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Multivariate qualitative methodology for semi-quantitative information. A case study: Adulteration of olive oil with sunflower oil

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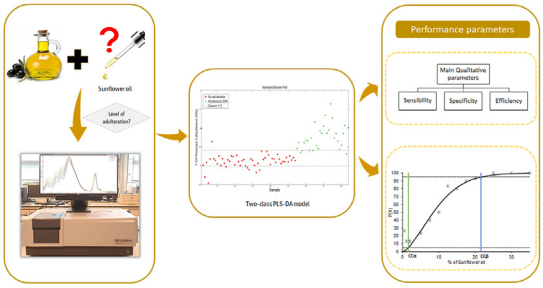
HIGHLIGHTS

- The adulteration of olive oil with sunflower oil has been studied by Fluorescence.
- Four levels of adulterant percentage have been considered.
- Two-class PLS-DA models have been established for each level of adulterant.
- Performance characteristic curves (PCC) were established for each model.
- The main and semi-quantitative performance parameters have been evaluated.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper proposes a strategy to assess the performance of a multivariate screening method for semi-quantitative purposes. The adulteration of olive oil with sunflower oil was considered as a case study using fluorescence spectroscopy and two-class Partial Least Squares Discriminant Analysis (PLS-DA). Building the proper screening methodology based on two-class multivariate classification model involve setting the cut-off value for the adulterated class (class 2). So, four classification models were established for four levels of adulterant (cut-off). Model validation involved calculating the main quality parameters (sensitivity, specificity and efficiency) and three additional semi-quantitative parameters (limit of detection, detection capability and unreliability region).

The probability of successfully recognizing non-adulterated samples as such was set by the main performance parameters of the two-class model. However, the probability of successfully recognizing adulterated samples as such was more accurately extracted from the performance characteristic curves (PCC) curves instead of just from the sensitivity of the adulterated class.

The main performance parameters of the PLS-DA models increased as the cut-off level increased although after a particular value the increase was less pronounced. As an example, when the cut-off was changed from 5% to 20%, sensitivity changed from 70 to 93%, specificity changed from 87 to 97%, and efficiency changed from 78 to 95%. The same can be stated for the semi-quantitative parameter's decision limit and detection capability, which moved from 0 to 1.6 and from 17.7 to 21.6 (% of adulterant), respectively.

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1. Introduction

There are different qualitative approaches that provide a binary response as output (for example, yes/no, accepted/not accepted, etc.). Qualitative methods can be classified in several ways depending on the information sought and the nature of the data used. Related to the information sought, it can be differentiated between non-quantifiable and semi-quantifiable methods. An example is when a qualitative method is developed to check whether the samples fit an overall property (i.e., geographical origin). Qualitative methods that give semi-quantifiable information determine whether a compound is present in a sample above or below a certain threshold or if a sample contains or does not contain it (i.e., samples has been adulterated or not). Related to data used, can be either a specific signal or as multiple non-specific variables known as univariate and multivariate qualitative methods, respectively. To develop a multivariate qualitative method, a chemometric approach is required by applying multivariate classification techniques.

Qualitative methods based on multivariate classification have proven to be faster, more economic and environmentally friendly, resulting in more sustaining methods. Consequentially they are currently in high demand. Examples are numerous in many fields, food science being one of them [1,2]. As in all newly developed methods, one of the steps is its validation. Considerable effort has been made in this matter and although there is still a lot to be done [3,4]. Nowadays the quality parameters sensitivity (SEN), specificity (SPC), efficiency (EFF) or accuracy (ACC) and precision (PR) are well established [3,5,6]. Recently, a new quality parameter called occurrence (OCURR) [7] and a work dealing with time stability of the classification models [8] have been reported. These performance parameters can be calculated by all of the classification approaches mentioned above. If the classification method is approached for semi-quantitative purposes, there are other parameters of great interest such as the detection limit (CCα), the detection capability (CCβ) and the unreliability region (UR). These parameters have hardly been addressed in the literature [3,9–13] although they are of the utmost importance when the qualitative method is to be used for screening purposes.

All the quality parameters mentioned are calculated from the probabilities that arise from the four well known binary responses [14]: True positive (TP) and True Negative (TN), when the qualitative method rightly considers a sample to be positive or negative that is indeed positive or negative, respectively; and False positive (FP) and False negative (FN) when the qualitative method wrongly gives a positive output for a sample that is negative or a negative output for a sample that is positive. Depending on the classification technique used, in a multi-class approach, some responses can be inconclusive, that is, a sample is assigned to no class or is assigned to more than one [15–17].

The present study proposes a strategy to assess the performance of a multivariate screening method for semi-quantitative purposes. It includes the calculation of the main quality parameters and the three additional semi-quantitative parameters. As a case study, an adulteration of olive oil with sunflower oil has been considered using fluorescence measurements.

To develop a multivariate screening, a decision must be made on using a class modelling or discriminating classification techniques. Discriminant methods require at least two classes and are applied to multi-class problems, while class modelling methods can be develop for just one-class or for more than two classes (multi-class). A review [18] has recently been published that describes and critically compares the main multivariate qualitative strategies. Deciding which one is the most appropriate in each case is not straight forward. Dealing with adulteration problem, some authors [19,20] prefer class modelling methods than discriminant ones considering the difficulties in acquiring a sample set representative of all possible types of adulterations. But, in case the adulterant under study is known, the use of a two-class discriminant technique has the advantage of making unambiguous assignments [21] In such case, in the prediction of a sample, it belongs or not to the adulterated class.

Given this and considering than the adulterant is known (sunflower oil), the proposed methodology consists of building a two-class PLS-DA model. Class 1 consisting of the non-adulterated samples and class 2 of the adulterated samples. The key point of the two-class strategy is that the adulteration level used to define the adulteration class must be specified. We will refer to this level as the cut-off level, since it is related to the threshold concentration below which the sample is acceptable (compliant) and above which it is not acceptable (non-compliant). Two situations can be addressed, when there is a reference or threshold value (concentration, %, etc.) and the goal is to verify whether the compound exceeds it or not. In the other, there is not a reference or threshold value. Therefore, the goal is to determine from what level of adulteration the method is able to determine if the sample is adulterated, with predetermined probabilities of success.

In the food area, this cut-off value is not usually known a priori. So, different values have to be tested so that the one that best fits the purpose of the application under consideration can be selected. The decision will also depend on considerations related to the nature of the problem ahead, whether the compound is a contaminant with an impact on health or the economy, etc.

Once the cut-off has been established, the discriminant model can be developed and validated on the basis of the main quality parameters (sensitivity, specificity and efficiency). If the main performance parameters are adequate, performance characteristic curves (PCC) will be established to determine the parameters related with the concentration (CCα, CCβ and unreliability region) by analyzing samples with adulteration percentages above and below the cut-off value. PCC are widely used in the validation of univariate qualitative methods [11,22,23] but not to the same extend in multivariate qualitative methods. The main drawback of PCC is the need for a large number of experiments for each level of adulterant. Despite this, PCC have proven to be a well-studied and tested tool in practice for estimating the parameters of qualitative methods, although their use for multivariate methods is still scarce. Recently, new ways to estimate the three semi-quantitative parameters has been proposed although, as the authors stated, they have not yet been fully investigated and validated in practice [4].

Nowadays, there are many studies, particularly on food, that establish multivariate qualitative methods to determine possible adulteration in food but very few of them determine parameters related to the concentration of the adulterant. This paper is a contribution in this sense, and it also demonstrates the usefulness of taking advantage of the information from the PCC curves in the unreliability range. Additionally, it shows, from a practical point of view, the benefits of allowing the analyst to set the cut-off value in each case.

2. Samples, instrumentation and software

A total of 60 virgin and extra virgin Arbequina oil samples from Catalonia were supplied by the Catalan Government's Official Tasting Panel of Virgin Olive Oils of Catalonia, which confirms the status of the oils. A further 24 samples were obtained by randomly mixing 5 original oils. The 84 unadulterated Arbequina samples were randomly divided into training (54 samples) and test set (30 samples). To obtain the adulterated samples, the 30 test set samples were adulterated with sunflower oil, each at 13 different levels (between 0.5% and 35%).

Fluorescence spectra were obtained using a Shimadzu RF-5301PC ((Shimadzu Corporation, Kyoto, Japan). The slit width was 5 nm for emission spectra, which were collected between 360 and 800 nm using an excitation wavelength of 350 nm. The integration time was 0.1s, and the increasing wavelength while scanning the spectrum was 10 nm. No sample pretreatment was applied.

The recorded data was treated by using MATLAB software, version 8.0.0.783 – R2012b (Natick, MA, USA) and PLS Toolbox 7.0.2 (Eigenvector Research Inc., Wenatchee, WA, USA).

3. Theoretical background

Principal component analysis (PCA) is one of the techniques most commonly used to compress, describe and interpret large sets of multidimensional data. It should always be used for a preliminary exploratory analysis of every dataset, even when the final aim is to perform a supervised classification for predictive purposes.

Partial Least Square Discriminant Analysis (PLS-DA) is a PLS regression technique adapted to a classification technique. It requires two matrices, one with independent variables (matrix X), which in our case are the fluorescence spectra and the other with dependent variables (matrix Y), which in our case is a binary code (0 and 1) where 1 indicates sample membership and 0 does not.

There is an extensive bibliography that describes theoretical and practical aspects of both PCA and PLS-DA. Without being exhaustive in the references, two recent reviews can be consulted, which in turn provide multiple references [18,24].

3.1. Performance parameters

To validate the classification models, the main performance parameters are typically sensitivity, specificity and efficiency, terms which have been extensively defined. Other parameters that are directly related with those mentioned (such as Youden's index, likelihood ratio …) are also reported. For further details on figures of merit and validation strategies, the interested reader is referred to [3,6,21,25].

Three additional performance parameters for semi-quantifiable qualitative methods have also been defined: decision limit, detection capability and unreliability region [3,12,13]. The three parameters can be obtained from the performance characteristic curve (PCC). The PCC is a plot of the probability of having a positive classification output, P(X), versus the corresponding concentration of the analyte [26–29]. Note that in the literature, PCC curves have also been referred to with other names.

Probability of positive classification (P(x)) is the probability of the classification PLS-DA method giving a positive result. This probability is dependent upon the amount of analyte present in the sample. In qualitative methods such as in this case, the probability of a positive classification should be zero or close zero when the analyte is not present and would increase to the final value of 100% as the adulterant concentration or mass increases. In this study, the probability of positive classification was experimentally obtained for the different adulterant levels (13 levels, between 0.5% and 35%). The PCC curve is obtained fitting the P(X) values to a sigmoid function by minimizing the root mean square of the residuals (RMSE) following Eq. (1) [3,29].

$$P(x) = 1 - e^{-(x/a)\hat{b}} \quad (1)$$

Where x is the adulterant concentration, and a and b are the regression coefficients fitted to minimize the RMSE.

The goodness parameters of the PCC non-linear fit are evaluated by means of the root mean square of errors (RMSE) (Eq. (2)), and the adjusted coefficient of determination (R^2_{adj}) which measures how accurately the calculated curve fits the original data (Eq. (3)) [12]:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n - m}} \quad (2)$$

$$R^2_{adj} = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2 \cdot (n-1)}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2 \cdot (n-m)} \quad (3)$$

Where n, is the number of adulteration levels (in that work n = 14, from 0% to 35%); i refers to specific level; y_i, is the value of P(X) for a given level; $\hat{y}$, is the P(X) predicted value for a given level; $\bar{y}$, the mean of values of the fourteen P(X) values and m, the number of equation parameters (in that case m = 2).

From the PCC curve, the three semi-quantitative performance parameters are calculated [3,12,13]:

Decision limit (CCα) is the minimum concentration of a compound that will give a positive output when indeed it is positive with a particular probability (usually P(X) = 5%). Below this limit, there is a 95% probability or higher of obtaining a negative output N(X) = 100-P(X) (that is to say, that the sample is not adulterated or adulterated at lower levels. *CCα* is obtained from the intersection of the PCC curve with the horizontal black dashed lines placed at $\alpha = 5\%$.

Detection capability (CCβ) is the concentration of a compound in a sample that can be reliably detected and/or identified with statistical certainty (usually (P(X) = 95%). Above this limit, there is a 95% probability or higher of obtaining a positive output for a sample adulterated at any level above it. *CCβ* is obtained from the intersection of the PCC curve with the horizontal black dashed lines placed at $1\text{-}\beta = 95\%$.

Unreliability region (UR) is the range of concentration between the two limits where there is certain probability (P(X) between 5% and 95%) of false negative errors. It means that there is certain probability that a sample is not adulterated when indeed it is.

4. Results and discussion

The flowchart of the screening strategy proposed is described in Fig. 1. Dealing with an adulteration problem, after selecting a multivariate (rather than a univariate) classification approach, the first step is to look at the spectra and the data structure (Fig. 1, preliminary studies). Fig. 2 shows the mean spectrum of the EVOOs non-adulterated and adulterated at the different sunflower oil percentages. It can be seen that the fluorescence increases as the percentage of adulterant does, mainly in the bands from 360 to 480 nm. Additionally, shifts are observed in the maximum at wavelengths around 520 nm (towards shorter wavelengths) and around 380, 440 and 475 nm (towards longer wavelengths). The appearance of a band can be perceived at 410 nm, which becomes more evident as the concentration of adulterant increases.

PCA was used to analyze the data structure and the presence of outliers. Fig. 3 shows the scores for the first two PCs, for all the samples analyzed no matter the % of adulteration. With a variance of 97%, it can be seen that there are no clear groups, but samples show a clear trend: the greater the percentage of sunflower oil, the higher the score is on PC1.

The next step (flowchart Fig. 1) is to decide on the type of classification technique to use, modelling or discriminant. In an adulteration problem in which the possible adulterant is known and will be studied (sunflower oil in this case study), a discriminant technique is the most appropriate since the class membership is

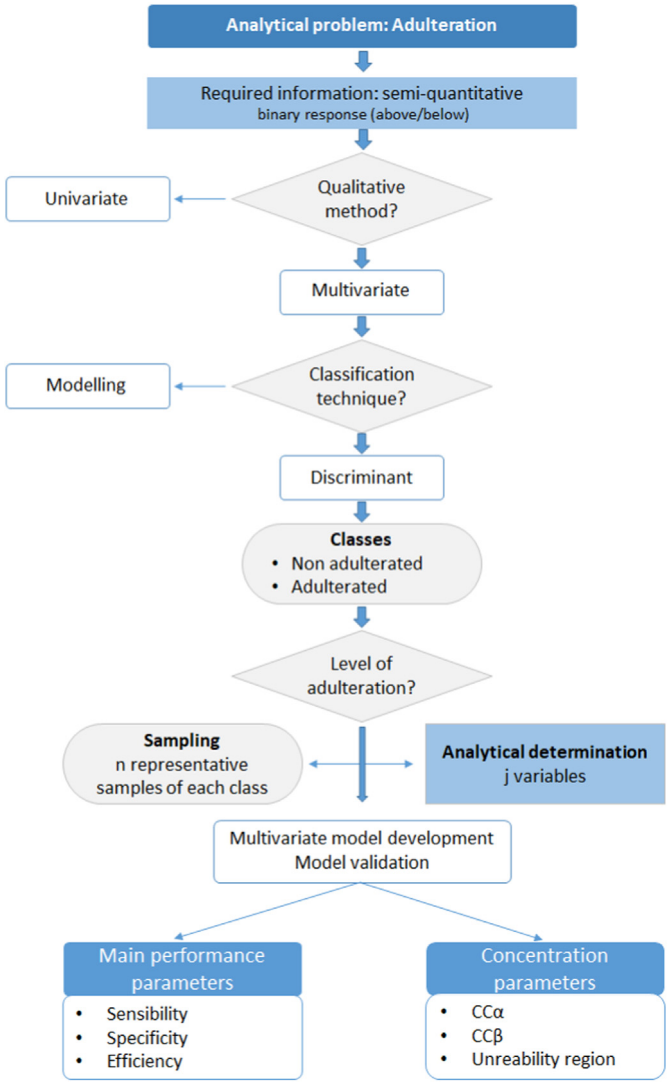

Fig. 1. Flow chart showing the steps to develop a qualitative multivariate method with semi-quantitative purpose.

assigned unambiguously. In this paper PLS-DA method was implemented and two classes were defined: class 1 for non-adulterated samples and class 2 for samples adulterated with sunflower oil. To establish the two-class model, the level of adulteration for class 2 (adulterated class) has to be defined. This level is called the cut-off level so the samples in class 2 have a percentage of adulterant equal to the cut-off value, but the non-adulterated class (class 1) is not changed.

The cut-off value that fit for the purpose of the present problem is unknown. Therefore, to define class 2 (adulterated class), various percentages of adulterant are studied. In this study, four cut-off values, had been considered: 5, 10, 15 and 20% of adulterant. So, four PLS-DA two-class models were established. In all four models, class 1 is the same and was built with the 54 non-adulterated training samples. The PLS-DA models differ in class 2, Model 1 was built with 30 samples adulterated at 5% sunflower. Likewise, Class 2 in Model 2, Model 3 and Model 4 was built with 30 samples adulterated at 10%, 15% and 20% of sunflower, respectively.

Before the model development, the spectra were mean-centered. A total of 4 LVs were kept in all four models, with the explained variance being around 98% in all four models. Then, the main quality parameters were calculated, all of which were based on the well-known TP, TN, TP and TN rates calculated from the model output. Table 1 shows the main performance parameters of the adulterated class (class 2) obtained with the four PLS-DA models. Since it is a two-class model, the main quality performance values for the non-adulterated class (class 1) are the same but swapping the value for sensitivity instead of specificity (and vice versa). As expected, all performance parameters increase as the cut-off level increases since it discriminates between non adulterated samples (class 1) and samples adulterated at higher percentages (class 2). This increase is less and less important and when the level of adulteration increases from 15% (Model 3) to 20% (Model 4) the value of the performance parameters was the same.

A closer look at the main quality performance values of one of the models (for instance, Model 1, cut-off set at 5%, Table 1) indicates that of every 100 non-adulterated samples, the model would properly recognize 87 as non-adulterated and wrongly recognize 13 as adulterated (specificity = 87%). Similarly, of 100 samples adulterated at or higher than 5% of sunflower oil, the model would properly recognize 70 as adulterated and wrongly

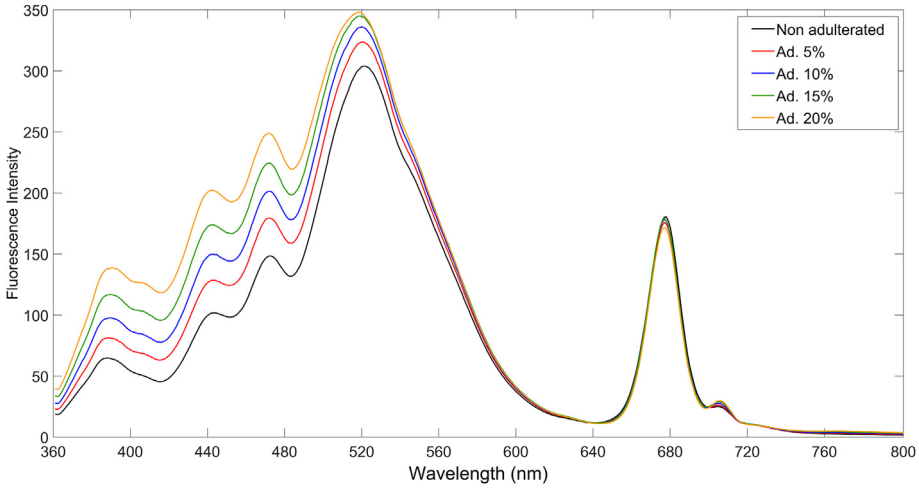

Fig. 2. Mean spectra of non-adulterated olive oil samples and samples adulterated at several percentage of sunflower oil.

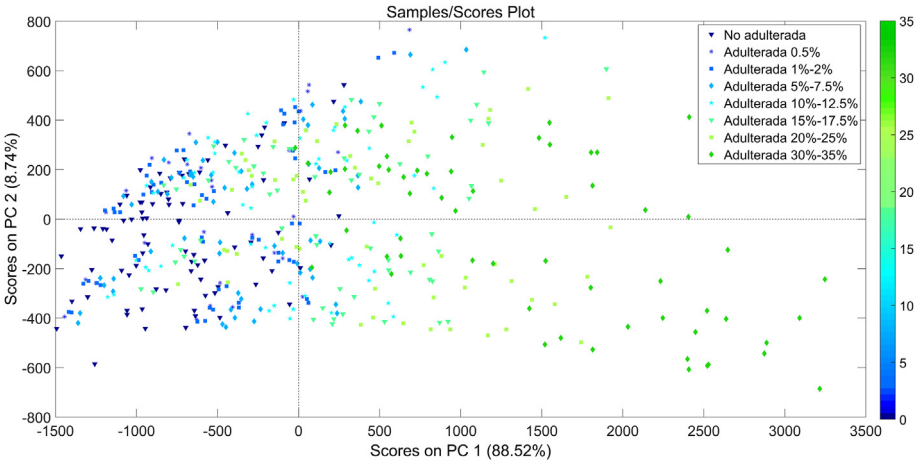

Fig. 3. PCA score plot for the non-adulterated olive oil samples and samples adulterated at several percentage of sunflower oil. Color bar representing the adulteration levels. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Main performance parameters of the adulterated class (class 2), for different adulterant levels. Model 1, Model 2, Model 3 and Model 4, class 2 built with samples adulterated at 5, 10, 15 and 20% of sunflower oil, respectively.

Parameter (%)	Model 1	Model 2	Model 3	Model 4
Sensitivity	70,0	76,7	93,3	93,3
Specificity	86,7	93,3	96,7	96,7
Efficiency	78,3	85,0	95,0	95,0

recognize 30 as non-adulterated. Similar conclusions can be drawn from the values obtained with the other three models.

From the main performance parameters (Table 1), useful information can be obtained for a fixed adulterant percentage (the cut-off values). To obtain information on levels of adulteration above and below the cut-off value, the performance characteristic curves (PCC) were adjusted. To build it, the developed two-class PLS-DA models (Models 1 to 4) were used to predict all the samples no matter the real level of adulterant they contained. Specifically, Fig. 4 shows the PCC curves for the four PLS-DA models. Each PCC curve was obtained from the PLS-DA model predicted values, express as P(X), of the whole data set. For instance, the first PCC curve (Fig. 4a) was obtained from the P(X) values obtained from Model 1 predictions of the 30 non-adulterated test samples (0% of adulterant) and all adulterated samples (30 samples at each of the 13 adulteration levels, from 0.5% to 35%), including the 30 samples of class 2. Likewise, PCC curves for Model 2, 3 and 4, from their model predictions of the whole data set (Fig. 4b, c, d).

Table 2 contains the corresponding fit parameters as well as the performance parameters for each PCC. In general, no big differences were found in the CCα, CCβ (intersection of the horizontal black dashed lines with the PCC curve, Fig. 4a, b, c, d) and unreliability regions obtained for the four PLS-DA models. Nevertheless, the CCα and CCβ values increased slightly with the cut-off value and the values were largest for the model built with an adulterant level of 20%.

It can be observed that as the cut-off value increases, the shape of the curve changes from exponential to sigmoidal (Fig. 4). Therefore, the slope of the PCC curve decreases as the cut-off value increases, the slope being highest at a cut-off value of 5%. This has implications for the performance parameters calculated, but more significantly for the probabilities of properly assigning an adulterated sample. When the shape of the PCC is exponential, the adulterant level corresponding to the cut-off value is placed in the first part of the curve within the unreliability region. However, when the shape is sigmoidal, the cut-off value is placed close to the CCβ value. This has considerable impact on the probability of properly classifying a sample whose level of adulteration is unknown. For instance, considering the fit equations of the PCC curves (Table 2) obtained with a cut-off value of 5% (Fig. 4a), an adulterated sample will have a probability higher than 50% (Fig. 4a, grey dotted line) of being properly classified as adulterated (class 2 assignation) when it is indeed adulterated with 1.6% of sunflower oil or higher (indicated with a dotted line). Similar conclusions can be drawn from the models obtained with a cut-off value of 10% (Fig 4b), 15% (Fig 4c) and 20% (Fig. 4d) when predicting samples adulterated at or higher than 4.6%, 6.3% and 8.5%, respectively. As expected, for a fixed probability of error, a low cut-off value enables adulterated samples to be discriminated from non-adulterated at lower adulteration levels.

The slope and shape of the PCC curves show that as the level of adulteration increases the percentage of correct classification also increases. As an example, for the model built at a cut-off value of 5% (Fig. 4a), the percentage of correct recognition of an adulterated sample is higher than 70% for an adulteration level above 4.1%; higher than 80% (Fig. 4a, grey dashed line) for an adulteration level above 6.8% and higher than 90% for an adulteration level above 12.5%. As the cut-off value increases, the adulterant level that will more probably be correctly recognized also increases. For instance, for the model built at a cut-off value of 20% (Fig. 4d), the percentage of correct recognition of an adulterated sample is higher than 70% for an adulteration level above 12%; higher than 80% (grey dashed line) for an adulteration level above 15% and higher than 90% for an adulteration level above 19%.

The PCC curve provides information on the behavior of the adulterated samples as a function of their level of adulteration. The behavior of the non-adulterated samples is indicated by the specificity of the class 2 model and is also PCC = 100-P(X) for an adulterant level equal to zero. Thus, to build a classification model with the appropriate cut-off value, a compromise must be reached between the probability of recognizing a non-adulterated sample as well as an adulterated sample. The first probability can be set by the main performance parameters of the two-class PLS-DA model. However, the second probability can be more accurately extracted from the PCC curves rather than from the sensitivity of the adulterated class. This approach involves checking different cut-off values with extra experimental data.

The proposed strategy makes it possible to adapt the screening methodology to the laboratory's requirements. To implement it,

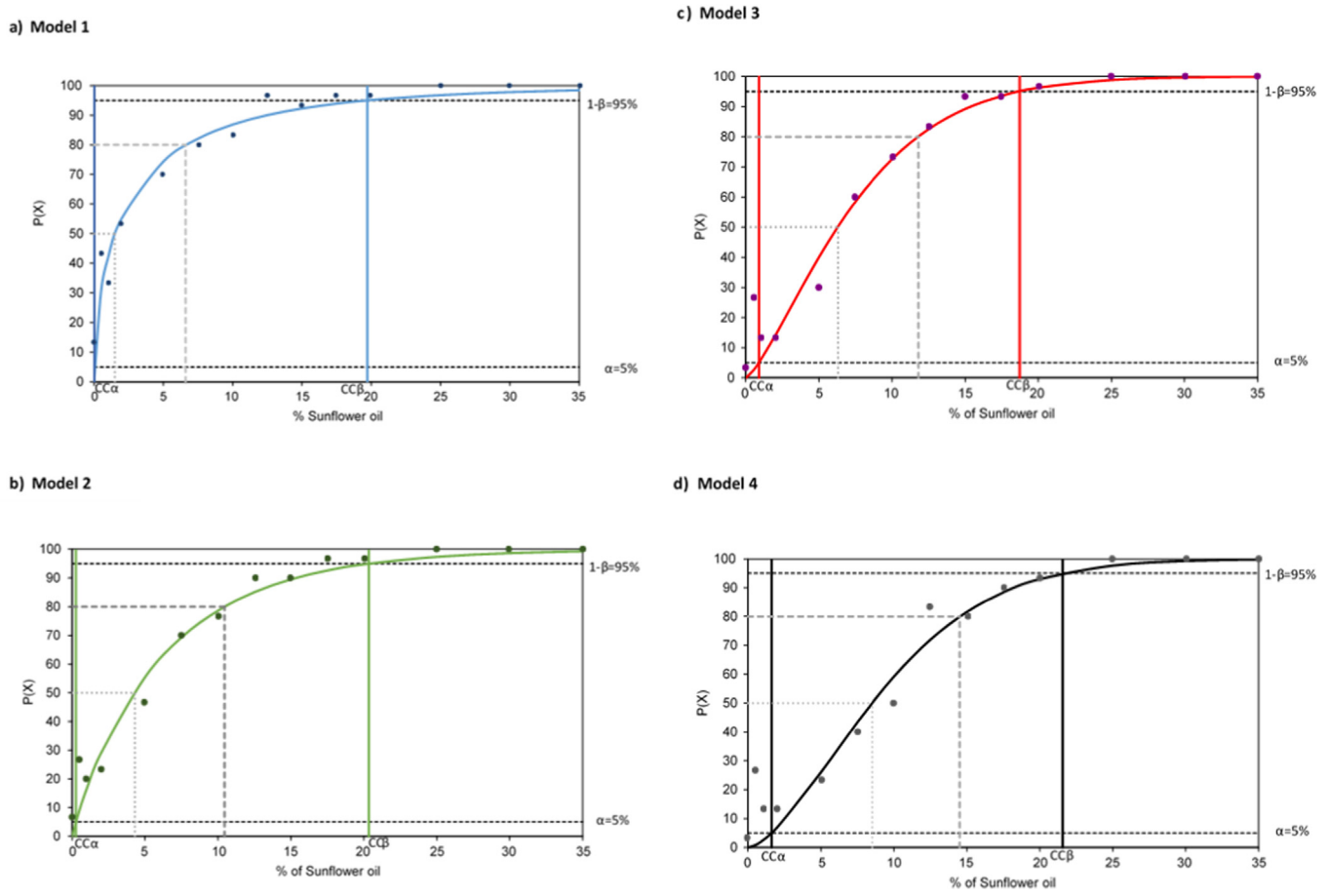

Fig. 4. Performance characteristic curves (PCC) and semi-quantitative parameters for obtained from the four PLS-DA Models: a) PCC for Model 1 (cut-off value at 5%), b) Model 2 (cut-off value at 10%), c) Model 3 (cut-off value at 15%) and d) Model 4 (cut-off value at 20%). CCα and CCβ values calculated from the intersection of the horizontal black dashed lines with the PCC curve, vertical grey lines indicate the adulteration level from which a sample will be detected as adulterated with 50% or higher probability (dotted line) and 80% or higher probability (dashed line).

Table 2
Fit parameters of the PCC curves and semi-quantitative performance parameters, for the four PLS-DA models. Model 1, Model 2, Model 3 and Model 4, class 1 built with 54 non-adulterated samples and class 2 built with samples adulterated at 5, 10, 15 and 20% of sunflower oil, respectively.

	Model 1	Model 2	Model 3	Model 4
R^2_{adj}	0,9468	0,9635	0,9541	0,9356
RMSE	0,0663	0,0669	0,0809	0,0951
Equation	$1-e^{-\left(\frac{x}{2,93}\right)^{0.57}}$	$1-e^{-\left(\frac{x}{6,27}\right)^{0.93}}$	$1-e^{-\left(\frac{x}{8,25}\right)^{1.34}}$	$1-e^{-\left(\frac{x}{10,72}\right)^{1.57}}$
CCα	0,0	0,3	0,9	1,6
CCβ	17,7	20,3	18,7	21,6
Uncertainty region	0,0–17,7	0,3–20,3	0,90–18,7	1,6–21,6

two types of adulterants should be considered: adulterants with an impact on health and adulterants for economic reasons. It is to be expected that the majority of cases would be of the second type.

In the case of adulteration for economic reasons, the choice of the cut-off point is determined by the value at which the adulteration is profitable This value and some close to it would be suitable cut-off values. What's more, several situations can be differentiated, for example, if adulteration is not expected, a laboratory might be interested in a screening approach that ensures that the non-adulterated samples are properly recognized. If that is the case, a high cut-off value is the best option. Another situation could be when the laboratory submits all samples classified as adulterated to a confirmatory analysis. In this case, it is more important that the adulterated samples be recognized as such, than wrongly classifying a non-adulterated sample as adulterated. Therefore, the best option will be to set a low-cut-off value.

In the case of a prohibited contaminant with an impact on health, the best option will be to set the cut-off value defining the adulterated class at low adulteration levels. As a result, the PCC will have an exponential shape and several non-adulterated samples will be submitted to a confirmatory analysis but only a small number of samples with an adulteration level below CCα will be erroneously assigned as non-adulterated. If the case study aims to discriminate samples that contain a compound below a specific threshold, therefore, the cut-off level should be placed below the threshold. For instance, if we want to discriminate samples

adulterated below 20% of sunflower oil, the best option is to set the cut-off value at 10% (Fig. 4b).

The errors that laboratories accept are different in every case. An error in non-adulterated samples means unnecessary confirmatory analysis and an error in adulterated samples means that there will be fraud with economic benefits or a health impact. In any case, the proposed strategy makes it possible to understand the risks and consequences that are being assumed and adapt them to the case under study by selecting the cut-off value.

Once the methodology has been established based on the above considerations, its validation can be carried out, first, from samples of known composition. Subsequently, with data obtained throughout its application, it can be readjusted through updating strategies.

5. Conclusions

A strategy using fluorescence measurements and two-class (adulterated and non-adulterated) PLS-DA classification model has been developed to determine adulteration of olive oil with sunflower oil. Dealing with two-class approach, it is a key point to have representative samples of the non-adulterated class and the adulterated class. Additionally, the adulterant concentration in the samples of the adulterated class (cut-off) should be properly defined. Even more, this work provides evidence that the choice of the level of adulteration is a relevant factor to consider in the design of the adulterated class.

Four PLS-DA models have been established at four cut-off levels. The performance of each model was evaluated by calculating the main quality parameters (sensitivity, specificity and efficiency) and the three additional semi-quantitative parameters (decision limit, detection capability and unreliability region) from the performance characteristic curve. All main performance parameters increase as the cut-off level increases since it discriminates between non adulterated (class 1) and adulterated samples at higher percentages (class 2), although above a certain percentage the increase is irrelevant. This trend is also observed in the semi-quantitative parameters.

This paper contributes to the implementation of the PCC curves as a tool to calculate figures of merit of qualitative methods. PCC are extensity used in univariate qualitative methods, but not in multivariate as in this case. Performance characteristic curves (PCC) has been shown as successful tool to provide information on the probability of properly assigning adulterated samples. Even more, it has been demonstrated that allows adapting the screening method to the laboratory requirements. For instance, setting a high cut-off value if adulteration is not expected, since one is more interested in recognizing the non-adulterated samples as such. Or setting a low cut-off value when it is more important to recognize adulterated samples as such. For example, when all samples classified as adulterated will be submitted to a confirmatory analysis.

CRediT authorship contribution statement

Itziar Ruisánchez: Writing – original draft, In charge of the whole process that has given rise to this work, Design of the experimental part, Chemometric treatment, Discussion of results and Drafting of the manuscript. **Glòria Rovira:** Participated in the selection of samples, Sample measures and the chemometric treatment of the data. **M. Pilar Callao:** Writing – original draft, In charge of the whole process that has given rise to this work, Design of the experimental part, Chemometric treatment, Discussion of results and Drafting of the manuscript.

Declaration of competing interest

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

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