Horizon 2020 European Research project OLEUM "Advanced solutions for assuring the authenticity and quality of olive oil at a global scale," which has received funding from the European Commission within the Horizon 2020 Programme (2014–2020), grant agreement no. 635690. The information expressed in this article reflects the authors' views; the European Commission is not liable for the information contained herein.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Anastasios Koidis https://orcid.org/0000-0002-9199-8704

REFERENCES

1. Mousavi, S., Mariotti, R., Stanzione, V., Pandolfi, S., Mastio, V., Baldoni, L., & Cultrera, N. G. M. (2021). Evolution of extra virgin olive oil quality under different storage conditions. *Foods*, *10*, 1–19. https://doi.org/10.3390/foods10081945
2. Breschi, C., Guerrini, L., Zanoni, B., Masella, P., Lunetti, L., & Parenti, A. (2022). Simulation of transport under different temperature conditions: Effects on extra virgin olive oil quality. *European Journal of Lipid Science and Technology*, *124*(9), 2100242. https://doi.org/10.1002/ejlt.202100242
3. García-González, D. L., Tena, N., Romero, I., Aparicio-Ruiz, R., Morales, M. T., & Aparicio, R. (2017). A study of the differences between trade standards inside and outside Europe. *Grasas Aceites*, *68*, e210. https://doi.org/10.3989/gya.0446171
4. Conte, L., Bendini, A., Valli, E., Lucci, P., Moret, S., Maquet, A., Lacoste, F., Brereton, P., García-González, D. L., Moreda, W., & Toschi, T. G. (2019). Olive oil quality and authenticity: A review of current EU legislation, standards, relevant methods of analyses, their drawbacks and recommendations for the future. *Trends in Food Science & Technology*, *105*, 483–493. https://doi.org/10.1016/j.tifs.2019.02.025
5. Nenadis, N., Mastralexi, A., & Tsimidou, M. Z. (2019). Physicochemical characteristics and antioxidant potential of the Greek PDO and PGI virgin olive oils (VOOs). *European Journal of Lipid Science and Technology*, *121*, 1800172. https://doi.org/10.1002/ejlt.201800172
6. Rey-Giménez, R., & Sánchez-Gimeno, A. C. (2022). Authenticity in olive oils from an Empeltre clonal selection in Aragon (Spain): How environmental, agronomic, and genetic factors affect sterol composition. *Foods*, *11*, 2587. https://doi.org/10.3390/foods11172587
7. Lozano-Castellón, J., López-Yerena, A., Domínguez-López, I., Siscart-Serra, A., Fraga, N., Sámano, S., López-Sabater, C., Lamuela-Raventós,

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

R. M., Vallverdú-Queralt, A., & Pérez, M. (2022). Extra virgin olive oil: A comprehensive review of efforts to ensure its authenticity, traceability, and safety. *Comprehensive Reviews in Food Science and Food Safety*, *21*, 2639–2664. https://doi.org/10.1111/1541-4337.12949

8. International Olive Council (IOC) (2022). COI/T.15/NC No 3/Rev. 19 November 2022. Trade standard applying to olive oils and olive pomace oils, Madrid. https://www.internationaloliveoil.org/what-we-do/chemistry-standardisation-unit/standards-and-methods/
9. European Union (2022). Commission Implementing Regulation (EU) 2022/2105 of 29 July 2022 laying down rules on conformity checks of marketing standards for olive oil and methods of analysis of the characteristics of olive oil (current consolidated text 04/11/2022). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32022R2105
10. Codex Alimentarius (1981). Standard for olive oils and olive pomace oils. CXS 33–1981. Adopted in 1981. Revised in 1989, 2003, 2015, 2017. Amended in 2009, 2013, 2021. Formerly CAC/RS 33–1970. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B33-1981%252FCXS_033e.pdf.
11. United States Department of Agriculture (USDA) (2010). United States standards for grades of olive oil and olive-pomace oil. Effective October 25, 2010. https://www.ams.usda.gov/grades-standards/olive-oil-and-olive-pomace-oil-grades-and-standards
12. Zaroual, H., Chénè, C., El Hadrami, E. M., & Karoui, R. (2022). Application of new emerging techniques in combination with classical methods for the determination of the quality and authenticity of olive oil: A review. *Critical Reviews in Food Science and Nutrition*, *62*, 4526–4549. https://doi.org/10.1080/10408398.2021.1876624
13. Quintanilla-Casas, B., Bustamante, J., Guardiola, F., García-González, D. L., Barbieri, S., Bendini, A., Gallina Toschi, T., Vichi, S., & Tres, A. (2020). Virgin olive oil volatile fingerprint and chemometrics: Towards an instrumental screening tool to grade the sensory quality. *LWT-Food Science and Technology*, *121*, 108936. https://doi.org/10.1016/j.lwt.2019.108936
14. Barbieri, S., Bubola, K. B., Bendini, A., Bučar-Miklavčič, M., Lacoste, F., Tibet, U., Winkelmann, O., García-González, D. L., & Gallina Toschi, T. (2020). Alignment and proficiency of virgin olive oil sensory panels: The oleum approach. *Foods*, *9*, 355. https://doi.org/10.3390/foods9030355
15. European Commission (EC) (2020). Directorate-General for Agriculture and Rural Development, Study on the implementation of conformity checks in the olive oil sector throughout the European Union, Publications Office, 2020. https://op.europa.eu/it/publication-detail/-/publication/606555af-46ff-11ea-b81b-01aa75ed71a1
16. Casadei, E., Valli, E., Aparicio-Ruiz, R., Ortiz-Romero, C., García-González, D. L., Vichi, S., Quintanilla-Casas, B., Tres, A., Bendini, A., & Gallina Toschi, T. (2021). Peer inter-laboratory validation study of a harmonized SPME-GC-FID method for the analysis of selected volatile compounds in virgin olive oils. *Food Control*, *123*, 107823. https://doi.org/10.1016/j.foodcont.2020.107823
17. Aparicio-Ruiz, R., Ortiz Romero, C., Casadei, E., García-González, D. L., Servili, M., Selvaggini, R., Lacoste, F., Escobessa, J., Vichi, S., Quintanilla-Casas, B., Golay, P. A., Lucci, P., Moret, E., Valli, E., Bendini, A., & Gallina Toschi, T. (2022). Collaborative peer validation of a harmonized SPME-GC-MS method for analysis of selected volatile compounds in virgin olive oils. *Food Control*, *135*, 108756. https://doi.org/10.1016/J.FOODCONT.2021.108756
18. Aparicio-Ruiz, R., Casadei, E., Ortiz Romero, C., García-González, D. L., Servili, M., Selvaggini, R., Lacoste, F., Escobessa, J., Vichi, S., Quintanilla-Casas, B., Tres, A., Golay, P. A., Lucci, P., Moret, E., Valli, E., Bendini, A., & Gallina Toschi, T. (2023). Method for the analysis of volatile compounds in virgin olive oil by SPME-GC-MS or SPME-GC-FID. *MethodsX*, *10*, 101972. https://doi.org/10.1016/j.mex.2022.101972
19. Jurado-Campos, N., Rodríguez-Gómez, R., Arroyo-Manzanares, N., & Arce, L. (2021). Instrumental techniques to classify olive oils according to their quality. *Critical Reviews in Analytical Chemistry*, *53*(1), 139–160. https://doi.org/10.1080/10408347.2021.1940829
20. Caporaso, N., Genovese, A., Pérez-Jiménez, M. A., Olivero-David, R., & Sacchi, R. (2021). Impact of olive harvesting date on virgin olive oil volatile composition in four Spanish varieties. *European Journal of Lipid Science and Technology*, *123*, 2000350. https://doi.org/10.1002/ejlt.202000350
21. Neugebauer, A., Schieberle, P., & Granvogl, M. (2021). Characterization of the key odorants causing the musty and fusty/muddy sediment off-flavors in olive oils. *Journal of Agricultural and Food Chemistry*, *69*, 14878–14892. https://doi.org/10.1021/acs.jafc.1c02228
22. Cecchi, L., Parenti, A., Bellumori, M., Migliorini, M., Mulinacci, N., & Guerrini, L. (2022). Clustering monovarietal extra virgin olive oil according to sensory profile, volatile compounds, and k-mean algorithm. *European Journal of Lipid Science and Technology*, *124*, 2200038. https://doi.org/10.1002/ejlt.202200038
23. Taverniers, I., de Loose, M., & van Bockstaele, E. (2004). Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *Trac, Trends in Analytical Chemistry*, *23*, 535–552. https://doi.org/10.1016/j.trac.2004.04.001
24. Horwitz, W. (1982). Evaluation of analytical methods used for regulation of food and drugs. *Analytical Chemistry*, *54*, 67A–76A. https://doi.org/10.1021/ac00238a765
25. Horwitz, W. (1995). Protocol for the design, conduct and interpretation of method-performance studies. *Pure and Applied Chemistry*, *67*, 331–343. https://doi.org/10.1351/pac199567020331
26. Peeler, J. T., Horwitz, W., & Albert, R. (1989). Precision parameters of standard methods of analysis for dairy products. *Journal—Association of Official Analytical Chemists*, *72*, 784–806. https://doi.org/10.1093/jaoac/72.5.784
27. International Organization for Standardization (ISO) (2019). ISO 5725-2:2019 Accuracy (trueness and precision) of measurement methods and results—Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method. Geneva, Switzerland. https://www.iso.org/standard/69419.html
28. International Organization for Standardization (ISO) (1999). ISO 78-2:1999 Chemistry—Layouts for standards—Part 2: Methods of chemical analysis. Geneva, Switzerland. Standard reviewed and confirmed in 2021. https://www.iso.org/obp/ui/#iso:std:iso:78:-2:ed-2:en
29. Fiorini, D., & Boarelli, M. C. (2016). Solid-phase microextraction may catalize hydrogenation when using hydrogen as carrier in gas chromatography. *Journal of Chromatography A*, *1453*, 134–137. https://doi.org/10.1016/j.chroma.2016.05.045
30. Sansom, C. E., & Perry, N. B. (2022). Analytical artefacts: H^2 carrier gas hydrogenation of plant volatiles during headspace solid-phase microextraction gas chromatography. *Phytochemical Analysis*, *33*, 386–391. https://doi.org/10.1002/pca.3096
31. Gallina Toschi, T., Bendini, A., & Valli, E. (2021). Securing olive oil quality—OLEUM project. *Food Science and Technology*, Online Version. https://doi.org/10.1002/fsat.3510_7.x
32. Thompson, M., Guffogg, S., Stangroom, S., Osborne, P., Key, P., & Wood, R. (2002). Precision estimates produced by specially-designed ruggedness tests compared with those derived from collaborative trials, in relation to estimation of measurement uncertainty. *Analyst*, *127*, 1669–1675. https://doi.org/10.1039/B208975K
33. Lobo-Prieto, A., Tena, N., Aparicio-Ruiz, R., Morales, M. T., & García-González, D. L. (2020). Tracking sensory characteristics of virgin olive oils during storage: Interpretation of their changes from a multiparametric perspective. *Molecules (Basel, Switzerland)*, *25*, 1686. https://doi.org/10.3390/molecules25071686
34. United Nations Office on Drugs and Crime (UNODC). (2009). Calibration/performance verification of instruments and equipment. In: *Guidance for the Validation of Analytical Methodology and Calibration of*

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens. United Nations Publication: New York, USA, 41–47. https://www.unodc.org/documents/scientific/validation_E.pdf
35. Ríos-Reina, R., Aparicio-Ruiz, R., Morales, M. T., & García-González, D. L. (2022). Contribution of specific volatile markers to green and ripe fruity attributes in extra virgin olive oils studied with three analytical methods. *Food Chemistry*, *399*, 133942. https://doi.org/10.1016/j.foodchem.2022.133942

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: García-González, D. L., Casadei, E., Aparicio-Ruiz, R., Ortiz Romero, C., Valli, E., Brereton, P., Koidis, A., Korytkowska, M., Servili, M., Selvaggini, R., Lacoste, F., Escobessa, J., Vichi, S., Quintanilla-Casas, B., Tres, A., Golay, P.-A., Lucci, P., Moret, E., Bendini, A., & Gallina Toschi, T. (2023). Multianalyte analysis of volatile compounds in virgin olive oils using SPME-GC with FID or MS detection: results of an international interlaboratory validation. *European Journal of Lipid Science & Technology*, 2300079. https://doi.org/10.1002/ejlt.202300079

14389312, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ejlt.202300079 by University Polit Delle Marche-Ancona Ctr Ateneo, Wiley Online Library on [22/05/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License