Screening tools combined with multivariate data analysis to predict or confirm virgin olive oil classification by the Panel test

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Running Title: Screening tools to support the Panel test

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Abbreviations: HS-GC-IMS, headspace-gas chromatography-ion mobility spectrometry**, FGC,** flash-gas chromatography**, EU,** European Union**, EVOO,** extra virgin olive oil**, VOO,** virgin olive oil**, LOO,** lampante olive oil**, ACN,** Alert and Cooperation Network**, IOC,** International olive council**, SDG,** sustainable development goal**, HS-SPME,** headspace solid-phase microextraction**, LOX,** lipoxygenases**, GA,** grant agreement **, FID,** flame ionization detector**, PLS-DA,** partial least squares discriminant analysis**, ICQRF,** Central Inspectorate of Quality Protection and Fraud Repression**, SM,** standard mixture**, LDA,** linear discriminant analysis**, kNN,** K-nearest-neighbor**, SVM,** support vector machine**, SPME-GC-MS,** solid-phase microextraction-gas chromatography-mass spectrometry.

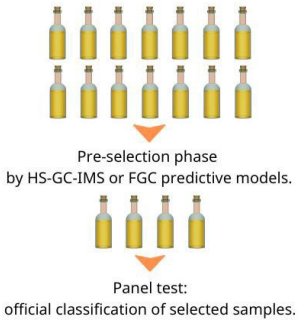
Abstract

A particular aspect of quality control of virgin olive oil (VOO) is the mandatory application, together with chemical and instrumental determinations, of a standardized and official method for sensory assessment. The latter, known as Panel test, is carried out by trained assessors and contributes to the classification of VOOs into three commercial categories (extra virgin, virgin, and lampante). One drawback of this method is related to the large number of samples to be analyzed, compared to the work capacity of a sensory panel, especially during the selection for purchase by companies that blend and market virgin oils and the quality control conducted by the authorities to verify the declared commercial category. For this reason, it is helpful to develop and validate robust and rapid screening methods, based on volatile fingerprints, to pre-classify each sample into one of the three commercial categories. Considering the strict relation between volatile compounds and the main sensory attributes (fruity and defects), a gas chromatographic volatile fingerprint can be the right choice. In this paper, the comparison of two emerging techniques, namely headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS) and flash gas chromatography (FGC), applied on a sample set of 49 VOOs, using calibrations previously built with a larger number of samples, is presented. The number of correctly classified samples, with respect to the commercial category determined by the Panel test, was satisfactory and comparable (92% for HS-GC-IMS, and 94% for FGC), confirming the effectiveness of both methods and the robustness of the predictive models.

Practical applications

The demand for rapid screening tools to reduce the number of samples to be assessed by the Panel test has increased in recent years. The validation of robust models and their joint adoption by companies that market VOOs as well as official control bodies could reduce non-conformities and increase the batches of VOO being controlled, thus better protecting the consumer. Therefore, it is desirable to have different tools available to analyze volatile compounds, together with the associated calibration models, along with detailed instructions for their application, to have different alternatives that suit the equipment of individual laboratories.

Graphical abstract



Rapid screening methods can support sensory analysis by pre-classifying samples to prioritize and/or reduce the number of VOOs to be assessed by the Panel test.

1 Introduction

The European Union (EU) produces annually around 68% of the world's olive oil (around 2 million tonnes), being the main producer, consumer, and exporter.[1] In particular, production is concentrated in the Mediterranean area, where Spain, Greece, Italy, and Portugal are the leading producers. In fact, the latter produced, respectively, 666, 345, 24, and 126 thousand tons of olive oil in 2022/2023 season which amounts to 99.8% of the total European production.[1]

Virgin olive oils (VOOs) can be classified in three commercial categories depending on their quality: extra virgin (EVOO), virgin (VOO) and lampante (LOO).[2] Each category has a different quality that also corresponds to a different price. Usually, a higher price will correspond to a higher quality.[3] For this reason, EVOO, which has the highest quality, has a higher price in the main European countries, which are increased by 18% with respect to VOO in Greece and up to +33% with respect to LOO in Italy in the 2022/2023 crop year.[1]

Because of the high price of EVOO, there is substantial temptation to commit concealment or mislabeling.[4] Indeed, despite the recent decrease of fraudulent cases declared in the ACN (Alert and Cooperation Network) Annual Report in 2022[5], in 2021 fats and oils were the third most reported category, as was number of requests to members in the system related to suspicions of fraud, in which most are related to mislabeling, and, in particular, to olive oil or VOO sold as EVOO.[6]

For this reason, analytical quality assessment classifies the product into three commercial categories and it is essential to verify the quality reported on the label and not mislead the consumer.[7] In the EU, to assess the quality of olive oil the European Commission sets limits and ranges for several parameters and components.[2]

In particular, the sensory evaluation of VOOs (presence/absence and intensity of sensory attributes),

together with chemical-physical analytical determinations, is crucial in determining the product category. The sensory analysis is performed by a Panel test[8], which is the official method to classify VOOs according to their sensory characteristics.[9]
Despite the widespread application of this official method for sensory assessment of VOOs, the Panel test has some weaknesses and limitations[11,12,13]. It is a time-consuming and costly procedure that small businesses cannot afford given that it requires a group of trained experts. Furthermore, the method may be affected by different sensory sensitivities between panels.[14,15] In addition, classifications of VOOs are sometimes debated and misalignments between different panels can be encountered.[16,17]

For these reasons, there is a strong and growing demand for rapid, easy-to use, and environmentally-friendly analytical procedures. In addition, the aforementioned limitations of the Panel test have promoted research in instrumental tools to support sensory analysis, mainly focusing on procedures to limit the number of samples to be evaluated by panelists.[18,19,20]
This can be made possible by adopting techniques that do not require solvents, as the case of the determination of volatile compounds by gas-chromatographic (GC) techniques involving headspace-solid phase microextraction (HS-SPME), ion mobility spectrometry (IMS), or flash-GC (FGC).[21,22] It has been shown that volatile compounds have a crucial role in determining the quality of VOOs, since they are directly responsible for the fruity and other pleasant olfactory notes as well as eventual negative attributes [23,24,25,26,27]; therefore, the application of methods for their determination can be used to support sensory analysis in the classification of VOOs based on quality.[18,20,28]
In particular, the main volatile molecules responsible for the unique positive aroma of VOOs are produced by the primary and secondary biosynthetic pathways of lipoxygenase (LOX).[29] In addition to these, undesirable compounds responsible for off-flavors can originate mainly due to fermentative and oxidation processes.[30,31] Such molecules are responsible for sensory defects in VOO and LOOs, including fusty-muddy sediment, musty-humid-earthy, winey-vinegary, rancid, and frostbitten olives: the perceived intensity of such defects determine a lower quality of the product, which can no longer be marketed as “extra virgin”, but is categorized as “virgin” or “lampante” depending on the intensity of the most perceived defect.

Regarding quality assessment of VOOs, even if the sector is highly regulated, with limits for many parameters, the “current legislation is still fragile and not always reliable, and therefore there is a need for improvements on the ground”.[32]

In particular, although several analytical solutions regarding the analysis of the volatile fraction have been proposed for quality control of VOOs, the acceptance by regulatory bodies involves the development of an internationally accepted and harmonized protocol that is amenable to be validated by an interlaboratory procedure to evaluate its performance. In particular, the main source of variability when comparing different analytical approaches concern the variability of the extraction technique to concentrate volatile compounds, which is the primary basis in their detection and quantification.[29] For this reason, different emerging techniques are now under investigation to develop new methods and protocols.
In this context, two different innovative gas-chromatographic techniques have been tested on a sample set of VOOs to verify the effectiveness of previously developed analytical tools.
One of the techniques adopted herein is HS-GC-IMS, which allows to identify ionized molecules at room temperature based on their mobilities in an electric field.[33] It has been recently used for the study of different food products, such as eggs,[34] sea bass[35] and in the olive oil sector represents a tool that can acquire a digital fingerprint of the aroma of VOOs samples in a relatively simple, rapid, and cost-effective way.[36] Recent studies used this instrument for the geographical origin determination[37] and for the discrimination of quality grades in VOOs.[38] In this work, a semi-targeted method, based on 15 volatile compounds selected as associated with positive and negative sensory attributes in VOOs,[26,39] was applied.[7]
Currently, a HS-GC-IMS screening method is already adopted by official control laboratories in Italy, not only

to reduce the number of the samples to be analyzed, but also to increase the percentage of irregular samples detected, widening the number of samples checked.

The other technique used is FGC, which is characterized by two metal capillary columns endowed with different polarity of the stationary phase and a flame ionization detector (FID) located at the end of each column. This technique gives results in a short time and its application combined with sensory analysis for calibration and chemometric tools are promising to support the Panel test in the discrimination of samples belonging to different commercial categories.[40] As for HS-GC-IMS, the applied method herein was developed within the framework of the European project H2020 OLEUM "Advanced solutions for assuring the authenticity and quality of olive oil at a global scale" (GA no. 635690). Recent studies have reported on the use of FGC for detection of adulterations in both EVOO[41] and milk[42]. In addition, this technique has also been applied to determine the geographical origin of EVOO[43] and other vegetable oils[44] in addition to the traceability and authenticity of plant-based foods.[45]

Both the methods adopted in this work are combined with multivariate classification and partial least squares discriminant analysis (PLS-DA) to classify VOO samples into the three commercial categories as previously published.[7,40] These rapid analytical techniques, combined with chemometric elaborations, can be adopted as screening methods to predict the commercial category of VOOs. The aim is to limit the number of samples that need to be sensory evaluated, thereby pre-classifying VOOs and simplifying quality control procedures. For this reason, to demonstrate the effectiveness of these two previously developed models, a set of 49 VOOs belonging to the three different commercial categories (EVOO, VOO, LOO) was analyzed. The results obtained, in terms of percentages of VOOs that were correctly classified with respect to the category established by four test panel, were compared to those obtained using the previously developed models.

2 Materials and methods

2.1 Samples

The sample set was composed of 49 VOOs obtained during 2021/2022 by the Central Inspectorate for fraud repression and quality protection of the Agrifood Products and Foodstuffs (ICQRF), part of the Italian Ministry of Agriculture. The volume of each sample was 2 L, that was equally divided into 0.5 L aliquots for each of the four sensory panels involved; one aliquot was used for both sensory and volatile compounds analysis, carried out at University of Bologna. These samples, on which a preliminary sensory analysis was carried out in order to have variety and balance between the three commercial categories, were stored at +-12 °C until sensory and volatile fraction analyses.

2.2 Sensory analysis

Four Italian sensory panels carried out the sensory analysis of all samples during the same period of time as the instrumental analyses. Each panel has different public authority recognitions (national authorities; International Olive Council, IOC; national accreditation bodies for EU standards)[46] and takes part in national and/or international interlaboratory proficiency tests (organized by private or public authorities) and/or IOC interlaboratory comparison.

The sensory analysis of the 49 samples was carried out by each panel following established methodology.[47], by 8-12 trained assessors. To obtain the most reliable sensory classification of samples in terms of quality and intensities of positive (fruity) and negative (main perceived defect/s) attributes, a decision tree was applied as described by Barbieri et al.[17] The decision tree is based on the agreement (more than 50% of panels) on the category and on the median of the intensity of the main perceived defect and/or of the fruity attribute. If one of these two agreements was not reached, a first or second type of misalignment occurred, and the sample was not classified.[17] Following the flow of the decision tree, the agreement on the main perceived defect was also verified.[17]

2.3 Sample preparation

Each sample was analyzed by introducing 2 ± 0.1 g of VOO into a hermetically closed 20 mL glass vial and working in a lab at room temperature (20–25°C). Each sample was analyzed in duplicate for both analytical techniques described below.

2.4 HS-GC-IMS analysis of the volatile fraction

2.4.1 Instrumentation

As reported by Valli et al.,[7] HS-GC-IMS analysis was performed with a HS-GC-IMS Flavourspec® instrument (G.A.S. Dortmund, Dortmund, Germany) connected to a nitrogen generator for carrier/drift gas production (Microprogel, Pordenone, Italy). For injection, 100 µL of each sample headspace was withdrawn using a 2.5 mL Hamilton syringe with a 51 mm needle using an HT2000H autosampler (HTA s.r.l., Brescia, Italy) and introduced in a splitless heated injector (2 mm ID, 6.5 mm OD x 78.5 mm fused quartz glass). The analytes passed into a low polar column FS-SE-54-CB-0.5 (30 m, 0.32 mm ID, film thickness 0.5 µm, 94% methyl-5% phenyl-1% vinylsilicone) for a first separation. The eluate was subjected to a second separation by IMS equipped with a tritium ionizing radioactive source at 5000 V and a 9.8 cm long drift tube (Gesellschaft für Analytische Sensorsysteme mbH, G.A.S.; Dortmund, Germany).

2.4.2 Selection of volatile compounds

Following the procedure of Valli et al.,[7] this study is based on a semi-targeted analytical approach that focused on 15 volatile compounds as the minimum number of markers related to fruity and the main negative sensory attributes in VOO, according to the literature[26,39]: 3-methyl-1-butanol (purity ≥ 98.5%), propanoic acid (≥ 99.8%), 6-methyl-5-hepten-2-one (≥ 97.0%), ethyl acetate (≥ 99.8%), (*E*)-2-heptenal (≥ 95.0%), ethyl propanoate (≥ 99.7%), (*E,E*)-2,4-hexadienal (≥ 95.0%), ethanol (≥ 99.9%), acetic acid (≥ 99.8%), 1-octen-3-ol (≥ 98.0%), hexanal (≥ 98.0%), nonanal (≥ 95.0%), (*E*)-2-hexenal (≥ 97.0%), (*Z*)-3-hexenyl acetate (≥ 98.0%), 1-hexanol (≥ 99.9%). All these analytical standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The volatile standards were dissolved in freshly refined olive oil to be analyzed individually, at a concentration of 50 mg kg^{-1}, for identification of each compound in the volatile profile.

2.4.3 Analysis of VOO samples

Samples were analyzed following a previously published method (Valli et al.[7]: the sample was incubated at 40 °C for 8 min and 100 µL of headspace was injected using a heated syringe (80 °C) into the injector (set at 80 °C). The conditions used for GC-IMS analysis (time of equilibrium, etc.) were set-up according to the results of previous trials, as described in Valli et al. [7] The carrier gas (nitrogen gas with inlet pressure of 4 bar) passed through the GC-IMS injector transferring the sample into the GC column using a flow ramp set as follows: the flow was initially set at 2 mL min^{-1} (default) for 2 min, increased to 17 mL min^{-1} for the next 8 min (70% of maximum flow), and maintained at this flow for another 20 min. Finally, the flow was reduced for the next 2 min to the predefined value (2 mL min^{-1}); the end of the program was set at 32 min. The analytes were separated in isothermal mode at 40 °C and introduced into the ionization chamber of the IMS where the tritium source (5000 V) ionized compounds eluting from the GC column and the ions reached the drift tube of the IMS through the shutter grid. The drift tube was maintained at a constant temperature of 45 °C. The gas flow rate of nitrogen introduced in the opposite direction of the sample into the IMS (drift gas) was 150 mL min^{-1}. For each sample, a heat map (3D chromatogram) was obtained: only the 15 selected volatile compounds were identified, thus highlighting their respective signals present as a monomer and/or dimer. The intensity of the signals was exported into a Microsoft Excel file using VOCal software (Gesellschaft für Analytische Sensorsysteme mbH, G.A.S.; Dortmund, Germany). The performance of the method, in terms of linearity and repeatability, was previously assessed by Valli et al.[7]

2.5 FGC analysis of the volatile fraction

2.5.1 Instrumentation and analysis of VOO samples

The FGC system (FGC-E-nose Heracles II, AlphaMos, Toulouse, France) is based on the ultra-fast gas-chromatography of the sample headspace. One of the two columns working in parallel is non-polar (MXT5: 5% diphenyl, 95% methylpolysiloxane, 10 m length and 180 µm diameter) and the other is polar (MXT-1701: 14% cyanopropylphenyl/86% dimethyl polysiloxane, 10 m length, 180 µm diameter). At the end of each column, an FID detector is placed and the acquired signal is digitalized every 0.01 s. As described by Barbieri et al., 2020[40], the analytical conditions applied were the same reported by Melucci et al., 2016.[48] The only difference was related to the temperature of the sample conditioning before injection: the auto-sampler (HS 100, CTC Analytics) moves the vial to an oven, in which it remains for 20 min at 40 °C, and is then shaken at 500 rpm. According to Barbieri et al.,[40] the full chromatogram, obtained from the first column (MXT5), was used for data elaboration.

2.6 Data analysis

2.6.1 HS-GC-IMS data

From the HS-GC-IMS analysis, a 3D chromatogram called a "heatmap", was obtained. Each point in the heatmap is characterized by the GC retention time (seconds), IMS drift time (milliseconds) and intensity of the ion current signal (mV). The raw 3D data were normalized on the reactant ion peak (RIP).[41] The RIP corresponds to the reactant ions or hydrated protons that are generated in the ion source of the IMS device. The analytes interact with the RIP to generate protonated species by the displacement of water.[49] Subsequently, the maximum intensity of the areas (monomer and dimer) belonging to the 15 markers were selected and used to develop the PLS-DA chemometric models (normalized and mean centered values). A total of 25 signals was used, since not all the 15 volatile compounds show both the monomeric and dimeric forms. The data matrix (49x25) was used to externally validate the calibration PLS-DA models developed by Valli et al.[7] In particular, four models were considered: EVOO *vs.* no-EVOO, VOO *vs.* LOO, LOO *vs.* no-LOO, and EVOO *vs.* VOO. The final classification was achieved by evaluating the probability (Bayes's rule) obtained with each model.

2.6.2 FGC data

Raw data from each chromatogram, for a total of 19,900 points, were aligned by a COW algorithm and auto scaled using the PLS Toolbox for Matlab (MatlabR2018a®). Subsequently, the noise was excluded and 8401 points were consecutively selected from the first to the last peak observed in the chromatogram. Similar to the approach used for HS-GC-IMS data, chromatograms were used to externally validate calibration PLS-DA models developed in previously (Barbieri et al., 2020).[40] Four models were also considered for FGC: EVOO *vs.* no-EVOO, VOO *vs.* LOO, LOO *vs.* no-LOO, and EVOO *vs.* VOO. The final classification was made by evaluating the probability (Bayes's rule) obtained with each model.

3 Results and Discussion

3.1 Sensory analysis results

The entire set of 49 VOO samples was sensory evaluated by the official Panel test method[47] by each of the 4 panels and classified as EVOOs, VOOs and LOOs. The commercial category was established by each panel depending on the median of the fruity and most perceived sensory defects, if present. Since the sensory analysis was performed by 4 different panels, the overall classifications were obtained through the application of a decision tree based on the agreement (more than 50% of panels) on the category and on the median of the intensity of the main perceived defect and/or of the fruity attribute.[17] After sensory assessment, 14 samples were classified as EVOO, 20 as VOO, and 15 as LOO. The main defect perceived for most of the VOO and LOO samples, for a total of 29, was fusty/muddy sediment; for 3 samples it was musty-humid-earthy, frostbitten olives for 2 samples, and rancid for 1 sample.

3.2 HS-GC-IMS

HS-GC-IMS analysis was performed on the same sample set. From the heatmaps obtained, the intensity of the 15 volatile compounds was used to predict the commercial category by applying the previously developed PLS-DA calibration models. For each PLS-DA model, the classification was reported as probability of belonging to a certain category (from the second to the fifth column of Table 1). Combining the probability results of each model, the commercial category of each sample was established (last column of Table 1): the samples were classified as 15 EVOO, 17 VOO, 16 LOO, and 1 "borderline" sample between VOO and LOO. 9

Table 1. Results, in terms of probability values, obtained by HS-GC-IMS analysis of the entire sample set applying the four PLS-DA models and relative category.

Sample	EVOO vs. no-EVOO	LOO vs. no-LOO	EVOO vs. VOO	LOO vs. VOO	Resulting category
S1	0.44	0.05	0.25	0.06	VOO
S2	0.13	0.17	0.13	0.12	VOO
S3	0.15	0.05	0.09	0.06	VOO
S4	0.45	0.05	0.23	0.06	VOO
S5	0.73	0.05	0.55	0.07	EVOO
S6	0.84	0.05	0.78	0.06	EVOO
S7	0.77	0.07	0.68	0.12	EVOO
S8	0.74	0.05	0.48	0.06	EVOO
S9	0.34	0.05	0.19	0.06	VOO
S10	0.82	0.06	0.81	0.07	EVOO
S11	0.86	0.05	0.87	0.06	EVOO
S12	0.89	0.07	0.99	0.18	EVOO
S13	0.11	0.15	0.11	0.08	VOO
S14	0.09	0.20	0.11	0.10	VOO
S15	0.11	0.76	0.16	0.31	VOO
S16	0.05	0.49	0.10	0.21	VOO
S17	0.79	0.09	0.48	0.10	EVOO
S18	0.74	0.06	0.60	0.06	EVOO
S19	0.74	0.06	0.59	0.06	EVOO
S20	0.00	1.00	0.05	0.97	LOO
S21	0.00	1.00	0.05	0.98	LOO
S22	0.00	1.00	0.05	0.97	LOO
S23	0.00	1.00	0.05	0.99	LOO
S24	0.07	0.53	0.10	0.43	VOO
S25	0.06	0.88	0.11	0.56	LOO
S26	0.00	1.00	0.05	1.00	LOO
S27	0.00	1.00	0.05	1.00	LOO
S28	0.01	0.39	0.05	0.31	VOO
S29	0.02	0.15	0.05	0.14	VOO
S30	0.00	1.00	0.05	1.00	LOO
S31	0.68	0.05	0.44	0.06	EVOO
S32	0.78	0.07	0.68	0.11	EVOO
S33	0.00	1.00	0.05	1.00	LOO
S34	0.69	0.05	0.44	0.06	EVOO

S35	0.49	0.05	0.21	0.06	VOO
S36	0.79	0.06	0.57	0.06	EVOO
S37	0.00	1.00	0.05	0.94	LOO
S38	0.03	1.00	0.06	1.00	LOO
S39	0.00	1.00	0.05	1.00	LOO
S40	0.00	1.00	0.05	1.00	LOO
S41	0.00	1.00	0.05	0.98	LOO
S42	0.00	1.00	0.05	1.00	LOO
S43	0.10	0.31	0.12	0.16	VOO
S44	0.00	1.00	0.05	1.00	LOO
S45	0.86	0.16	0.98	0.06	EVOO
S46	0.09	0.65	0.11	0.20	VOO
S47	0.20	0.06	0.14	0.06	VOO
S48	0.18	0.18	0.17	0.11	VOO
S49	0.05	0.78	0.10	0.47	Borderline

Borderline samples had uncertain classification, since the probability of belonging to a certain category is around 50% or showed misalignment in the results. Misalignment occurs in case of discordant classifications among the PLS-DA models. In this case, based on the probabilities obtained by each PLS-DA model, it is impossible to assign a specific commercial category and, consequently, the sample was classified at the boundary between two categories. Sample S49 was between the VOO and LOO categories, showing a probability of 78% of belonging to LOO *vs.* no-LOO, but a 47% of belonging to LOO *vs.* VOO. Considering the sensory classification, this was a VOO sample with an average value of the medians of the intensity of the main perceived defect, fusty-muddy, of 2.5 ± 0.7, which is at the border between the limits of the two categories (according to the EU regulations, a value ≤3.5 determines the VOO category, and with a value >3.5 the sample is classified as LOO[2]). In other cases, the probability of belonging to LOO category was 100% (e.g. S26 and S27), in both the models LOO *vs*. no-LOO and LOO *vs.* VOO, with zero or low probability of belonging to the EVOO category, giving no misalignment in the results.

For each PLS-DA model, the results, in terms of percentage of correctly classified samples, are reported in the second column of Table 2, were compared to the sensory results obtained from the 4 panels. results, considering the four models, ranged from 85% to 100%. The best outcome was obtained for the EVOO *vs*. no-EVOO model (100%, see Fig. 1), while the worst was the LOO *vs*. no-LOO model (85%). As shown in Table 2, these values were similar or higher to those reported by Valli et al.[7] (third column) for the external validation of the same PLS-DA calibration model. In this case, the worst result was obtained for EVOO *vs*. VOO (67%). This is probably due to the fact that in some samples the volatile profile is quite similar between VOO and EVOO, which could lead to a difficultly in discriminating between the two categories by the predictive model.

Table 2. Results of HS-GC-IMS analysis: percentages of correctly classified samples considered in this study (49) (second column) and percentages of samples correctly classified in external validation according to the study by Valli et al.[7] (third column).

PLS-DA model	% correctly classified samples	% reported by Valli et al.[7]
EVOO	100	74
no-EVOO	97	77
LOO	100	73

no-LOO	85	95
EVOO	95	70
VOO	91	67
LOO	100	77
VOO	94	87

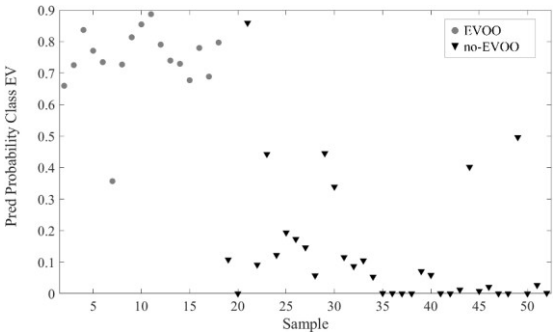


Figure 1. Class prediction probability by the single model EVOO *vs*. no-EVOO for HS-GC-IMS. EVOO samples: gray circles; no-EVOO samples: black triangles.

These results were comparable with those reported in similar studies, such as Contreras et al.,[38] in which PLS-DA predictive models were applied to HS-GC-IMS data as well. In particular, their targeted approach included EVOO vs. no-EVOO and LOO vs. no-LOO models, with the latter showing 90% of samples as correctly classified, [38] which is very close to those reported herein.

In addition, the studies by Gerhardt et al.[50] and García-Nicolás et al.,[51] with the same aim in which similar chemometric approaches were applied, showed comparable results. In the first study, LDA, kNN, and SVM methods were used to develop predictive models for olive oil classification into the three commercial categories through the identification and quantification of 26 target compounds. The LDA, kNN, and SVM models correctly classified 83.3%, 73.8%, and 88.1% of samples analyzed by HS-GC-IMS, respectively.[50] In the second study, a kNN approach was applied to HS-GC-IMS data and 85.7% of samples in the external validation set were correctly classified.[51]

3.3 FGC

FGC was performed on the same sample set, applying an untargeted method based on the full chromatograms and applying a fingerprinting approach of the entire profiles of volatile molecules without identification and quantification.

Calibration PLS-DA models (EVOO *vs.* no-EVOO, VOO *vs.* LOO, LOO *vs.* no-LOO, and EVOO *vs.* VOO), previously developed by Barbieri et al.,[40] were validated by the new data set (49 samples). To define the commercial category, the same probabilistic approach adopted for the HS-GC-IMS data was used.

The results of the four PLS-DA models are shown in Table 3, reported as probabilities of belonging to a specific category. In particular, 14 samples were classified as EVOO, 20 as VOO, 13 as LOO, and 2 as borderline.

Table 3. FGC analysis applying the four PLD-DA models, reported as probability of belonging to a commercial category. 14 samples are classified as EVOO, 20 as VOO, 13 as LOO, and 2 as borderline.

Sample	**EVOO vs. no-EVOO**	**LOO vs. no-LOO**	**EVOO vs. VOO**	**LOO vs. VOO**	**Resulting category**
S1	0.40	0.04	0.29	0.07	VOO

S2	0.26	0.18	0.23	0.15	VOO
S3	0.42	0.05	0.19	0.08	VOO
S4	0.53	0.04	0.48	0.07	VOO
S5	0.84	0.04	0.84	0.09	EVOO
S6	0.99	0.20	0.96	0.12	EVOO
S7	0.95	0.17	0.95	0.12	EVOO
S8	0.86	0.20	0.80	0.12	EVOO
S9	0.39	0.04	0.16	0.07	VOO
S10	0.95	0.11	0.93	0.12	EVOO
S11	0.99	0.20	0.95	0.12	EVOO
S12	1.00	0.20	0.98	0.12	EVOO
S13	0.37	0.04	0.10	0.07	VOO
S14	0.33	0.06	0.13	0.09	VOO
S15	0.25	0.07	0.20	0.08	VOO
S16	0.22	0.09	0.05	0.10	VOO
S17	0.88	0.05	0.79	0.11	EVOO
S18	0.92	0.04	0.94	0.07	EVOO
S19	0.98	0.06	0.96	0.10	EVOO
S20	0.03	1.00	0.00	1.00	LOO
S21	0.04	1.00	0.00	1.00	LOO
S22	0.12	0.98	0.00	1.00	LOO
S23	0.13	1.00	0.00	1.00	LOO
S24	0.30	0.04	0.01	0.09	VOO
S25	0.20	0.05	0.00	0.08	VOO
S26	0.00	1.00	0.00	0.89	LOO
S27	0.65	1.00	0.48	0.12	LOO
S28	0.26	0.40	0.00	0.87	Borderline
S29	0.43	0.07	0.00	0.28	VOO
S30	0.14	0.68	0.00	1.00	LOO
S31	0.88	0.04	0.75	0.07	EVOO
S32	0.98	0.07	0.91	0.10	EVOO
S33	0.19	1.00	0.00	1.00	LOO
S34	0.93	0.06	0.82	0.12	EVOO
S35	0.62	0.04	0.15	0.07	VOO
S36	1.00	0.20	0.95	0.12	EVOO
S37	0.20	0.70	0.00	1.00	LOO
S38	0.13	0.13	0.00	0.12	VOO
S39	0.18	0.04	0.00	0.07	VOO
S40	0.52	0.97	0.00	1.00	LOO
S41	0.17	0.63	0.00	0.18	LOO
S42	0.58	1.00	0.39	1.00	LOO
S43	0.25	0.08	0.05	0.11	VOO
S44	0.14	0.83	0.00	0.48	LOO
S45	0.16	0.26	0.63	0.12	Borderline
S46	0.04	0.33	0.00	0.12	VOO
S47	0.36	0.06	0.56	0.08	VOO

S48	0.37	0.07	0.60	0.09	VOO
S49	0.21	0.10	0.21	0.08	VOO

Specifically, two borderline samples were characterized by contradictory probability values that do not allow classification of the sample in a single category. S28 sample showed a high probability of belonging to the LOO category (LOO *vs*. VOO), but this was not confirmed by the values of the other models. Considering sensory evaluation, this sample was classified by the decision tree as VOO, with an average value among the median of the main perceived defect (fusty-muddy) of 2.0 ± 0.5. For S45, the probability of belonging to the EVOO category compared to VOO is high, but was not sufficient to allow definite classification in this category, considering the probabilities given by the other models. In this case, considering the sensory evaluation, the main perceived defect was frostbitten olives with an average value among the medians of 1.8 ± 0.4, and was classified as VOO.

Table 4 shows the percentages of correctly classified samples, comparing the results obtained considering the 4 PLS-DA models individually and the results of sensory analysis. The highest percentage was obtained for the EVOO *vs*. no-EVOO model (100%, Fig. 2). The correct prediction of LOOs showed lower percentages, both in the second and in the fourth models (LOO *vs* no-LOO and LOO *vs* VOO) in which there were the lowest percentage (87%, for the second, and 67%, for the fourth). Comparing these results with those obtained in the external validation in the study by Barbieri et al.,[40] it is possible to affirm that, in general, the percentages of correctly classified samples, within the range of 67-100%, are comparable to those obtained in the previous study[40] in which they ranged from 72% to 85%.

Table 4. FGC analysis: percentages of correctly classified samples considered in this study (49) (second column) and percentages of correctly classified samples in external validation according to Barbieri et al. (third column).

PLS-DA model	% correctly classified samples	% reported by Barbieri et al.[40]
EVOO	100	81
no-EVOO	94	77
LOO	86	85
no-LOO	100	85
EVOO	100	73
VOO	91	85
LOO	67	72
VOO	94	85

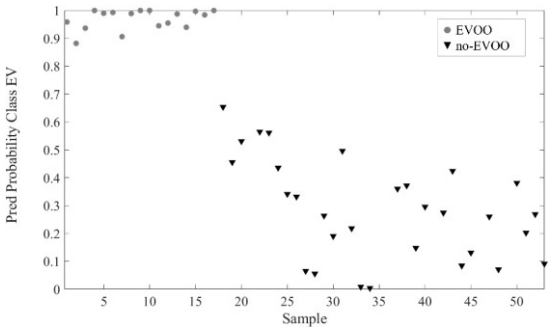


Figure 2. Class prediction probability by the single model EVOO *vs.* no-EVOO for FGC. EVOO samples: gray circles; no-EVOO samples: black triangles.

While FGC has been used for different purposes in the framework of analysis of VOO, such as the detection of adulterations[41] and for determination of geographical origin[43,38] in EVOOs, to our knowledge only the study by Barbieri et al.[40] described a quality classification based on predictive models. For this reason, this approach is considerably innovative, and use of FGC analysis to predict quality needs to be studied further, possibly using a larger sample set, to confirm the effectiveness of the method and the results obtained herein.

3.4 HS-GC-IMS and FGC: correctly classified samples for each commercial category

In Table 5 the results obtained by FGC and HS-GC-IMS are reported as number and percentage of correctly classified samples for each commercial category with respect to sensory assessment. For HS-GC-IMS, the highest percentage of correctly classified samples was for EVOO (100%) and the lowest for VOO (85%). For FGC, the highest percentage of correctly classified samples was for EVOO (100%) and the lowest for LOO (86%). The percentage of the overall total number of correctly classified samples was comparable between the two techniques: 92% for HS-GC-IMS and 94% for FGC, thus showing satisfactory and comparable reliability. As noted above, the percentages of correctly classified samples herein were higher than those obtained in previous reports.[5,38] These results demonstrate that the previously developed models are effective when applied to a new set of VOOs, despite the time passed between this investigation and the previous analyses.

Table 5. Comparison of correctly classified samples for each commercial category by HS-GC-IMS and FGC.

	HS-GC-IMS		***FGC***	
COMMERCIAL CATEGORY	**CORRECTLY CLASSIFIED SAMPLES**	**%**	**CORRECTLY CLASSIFIED SAMPLES**	**%**
EVOO	14/14	100	14/14	100
VOO	17/20	85	19/20	95
LOO	14/15	93	13/15	86
TOTAL	**45/49**	**92**	**46/49**	**94**

The results obtained were comparable with those reported by similar studies.[52,53] For example, Quintanilla-Casas et al.[52] applied EVOO *vs.* no-EVOO and VOO *vs.* LOO PLS-DA models on SPME-GC-MS data and 84% of samples were correctly classified (as mean % among the three commercial categories). Furthermore, Cecchi et al.[53] reported the best results with 83.5% of samples correctly classified using a PCA-LDA approach on SPME-GC-MS data to discriminate EVOOs *vs.* defective samples.

Comparison with these studies revealed satisfactory results, since the percentages (of correctly classified samples obtained herein were higher, also considering that FGC is a rapid technique which requires substantially less time compared to other gas chromatographic techniques, such as SPME-GC-MS.

In addition, a more innovative approach might be achieved by low-level data fusion between HS-GC-IMS and FGC data as in the study reported by Tata et al.[54] in which promising results were reported for detection of soft-refined oils in EVOO. This type of approach could also be applied to classify VOOs in the three commercial categories.

Finally, to support the panel test, the use of different spectroscopic techniques that investigate the non-volatile fraction of VOOs to determine their quality may be promising. In particular, non-destructive analysis like near-infrared spectroscopy (NIR) can be useful to provide chemical information on the quality of VOOs.[55] These techniques may be useful in providing additional information to allow more robust classification of rancid samples.

4 Conclusions

Analytical approaches using HS-GC-IMS and FGC with chemometric elaborations and established calibrations, both previously tested to screen the volatile fraction of VOOs, set of 49 VOOs to compare the commercial category predicted with that determined by sensory panels.

The mean percentage of correctly classified samples was higher than 92% for both techniques. This result may represent a cross validation for these screening tools, demonstrating that both are robust enough to support the daily work of sensory panels.

For HS-GC-IMS, a semi-targeted approach was applied, through the selection of specific volatile compounds. This screening tool is already used by official control laboratories to increase the percentage of irregular samples detected, by increasing the number of samples checked daily. On the other hand, the FGC method, by applyingied with an untargeted approach, ensuresing even faster chromatographic separation (about 200 s). The calibrations of such screening methods can also be linked to those obtained with a targeted method, already validated and officially recognizable in the future, to support the Panel test. Indeed, in case of disagreement between panels, one or more validated signals, associated with specific defects (i.e. volatile compounds), can be useful to reach indisputable resolution of the disaccord.

Further efforts to improve the robustness and prediction capability of the models, i.e. by increasing the number of samples analyzed, sharing data and calibration models between official labs , and adopting data fusion approaches with other techniques, can be also of value.

The authors have declared no conflict of interest.

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Tables ((including table captions))

Table 1. Results, in terms of probability values, obtained by HS-GC-IMS analysis of the entire sample set applying the four PLS-DA models and relative category.

Sample	EVOO vs. no-EVOO	LOO vs. no-LOO	EVOO vs. VOO	LOO vs. VOO	Resulting category
S1	0.44	0.05	0.25	0.06	VOO
S2	0.13	0.17	0.13	0.12	VOO
S3	0.15	0.05	0.09	0.06	VOO
S4	0.45	0.05	0.23	0.06	VOO
S5	0.73	0.05	0.55	0.07	EVOO

S6	0.84	0.05	0.78	0.06	EVOO
S7	0.77	0.07	0.68	0.12	EVOO
S8	0.74	0.05	0.48	0.06	EVOO
S9	0.34	0.05	0.19	0.06	VOO
S10	0.82	0.06	0.81	0.07	EVOO
S11	0.86	0.05	0.87	0.06	EVOO
S12	0.89	0.07	0.99	0.18	EVOO
S13	0.11	0.15	0.11	0.08	VOO
S14	0.09	0.20	0.11	0.10	VOO
S15	0.11	0.76	0.16	0.31	VOO
S16	0.05	0.49	0.10	0.21	VOO
S17	0.79	0.09	0.48	0.10	EVOO
S18	0.74	0.06	0.60	0.06	EVOO
S19	0.74	0.06	0.59	0.06	EVOO
S20	0.00	1.00	0.05	0.97	LOO
S21	0.00	1.00	0.05	0.98	LOO
S22	0.00	1.00	0.05	0.97	LOO
S23	0.00	1.00	0.05	0.99	LOO
S24	0.07	0.53	0.10	0.43	VOO
S25	0.06	0.88	0.11	0.56	LOO
S26	0.00	1.00	0.05	1.00	LOO
S27	0.00	1.00	0.05	1.00	LOO
S28	0.01	0.39	0.05	0.31	VOO
S29	0.02	0.15	0.05	0.14	VOO
S30	0.00	1.00	0.05	1.00	LOO
S31	0.68	0.05	0.44	0.06	EVOO
S32	0.78	0.07	0.68	0.11	EVOO
S33	0.00	1.00	0.05	1.00	LOO
S34	0.69	0.05	0.44	0.06	EVOO
S35	0.49	0.05	0.21	0.06	VOO
S36	0.79	0.06	0.57	0.06	EVOO
S37	0.00	1.00	0.05	0.94	LOO
S38	0.03	1.00	0.06	1.00	LOO
S39	0.00	1.00	0.05	1.00	LOO
S40	0.00	1.00	0.05	1.00	LOO
S41	0.00	1.00	0.05	0.98	LOO
S42	0.00	1.00	0.05	1.00	LOO
S43	0.10	0.31	0.12	0.16	VOO
S44	0.00	1.00	0.05	1.00	LOO
S45	0.86	0.16	0.98	0.06	EVOO
S46	0.09	0.65	0.11	0.20	VOO
S47	0.20	0.06	0.14	0.06	VOO
S48	0.18	0.18	0.17	0.11	VOO
S49	0.05	0.78	0.10	0.47	Borderline

Table 2. Results of HS-GC-IMS analysis: percentages of correctly classified samples considered in this study (49) (second column) and percentages

of samples correctly classified in external validation according to the study by Valli et al.[7] (third column).

PLS-DA model	% correctly classified samples	% reported by Valli et al.[7]
EVOO	100	74
no-EVOO	97	77
LOO	100	73
no-LOO	85	95
EVOO	95	70
VOO	91	67
LOO	100	77
VOO	94	87

Table 3. FGC analysis applying the four PLD-DA models, reported as probability of belonging to a commercial category. 14 samples are classified as EVOO, 20 as VOO, 13 as LOO, and 2 as borderline.

Sample	EVOO vs. no-EVOO	LOO vs. no-LOO	EVOO vs. VOO	LOO vs. VOO	Resulting category
S1	0.40	0.04	0.29	0.07	VOO
S2	0.26	0.18	0.23	0.15	VOO
S3	0.42	0.05	0.19	0.08	VOO
S4	0.53	0.04	0.48	0.07	VOO
S5	0.84	0.04	0.84	0.09	EVOO
S6	0.99	0.20	0.96	0.12	EVOO
S7	0.95	0.17	0.95	0.12	EVOO
S8	0.86	0.20	0.80	0.12	EVOO
S9	0.39	0.04	0.16	0.07	VOO
S10	0.95	0.11	0.93	0.12	EVOO
S11	0.99	0.20	0.95	0.12	EVOO
S12	1.00	0.20	0.98	0.12	EVOO
S13	0.37	0.04	0.10	0.07	VOO
S14	0.33	0.06	0.13	0.09	VOO
S15	0.25	0.07	0.20	0.08	VOO
S16	0.22	0.09	0.05	0.10	VOO
S17	0.88	0.05	0.79	0.11	EVOO
S18	0.92	0.04	0.94	0.07	EVOO
S19	0.98	0.06	0.96	0.10	EVOO
S20	0.03	1.00	0.00	1.00	LOO
S21	0.04	1.00	0.00	1.00	LOO
S22	0.12	0.98	0.00	1.00	LOO
S23	0.13	1.00	0.00	1.00	LOO
S24	0.30	0.04	0.01	0.09	VOO
S25	0.20	0.05	0.00	0.08	VOO
S26	0.00	1.00	0.00	0.89	LOO
S27	0.65	1.00	0.48	0.12	LOO
S28	0.26	0.40	0.00	0.87	Borderline

S29	0.43	0.07	0.00	0.28	VOO
S30	0.14	0.68	0.00	1.00	LOO
S31	0.88	0.04	0.75	0.07	EVOO
S32	0.98	0.07	0.91	0.10	EVOO
S33	0.19	1.00	0.00	1.00	LOO
S34	0.93	0.06	0.82	0.12	EVOO
S35	0.62	0.04	0.15	0.07	VOO
S36	1.00	0.20	0.95	0.12	EVOO
S37	0.20	0.70	0.00	1.00	LOO
S38	0.13	0.13	0.00	0.12	VOO
S39	0.18	0.04	0.00	0.07	VOO
S40	0.52	0.97	0.00	1.00	LOO
S41	0.17	0.63	0.00	0.18	LOO
S42	0.58	1.00	0.39	1.00	LOO
S43	0.25	0.08	0.05	0.11	VOO
S44	0.14	0.83	0.00	0.48	LOO
S45	0.16	0.26	0.63	0.12	Borderline
S46	0.04	0.33	0.00	0.12	VOO
S47	0.36	0.06	0.56	0.08	VOO
S48	0.37	0.07	0.60	0.09	VOO
S49	0.21	0.10	0.21	0.08	VOO

Table 4. FGC analysis: percentages of correctly classified samples considered in this study (49) (second column) and percentages of correctly classified samples in external validation according to Barbieri et al. (third column).

PLS-DA model	% correctly classified samples	% reported by Barbieri et al.[40]
EVOO	100	81
no-EVOO	94	77
LOO	86	85
no-LOO	100	85
EVOO	100	73
VOO	91	85
LOO	67	72
VOO	94	85

Table 5. Comparison of correctly classified samples for each commercial category by HS-GC-IMS and FGC.

	HS-GC-IMS		*FGC*	
COMMERCIAL CATEGORY	**SAMPLES CORRECTLY CLASSIFIED**	**%**	**SAMPLES CORRECTLY CLASSIFIED**	**%**
EVOO	14/14	100	14/14	100
VOO	17/20	85	19/20	95
LOO	14/15	93	13/15	86
TOTAL	**45/49**	**92**	**46/49**	**94**

Figures ((please label the figures))

Figure 1

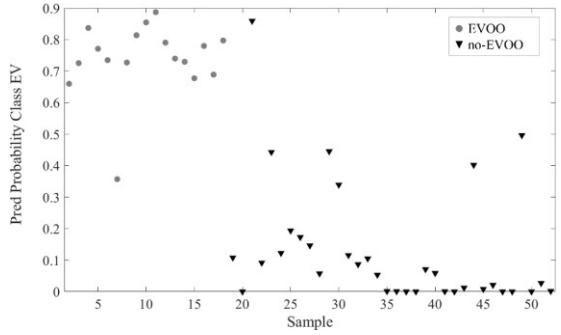

Figure 2

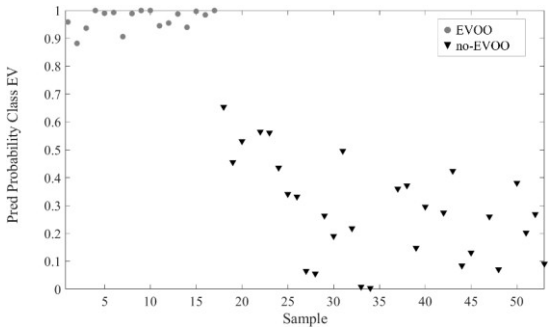

Figure legends

Figure 1. Class prediction probability by the single model EVOO vs. no-EVOO for HS-GC-IMS. EVOO samples: gray circles; no-EVOO samples: black triangles.

Figure 2. Class prediction probability by the single model EVOO vs. no-EVOO for FGC. EVOO samples: gray circles; no-EVOO samples: black triangles.