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Robust application of a chemometric model based on the relationships between 10 volatile compounds and sensory attributes to support the panel test in virgin olive oil quality classification in olive oil companies

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ABSTRACT

Several approaches have been proposed to support the panel test in virgin olive oil classification, but none of them is currently applied in olive oil companies. Aim of this study was the robust application of a chemometric model in a big olive oil company. The application on 244 samples of the PCA-LDA model developed in 2019, based on volatile profile by HS-SPME-GC-MS, gave unsatisfactory results, pointing out critical issues relating to the training-set, variable selection and validation. Therefore, a new *t-test-FwS-LDA* model was developed; it was based on a very wide dataset (approx. 1800 samples from 6 different production years) and on an algorithm for a stepwise selection of variables. The crucial role of the production year has been proven and included in the model. Ten volatile molecules were thus selected coming from both the lipoxygenase pathway and several virgin olive oil sensory defects. The new model was two-fold validated with 53 and 273 samples coming from production years belonging and not belonging to the training-set, respectively, with very satisfactory results (>90 % and 80 % correct classification, respectively). Finally, the study indicated that for routinary application of the model, year-by-year updating of training-set and variable selection is required.

1. Introduction

Virgin olive oil (VOOx) is the principal fat of the Mediterranean Diet. Since it is obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions that do not lead to alteration to the oil, VOOx has unique nutritional and sensory values for the consumers (Rodrigues et al., 2020; Cecchi et al., 2021; COI, 2024). Regulatory bodies such as the International Olive Council (IOC), the European Union Commission (EU) and the Codex Alimentarius have defined several inherent characteristics to classify VOOx in commercial categories with decreasing quality as follows: extra virgin olive oil (EVOO), virgin olive oil (VOO) and lampante virgin olive oil (LVOO) (Conte et al., 2020). Oils belonging to the latter category (LVOO) are not edible but must be refined and mixed with VOO or EVOO to obtain the common olive oil category (OO).

Unique case in the food sector, the conformity with specific values of negative and positive sensory attributes is mandatory in the EU for the above categories (Conte et al., 2020; Gerhardt et al., 2019; Peres et al., 2013; Cecchi et al., 2021). A VOOx with no sensory defects (i.e., median = 0) is classified as EVOO, while it is downgraded to VOO or LVOO category when it presents sensory defects smaller or greater than 3.5, respectively (Aparicio-Ruiz et al., 2019). Based on literature data (Wittes and Turk, 1968; Gutierrez Rosales et al., 1984), the sensory analysis procedure was standardized by the IOC, and it was included in the EU olive oil regulation with Reg EEC No 2568/91 (Fernandes et al., 2018; Amelio, 2016), which was recently repealed by the Regulations

Abbreviations: EVOO, Extra Virgin Olive Oil Sub-category; FwS, Forward selection; HS-SPME-GC-MS, Head Space-Solid Phase Micro Extraction-Gas Chromatography-Mass Spectrometry; IOC, International Olive Council; LDA, Linear Discriminant Analysis; LOX, lipoxygenase; LVOO, lampante virgin olive oil sub-category; MISN, Multiple Internal Standard Normalization; OO, olive oil; PCA, Principal Component Analysis; VOCs, Volatile Organic Compounds; VOO, Virgin Olive Oil Sub-category; VOOx, Virgin Olive Oil Category.

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(EU) No 2104/2022 and No 2105/2022 (European Parliament and the Council (EC), 2022a, 2022b). An essential role on measurement of the sensory attribute intensity is played by the Panel Test, which must be carried out by a group of tasters selected, trained and monitored as a panel (Conte et al., 2020; Gerhardt et al., 2019; Peres et al., 2013; Cecchi et al., 2021). However, some critical issues were raised by the literature concerning the Panel Test (Aparicio-Ruiz et al., 2019; Conte et al., 2020; Gerhardt et al., 2019; Quintanilla-Casas et al., 2020; Cecchi et al., 2021). The Panel test is not very time- and cost-effective due to the limited number of samples that can be evaluated per day, the need for a permanent group of qualified tasters, and the lack of suitable reference standards. Moreover, the oil samples with a median of defect lower than 1.5 (i.e., the so-called "borderline" samples between the VOO and EVOO categories) are recognized as difficult to be classified by the Panel Test (Cecchi et al., 2019; Valli et al., 2020).

These critical issues are very important for those medium to large enterprises operating in the filtration, blending, storage and packaging of VOOx, which widely use the Panel Test as a decision-making tool for both the supply and blending of olive oil. Therefore, companies can benefit particularly from having predictive models to support the Panel Test in VOOx quality grade evaluation by significant relationships with inherent chemical characteristics. Some predictive models have been developed (del Mar Contreras et al., 2019; Cecchi et al., 2019; Gerhardt et al., 2019; Quintanilla-Casas et al., 2020; Valli et al., 2020; Grigoletto et al., 2024), based generally on the relationship between volatile organic compounds (VOCs) content and intensity of virgin olive oil sensory attributes (Cecchi et al., 2021; Gerhardt et al., 2019; Mascrez et al., 2024; Quintanilla-Casas et al., 2020; Luna et al., 2006; Oliver-Pozo et al., 2019; Valli et al., 2020). The Head Space-Solid Phase Micro Extraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS) was more often applied for the analysis of VOCs (Cecchi et al., 2019; Romero et al., 2015; Lioupi et al., 2022; Aparicio-Ruiz et al., 2018; Quintanilla-Casas et al., 2020), due to the several advantages described in the literature (Cecchi et al., 2021; Oliver-Pozo et al., 2015; Calamai et al., 2012; Pawliszyn, 1999).

However, none of the proposed predictive models have been recognized by EU/IOC and routinely applied in companies, likely due to the need to contemporary fulfil the following non-trivial requirements for the development of the predictive model: i) HS-SPME is a sampling technique at equilibrium, and when the goal is quantitation of many VOCs, suitable analytical approaches should be used to avoid unbiased quantitation (Fortini et al., 2017; Romero et al., 2015; Oliver-Pozo et al., 2015; Calamai et al., 2012); ii) the database shall consist of data concerning VOCs and sensory attributes from as many VOOx samples as possible from different production years and geographical origins; iii) since the above database includes huge volumes of highly complex data (Cecchi et al., 2021), suitable chemometric tools are required for the development of the predictive model; iv) the model must be validated by routine application in the following years to check its reliability in VOOx samples' classification, and adjusted if necessary. Cecchi et al. (2019) created a database including data of VOCs contents and sensory attributes intensity measured on more than 1200 VOOx samples from 3 consecutive olive oil production years and from the main producing countries. The analysis of VOCs was carried out through a reliable HS-SPME-GC-MS quantitative method based on the Multiple Internal Standard Normalization (MISN) approach according to Fortini et al. (2017). Finally, a chemometric approach was adopted and applied to such a data-set to develop a model to support the work of the Panel Test in olive oil company (Cecchi et al., 2019).

The aim of the present study is the robust application of the above model in olive oil blending companies for the validation and improvement of its ability in supporting the Panel Test in virgin olive oil classification. 1796 VOOx samples from the 2016–17, 2017–18, 2018–19, 2021–22, 2022–23 and 2023–24 olive oil production years were analysed by Panel Test and HS-SPME-GC-MS methods and used as data-set.

2. Materials and methods

2.1. Chemicals and standards preparation

All commercial standards used for VOCs analysis by HS-SPME-GC-MS were from Merck (Darmstadt, Germany); they included 8 internal standards and 72 external standards, and their purity is reported in Table S1 of Supplementary Material. Two standard solutions (one with the external standards and one with the internal standards listed in Table S1) were prepared according to the Multiple Internal Standard Normalization (MISN) approach (Fortini et al., 2017), by dissolving suitable amounts of the commercial standards in a refined olive oil sample, which was previously verified as free from interfering VOCs. The solutions were stored at −20 °C until used for HS-SPME-GC-MS analysis.

2.2. Samples

All the 1796 virgin olive oil samples used in the study were collected from Carapelli Firenze S.p.A. (Tavarnelle Val di Pesa, Florence, Italy), a company operating in bottling VOOx purchased from all over the world. In particular, the sample-set included the 1223 virgin olive oil samples studied by Cecchi et al. (2019) during the 2016–17, 2017–18 and 2018–19 olive oil production years and 573 new VOOx samples as follows: (i) 297 samples from the 2021–2022 and 2022–2023 olive oil production years; (ii) 276 samples from the 2023–2024 olive oil production year (Fig. 1). The 573 new VOOx samples were analysed as described in the following paragraphs, and the obtained data were added to the data of Cecchi et al. (2019). All the samples were from the main worldwide producing countries (i.e., Spain, Italy, Greece, Portugal and Tunisia). Both the chemical and sensory analyses described below were carried out simultaneously, as soon as the samples arrived in the laboratory.

2.3. Analysis of legal chemical parameters

The sample legal quality parameters such as free acidity, peroxide value and UV spectrophotometric indices were measured according to the European Regulations 2104/2022 and 2105/2022 for the virgin olive oil classification. Briefly, free acidity (expressed as percentage of oleic acid) was measured by titrating 10 g of oil sample dissolved in a neutralized EtOEt:EtOH 50:50 v/v solution with a 0.1 M solution of KOH, using phenolphthalein as indicator. Peroxide value (expressed as milliequivalents of oxygen per kg of oil) was measured by titrating 3 g of oil samples (dissolved in a CH_3COOH:$CHCl_3$ 3:2 v/v and left to react for 5 min (after 1-minute shaking) in the presence of 1 mL of a saturated aqueous KI solution) with a 0.01 M solution of $Na_2S_2O_3$ using a starch solution as indicator. UV spectrophotometric indices were evaluated by dissolving 0.25 g of filtered oil sample in cyclohexane, filling with it a quartz cell and measuring the extinctions at 232, 266, 270 and 274 nm against the solvent used as a reference; the following indices were calculated: $K_{232} = E_{232}/(c \times s)$; $K_{270} = E_{270}/(c \times s)$; $\Delta K = K_{270}-((K_{266}+K_{274})/2)$, where K_λ = specific extinction at wavelength λ, E_λ = extinction measured at wavelength λ, c = concentration of the solution in g/100 mL, s = path length of the quartz cell in cm.

2.4. HS-SPME-GC-MS analysis of VOCs

The samples' volatile fraction, including 72 VOCs, was analyzed by a validated HS-SPME-GC-MS method, based on the MISN approach for quantitative purposes (Fortini et al., 2017; Cecchi et al., 2019). Briefly, 4.3 g of sample were weighed into a 20-mL screw cap vial in which was then added 0.1 g of internal standards solution. A DVB/CAR/PDMS SPME fiber (1 cm, 50/30 μm, Agilent, Palo Alto, CA, USA) was exposed at 45 °C for 20 min in the vial headspace to pre-concentrate the VOCs. The adsorbed VOCs were then desorbed at 260 °C for 1.7 min in the

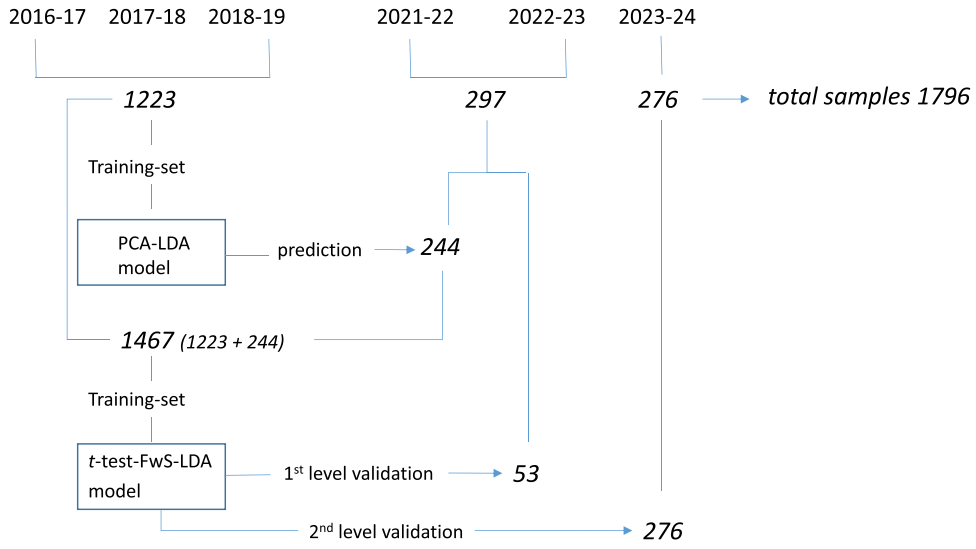

Fig. 1. The samples used in the study.

injection port of the GC system (6890 N model), where they were separated using a capillary column that was a HP-Innowax (50 m × 0.2 mm i.d., 0.4 μm film thickness), and then detected by a Mass Spectrometer Detector (5975-model), all from Agilent (Palo Alto, CA, USA). The oven with the column was set at a starting temperature of 40 °C, which stayed at this temperature for 2 min and then increased at 4 °C/min up to 156 °C and at 10 °C/min up to 260 °C. The carrier gas was helium at 1.2 mL/min; the temperature of the transfer line was 250 °C; the temperature of the ion source was 230 °C. The working range of the Mass Spectrometer Detector was *m/z* 30–350 in scan mode at 1500 (*m/z*)/s, with an IE energy of 70 eV. The identification of the 72 VOCs was carried out by comparing mass spectra and retention times of the analytical peaks with those of the commercial standards (Table S1). Since 2-methylbutanol and 3-methylbutanol were co-eluting molecules, they were integrated together and the output for each sample was made of 71 quantitative data.

For quantitative analysis, starting from the internal and external standard solutions (see paragraph 2.1), 6 diluted solutions were prepared by adding in each of them the same content of internal standard solution and increasing contents of external standard solution. Six-point linear least squares calibration lines (i.e., one for each of the 71 VOCs) were built at the beginning of each analytical sequence by analyzing the 6 diluted solutions in the same conditions of samples. For each VOC, the most suitable internal standard for area normalization was selected among the available ones (Table S1), the calibration line was built by plotting the area ratio (i.e., peak area of the analyte over peak area of the selected internal standard) *vs* the amount ratio (i.e., injected amount of analyte over injected amount of internal standard) and used for quantitation purposes. The area used for each analyte (both external and internal standards) were from a specific quantifier ion chromatogram; the list of quantifier and qualifier ions for each analyte is reported in Supplementary file (Table S2).

2.5. Sensory analysis by the panel test

The sensory analysis of samples was carried out through the official method, that is the so-called Panel Test, for their classification in one of the three commercial categories (i.e., extra virgin olive oil, virgin olive oil, lampante virgin olive oil). According to European Regulations 2104/2022 and 2105/2022, the Panel Test is carried out following the official method of the IOC (https://www.internationaloliveoil.org/wp-content/uploads/2024/07/III-2.2.COI-T20-Doc.-15-REV-11–2024_EN-1.pdf): a panel leader and 8–12 trained panellists comprised the Panel (which was acknowledged by the Italian Ministry of Agricultural Policies (MIPAAF)) and the profile sheet suggested by the official method was used (the profile sheet is reported in Figure S1 of the Supplementary file). Samples were classified as EVOO if the medians of defect and fruity were 0 and > 0, respectively; they were classified as VOO if the median of defect was between 0 and 3.5 and the median of fruity was > 0; they were classified as LVOO if the median of defect was > 3.5 and/or the median of fruity was 0. The tasting panel employed in this research was approved by the Italian Ministry of Agricultural Policies (MIPAAF)); to get the approval, the panel group takes part in a national proficiency ring test every year, and continued approval depends on the performance at an annual review of the tasting panel by the Member State (i.e., the acknowledgement is renewed only if all the parameters to assess the alignment with the other national panels are fulfilled).

2.6. Logical sequence of chemometric methods applied in data processing

Principal Component Analysis (*PCA*), Linear Discriminant Analysis (*LDA*), *t*-test and Forward-Selection (*FwS*) statistical tools were applied (R software, version 4.3.1 (The R foundation for Statistical Computing).

First, the prediction ability of the model previously developed by Cecchi et al. (2019) using the 1223 samples from 2016 to 17–2018–19 olive oil production years was tested by mean of *PCA-LDA* on the 244 samples from 2021 to 22 and 2022–23 olive oil production years (Fig. 1). Since the prediction was unsatisfactory (see paragraph 3.1), a new model was developed using a combination of *t-test*, *FwS* and *LDA* on 1467 samples (i.e., the training-set), that is the set of the above 1223 and 244 samples (Fig. 1). The model was developed searching for relationships between the most significant combination of variables (i.e., the VOCs contents) and the classification of samples by the Panel Test in EVOO and non-EVOO categories.

The first step of the model setup aimed at the selection of the variables according to the algorithm in Figure S2 of the Supplementary Material. A *t-test* was run on the experimental data of the 1467 samples for ranking the variables as a function of the *p*-value which explained their ability to discriminate among EVOO and non-EVOO samples: the lower the *p*-value of a variable, the higher its rank. The *FwS* combined with *LDA* was then applied. The variable with the lowest *p*-value was used as the variable to run the *LDA*, which was applied in 10-fold cross-validation mode on the data-set of the 1467 samples; it resulted in a score given by the percentage of correctly classified samples (i.e., accuracy %). Then, the variable with the second lowest *p*-value was added to the previous variable, and the *LDA* was run again. If the score

increased of at least 1 % in comparison with the previous *LDA* run, the variable was retained, otherwise it was discarded. The process was reiterated until all variables were tested, creating a first set of significant variables in predicting EVOO or non-EVOO commercial categories (Figure S2). The entire *FwS-LDA* process was repeated 20 times in relation to a different random division in ten blocks of the training-set, and each time a slightly different set of significant variables was obtained. The variables were finally ranked based on the frequency they were present in the above 20 combinations, and thus the best set of significant variables in predicting EVOO or non-EVOO commercial categories was created, that is the set of variables to develop the new *LDA* model.

The new *LDA* model was validated in two steps. In the first step, the external test-set was given by 53 samples from 2021 to 22 and 2022–23 olive oil production years. In the second step, the external test-set was given by the 276 samples from 2023 to 2024 olive oil production year (Fig. 1). A Decision Boundary Threshold (DBT) of 55 % was applied to the posterior probability, and the prediction of samples classification leads the model to work in a two-stage manner: first, the samples for each none of the two commercial classes reached the above DBT were classified as "unassigned samples" and the percentage of unassigned samples was calculated; then, the assigned samples (i.e., the samples for which the above DBT was reached) were classified in one of the two commercial classes and involved in calculating the % of correctly classified samples.

3. Results and discussion

The quantitative HS-SPME-GC-MS method with MISN approach used to obtain the data used in this research is a validated method, and the parameters of validation (retention time, range of linear calibration, slope and intercept of the calibration curve, its R^2_{adj}, LOD, LOQ, and accuracy (in terms of trueness and precision at low and high concentrations)) along with further useful information (selected internal standard, quantifier/qualifier ions, molecular weight, chemical class) for each analyte are reported in Table S2 of Supplementary file. Furthermore, Table S3 of Supplementary file shows a comparison of validation parameters (linear working range, LOD, LOQ, R^2_{adj}) and retention time of the method used in this study (HS-SPME-GC-MS using Multiple Internal Standard Normalization approach for quantitative purposes) in comparison with literature HS-SPME-GC-MS method not using multiple internal standards (Romero et al., 2015). The data in the table shows that the analysis time of the method used in this study is shorter, and this was possible thanks to using target quantifier ions for quantitation, which ensured separation of the analyte peaks without interferences also in those cases in which partial co-elution occurred. The data in the table also shows that having several internal standards available and selecting the suitable one for each analyte to be quantitated has allowed the LOQ to be lowered and the range of linear calibration to be much widened, thus making the method more suitable for VOOx samples of different origin with their different volatile profile (Cecchi et al., 2021).

3.1. Critical issues in application of the developed PCA-LDA model by Cecchi et al. (2019)

Cecchi et al. (2019) showed that the developed *PCA-LDA* model correctly classified 83.5 % of external samples. Only after the Covid-19 pandemic, the model was applied again to classify 244 new samples from the 2021–2022 and 2022–2023 olive oil production years (Fig. 1), but more than half of the samples was misclassified, showing that the model no longer had any predictive ability (Table 1A). These unsatisfactory results were truly unexpectedly, since the model was thought to be robust, being based both on a reliable, validated HS-SPME-GC-MS method for VOCs analysis (Fortini et al., 2017) and on a very large data-set (i.e., 1223 VOOx samples). The performance of a linear PLS-DA model was also tested to assess whether the unsatisfactory results depended on the statistical approach; more than half of the samples was again misclassified. An effect of interannual variations in the VOCs profile was hypothesized; when the *PCA-LDA* model was rebuilt using only the 244 samples, randomly divided into size-variable training-sets and test-sets, the prediction ability of the model was good and comparable with Cecchi et al. (2019) (Table 1B).

Table 1
Classification of the 244 virgin olive oil samples from the 2021–22 and 2022–23 olive oil production years. A) The 244 samples were classified according to the *PCA-LDA* model developed by Cecchi et al. (2019), based on the training-set of samples from 2016 to 2019 olive oil production years. B) The *PCA-LDA* model was rebuilt using random training sets and test sets from the 244 samples.

A

Samples (n°)	Unassigned samples	Among assigned samples	
		Correctly classified	Error rate (%)
244	9 (3.7 %)	98 (41.7 %)	58.3 %

B

Trial	Training set (n° samples)	Test set (n° samples)	unassigned samples	Among assigned samples	
				Correctly classified	Error rate (%)
1	89	155	5 (3.2 %)	122 (81.3 %)	18.7 %
2	135	109	6 (5.5 %)	85 (82.5 %)	17.5 %
3	180	64	7 (10.9 %)	46 (80.7 %)	19.3 %

Literature data have recently shown that the application of inappropriate statistical procedures (i.e., incomplete validation tests) leads to overoptimistic results from the model (Aparicio-Ruiz et al., 2019). The emphasis was also on the fact that models based on *LDA* must be combined with a suitable step for the selection of variables (Aparicio-Ruiz et al., 2019). Therefore, it was necessary to improve the above model in terms of training-set, selection of significant combination of variables and suitable test-sets (i.e., with samples from olive oil production years different from those belonging to the training-set).

3.2. The new t-test-FwS-LDA model and first level of validation

The first step of the model development is aimed at selecting the variables. The *t-test* was run on the experimental data of the 1467 samples (i.e., the training-set as sum of 1223 and 244 samples from 2016 to 2019 and from the 2021–2022 and 2022–2023 olive oil production years, respectively) for ranking the variables as a function of the *p*-value which explained their capability to discriminate among EVOO and non-EVOO samples; the lower the *p*-value of a variable, the higher its rank (Table 2). Considering the top 15 ranked VOCs (i.e., *p*-value $< 10^{-19}$), a variety of molecules was present that literature data has associated both with the positive attributes of EVOO samples and with the sensory defects of non-EVOO samples (Cecchi et al., 2021; Cecchi et al., 2019; Morales et al., 2005; Purcaro et al., 2014; Dierkes et al., 2012; Quintanilla-Casas et al., 2020; Vichi et al., 2009; Angerosa et al., 1997). Considering the positive attributes, (*E*,*E*)-2,4-hexadienal has been associated to green, floral, cut grass and fresh notes, whereas 1-penten-3-ol, (*Z*)-3-hexenal, (*E*)-2-penten-1-ol and (*E*)-2-hexenal are volatile molecules originating from different branches of the lipoxygenase pathway, and they have been associated with generic fresh and green notes of EVOOs. The remaining 10 VOCs have been associated with EVOO sensory defects. Octane has been mainly associated to the rancid defect, and sometime to other defects (e.g., musty, fusty/muddy sediment), as well as heptanal, 1-heptanol and pentanal have been associated with the rancid defect. 1-Propanol, 2-pentanol, 6-methyl-5-hepten-2-one, 4-ethylphenol and 1-octen-3-ol have been associated with defects such as musty and fusty-muddy sediment, while ethanol (in combination to acetic acid and ethyl acetate) has been associated with the winey-vinegary defect. These last sensory defects

Table 2
Volatile organic compounds (i.e., the model variables) measured in the virgin olive oil samples, ranked according to the *t*-test in relation with the capability of the variables to discriminate among EVOO and non-EVOO samples.

Rank	*p*-value	VOCs Name	Rank	*p*-value	VOCs Name	Rank	*p*-value	VOCs Name	Rank	*p*-value	VOCs Name
1	6.71E−48	(*E*,*E*)−2,4-Hexadienal	19	1.32E−15	4-Ethylguaiacol	37	4.15E−07	Benzaldehyde	55	1.66E−02	1-Hexanol
2	1.39E−38	Octane	20	3.37E−15	1-Nonanol	38	1.44E−06	Ethyl butanoate	56	2.14E−02	(*E*)−2-Hexen−1-ol
3	8.7E−38	1-Penten−3-ol	21	6.67E−15	(*E*)−2-Octenal	39	3.34E−06	Hexyl acetate	57	2.60E−02	3-Pentanone
4	1.56E−33	(*Z*)−3-Hexenal	22	2.07E−14	(*E*)−2-Nonenal	40	5.82E−06	(*E*)−2-Decenal	58	2.60E−02	Methyl propanoate
5	3.59E−31	Heptanal	23	2.12E−13	Acetic acid	41	7.62E−06	2-Octanone	59	3.70E−02	2 + 3-Methyl−1-butanol
6	5.98E−27	1-Octen−3-ol	24	5.32E−13	Propanoic acid	42	1.26E−05	Methyl acetate	60	4.24E−02	Decanal
7	8.37E−27	6-Methyl−5-hepten−2-one	25	1.59E−12	Ethyl propanoate	43	2.65E−05	1-Octen−3-one	61	5.36E−02	(*Z*)−2-Hexen−1-ol
8	2.78E−24	(*E*)−2-Penten−1-ol	26	2.01E−12	3-Methyl butanal	44	3.22E−05	2-Phenylethanol	62	7.80E−02	(*E*)−2-Hexenyl acetate
9	8.02E−24	4-Ethylphenol	27	2.31E−12	Isobutanol	45	3.53E−05	Butanoic acid	63	1.12E−01	(*Z*)−3-Hexen−1-ol
10	3.38E−23	2-Pentanol	28	2.36E−12	(*Z*)−2-Penten−1-ol	46	8.03E−05	(*Z*)−3-Hexen−1-yl, acetate	64	1.50E−01	Methanol
11	5.04E−23	1-Heptanol	29	6E−12	Ethyl Acetate	47	9.59E−05	2-Heptanone	65	1.93E−01	2-Butanone
12	3.31E−22	(*E*)−2-Hexenal	30	1.97E−11	2-Methyl butanal	48	1.18E−04	(*E*)−3-Hexen−1-ol	66	2.72E−01	1-Pentanol
13	8.14E−21	Ethanol	31	5.97E−11	Guaiacol	49	1.64E−03	2-Octanol	67	3.06E−01	2-Butanol
14	1.6E−20	1-Propanol	32	6.7E−11	2-Heptanol	50	2.33E−03	(*E*,*E*)−2,4-Heptadienal	68	3.15E−01	1-Penten−3-one
15	3.29E−20	Pentanal	33	1.3E−10	(*E*,*E*)−2,4-Nonadienal	51	2.72E−03	Pentanoic acid	69	4.19E−01	Heptane
16	3.06E−19	Nonanal	34	4.6E−10	(*E*)−2-Pentenal	52	2.79E−03	Hexanoic acid	70	4.34E−01	2-Nonanone
17	1.05E−17	(*E*)−2-Heptenal	35	1.62E−09	Octanal	53	3.59E−03	Butyl acetate	71	4.69E−01	Hexanal
18	5.25E−16	(*E*,*E*)−2,4-Decadienal	36	3.43E−09	Phenol	54	1.41E−02	1-Octanol			

have often been related to spoilage microorganisms.

As reported in paragraph 2.6, the *FwS* combined with *LDA* was applied to develop the new model, which was based on searching the best combinations of VOCs that provided predictions with acceptable errors on the training-set of 1467 samples, and repeating this searching up to 20 times, thus obtaining 20 combinations of variables. Fig. 2 shows the frequency with which the VOCs were present in the above 20 combinations. Only 22 out of the 71 VOCs were present at least once, including 4 VOCs associated with EVOO positive sensory attributes (3 of which from the LOX pathway), and 18 VOCs associated with the EVOO sensory defects. Interestingly, the *FwS* has selected as significant 12 VOCs among the 15 VOCs with the lowest *p*-values, but also 10 VOCs among those with a *p*-value greater than 10^{-19}.

To make a final selection of variables, which also simplify the model, a validation was carried out using the external test-set of 53 samples from 2021 to 22 and 2022–23 olive oil production years (Fig. 1). The 22 VOCs were revised based on their frequency in the above 20 combinations (Fig. 2), and only those with a frequency of at least 20 % were chosen. Therefore, the model was run 12 times, as follows; (i) first, only the two VOCs with 100 % frequency (i.e., (*E*,*E*)-2,4-hexadienal and (*Z*)-2-penten-1-ol) were used as the variables; (ii) then, one VOC at a time was added to the previous series, following the decreasing order of their frequency. Fig. 3 shows the prediction ability of the model in relation to the increasing number of selected VOCs. The model prediction ability to correctly classify the assigned samples was already good even with only the first two and three selected variables, with 85.3 % and 83.8 % of

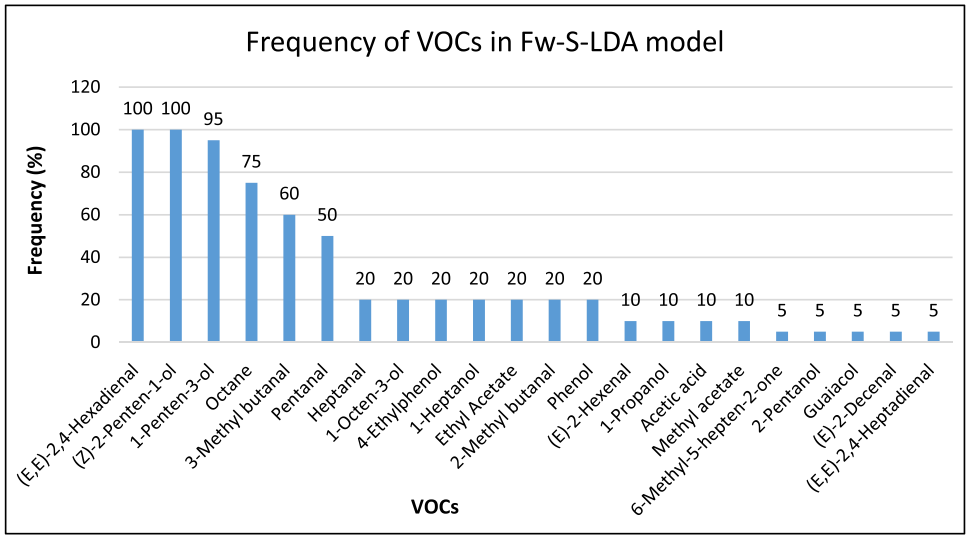

Fig. 2. Bar chart representing the frequency with which each volatile compound was present in the 20 combinations obtained when the *FwS-LDA* was applied on the training-set of 1467 samples.

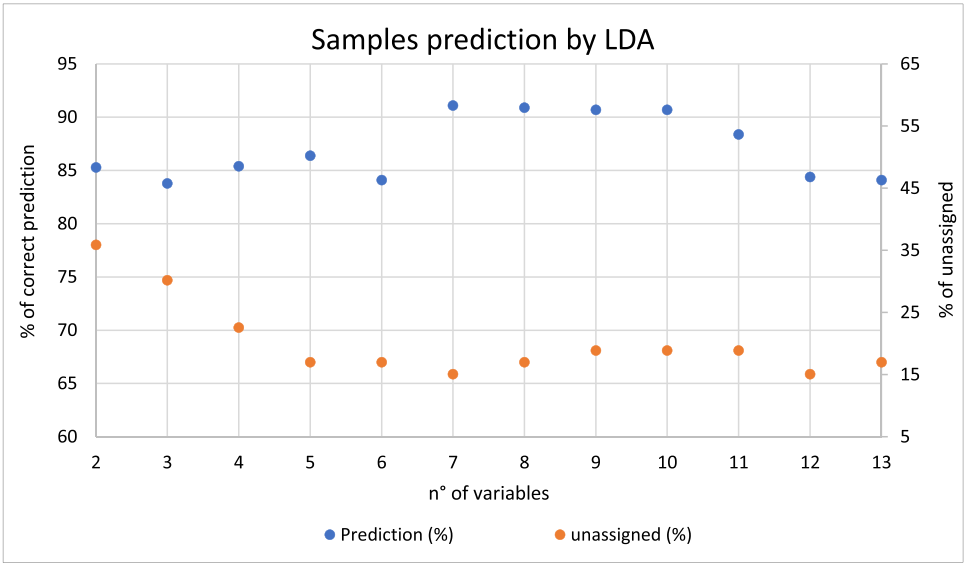

Fig. 3. Effect of the increasing number of selected volatile compounds on percentages of unassigned samples (in orange) and correctly classified samples among the assigned ones (in light blue) when the *t-test-FwS-LDA* model was validated using the external test-set of 53 samples from 2021 to 22 and 2022–23 olive oil production years.

correct prediction, respectively. However, the percentage of unassigned samples was high (35.9 % and 30.2 % with the first two and three selected VOCs, respectively), while it decreased in the range 15.1–18.9 % when the number of variables was greater than 4. The prediction ability reached the maximum values with 7–10 VOCs (i.e., from 90.7 % to 91.1 % of correctly classified samples), and the percentage of correctly classified samples decreased with the next increase of variables. Since only one out of the first 7 VOCs, that is 3-methyl butanal, is associated with the several sensory defects caused by microbial phenomena, it was reasonable to expand the number of the selected VOCs in relation to the microbial sensory defects (i.e., fusty/muddy sediment, winey/vinegary, moldy). Therefore, the 8th, 9th and 10th VOCs were also included among the selected variables to define the final *LDA* model (Cecchi et al., 2021; Angerosa et al., 1996; Morales et al., 2005).

Summarizing, the model for predicting commercial categories (i.e., EVOO or non-EVOO) of the samples was based on a final set of 10 selected VOCs, as follows: (i) (*E*,*E*)-2,4-hexadienal, (*Z*)-2-penten-1-ol and 1-penten-3-ol to predict the EVOO sensory positive attributes; (ii) octane, pentanal and heptanal to predict the rancid defect; (iii) 3-methyl butanal, 1-octen-3-ol, 4-ethylphenol and 1-heptanol to predict the sensory defects with microbial origin.

3.3. *Effect of the training-set on the prediction ability of the model and second level of validation*

The effect of the training-set on the prediction ability of the *LDA* model based on 10 variables (i.e., the 10 selected VOCs) was studied in order to verify the effect of the VOCs profile variations over olive oil production years as hypothesized in paragraph 3.1.

The external test-set of 53 VOOx samples from 2021 to 22 and 2022–23 olive oil production years was used once again, but some variations were adopted in the training-set of the 1467 samples from 2016 to 2023 olive oil production years. In a first trial, the training-set

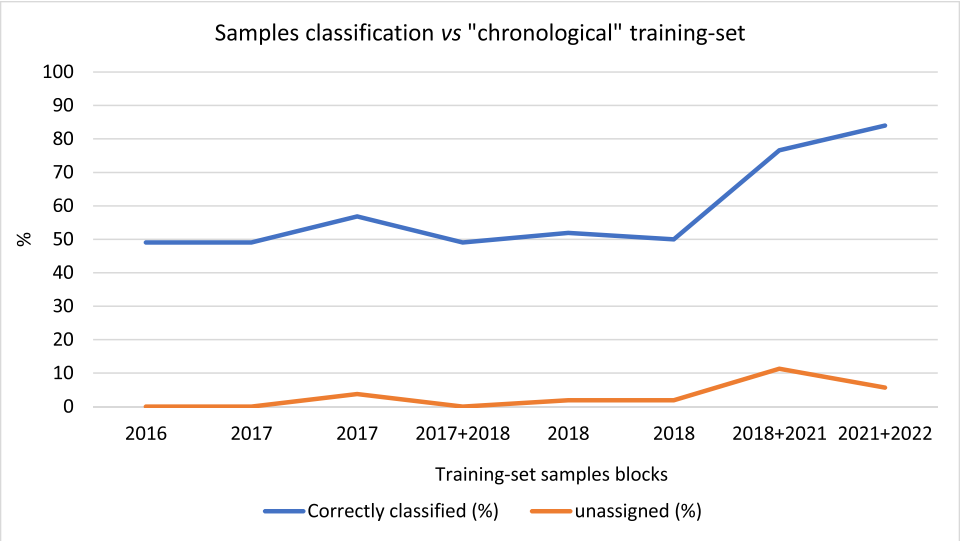

Fig. 4. Percentage of unassigned samples and correctly classified samples (among the assigned ones) samples by *t-test-FwS-LDA*, when the training-set was divided in 8 sub-training-sets with samples in ascending chronological order from the oldest to the newest olive oil production years.

was divided into 8 sub-training-sets including, in each consecutive sub-training-set, samples from the oldest (the sub-training-set #1 from sample 1–183) to the newest (the sub-training-set #8 from sample 1285–1467) olive oil production years. In a second trial, the training-set was divided again into 8 sub-training-sets, but this time the samples were randomly distributed in the 8 sub-training-sets; the second trial was repeated three times. Figs. 4 and 5 show the results in terms of both unassigned samples and correctly classified samples among the assigned ones for the trial 1 and 2, respectively. When the external test-set of 53 samples was classified only using in the training-set samples from the oldest olive oil production years (i.e., 1–183–918–1100 samples), the percentage of correctly classified samples was about 50 % (Fig. 4), meaning that the model had no prediction ability, thus confirming the results in Table 1A. Instead, when the training-set was composed of samples from both the oldest and the newest olive oil production years (i.e., 1101–1284 samples), the percentage of correctly classified samples increased to approx. 79 %, and it further increased to approx. 83 % when the training-set was composed of only samples from the newest olive oil production year (i.e., the same production year of the sample in test-set; 1285–1467). When the 1467 samples were instead randomly distributed in the 8 × 3 sub-training-sets (Fig. 5), a good prediction ability was obtained most of the time; in fact, only when using three out of the 24 sub-training-sets, the percentage of correctly classified samples was below 80 %. Therefore, the training-set composed of samples from the highest possible number of olive oil production years played an essential role in the prediction ability of the *t-test-FwS-LDA* model.

This evidence was further confirmed during the second and final validation of the model, which was carried using the external test-set of 276 samples from the 2023–24 olive oil production year (Fig. 1). The effect of the olive oil production years in affecting the prediction ability of the model was confirmed again (Table 3): 80 % of the assigned samples were correctly classified in this second validation using an external test-set composed of samples from a different olive oil production year than those present in the training-set, while approx. 91 % of the assigned samples were correctly classified in the first validation (see paragraph 3.2) using an external test-set was composed of samples from olive oil production years included in the training-set. However, the prediction ability of model can be considered satisfactory, showing that the logical sequence of the chemometrics methods in data processing applied in this study (including a large and robust training-set, a suitable approach to select the variables to be used in the *LDA* model) had undoubtedly improved the ability of the *t-test-FwS-LDA* model in predicting EVOO or non-EVOO commercial categories of samples in comparison to the *PCA-LDA* model by Cecchi et al. (2019).

Table 3
Classification of 276 virgin olive oil samples from the 2023–24 olive oil production year during external validation of the *t*-test-FwS-LDA set using the training-set of 1467 samples and 10 variables.

Samples (n°)	Unassigned samples	Among assigned samples	
		Correctly classified	Error rate (%)
276	26 (9.4 %)	200 (80.0 %)	20.00 %

4. Conclusion

This study has proposed a new *t-test-FwS-LDA* model to support the Panel Test in virgin olive oil classification that presents several strengths and novelties. First, the very great number of samples (i.e., approx. 1800) from six different olive oil production years was the key point that allowed minimizing the error source in sample volatile profiles. Second, the approach for the variable selection was not based on a pre-assembled choice of a few VOCs, but, starting from data of 71 VOCs × 1467 VOOx samples, it used the statistical significance of each VOC in discriminating among EVOO and non-EVOO samples to select ten VOCs whose best combination maximized the prediction ability of the model while avoiding overlapping of the information thus removing noise. Third, the crucial role of the olive oil production year for the prediction ability of the model has been proved; year-by-year, the training-set must be updated by adding samples from the last olive oil production year and the variables (i.e., the VOCs amounts) must be selected select again.

The model has shown a good prediction ability, and it was ready to be routinely applied to support the Panel Test in the olive oil companies, discriminating among EVOO and defective non-EVOO samples also in the case of borderline samples. However, the model was not developed to discriminate the LVOO non-edible samples, requesting future research on.

Ethics & standards requirements for sensory evaluation

Sensory evaluation was carried out in accordance with the principles set forth in the Declaration of Helsinki for medical research involving humans and, it was subject to ethical standards that promote and ensure respect for all human participants and protect their health and rights. Since the research study was not of a medical nature, the research protocol was not submitted to an ethics committee for approval, in line

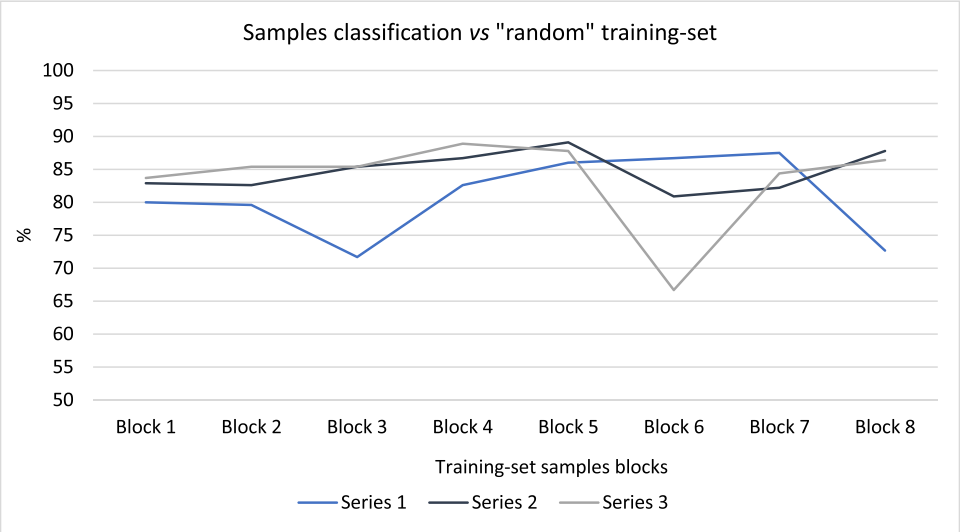

Fig. 5. Percentage of correctly classified samples by *t-test-FwS-LDA* model, when the training-set was divided in 8 sub-training-set with samples randomly allocated.

with national regulations. Written informed consent was obtained from all participants in accordance with the GDPR (General Data Protection Regulation) 2016/679. Participants could withdraw from the study at any time without giving a reason. The tested products were safe for consumption.

Consent for publication

All authors have reviewed and approved the final version of the manuscript for publication.

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Author statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

CRediT authorship contribution statement

Digiglio Irene: Writing – original draft, Visualization. **Migliorini Marzia:** Methodology, Investigation, Formal analysis, Conceptualization. **Trapani Serena:** Resources, Formal analysis. **Ugolini Tommaso:** Formal analysis, Data curation. **Cecchi Lorenzo:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Melani Fabrizio:** Writing – original draft, Validation, Software, Methodology, Investigation, Data curation, Conceptualization. **Mulinacci Nadia:** Writing – review & editing, Supervision, Project administration, Investigation. **Zanoni Bruno:** Writing – review & editing, Resources, Funding acquisition.

Declaration of Competing Interest

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.107362.

Data availability

The data that has been used is confidential.

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