In-house validation of a visible and near infrared spectroscopy non-targeted method to support panel test of virgin olive oils

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ABSTRACT

In this study, an in-house validation of Visible and Near Infrared Spectroscopy was performed to distinguish between extra virgin olive oil (EVOO) and virgin olive oil (VOO). A total of 161 samples of olive oil of three different categories (EVOO, VOO and lampante (LOO)) were analysed by transflectance using a monochromator instrument. One-class models were initially developed using Partial Least Squares (PLS) Density Modelling to characterize EVOO and VOO category. Once the LOO samples were discriminated, linear and non-linear discriminant models were built to classify EVOO and VOO. Different data pre-treatments and variable selection algorithms were evaluated to establish the best models in terms of Correct Classification Rate (CCR). The best model, obtained after variable selection using PLS Discriminant Analysis, yielded CCR values of 82.35 % for EVOO and 66.67 % for VOO in external validation. These results confirmed that VIS + NIRS technology may be used to provide rapid, non-destructive preliminary screening of olive oil samples for categorization; suspect samples may then be analysed by official analytical methods.

1. Introduction

The soaring price of extra virgin olive oil on the market has rendered it susceptible to fraud, necessitating the availability of analytical methodologies that ensure the product's integrity for consumers. In recent years, the technological advances in the analysis of fats and oils have gone hand in hand with a growing awareness of the need for even more effective control of the quality, purity and authenticity of the olive oil that is sold, especially in countries which have only recently become olive oil consumers (IOC, 2022). Delegated Regulation (EU) 2022/2104 (OJEU, 2022) establishes standards which can be applied to all olive oil which is destined for international trade. These standards include, among others, that the competent authorities should carry out conformity checks, based on a risk analysis, to maximize consumer protection. Conformity tests should include assessment of physicochemical parameters, complemented by the evaluation of organoleptic characteristics by panels of selected and trained tasters (Panel Test). These panels play a critical role in providing sensory evaluations that inform the classification and labelling guidelines for olive oil finally decide the classification and labelling of the olive oils (IOC, 2021).

Since 2013, the International Olive Council (IOC), the European Union (EU) and the Spanish Ministry of Agriculture, as well as standardization bodies from other traditional and non-traditional producer countries, have taken different steps aimed at updating the existing knowledge about novel analytical methods which could complement the Panel Test (PT). This is due to the limitations of the PT: it is slow, costly to implement, subjective, variable between and within panels, and also has other weakness such as the difficulty to classify borderline types of oil (e.g., extra virgin *versus* virgin olive oil), and the limitation in terms of the low number of samples (12) which can be analysed by one expert panel per day (Conte et al., 2020; Barbieri et al., 2020a). Moreover, virgin olive oil (VOO) companies are also seeking to support research into new instrumental methods to improve and complement the PT, since this method is not accessible to many producers and retailers. Moreover, the considerable expense and time needed to gather such data result in only a small fraction of OOs being inspected annually relative to total production.

During the past ten years, a number of papers have focused on the weak points of the current regulations and on analytical methods which can be used to fight against the increasing cases of fraud in the olive oil

(OO) sector and the current state of the official methods used to prevent it (Circi et al., 2017; Barbieri et al., 2020a; Conte et al., 2020; Quintanilla-Casas et al., 2020; Valli et al., 2020; Casadei et al., 2021). More recently, the IOC, probably as a consequence of the results of the study carried out by the European Commission (EC) in 2020 on the flaws in the conformity checks used in the olive oil sector across the European Union (EC, 2020), invited experts from its member countries to collaborate in the search for new, more accurate and reliable methods, particularly to detect fraud, based on the latest technical advances. García-González et al. (2017) reviewed the differences between EU and non-EU trade standards, providing detailed information about the differences. They recommended improving the current IOC methods, wherever possible, but also opened the door to exploring non-targeted methods (NTMs), although some researchers (Riedl, Esslinger & Fauhl-Hassek, 2015; McGrath et al., 2018; Nichani et al., 2023) have pointed to the absence of internationally-accepted validation protocols for the use of NTMs in food authentication issues.

Over the last few years, Near Infrared Spectroscopy (NIRS) has started to be taken seriously as the go-to NTM in the fight against many types of food fraud (Karunathilaka, Kia, Srigley, Chung, & Mossoba, 2016; García-González et al., 2017; McGrath et al., 2018; Pérez-Marín et al., 2019; Baeten & Dardenne, 2021; Rodionova, Pierna, Baeten & Pomerantsev, 2021; Moghaddam, Tamiji, Lakeh, Khoshayand, & Mahmoodi, 2022; Pérez-Marín & Garrido-Varo, 2023). Most of the published papers concerning the NIRS qualitative analysis (now termed 'non-targeted analysis') of commercialized olive oil focus on differentiating between types of olive oil mixed with other vegetable oils, as well as those which come from different varieties or geographical origins, and which have quality grades (Garrido-Varo, García-Olmo & Pérez-Marin, 2004; Armenta, Moros, Garrigues & Guardia, 2010; Casale & Simonetti, 2014; Gómez-Caravaca, Maggio & Cerretani, 2016). Other authors (Ortiz-Romero et al., 2023) have attempted to use NIRS to authenticate VOOs, but not to distinguish between EVOO and VOO, which, as well as being the two main categories, are those which PT has the most difficulty categorizing, due to their overlapping attributes. As regards the prediction of PT scores in OO, Garrido-Varo et al. (2000) showed, for the first time, the capabilities of visible and near infrared spectroscopy (VIS + NIRS) using a quantitative approach. Willenberg, Matthäus, & Gertz (2019) emphasized the challenges involved in the adoption of NIRS technology as a NTM, but based their study on NIRS prediction of seven individual compounds, such as acidity or the *p*-anisidine value, using the latter in different classification algorithms. However, according to their definition, this cannot be considered as a NTM or "fingerprint method", (Riedl et al., 2015; Nichani et al., 2023). Furthermore, many of these cited works should be considered as viability studies, because they use a low number of samples, or the samples did not originate from producers, but were acquired from local retailers, or were mixed or adulterated in the researchers' own laboratories using spiked samples, without a satisfactory statistical mixture design.

Several publications (Oliveri & Downey, 2013; Oliveri, 2017; Jiménez-Carvelo, González-Casado, Bagur-González, & Cuadros-Rodríguez, 2019; Biancolillo, Marini, Ruckebusch, & Vitale, 2020; Zeng et al., 2021) have summarized the main pattern recognition (PR) methods for use in cases of food adulteration and authentication, and distinguished two main types, discrimination and class-modelling, stressing that the differences between them have enormous practical implications. In addition, PR methods can range from traditional algorithms, such as Partial Least Squares-Discriminant analysis (PLS-DA), Soft Independent Modelling by Class Analogy (SIMCA), Fisher Discriminant Analysis to other newer, more sophisticated methods such as Bayesian Discriminant Analysis, Logistic Regression, Artificial Neural Network (ANN), Classification and Regression Tree (CART), or Partial Least Squares Density Modeling (PLS-DM), etc. However, although one of the recommendations in Oliveri and Downey (2013) was that the choice of the method should be guided by the criterion of simplicity, some of the recent published works that apply new discrimination or class-modelling

approaches have reported highly efficient models, with a good degree of balance between sensitivity and specificity values, as well as high stability over time when compared with classical class-modeling methods (Devos, Downey & Duponchel, 2014; Oliveri et al., 2014). These results are of great interest to encourage further research into the use of this algorithm in commercialized VOOs to discriminate them according to the commercial category and, therefore, the quality grade.

In view of the above and taking into account to the state of the art concerning the needs for a standardization of validated NTM, the main goal of this study was to contribute further to our knowledge and generate new methodology for the future validation of VIS + NIRS technology as an NTM. Following the recommendations by Nichani et al., (2023), we performed an in-house validation (single-lab) to distinguish between EVOO and VOO, to support the conformity checks requested in the international trade of virgin olive oil. The three specific objectives were: a) to set up a methodology for instrument performance control and sample analysis optimization; b) to evaluate a two-step authentication strategy combining one-class classification and the discriminant approach to authenticate EVOO and VOO; c) to study the effect of different mathematical pre-treatments on the classification performance of the two-step modelling approach and on two well-established methods, one lineal (PLS-DA) and another non-lineal (Support Vector Machine-Discriminant Analysis, SVM-DA).

2. Materials and methods

2.1. Sampling

In this study, a total of 161 OO samples from different cultivars ('Picual', 'Hojiblanca', 'Arbequina', 'Manzanilla', 'Picudo') in the municipal areas of Cordoba, Granada and Jaen (Andalusia, Spain), made up of three different categories, EVOO (N = 66), VOO (N = 62) and LOO (N = 33), as classified according to Regulation (UE) N° 2019/1604 was used (OJEU, 2019). They were collected during the 2020/21 production season and were received in sealed, correctly-labelled plastic bottles of about 250 mL. Each bottle was then divided into small airtight bottles of about 50 mL, one of which was used for acquiring NIRS spectra, while the rest were stored protected from the light at −20 °C.

2.2. *Reference data.*

The organoleptic assessment of the olive oil samples was performed by a single Sensory Panel of Fundacion Citoliva (Jaen, Spain). The official procedure described in Commission Regulation (EEC) No 2568/91 (OJEC, 1991), with later modifications (OJEU, 2019; OJEU, 2022) was followed. The panel consisted of 12 members. The sensory data were expressed as the mean of medians provided by each member and, based on these data, the PT assigned a category to each sample. All procedures for sensory evaluation were authorized by the Spanish Ministry of Agriculture, Fisheries and Food and accredited by the Spanish National Accreditation Body (ENAC) according to the criteria of the standard UNE-EN ISO/IEC 17025. Informed consent was obtained from all individual participants included in the study.

2.2. NIRS analysis, instrument performance and spectral repeatability

2.2.1. Spectra collection

The spectra were collected for all the samples in transflectance mode (log (1/R)) using an NIRS™ DS2500 monochromator (FOSS Analytical, Hillerød, Denmark), equipped with a folding transmission cup with a 0.1 mm gold reflector. This instrument provides absorbance readings between 400 and 2500 nm, in 2 nm steps. Two spectra were collected per sample and averaged to provide a mean spectrum. The data acquisition was performed using ISIscan Nova software (FOSS Analytical, Hillerød, Denmark).

2.2.2. Selection of the optimal region of the instrument

Before the spectral data were processed, one important step was to

select the most suitable spectral range of the instrument for the analysis of olive oil. To achieve this, a first-order derivative, with a single-unit derivation segment and without smoothing, (1,1,1,1) was applied, which allows to highlight the areas of the spectrum where the signal to noise ratio is degraded (Hruschka, 1987; McClure, 1992). To achieve this, the WinISI II software package, version 1.50 (Infrasoft International, Port Matilda, PA, USA) (ISI, 2000) was used.

2.2.3. Spectral repeatability

Spectrum quality was evaluated using the root mean square (RMS) (Shenk & Westerhaus, 1995; Shenk and Westerhaus, 1996) included in WinISI II software (ISI, 2000). This statistic shows the similarity between different spectra of a single sample; in this case, between the two spectra collected per sample. The RMS for an individual subsample (*j*) of sample (*k*), and the MEAN and STD values for a given *k* sample were calculated according to the formulae provided by Martínez, Garrido-Varo, De Pedro & Sánchez (1998):

$$RMS_{j,k} = \sqrt{\frac{\sum_{i=1}^{n} D_{ij}^2}{n}}; \quad D_{ij} = y_{ij} - \bar{y}_i;$$

$$MEAN_k = \sqrt{\frac{\sum_{j=1}^{N} (RMS_j)^2}{N}};$$

$$STD_k = \sqrt{\frac{\sum_{j=1}^{N} (RMS_j)^2}{N-1}}$$

where y_{ij} is log $(1/R)$ at wavelength i for subsample j, $\bar{y}_i$ is log $(1/R)$ at wavelength i for the average spectrum of N subsamples of a sample k and n is the number of data points collected by the instrument (here, 1050 data points for the instrument under study). The RMS value obtained in each case was multiplied by 10^6 to simplify the calculations.

The usefulness of the STD RMS statistic is that, when known, it can be used to calculate an STD limit for subsamples of the same sample using the following formula:

$$STD_{limit} = 1.036 \sqrt{\frac{\sum_{k=1}^{k=m} STD_k^2}{m}} = 1.036 \sqrt{\overline{STD^2}}$$

where STD is the standard deviation per sample and m is the number of samples.

After calculating the standard deviation (STD) limit from the RMS statistic, an admissible limit for spectrum quality and repeatability was set, ensuring the highest quality of the OO NIR spectra.

2.3. Data analysis

All the data pre-processing and chemometric treatments were performed using PLS_Toolbox software (Eigenvector Research, Inc.,

Manson, WA, USA) and in-house Matlab routines (The MathWorks, Inc., Natick, MA, USA).

2.3.1. Definition of the training and validation sets

Before developing the classification models, the data were randomly divided into training (75 % of samples) and test sets (25 % of samples), ensuring that each category was divided proportionally in each set. Finally, the training set (N = 121) used for the construction and optimisation of the model was composed of 49 EVOO, 47 VOO and 25 Lampante olive oil (LOO) samples. The test set (N = 40) was made up of 17 EVOO, 15 VOO and 8 LOO samples, and was used to assess the performance of the final model.

2.3.2. Development and validation of non-targeted modelling methods

A two-step authentication, combining the one-class classification and the discriminant approach, was used to classify olive oils into EVOO, VOO and LOO categories. The procedure followed is shown in Fig. 1.

2.3.2.1. One-class classification. To develop the one-classification models, the combination of N = 49 EVOO and N = 47 VOO samples was used as the target class. The one-class modelling method proposed by Oliveri et al. (2014), called Partial Least Squares Density Modelling (PLS-DM) was used. Several parameters were settled in order to define the optimal model – namely, the pre-processing, *k* (nearest neighbours), *L* (latent variables) and *a* (smoothing coefficient). In more detail, four possibilities were considered for variable pre-processing: without pre-processing, mean centering, scaling and autoscaling. For the *k* parameter, integer values from 1 to 6 were considered. The number *L* of latent variables varied from 1 to 10. Finally, the smoothing coefficient *a* ranged from 0.3 to 0.8, with 0.1 increments. These parameters vary within the specified ranges, so that the algorithm calculates models with all of the different parameter combinations using a cross-validation procedure with five deletion groups (Venetian-blind scheme). In the end, the outcomes of all of the combinations are evaluated in terms of sensitivity and specificity, by means of a Pareto diagram, which is useful to choose the optimal conditions, looking for the best compromise between the two parameters. The sensitivity indicates the percentage of samples correctly assigned to the target class, and the specificity indicates the percentage of samples correctly discarded from that class. Finally, the optimal class model was externally validated to obtain final sensitivity and specificity estimations. The test set was composed of 32 samples of the target class (EVOO + VOO) and 8 samples of the non-target class (LOO).

2.3.2.2. Discriminant analysis. Next, the EVOO and VOO samples were divided into the above categories. The discriminant models were built using the traditional linear Partial Least Squares Discriminant Analysis (PLS-DA) and the non-linear Support Vector Machine Discriminant Analysis (SVM-DA). To obtain models with a reduced complexity, different variable selection methods were tested. Using these methods
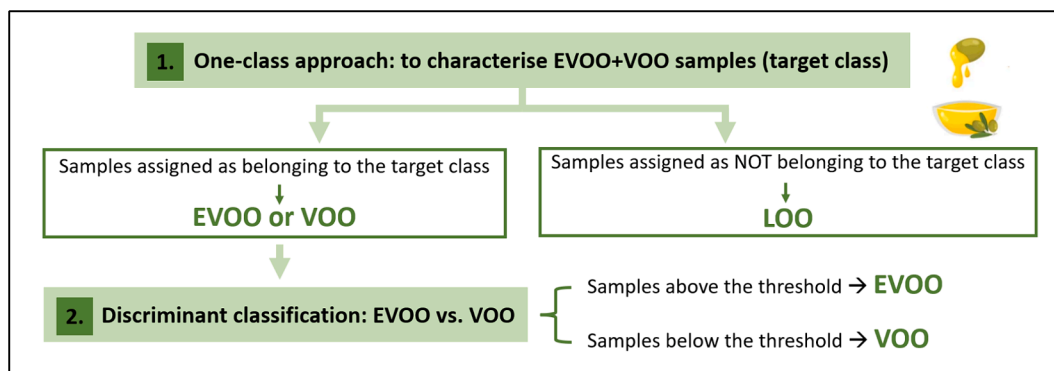

Fig. 1. Flow chart of the two-step authentication approach to classify virgin olive oil into EVOO, VOO and LOO categories.

allows to discard variables with low information content and select the variables that maximize the predictive ability of the models. The methods used were the Genetic Algorithm (GA) (Niazi & Leardi, 2012), Interval PLS (iPLS) (Nørgaard et al., 2000), with different interval widths (10, 50 and 100 wavelengths) and an automatic variable selection tool included in Matlab's PLS Toolbox, known as 'Selectvars'. All the models were built with five cross-validation groups, using the Venetian-blind technique.

In order to compare the results obtained by the different discriminant models developed, McNemar's test (McNemar, 1947; Roggo, Duponchel, Ruckebusch & Huvenne, 2003), a paired version of the χ^2 test, was used. The McNemar test value is calculated as follows:

$$McNemar's\,value = \frac{(|n_{01} - n_{10}| - 1)^2}{n_{01} + n_{10}}$$

where n_{01} is the number of samples misclassified only by the first model and n_{10} is the number of samples misclassified only by the second model. This statistic is distributed (approximately) as χ^2 within one degree of freedom (if the number of samples is higher than 20), and the critical value for 5 % significance level is 3.841. If the McNemar test value is greater than this critical value, the two models are considered significantly different.

Finally, the performance of the discriminant models was evaluated based on the results obtained in the test set, in terms of the correct classification rate (CCR), that is, the percentage of correctly classified samples (Oliveri & Downey, 2013).

2.3.2.3. Spectra pre-processing. In both steps, twelve different combinations of data pre-treatments were applied in order to select the most suitable pre-processing strategy to obtain the best classification model. 7 data transformations (two column and five row pre-processing algorithms) were applied, using the following combinations: Standard Normal Variate (SNV) transform + column mean centering (MC); First derivative with the Savitzky-Golay filter (SG1) + MC; Second derivative with the Savitzky-Golay filter (SG2) + MC; SNV + Detrend (DT) + SG2 + MC; Orthogonal Signal Correction (OSC) + MC; OSC + SG2 + MC; SNV + column autoscaling; SG1 + column autoscaling; SG2 + column autoscaling; SNV + DT + SG2 + column autoscaling; OSC + column autoscaling; OSC + SG2 + column autoscaling. SNV and DT for scatter correction (Barnes, Dhanoa & Lister 1989). OSC was used to remove spectral information not related to the reference data (Wold, Antti, Lindgren & Öhman, 1998), and SG derivatives were employed to remove

both additive and multiplicative effects in the spectra (Rinnan, Van Den Berg & Engelsen, 2009). Finally, column pre-processing (MC and autoscaling) was used to eliminate systematic location and dispersion differences among variables (Oliveri & Downey, 2013).

3. Results and discussion

3.1. Instrument performance and optimization of sample presentation

One key aspect in the fine-tuning of a given NIRS application is the graphical observation of the spectra and the detection of high-noise regions. Fig. 2 shows the raw spectra (log 1/R) of the entire sample collection used.

In the visible region, absorbance peaks occurred between 420–460 nm and also at 668 nm, due to the presence of pigments such as carotenoids, anthocyanins and chlorophyll, which are the source of the colour in virgin olive oil (McClure, 1992). In the NIR region, the highest absorption peaks observed (1208, 1390, 1414, 1724, 1760, 2308 and 2348 nm) have been associated by different authors to the absorption of fats and oils (Shenk & Westerhaus, 1995; Garrido-Varo et al., 2004; Garrido-Varo, Sánchez, De la Haba, Torres & Pérez-Marín, 2017). Within the collection of samples analysed, one of them presented a very broad absorption band around 1940 nm. This particular sample was a dark LOO with many suspended particles. A few other samples also presented very sharp peaks at 422 nm, 452 nm and 668 nm, which were attributed to carotenoids and chlorophylls. Garrido-Varo et al. (2004) pointed out that the absorptions found in those regions should be taken into account not only for colour determination, but also for studies of oxidation in VOOs.

Another critical aspect in the evaluation of a specific NIRS instrument or analysis mode is to ascertain whether the total range available in the spectrometer is appropriate or whether there are regions that exhibit inappropriate levels of noise and, consequently, low signal quality levels (Garrido-Varo, Sánchez-Bonilla, Maroto-Molina, Riccioli & Pérez-Marín, 2018). As can be seen in Fig. 3, no areas with a high noise level could be detected, so it was decided to use the whole spectral region (400–2500 nm) for the present work.

The spectral repeatability was evaluated using the RMS statistic. Table 1 shows the MEAN and STD RMS values for the whole sample collection. The MEAN statistic range showed values as high as 7,954 μlog (1/R) and as low as 284 μlog (1/R). This led to believe that this wide variability could be caused by some unknown aspect of the methodology and/or physicochemical, sensory and/or process attributes, such as its
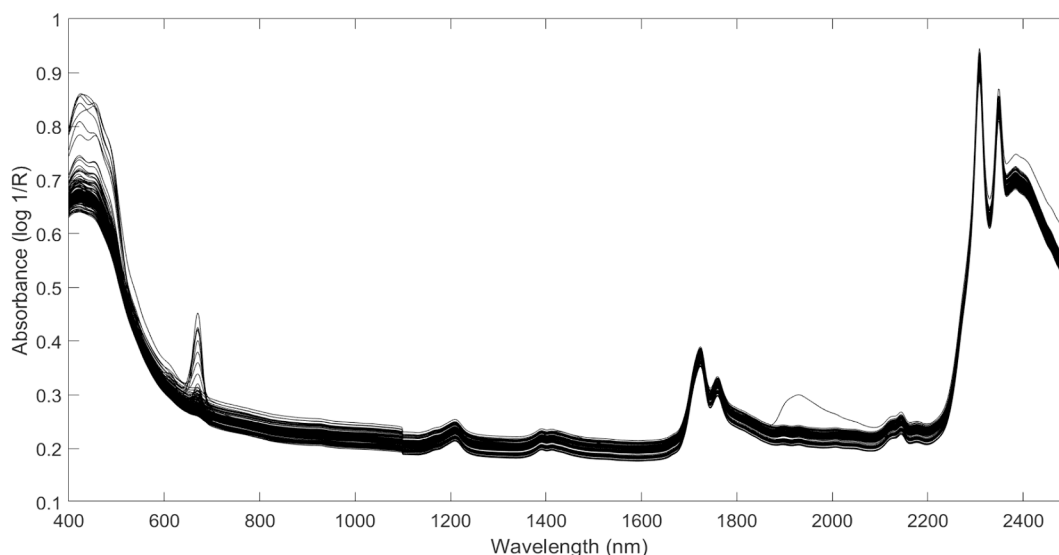
Fig. 2. Raw spectra of the collection of olive oil samples. DS2500 instrument.
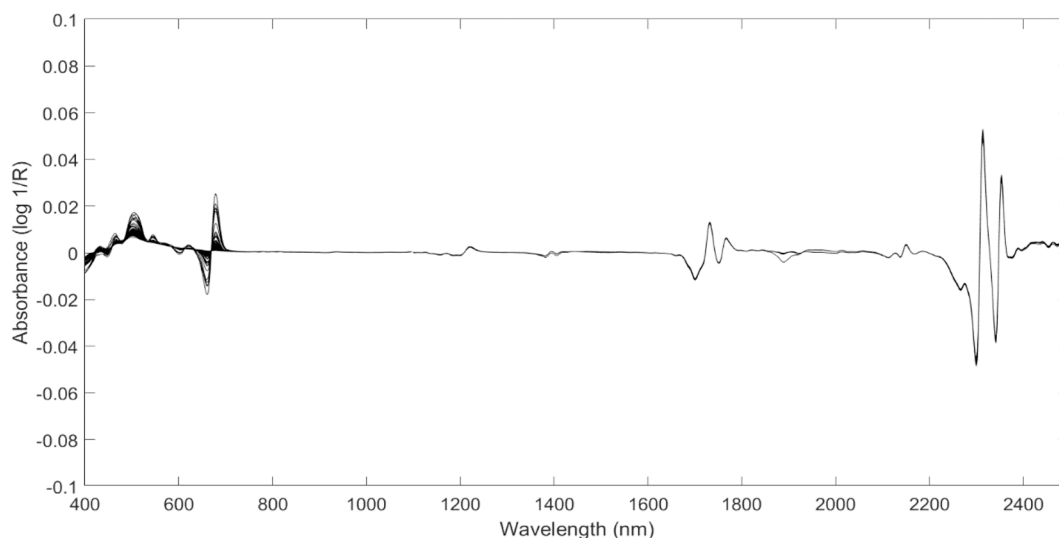
Fig. 3. Pre-processed spectra with a 1st-order derivative.

Table 1
MEAN and STD RMS (μlog (1/R)) values in olive oil.

	MEAN (RMS)			STD (RMS)		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
TOTAL	284	7,954	1,390	402	11,248	1,966
EVOO	352	4,090	1,366	498	5,784	1,932
VOO	284	5,828	1,340	402	8,242	1,894
LOO	340	7,954	1,534	481	11,248	2,170

EVOO: Extra Virgin Olive Oil; VOO: Virgin Olive Oil; LOO: Lampante Olive Oil; RMS: Root Mean Square statistic; STD: standard deviation of the RMS statistic.

Table 2
Mean values of the sensory attributes of the olive oil samples.

	EVOO (N = 66)	VOO (N = 62)	LOO (N = 33)
Fruity median	4.13	2.52	0.00
Bitter median	4.00	3.79	1.59
Pungent median	5.33	4.93	2.22
Median of defect	0.00	2.34	4.83

EVOO: Extra Virgin Olive Oil; VOO: Virgin Olive Oil; LOO: Lampante Olive Oil; N: number of samples.

colour, impurities, and the category of olive oil, etc. As it is known, NIRS analysis of liquid and semi-liquid samples is influenced by minor variations in the effective optical path (Garrido-Varo et al., 2004), so the greater or lesser degree of impurities (or particles in suspension) at the time of analysis could be responsible for these marked differences in RMS among the samples. Additionally, it was decided to calculate the RMS statistics for each category (Table 1). The MEAN and STD values obtained for LOO (1,534 and 2,170 μlog (1/R), respectively) showed a lower spectral repeatability than that obtained for EVOO (1,366 and 1,931 μlog (1/R), respectively) and VOO (1,339 and 1,894 μlog (1/R), respectively). This difference could be attributed again to the presence of suspended particles in the LOO samples.

The RMS cut-off limit is an important factor in this routine, as it indicates the repeatability value above which the spectral analysis of a given sample probably needs to be repeated. This value was calculated for each category, following the procedure mentioned in the Materials and Methods section, with values of 2,298, 2,424 and 3,021 μlog (1/R) for EVOO, VOO and LOO respectively. Any sample in which the duplicated screening scans yielded an RMS above its corresponding limit was rejected and the process was repeated until values fell below these limits, thus ensuring a high degree of spectrum repeatability. Finally, the mean spectrum of the two replicates of each sample was used for further analysis.

3.2. Development of non-targeted approaches

3.2.1. Characterization of the sample set

In this study, a total of 161 OO samples were used. The Panel test assigned a category to each sample based on the average scores of the different sensory attributes. This resulted in 66 EVOO samples, 62 VOO samples, and 33 LOO samples. Table 2 shows the mean values of the

positive (fruitiness, bitterness, and pungency) and negative attributes of the samples. The data in table 2 indicate a clear differentiation in the sensory attributes among the three categories of olive oil. EVOO samples exhibited the highest mean values for positive attributes, and had no detectable defects. VOO samples showed moderate mean values for the positive attributes, and some level of sensory defects. LOO samples, on the other hand, had the lowest mean values for positive attributes, and the highest mean defect value, reflecting significant sensory defects. These data provide an overview of the predominant sensory characteristics in each category of olive oil studied, allowing for a clear and objective comparison among them.

3.2.2. One-class models

Firstly, one-class models were developed to characterize EVOO and VOO categories (target class) using Partial Least Squares Density Modelling (PLS-DM). In this case, the training set was composed of 96 samples of the target class (EVOO and VOO) and 25 samples of the non-target class (LOO). The test set was made up of 32 samples from the target class and 8 samples from the non-target class.

The first step was to select the optimal number of latent variables (LVs) for each condition, by considering the maximum efficiency of the resulting class model, evaluated by cross-validation. Then, models at fixed LVs were evaluated by the Pareto diagram (Fig. 4), whose axes correspond to sensitivity and specificity of class models. Pareto optimal solutions, which define the Pareto front, are represented by points connected by the red line. The final model was selected among the solutions lying on the Pareto front, and we aimed for a balance between sensitivity and specificity.

The most suitable solution had 83.33 % sensitivity and 74.40 % specificity in cross-validation, and the following conditions: $k = 3$, column pre-processing = autoscaling, $L = 10$ and $a = 0.8$. Also, to correct undesirable effects in the spectra, this solution included data pre-processing with a first order derivative according to the Savitzky-
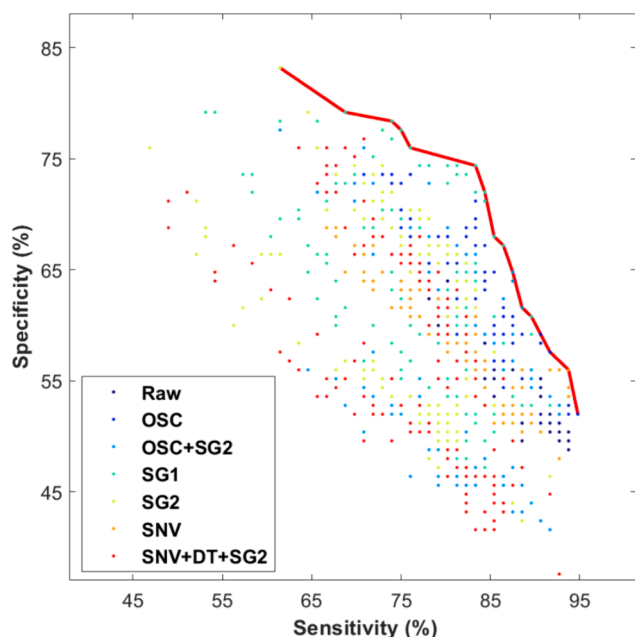
Fig. 4. Pareto diagram – PLS-DM. Each point represents a model obtained varying PLS-DM parameters; optimal solutions are connected by the red line (Pareto front). Each point represents a model obtained under different settings of relevant parameters. Colours are used to code the different data pre-treatments: Raw; Orthogonal Signal Correction (OSC); OSC + Second derivative with the Savitzky-Golay filter (SG2); First derivative with the Savitzky-Golay filter (SG1); SG2; Standard Normal Variate (SNV) transform; SNV + Detrend (DT) + SG2.

Golay method, with 15 smoothing points and third order polynomial adjustment.

The best one-classification model selected, was applied to the test set in order to evaluate its performance in predicting new samples, obtaining, in this case, 90.63 % sensitivity and 62.50 % specificity. This shows that almost all the EVOO and VOO samples had been correctly assigned to the target class. Nevertheless, the ability of the model to recognise samples that did not belong to the target class (LOO samples) was lower, which can be attributed to the unbalance of samples between the target and non-target classes. In classification problems, one consequence of an unbalanced class distribution is that samples belonging to the smaller classes are misclassified more often than those belonging to the more prevalent classes (Sun, Wong & Kamel, 2009).

3.2.3. Discriminant models

Once the LOO samples had been identified, the EVOO and VOO samples were then divided into the corresponding categories. Discriminant models were built using PLS-DA and SVM-DA. To do this, a training set of 96 samples (49 EVOO and 46 VOO) and a test set of 32 samples (17 EVOO and 15 VOO) were used. The cross-validated results obtained by discriminant models, using the different pre-treatments mentioned in the Materials and Methods section, are shown in Table 3.

The best PLS-DA model was built with the pre-processing combination of SNV + DT + SG2 + MC, because it led to the highest average percentage of correct classifications in cross-validation. The model correctly classified 83.33 % (80/96) of the samples, 83.67 % (41/49) as EVOO and 82.98 % (39/47) as VOO. In contrast, the best SVM-DA model was obtained with the pre-processing combination of SG2 + autoscaling. This model correctly classified 86.46 % (83/96) of the samples, 87.76 % (43/49) as EVOO and 85.11 % (40/47) as VOO. These discriminant models were not significantly different ($p < 0.05$) (McNemar's value = 0.364). From the data reported in Table 3, it can be concluded that it is key to consider that the improvement in model accuracy does not depend solely on the use of the non-linear algorithm, as reported by

Table 3

Cross-validation results obtained by discriminant models (PLS-DA and SVM-DA) with different pre-processing methods.

Pre-treatments	PLS-DA		SVM-DA	
	CCR (%) EVOO	CCR (%) VOO	CCR (%) EVOO	CCR (%) VOO
SNV + MC	73.47	78.72	87.76	78.72
SG1 + MC	69.39	85.11	79.59	72.34
SG2 + MC	83.67	78.72	100.00	0.00
SNV + DT + SG2 + MC	83.67	82.98	67.35	89.36
OSC + MC	71.43	78.72	73.47	82.98
OSC + SG2 + MC	75.51	85.11	100.00	0.00
SNV + autoscaling	65.31	72.34	75.51	80.85
SG1 + autoscaling	81.63	72.34	87.76	76.60
SG2 + autoscaling	81.63	80.85	87.76	85.11
SNV + DT + SG2 + autoscaling	77.55	78.72	83.67	85.11
OSC + autoscaling	65.31	72.34	79.59	78.72
OSC + SG2 + autoscaling	77.55	80.85	83.67	85.11

PLS-DA: Partial Least Squares-Discriminant analysis; SVM-DA: Support Vector Machine-Discriminant Analysis; CCR: Correct Classification Rate; EVOO: Extra Virgin Olive Oil; VOO: Virgin Olive Oil; SNV: Standard Normal Variate; MC: column mean centering; SG1: First derivative with the Savitzky-Golay filter; SG2: Second derivative with the Savitzky-Golay filter; DT: Detrend; OSC: Orthogonal Signal Correction.

Devos et al. (2014). Therefore, the combination of both data pre-treatment and PR method should be taken into consideration when developing predictive non-targeted NIRS models.

Different variable selection techniques (GA, iPLS and 'Selectvars') were applied to discard variables with little information in order to improve the predictive ability of the model. Table 4 shows the best models obtained by each variable selection method tested. In general, the application of variable selection methods improved the results of the discriminant models, especially for the VOO category. This means that the application of these methods is useful to identify wavelengths that best define the class of the sample and allow to build models which are more stable, robust, and easy to interpret, and which are less susceptible

Table 4

Best models obtained by different variable selection methods.

Discriminant model	Variable Selection Method	Pre-processing	CCR (%) EVOO	CCR (%) VOO
PLS-DA	–	SNV + DT + SG2 + MC	83.67	82.98
	GA	SG2 + autoscaling	87.76	87.23
	iPLS 10 (width = 10)	OSC + MC	79.59	80.85
	iPLS (width = 50)	OSC + autoscaling	83.67	87.23
	iPLS (width = 100)	OSC + MC	79.59	87.23
	Selectvars	SG2 + autoscaling	81.63	85.11
SVM-DA	–	SG2 + autoscaling	87.76	85.11
	GA	SG2 + autoscaling	91.84	87.23
	iPLS (width = 10)	SNV + autoscaling	83.67	82.98
	iPLS (width = 50)	SG1 + autoscaling	85.71	82.98
	iPLS (width = 100)	SNV + DT + SG2 + autoscaling	85.71	89.36
	Selectvars	SNV + autoscaling	89.80	85.11

PLS-DA: Partial Least Squares-Discriminant analysis; SVM-DA: Support Vector Machine-Discriminant Analysis; CCR: Correct Classification Rate; EVOO: Extra Virgin Olive Oil; VOO: Virgin Olive Oil; SNV: Standard Normal Variate; MC: column mean centering; SG1: First derivative with the Savitzky-Golay filter; SG2: Second derivative with the Savitzky-Golay filter; DT: Detrend; OSC: Orthogonal Signal Correction; GA: Genetic Algorithm; iPLS: Interval PLS.

to external variation (Xiaobo, Jiewen, Povey, Holmes & Hanpin, 2010). Due to the lack of significant differences (McNemar's test) found between the best models obtained by the different discriminant methods tested (PLS-DA and SVM-DA, with and without variable selection), all of them were validated for the test set (Table 5).

The model with the highest average percentage of correct classifications in external validation was a PLS-DA model with the pre-processing combination of SG2 + autoscaling and GA as variable selection method. GA selected 254 variables for inclusion. The model correctly classified 75 % (24/32) of the samples, 82.35 % (14/17) as EVOO and 66.67 % (10/15) as VOO. Examining the PLS-DA scores of the model, it can be seen that the first and second latent variables (LVs) were effective in distinguishing between EVOO and VOO (Fig. 5). However, some overlap exists between the two groups, which indicates that while the model generally distinguishes between EVOO and VOO, there are some cases where the differentiation is less clear.
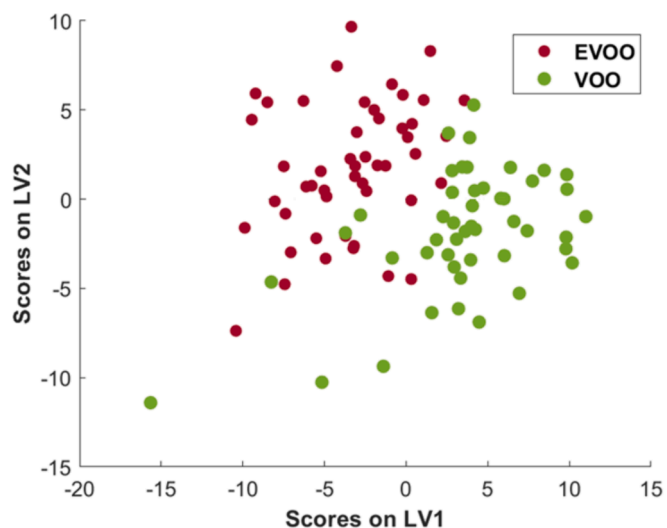
The results from the external validation were plotted in Fig. 6 for further detailed analysis. The class-boundary was fixed at around 0.5. Samples falling above this boundary were classified as EVOO, while those below were classified as VOO. It can be seen that 3 EVOO and 5 VOO samples were misclassified. However, the misclassified EVOO samples were all near the threshold between the two categories. One crucial methodological factor in the validation of multivariate quantitative or qualitative NIRS models is not only to detect the number of samples that do not fit the model, but also to understand the reason for this uniqueness. We therefore explored further the reason for the uniqueness of the 8 misclassified samples: as the present research is part of two large R&D Spanish projects, the physicochemical composition (free acidity, peroxide index, spectrophotometric evaluation (K_{232}, K_{270}, ΔK) and fatty acid ethyl esters (FAEE)) of the samples were known. Therefore, the study of the misclassified samples was carried out taking into account this information together with the PT characterization (Table 6). It can be seen that the 3 EVOO samples classified as VOO by NIRS presented values of the physicochemical parameters established in the legislation (OJEU, 2022) that would allow them to be classified as EVOO. Misclassifying an EVOO as VOO may entail economic consequences for the industry. However, the reverse scenario is more critical. Given that EVOO represents the highest quality in olive oil, misclassifying a VOO sample as EVOO could lead to significant quality and authenticity issues. Table 6 also shows that 3 of the 5 misclassified VOO samples presented values of the physicochemical parameters established in the legislation that would allow them to be classified as EVOO. The remaining two misclassified VOO samples presented values for all the physicochemical parameters that would allow them to be classified as

Fig. 5. Score plot for the first and second latent variable of the olive oil samples.

EVOO, except the FAEE content (FAEE > 35 mg/kg).

A recent comparative study of 9 official IOC laboratories (Circi et al., 2017), involving 16 samples from Italy and USA, reported that the sensory Panel Test seems to work well for extremely high-quality olive oils, whereas in the case of common commercial EVOOs may give discordant results.

The lack of NIRS studies which test using NIRS as an NTM to distinguish only between the two main categories of EVOO and VOO, as is the case of the present paper, does not allow to compare the results obtained in this study with other published findings. However, with the aim of advancing our knowledge of VIS + NIRS technology as a potential NTM, along with others that are currently under investigation, we have compared the CCR values obtained by NIRS with those obtained by other authors using different chromatographic methods (Barbieri et al., 2020b; Quintanilla-Casas et al., 2020; Valli et al., 2020; Ortiz-Romero et al., 2023). In a strictly quantitative and classical approach, we should conclude that the CCR values obtained in the present work (82.35 % for EVOO and 66.67 % for VOO) are similar or even better than those obtained by Barbieri et al. (2020b) (73 % for EVOO and 85 % for VOO), Quintanilla-Casas et al. (2020) (82.3 % for EVOO and 74.6 % for VOO), Valli et al. (2020) (70 % for EVOO and 67 % for VOO) and Ortiz-Romero et al. (2023) (90 % for EVOO and 64.29 % for VOO). Furthermore, as recommended by Riedl et al. (2015); Quintanilla-Casas et al. (2020) and Nichani et al. (2023), it is urgent need to establish standardized NTM validation protocols for any analytical method based on fingerprints.

Finally, it is important to highlight the advantages of NIRS technology over the chromatographic methods mentioned above in terms of speed of analysis, ease of use for non-experts and cost per sample. Chromatographic methods often have some major disadvantages, such as not being available to many laboratories due to the expensive instrumentation used, the need for highly specialized staff, and the need for sample pre-treatment prior to analysis. Additionally, some of them involve the use chemical reagents and may produce chemical residues, which can be harmful to the environment. Over the last few years, a number of experts in OO analysis have warned about the drawbacks, unsuitability and potential regulatory shortcomings of most IOC and EU protocols for analysing OOs, particularly concerning the use of toxic solvents (Conte et al., 2020; Milani et al., 2020).

4. Conclusions

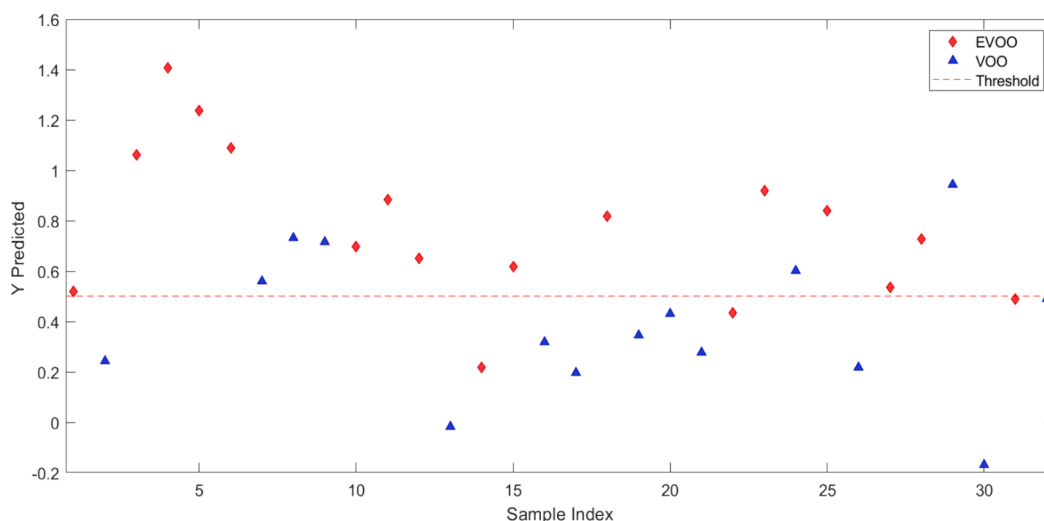The results obtained demonstrate, for the first time to the authors'

Table 5
External validation of the best models obtained by the different methods tested.

Discriminant model	Variable Selection Method	Pre-processing	CCR (%) EVOO	CCR (%) VOO
PLS-DA	–	SNV + DT + SG2 + MC	88.24	60.00
	GA	SG2 + autoscaling	82.35	66.67
	iPLS (width = 50)	OSC + autoscaling	70.59	40.00
	Selectvars	SG2 + autoscaling	88.24	60.00
SVM-DA	–	SG2 + autoscaling	70.59	46.67
	GA	SG2 + autoscaling	88.24	60.00
	iPLS (width = 100)	SNV + DT + SG2 + autoscaling	76.47	60.00
	Selectvars	SNV + autoscaling	70.59	46.67

PLS-DA: Partial Least Squares-Discriminant analysis; SVM-DA: Support Vector Machine-Discriminant Analysis; CCR: Correct Classification Rate; EVOO: Extra Virgin Olive Oil; VOO: Virgin Olive Oil; SNV: Standard Normal Variate; MC: column mean centering; SG2: Second derivative with the Savitzky-Golay filter; DT: Detrend; OSC: Orthogonal Signal Correction; GA: Genetic Algorithm; iPLS: Interval PLS.

Fig. 6. Graphic representation of the best model in external validation.

Table 6
Physicochemical characterization of the misclassified samples by NIRS.

Sample	Category given by Panel Test	Category predicted by NIRS	Acidity (%)	Peroxide value (mEq O_2/kg)	K_{232}	K_{270}	FAEE (mg/kg)	Category given by physicochemical characterization
001	VOO	EVOO	0.14	8.90	2.00	0.12	<10	EVOO
002	VOO	EVOO	0.14	7.40	2.16	0.12	24	EVOO
020	EVOO	VOO	0.15	8.00	1.62	0.12	13	EVOO
027	VOO	EVOO	0.51	6.80	1.63	0.13	60*	no EVOO
037	VOO	EVOO	0.18	8.90	1.78	0.15	<10	EVOO
119	EVOO	VOO	0.19	5.80	1.68	0.14	12	EVOO
170	EVOO	VOO	0.14	11.50	1.90	0.13	12	EVOO
187	VOO	EVOO	0.25	10.60	1.65	0.13	71*	no EVOO

* values which exceed the limits established by legislation for the EVOO category.
EVOO: Extra Virgin Olive Oil; VOO: Virgin Olive Oil; FAEE: Fatty acid ethyl esters.

knowledge, that VIS + NIRS used as an NTM can distinguish between the two most expensive categories of OO (EVOO and VOO) with a high classification rate. Therefore, NTM-NIRS should be considered as an affordable, mass-screening sampling method which could be of great help to support the final decision in conformity checks based on the Panel Test, for the categorization of VOOs. Nevertheless, these results must be considered as a preliminary study, the research path required to make VIS + NIRS into a validated NTM method for VOO authentication has a long way to go due to the absence of internationally-accepted validation protocols for the use of NTMs in food authentication issues but has definitely started. The results shown here are only a small piece of a large R&D line undertaken by the authors' Research Group and funded by two national projects. Looking ahead, it is crucial to emphasize the necessity for more balanced and numerous sample sets in future investigations to substantiate the preliminary and promising outcomes presented. Additionally, further work is in progress to achieve the *in situ* validation of different novel *at line* and *on-site* portable NIRS instruments.

In the light of the encouraging results obtained here using VIS + NIRS, it would be a very welcome step for the NIRS scientific community if the IOC extended its invitation to collaborate in the search for new, more accurate and reliable methods, particularly to detect fraud, based on NIRS, and in particular, to consider NIRS as the go-to candidate for any project or inter-laboratory validation study.

CRediT authorship contribution statement

María-del-Mar Garrido-Cuevas: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Ana-María Garrido-Varo:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Paolo Oliveri:** Validation, Software, Methodology, Investigation. **María-Teresa Sánchez:** Writing – review & editing, Visualization, Resources, Methodology, Investigation. **Dolores Pérez-Marín:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

References

Armenta, S., Moros, J., Garrigues, S., & Guardia, M. D. L. (2010). The use of near-infrared spectrometry in the olive oil industry. *Critical Reviews in Food Science and Nutrition, 50*(6), 567–582. https://doi.org/10.1080/10408390802606790

Baeten, V., & Dardenne, P. (2021). Application of NIR in agriculture. In Y. Ozaki, C. W. Huck, S. Tsuchikawa, & S. B. Engelsen (Eds.), *Near-infrared Spectroscopy* (pp. 331–345). Springer.

Barbieri, S., Brkić Bubola, K., 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*(3), Article 355. https://doi.org/10.3390/foods9030355

Barbieri, S., Cevoli, C., Bendini, A., Quintanilla-Casas, B., García-González, D. L., & Gallina Toschi, T. (2020). Flash gas chromatography in tandem with chemometrics: A rapid screening tool for quality grades of virgin olive oils. *Foods, 9*(7), Article 862. https://doi.org/10.3390/foods9070862

Barnes, R. J., Dhanoa, M. S., & Lister, S. J. (1989). Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Applied Spectroscopy, 43*(5), 772–777. https://doi.org/10.1366/0003702894202201

Biancolillo, A., Marini, F., Ruckebusch, C., & Vitale, R. (2020). Chemometric strategies for spectroscopy-based food authentication. *Applied Sciences, 10*(18), Article 6544. https://doi.org/10.3390/app10186544

Casadei, E., Valli, E., Panni, F., Donarski, J., Gubern, J. F., Lucci, P., Conte, L., Lacoste, F., Maquet, A., Brereton, P., Bendini, A., & Gallina Toschi, T. (2021). Emerging trends in olive oil fraud and possible countermeasures. *Food Control, 124*, Article 107902. https://doi.org/10.1016/j.foodcont.2021.107902

Casale, M., & Simonetti, R. (2014). Review: Near infrared spectroscopy for analysing olive oils. *Journal of Near Infrared Spectroscopy, 22*(2), 59–80. https://doi.org/10.1255/jnirs.1106

Circi, S., Capitani, D., Randazzo, A., Ingallina, C., Mannina, L., & Sobolev, A. P. (2017). Panel test and chemical analyses of commercial olive oils: A comparative study. *Chemical and Biological Technologies in Agriculture, 4*, 1–10. https://doi.org/10.1186/s40538-017-0101-0

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. (2020). 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

Devos, O., Downey, G., & Duponchel, L. (2014). Simultaneous data pre-processing and SVM classification model selection based on a parallel genetic algorithm applied to spectroscopic data of olive oils. *Food Chemistry, 148*, 124–130. https://doi.org/10.1016/j.foodchem.2013.10.020

European Commission, Directorate-General for Agriculture and Rural Development. (2020). Study on the implementation of conformity checks in the olive oil sector throughout the European Union. *Publications Office*. https://doi.org/10.2762/274137

García-González, D. L., Tena-Pajuelo, N., Aparicio-Ruiz, R., Morales-Millán, M. T., Romero del Río, I., & Aparicio-López, R. (2017). A study of the differences between trade standards inside and outside Europe. *Grasas y Aceites, 68*(3), 1–22. https://doi.org/10.3989/gya.0446171

Garrido-Varo, A., Cobo, C., García-Olmo, J., Sánchez-Pineda, M. T., Alcalá, R., Horcas, J. M., & Jiménez, A. (2000). In A. M. C. Davies, & R. Giangiacomo (Eds.), *The feasibility of near infrared spectroscopy for olive oil quality control* (pp. 867–871). NIR Publications.

Garrido-Varo, A., García-Olmo, J., & Pérez-Marín, M. D. (2004). Applications in fats and oils. *Near-Infrared Spectroscopy in Agriculture, 44*, 487–558. https://doi.org/10.2134/agronmonogr44.c19

Garrido-Varo, A., Sánchez, M. T., De la Haba, M. J., Torres, I., & Pérez-Marín, D. (2017). Fast, low-cost and non-destructive physico-chemical analysis of virgin olive oils using near-infrared reflectance spectroscopy. *Sensors, 17*(11). https://doi.org/10.3390/s17112642. Article 2642.

Garrido-Varo, A., Sánchez-Bonilla, A., Maroto-Molina, F., Riccioli, C., & Pérez-Marín, D. (2018). Long-length fiber optic near-infrared (NIR) spectroscopy probes for on-line

quality control of processed land animal proteins. *Applied Spectroscopy, 72*(8), 1170–1182. https://doi.org/10.1364/AS.72.001170

Gómez-Caravaca, A. M., Maggio, R. M., & Cerretani, L. (2016). Chemometric applications to assess quality and critical parameters of virgin and extra-virgin olive oil. *A review. Analytica Chimica Acta, 913*, 1–21. https://doi.org/10.1016/j.aca.2016.01.025

Hruschka, W. R. (1987). Data analysis: Wavelength selection methods. In P. Williams, & K. Norris (Eds.), *Near-infrared technology in the agricultural and food industries* (pp. 35–55). American Association of Cereal Chemists Inc.

International Olive Council. (2021). Trade standard applying to olive oils and olive pomace oils. COI/T.15/NC, No 3/Rev. 16.

ISI. (2000). *The complete software solution using a single screen for routine analysis, robust calibrations, and networking.* Manual, FOSS NIRSystems/TECATOR: Infrasoft International, LLC, Sylver Spring, MD, USA.

Jiménez-Carvelo, A. M., González-Casado, A., Bagur-González, M. G., & Cuadros-Rodríguez, L. (2019). Alternative data mining/machine learning methods for the analytical evaluation of food quality and authenticity–A review. *Food Research International, 122*, 25–39. https://doi.org/10.1016/j.foodres.2019.03.063

Karunathilaka, S. R., Kia, A. R. F., Srigley, C., Chung, J. K., & Mossoba, M. M. (2016). Nontargeted, rapid screening of extra virgin olive oil products for authenticity using near-infrared spectroscopy in combination with conformity index and multivariate statistical analyses. *Journal of Food Science, 81*(10), C2390–C2397. https://doi.org/10.1111/1750-3841.13432

Martínez, M. L., Garrido-Varo, A., De Pedro, E., & Sánchez, L. (1998). Effect of sample heterogeneity on near infrared meat analysis: The use of the RMS statistic. *Journal of Near Infrared Spectroscopy, 6*(201), A313–A320. https://doi.org/10.1255/jnirs.214

McClure, W. F. (1992). Making light work: Lighting new frontiers. In I. Murray, & I. A. Cowe (Eds.), *Making light work: Advances in near-infrared spectroscopy* (pp. 1–13). VCH.

McGrath, T. F., Haughey, S. A., Patterson, J., Fauhl-Hassek, C., Donarski, J., Alewijn, M., van Ruth, S., & Elliott, C. T. (2018). What are the scientific challenges in moving from targeted to non-targeted methods for food fraud testing and how can they be addressed? – Spectroscopy case study. *Trends in Food Science & Technology, 76*, 38–55. https://doi.org/10.1016/j.tifs.2018.04.001

McNemar, Q. (1947). Note on the sampling error of the difference between correlated proportions or percentages. *Psychometrika, 12*(2), 153–157. https://doi.org/10.1007/BF02295996

Milani, A., Lucci, P., Sedran, M., Moret, E., Moret, S., & Conte, L. (2020). Improved method for determination of waxes in olive oils: Reduction of silica and use of a less hazardous solvent. *OCL - Oilseeds and fats, Crops and Lipids, 27*, Article 20. doi: 10.1051/ocl/2020016.

Moghaddam, H. N., Tamiji, Z., Lakeh, M. A., Khoshayand, M. R., & Mahmoodi, M. H. (2022). Multivariate analysis of food fraud: A review of NIR based instruments in tandem with chemometrics. *Journal of Food Composition and Analysis, 107*, Article 104343. https://doi.org/10.1016/j.jfca.2021.104343

Niazi, A., & Leardi, R. (2012). Genetic algorithms in chemometrics. *Journal of Chemometrics, 26*(6), 345–351. https://doi.org/10.1002/cem.2426

Nichani, K., Uhlig, S., Stoyke, M., Kemmlein, S., Ulberth, F., Haase, I., Döring, M., Walch, S. G., & Gowik, P. (2023). Essential terminology and considerations for validation of non-targeted methods. *Food Chemistry: X, 17*, Article 100538. https://doi.org/10.1016/j.fochx.2022.100538

Nørgaard, L., Saudland, A., Wagner, J., Nielsen, J. P., Munck, L., & Engelsen, S. B. (2000). Interval partial least-squares regression (iPLS): A comparative chemometric study with an example from near-infrared spectroscopy. *Applied Spectroscopy, 54*(3), 413–419. https://doi.org/10.1366/0003702001949500

Official Journal of the European Communities (OJEC). (1991). Commission Regulation (EEC) No 2568 /91 of 11 July 1991 on the Characteristics of Olive Oil and Olive-Residue Oil and on the Relevant Methods of Analysis. L, 248, 1–83.

Official Journal of the European Union (OJEU). (2019). Commission Implementing Regulation (EU) 2019/1604 of 27 September 2019 Amending Regulation (EEC) No 2568/91 on the Characteristics of Olive Oil and Olive-Residue Oil and on the Relevant Methods of Analysis. L, 250, 14–48.

Official Journal of the European Union (OJEU). (2022). Commission Delegated Regulation (EU) 2022/2104 of 29 July 2022 Supplementing Regulation (EU) No 1308/2013 of the European Parliament and of the Council as Regards Marketing Standards for Olive Oil, and Repealing Commission Regulation (EEC) No 2568/91 and Commission Implementing Regulation (EU) No 29/2012. L, 284, 1–22.

Oliveri, P. (2017). Class-modelling in food analytical chemistry: Development, sampling, optimisation and validation issues–a tutorial. *Analytica Chimica Acta, 982*, 9–19. https://doi.org/10.1016/j.aca.2017.05.013

Oliveri, P., & Downey, G. (2013). Chapter 13 - Discriminant and class-modelling chemometric techniques for food PDO verification. In M. de la Guardia, & A. Gonzálvez (Eds.), *Comprehensive analytical chemistry* (pp. 317–338). Elsevier.

Oliveri, P., López, M. I., Casolino, M. C., Ruisánchez, I., Callao, M. P., Medini, L., & Lanteri, S. (2014). Partial least squares density modeling (PLS-DM) – A new class-modeling strategy applied to the authentication of olives in brine by near-infrared spectroscopy. *Analytica Chimica Acta, 851*, 30–36. https://doi.org/10.1016/j.aca.2014.09.013

Ortiz-Romero, C., Ríos-Reina, R., García-González, D. L., Cardador, M. J., Callejón, R. M., & Arce, L. (2023). Comparing the potential of IR-spectroscopic techniques to gas chromatography coupled to ion mobility spectrometry for classifying virgin olive oil categories. *Food Chemistry: X, 19*, Article 100738. https://doi.org/10.1016/j.fochx.2023.100738

Pérez-Marín, D., Calero, L., Fearn, T., Torres, I., Garrido-Varo, A., & Sánchez, M. T. (2019). A system using in situ NIRS sensors for the detection of product failing to meet quality standards and the prediction of optimal postharvest shelf-life in the case

of oranges kept in cold storage. *Postharvest Biology and Technology, 147*, 48–53. https://doi.org/10.1016/j.postharvbio.2018.09.009

Pérez-Marín, D., & Garrido-Varo, A. (2023). Near-Infrared spectroscopy and chemometrics in food and agriculture. In R. A. Meyers (Ed.), *Encyclopedia of analytical chemistry* (pp. 1–39). Wiley.

Quintanilla-Casas, B., Marin, M., Guardiola, F., García-González, D. L., Barbieri, S., Bendini, A., Gallina-Toschi, T., Vichi, S. & Tres, A. (2020). Supporting the sensory panel to grade virgin olive oils: An in-house-validated screening tool by volatile fingerprinting and chemometrics. *Foods*, *9*(10), Article 1509. doi: 10.3390/foods9101509.

Riedl, J., Esslinger, S., & Fauhl-Hassek, C. (2015). Review of validation and reporting of non-targeted fingerprinting approaches for food authentication. *Analytica Chimica Acta, 885*, 17–32. https://doi.org/10.1016/j.aca.2015.06.003

Rinnan, Å., Van Den Berg, F., & Engelsen, S. B. (2009). Review of the most common pre-processing techniques for near-infrared spectra. *Trends in Analytical Chemistry, 28* (10), 1201–1222. https://doi.org/10.1016/j.trac.2009.07.007

Rodionova, O. Y., Pierna, J. F., Baeten, V., & Pomerantsev, A. L. (2021). Chemometric non-targeted analysis for detection of soybean meal adulteration by near infrared spectroscopy. *Food Control, 119*, Article 107459. https://doi.org/10.1016/j.foodcont.2020.107459

Roggo, Y., Duponchel, L., Ruckebusch, C., & Huvenne, J. P. (2003). Statistical tests for comparison of quantitative and qualitative models developed with near infrared spectral data. *Journal of Molecular Structure, 654*(1–3), 253–262. https://doi.org/10.1016/S0022-2860(03)00248-5

Shenk, J. S., & Westerhaus, M. O. (1995). *Analysis of agriculture and food products by near infrared reflectance spectroscopy*. Monograph: NIRSystems Inc.

Shenk, J. S., & Westerhaus, M. O. (1996). Calibration the ISI way. In A. M. C. Davies, & P. Williams (Eds.), *Near infrared spectroscopy: the future waves* (pp. 198–202). NIR Publications.

Sun, Y., Wong, A. K., & Kamel, M. S. (2009). Classification of imbalanced data: A review. *International Journal of Pattern Recognition and Artificial Intelligence, 23*(04), 687–719. https://doi.org/10.1142/S0218001409007326

Valli, E., Panni, F., Casadei, E., Barbieri, S., Cevoli, C., Bendini, A., … Gallina Toschi, T. (2020). An HS-GC-IMS method for the quality classification of virgin olive oils as screening support for the panel test. *Foods, 9*(5), Article 657. https://doi.org/10.3390/foods9050657

Willenberg, I., Matthäus, B., & Gertz, C. (2019). A new statistical approach to describe the quality of extra virgin olive oils using near infrared spectroscopy (NIR) and traditional analytical parameters. *European Journal of Lipid Science and Technology, 121*(2), Article 1800361. https://doi.org/10.1002/ejlt.201800361

Wold, S., Antti, H., Lindgren, F., & Öhman, J. (1998). Orthogonal signal correction of near-infrared spectra. *Chemometrics and Intelligent Laboratory Systems, 44*(1–2), 175–185. https://doi.org/10.1016/S0169-7439(98)00109-9

Xiaobo, Z., Jiewen, Z., Povey, M. J. W., Holmes, M., & Hanpin, M. (2010). Variables selection methods in near-infrared spectroscopy. *Analytica Chimica Acta, 667*(1–2), 14–32. https://doi.org/10.1016/j.aca.2010.03.048

Zeng, J., Guo, Y., Han, Y., Li, Z., Yang, Z., Chai, Q., … Fu, C. (2021). A review of the discriminant analysis methods for food quality based on near-infrared spectroscopy and pattern recognition. *Molecules, 26*(3), Article 749. https://doi.org/10.3390/molecules26030749