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# Multivariate modeling for detecting adulteration of extra virgin olive oil with soybean oil using fluorescence and UV–Vis spectroscopies: A preliminary approach

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ABSTRACT

This work presents a comparative study of chemometric methods used to quantify adulteration of extra virgin olive oil (EVOO) with soybean edible oil using fluorescence and UV–Vis spectroscopies. The adulteration was prepared by adding soybean edible oil in different concentrations (10, 50, 100, 150, 200, 250 and 300 g/kg). Different multivariate regression strategies were evaluated: partial least squares (PLS) using full spectrum; PLS with significant regression coefficients selected by the Jack-Knife algorithm (PLS-JK) and multiple linear regression (MLR) with previous selection of variables by stepwise algorithms (SW-MLR); successive projections algorithm (SPA-MLR); and genetic algorithm (GA-MLR). The predictive ability of the models was assessed, for each spectroscopic technique. For fluorescence spectroscopy, satisfactory prediction results were obtained for all the regression models with Root Mean Square Error of Prediction (RMSEP) values varying from 14.0 to 17.5 g/kg. When the regression methods were evaluated for UV–Vis spectra, higher RMSEP values were found, varying from 13.3 to 30.4 g/kg. The results indicate that the two spectroscopic techniques have similar performances with respect to predictive ability of the regression models.



## 1. Introduction

Extra virgin olive oil (EVOO) consumption is widespread due to its high nutritional value and pleasant sensory characteristics, obtained by labor-intensive techniques from the cultivation of olive trees to oil production (Lerma-García, Ramis-Ramos, Herrero-Martínez, & Simó-Alfonso, 2010; Šmejkalová & Piccolo, 2010). These characteristics confer high quality and high commercial value to the product, making it a frequent target of adulteration by the addition of vegetable oils or olive oils of lower quality (Johnson, 2015). For this reason, it is essential to ensure its quality and identify fraudulent practices in commercially available EVOOs, mainly with regard to the health and economic needs of consumers.

Internationally, the regulation and supervision of the EVOOs are carried out by the European Union Commission (EUC, 2015), Codex Committee on Fats and Oils (CCFO, 2017) and the International Olive Council (IOC, 2015). These also designate the official methods to be used in the quality control of EVOOs. However, some of these methods are time-consuming, complex, involve preparation of samples before analysis, and use expensive and toxic reagents (Valli et al., 2016).

A number of recent studies have reported simple, fast and inexpensive analytical methodologies to verify the authenticity of EVOOs efficiently and safely (Valli et al., 2016). These methodologies are used as an alternative to classical chromatographic methods (Jiménnez-Carvelo, Pérez-Castaño, González-Casado, & Cuadros-Rodríguez, 2017; Ruiz-Samblás, Marini, Cuadros-Rodríguez, & González-Casado, 2012). They include: infrared

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spectroscopy (Lerma-García et al., 2010; Georgouli, del Rincon & Koidis, 2017), voltammetric e-tongue (Apetrei & Apetrei, 2014), digital images (Milanez & Pontes, 2015), UV–Vis spectroscopy (Ferreiro-González et al., 2017; Torrecilla, Cancilla, Matute, Díaz-Rodríguez, & Flores, 2013), and fluorescence spectroscopy (Mabood et al., 2015; Poulli, Mousdis, & Georgiou, 2007).

Fluorescence spectroscopy and UV–Vis spectroscopy are distinguished because they are simple, cheap, fast and require little or no pretreatment of samples. In the case of fluorescence spectroscopy, moreover, there is high sensitivity and selectivity of the technique (Guzman, Baeten, Pierna, & Garcia-Mesa, 2015).

Normally, spectral data is associated with multivariate regression methods to associate the predictor variables (or instrumental response) with the dependent variables (or properties of interest) (Gómez-Caravaca, Maggio, & Cerretani, 2016). The partial least squares regression (PLS) (Wold, 2001) and multiple linear regression (MLR) methods (Martens & Naes, 1993, pp. 73–97) have been widely used in different analytical applications (Pontes, Rocha, Pimentel, & Pereira, 2011; de Paulo, Barros, & Barbeira, 2016). Poulli et al. (2007) used the synchronous fluorescence method and the PLS model to quantify virgin olive oil adulterated with different concentrations of olive-pomace and other vegetable oils. Mabood et al. (2015) investigated the effect of thermal treatment on the discrimination of pure EVOO samples from EVOO samples adulterated with sunflower oil. In addition, the level of adulteration was quantified using the PLS regression method with a prediction error of 1.75% of adulteration.

The PLS method is based on the simultaneous decomposing of predictors (matrix **X** of instrumental response) and dependent variables (matrix **Y** of the reference values of the parameters) in factors, or latent variables, as expressed in Equations (1) and (2).

$$\mathbf{X} = \mathbf{T}\mathbf{P}^{\mathbf{T}} + \mathbf{E} \tag{1}$$

$$\mathbf{Y} = \mathbf{U}\mathbf{Q}^{\mathbf{T}} + \mathbf{F} \tag{2}$$

where **T** and **U** are the score matrices and **P** and **Q** are the loading matrices for **X** and **Y**, respectively; **E** and **F** are the residual matrices (da Silva et al., 2015). Thereafter, a relationship is established between the scores of both variables sets thus making it possible to obtain regression coefficients for each factor, as described in Equation (3).

$$\widehat{\mathrm{Y}} = \mathbf{T}\mathbf{Q}^{\mathbf{T}} + \mathbf{G} = \mathbf{X}\mathbf{W}\left(\mathbf{P}^{\mathbf{T}}\mathbf{W}\right)^{-1}\mathbf{Q}^{\mathbf{T}} + \mathbf{G} = \mathbf{X}\mathbf{b} + \mathbf{G} \tag{3}$$

In Equation (3), $\widehat{Y}$ represents the estimate of the interest parameter for a set of samples, **W** is the weight matrix as determined in the PLS algorithm, **G** represents **Y**-residual matrix residuals and **b** corresponds to the regression coefficients (da Silva et al., 2015). The goal is to search latent variables that can express the variances of the **X** considering the prediction of the dependent variables **Y** (de Almeida, Correa, Rocha, Scafi, & Poppi, 2013). Usually the PLS model is developed using the full set of predictor variables. However, the Jack-Knife algorithm (JK) (Esbensen, 2002, pp. 483–488) can be used to select a reduced number of predictor variables that participate in the PLS regression model. In this way, confidence intervals can be calculated for the regression coefficients of the factors to evaluate the significance of each variable used in the model (Honorato, Barros Neto, Martins, Galvão, & Pimentel, 2007).

MLR method is simpler and can be more easily interpreted because it does not decompose the **X** and **Y** matrices into latent variables, as do the PLS methods (Hemmateenejad, Miri, Akhond, & Shamsipur, 2002). In this case, the original variables are used in the regression models and the concentration of the interest parameter (Y) can be predicted as follows:

$$\mathrm{Y} = \mathbf{X}\mathbf{b} \tag{4}$$

where the **b** vector of regression coefficients can be estimated as (Beebe, Pell, & Seasholtz, 1998, pp. 245–278)

$$\widehat{\mathbf{b}} = \left(\mathbf{X}^{\mathbf{T}}\mathbf{X}\right)^{-1}\mathbf{X}^{\mathbf{T}}\mathrm{Y} \tag{5}$$

The use of the MLR method, however, is limited to data with a reduced number of variables and low correlation coefficient (Riahi, Ganjali, Norouzi, & Jafari, 2008). When this is not the case, it is necessary to select the variables that can be used by the model. The successive projections algorithm (SPA) (Araújo et al., 2001; Galvão et al., 2007) the stepwise algorithm (Montgomery & Peck, 1982, pp. 344–351) and the genetic algorithm (GA) (Devillers, 1996) have been widely used for this purpose in many calibration problems (Gonçalves, Vilar, Medeiros, & Pontes, 2016; Pourbasheer, Aalizadeh, Ganjali, Norouzi, & Shadmanesh, 2014; Roy & Roy, 2009).

In the present study, we made a comparative study of different chemometric models used to quantify adulteration of EVOO samples with soybean edible oil, using fluorescence and UV–Vis spectroscopies. The following regression strategies were evaluated: partial least squares (PLS) using full spectrum, PLS with significant regression coefficients selected by the Jack-Knife algorithm (PLS-JK) and multiple linear regression (MLR) with previous selection of variables by stepwise algorithm (SW-MLR), algorithm of successive projections (SPA-MLR) and genetic algorithm (GA-MLR). The predictive ability of the models was assessed, for each spectroscopic technique, according to the values of RMSEC (Root Mean Square Error of Calibration), RMSEP (Root Mean Square Error of Prediction) and determination ($R^2$) coefficients.

## 2. Material and methods

### 2.1. Samples

A total of 39 adulterated EVOO samples were used. The adulteration was prepared by adding soybean edible oil in different concentrations (10 g/kg, 50 g/kg, 100 g/kg, 150 g/kg, 200 g/kg, 250 g/kg, 300 g/kg). The procedure of adulteration was performed at random to include variability in the data set. After addition of the soybean oil, the blends were only manually shaken for about 30 s and stored until the analysis. The unadulterated samples submitted to adulteration (a total of 7 samples) were acquired in local markets from a manufacturer and with different lots. The two soybean oils used as adulterants were also acquired in a local market from the same manufacturer and lot. Both the EVOOs and soybean oil used in this study were purchased from manufacturers with guaranteed quality and reliability.

All samples were stored in amber glass bottles for a period of 15 days, protected from light and kept at a temperature of approximately 23 ± 2 °C to retard the oxidative process until time of analysis. The samples were analyzed in crude form, without any pretreatment or dilution with use of chemical solvents.

### 2.2. Fluorescence spectra acquisition

Fluorescence measurements were performed with a Jasco FP-6500 Spectrofluorometer (Japan Spectroscopic Corporation, Tsukuba, Japan). This is a fully computer controlled instrument using a double-grating monochromator for excitation and a single-grating emission monochromator. The slit width was 3 nm to excitation and 5 nm for emission. The acquisition interval and integration

time was maintained at 1 nm and 0.5 s, respectively. A PMT lamp 400 W and a quartz cell 10 × 10 × 45 mm were used for a right-angle geometry.

The fluorescence emission spectra were collected between 300 and 750 nm. An excitation wavelength of 340 nm was selected for the development of the multivariate models. The excitation wavelength range from 280 to 480 nm was evaluated in 10 nm steps.

### 2.3. UV–Vis spectra acquisition

A Hewllet Packard model HP 8453 UV–VIS spectrophotometer (Agilent, Santa Clara, CA, US) equipped with a quartz cell (10 mm optical path) was employed for the spectral measurements. The spectrum was registered in the range between 190 and 1100 nm with 1 nm resolution. The adjustment of the transmittance signal was performed using isooctane as blank.

In the two spectroscopic techniques, all measurements were performed in triplicate.

### 2.4. Software and chemometric procedures

For each spectroscopic technique, multivariate calibration models based on PLS, PLS-JK, SPA-MLR, SW-MLR and GA-MLR regression methods were developed to quantify adulteration in EVOO samples. Adulteration levels (g/kg) were used as the dependent variable *y*. Before the construction of these models, the datasets were divided into calibration (70%) and prediction (30%) subsets by using the algorithm SPXY (Sample set partitioning based on joint X-y distances) (Galvão et al., 2005).

Full cross-validation leave-one-out was employed as the validation technique. The calibration samples were used in the modeling procedures (cross-validation) including SPA, SW and GA variable selection for MLR and determination of factors (latent variables) in PLS models. The prediction samples were only employed in the final evaluation and for the comparison of the resulting models. Model performances were assessed according to the values of RMSEC, RMSEP and determination ($R^2$) coefficients.

An *F*-test, at a 95% confidence level, was carried out to assess the existence of statistically significant differences between the values of the RMSEP obtained by the calibration models. The *F*-values were calculated as the ratio of the squares of the largest and smallest RMSEP values (Bhandare et al., 1993; Skoog, West, Holler, & Crouch, 2013, pp. 130–149) as shown below:

$$F(n_1, n_2) = (RMSEP_1)^2 \Big/ (RMSEP_2)^2 \quad (6)$$

In Equation (6), $n_1$ and $n_2$ are the number of prediction samples, and $RMSEP_1$ and $RMSEP_2$ are the higher and lower root mean square error, respectively. This ratio was compared with the critical $F(0.95, n, n)$ value, where $n$ is the number of prediction samples. A paired *t*-test, at a 95% confidence level, was carried out to assess whether there were significant differences between the predicted values by models and reference values.

The SPXY, SPA-MLR, SW-MLR and GA-MLR algorithms were coded in Matlab (Mathworks, USA). PLS and PLS-JK was carried out using Unscrambler X.1 (CAMO S/A).

## 3. Results and discussion

### 3.1. Spectral analysis

Fig. 1a shows the fluorescence emission spectra (between 350 and 700 nm) to the average of EVOOs adulterated at different levels. It can be seen that as the amount of soybean oil added increases, fluorescence intensity also increases.

The fluorescence of EVOOs has been attributed to some natural fluorophores such as: phenolic compounds and tocopherols (between 300 and 390 nm), oxidation products of fatty acids (two smooth peaks at 445 and 475 nm), vitamin E (525 nm), and degradation products of chlorophyll *a and b* (peak at approximately 681 nm) (Guzman et al., 2015; Sikorska, Khmelinskii, & Sikorski, 2012; Zandomeneghi, Carbonaro, & Caffarata, 2005). Studies indicate that main fluorophores of vegetable oils are the same as those of EVOOs (Magalhães, Caires, Silva, Alcantara, & Oliveira, 2014). However, refined oils have higher concentrations of oxidation products of fatty acids usually related to the refining process, resulting in higher fluorescence intensities between 400 and 500 nm (Kongbonga et al., 2011).

This characteristic can be observed in Fig. 1a, which presents only the spectra corresponding to some concentration levels of soybean oil in the blend. As vegetable soybean oil was added to EVOO, it increased the amount of oxidation products of fatty acids in these samples and, consequently, increased the fluorescence intensity in the region between 400 and 475 nm, which corresponds to the emission wavelength of oxidation products of fatty acids. The increase of the intensity to approximately 525, can be associated to the increase in the amount of vitamin E in the blend (Zandomeneghi et al., 2005).

Fig. 1b presents the UV–Vis absorbance spectra between 364 and 706 nm. Three clearly defined peaks, near the ultraviolet and blue ranges, can be observed at 410, 450 and 470 nm, corresponding to carotenoid absorption. The peak at approximately 660 nm corresponds to chlorophyll compounds absorption (Tarakowski, Malanowski, Kościesza, & Siegoczyński, 2014). In contrast to what happened with the fluorescence emission spectra, the measure to which the amount of soybean oil in the EVOO is increased, the absorbance decreases, which is particularly true for the regions where the absorption bands of the carotenoids and chlorophyll compounds occur.

### 3.2. Multivariate calibration models

#### 3.2.1. Fluorescence data

Table 1 presents the statistical parameters calculated for PLS, PLS-JK, SPA-MLR, GA-MLR and SW-MLR models applied to the fluorescence data.

As can be seen, satisfactory values of RMSEP were obtained by all regression methods, especially by SPA-MLR, GA-MLR and SW-MLR models.

The predictive ability of the models was evaluated according to the RMSEP obtained for the prediction set. At a 95% confidence level, the test-*F* indicated the absence of statistically significant differences among the values of the RMSEP of the models, as shown in Table 1.

As can be seen in Table 2, all *F*-values calculated for fluorescence data were below the critical $F(0.95, 12, 12)$ value = 2.69. The same happened with the *t*-test: when applied to the prediction set, at a 95% confidence level, it did not indicate significant differences between the predicted and reference values.

Fig. 2 shows the variables selected for the SPA, SW and GA algorithms and the predicted versus reference value plots for the MLR models obtained using these variables. As can be seen, variables were selected along the whole spectrum, especially in the bands between 400 and 550 nm, where emissions of oxidation products of fatty acids and vitamin E occur. In the plots, there was acceptable agreement between predicted and reference values for both the calibration and prediction sets. Moreover, no systematic error was present, as the points can be seen to be distributed on both sides of the bisectrix line along the entire range of *y*-values.

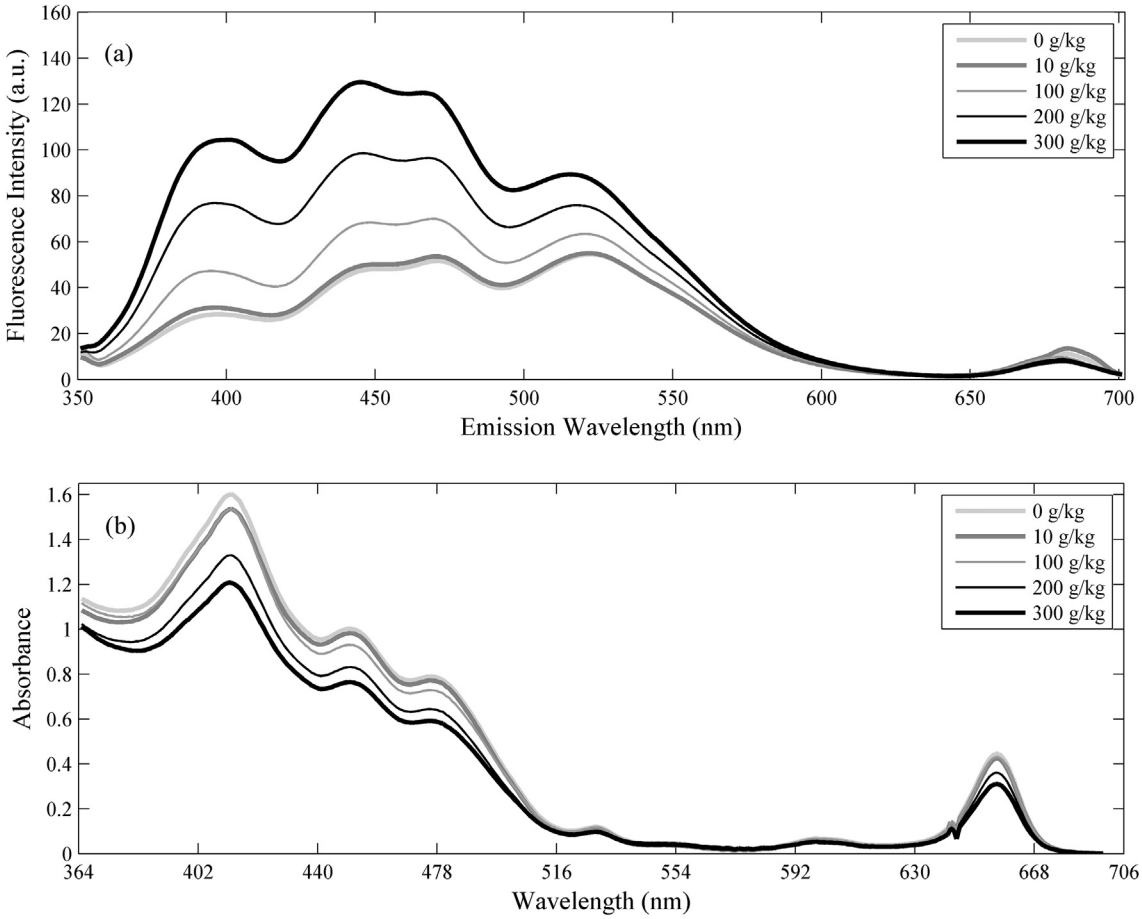


**Fig. 1.** (a) Average spectra of fluorescence emission of EVOO samples. (b) Average spectra of absorbance in the UV–Vis of EVOO samples. The lines in each spectrum represent the averages for some adulteration concentration, expressed in grams of soybean oil for kg of blend. The unadulterated sample (0 g/kg) was presented only for the purpose of comparison.

**Table 1**
Final prediction results in terms of RMSEC, RMSEP, determination ($R^2$) coefficients of level of adulteration (g/kg) and number of variables (for MLR models) or latent variables (for PLS models). *N* is the number of prediction samples analyzed by fluorescence spectroscopy.

| Statistical methods | *N* | Fluorescence Measurement | | | |
|---|---|---|---|---|---|
| | | RMSEC (g/kg) | RMSEP (g/kg) | $R^2$ | Variables or latent variables |
| PLS | 12 | 19.0 | 17.5 | 0.95 | 2 |
| PLS-JK | 12 | 19.0 | 17.1 | 0.95 | 2 |
| SPA-MLR | 12 | 13.0 | 14.6 | 0.99 | 11 |
| GA-MLR | 12 | 8.42 | 14.3 | 0.99 | 13 |
| SW-MLR | 12 | 12.0 | 14.0 | 0.99 | 5 |

Calibration range: 10–300 g/kg of soybean oil in EVOO samples.
PLS: Partial Least Squares.
PLS-JK: Partial Least Squares – Jack-Knife.
MLR: Multiple Linear Regression.
SPA-MLR: Successive Projections Algorithm - Multiple Linear Regression.
GA-MLR: Genetic Algorithm - Multiple Linear Regression.
SW-MLR: Stepwise - Multiple Linear Regression.
EVOO: Extra Virgin Olive Oil.
RMSEC: Root Mean Square Error of Calibration.
RMSEP: Root Mean Square Error of Prediction.

### 3.2.2. UV–Vis data

Table 3 summarizes the results obtained from the calibration models applied to the UV–Vis spectra.

As can be seen in Table 3, satisfactory results were found for all models. More specifically, lower values of RMSEP were found when the PLS (full spectrum) and PLS-JK were used with three latent variables. Moreover, these models showed slightly higher determination ($R^2$) coefficients values than those found by MLR models with selection of variables. The superiority of the PLS models over the MLR models was demonstrated with a *F*-test performed at a confidence level of 95% (Table 2), indicating statistically significant differences between the values of RMSEP obtained by the PLS models (both full spectrum and to the regression coefficients selection by JK) and the MLR (SPA, GA and SW).

**Table 2**
Results obtained for *F*-test and *t*-test for fluorescence and UV–Vis data.

| Models | Fluorescence data | UV–Vis data | *F* - critical | Models | Fluorescence data | UV–Vis data | *t* - critical |
|---|---|---|---|---|---|---|---|
| | *F* - values | *F* - values | | | *t* - values | *t* - values | |
| PLS and PLS-JK | 1.06 | 1.02 | 2.69 | PLS | 1.08 | 0.53 | 1.79 |
| PLS and SPA-MLR | 1.44 | 3.15 | | | | | |
| PLS and GA-MLR | 1.5 | 3.31 | | PLS-JK | 0.91 | 0.55 | |
| PLS and SW-MLR | 1.57 | 5.24 | | | | | |
| PLS-JK and SPA-MLR | 1.36 | 3.11 | | SPA-MLR | 1.48 | 1.62 | |
| PLS-JK and GA-MLR | 1.42 | 3.27 | | | | | |
| PLS-JK and SW-MLR | 1.48 | 5.16 | | GA-MLR | 1.35 | 4.05 | |
| SPA-MLR and GA-MLR | 1.04 | 1.05 | | | | | |
| SPA-MLR and SW-MLR | 1.09 | 1.66 | | SW-MLR | 1.22 | 1.43 | |
| SW-MLR and GA-MLR | 1.04 | 1.58 | | | | | |

PLS: Partial Least Squares.
PLS-JK: Partial Least Squares – Jack-Knife.
MLR: Multiple Linear Regression.
SPA-MLR: Successive Projections Algorithm - Multiple Linear Regression.
GA-MLR: Genetic Algorithm - Multiple Linear Regression.
SW-MLR: Stepwise - Multiple Linear Regression.

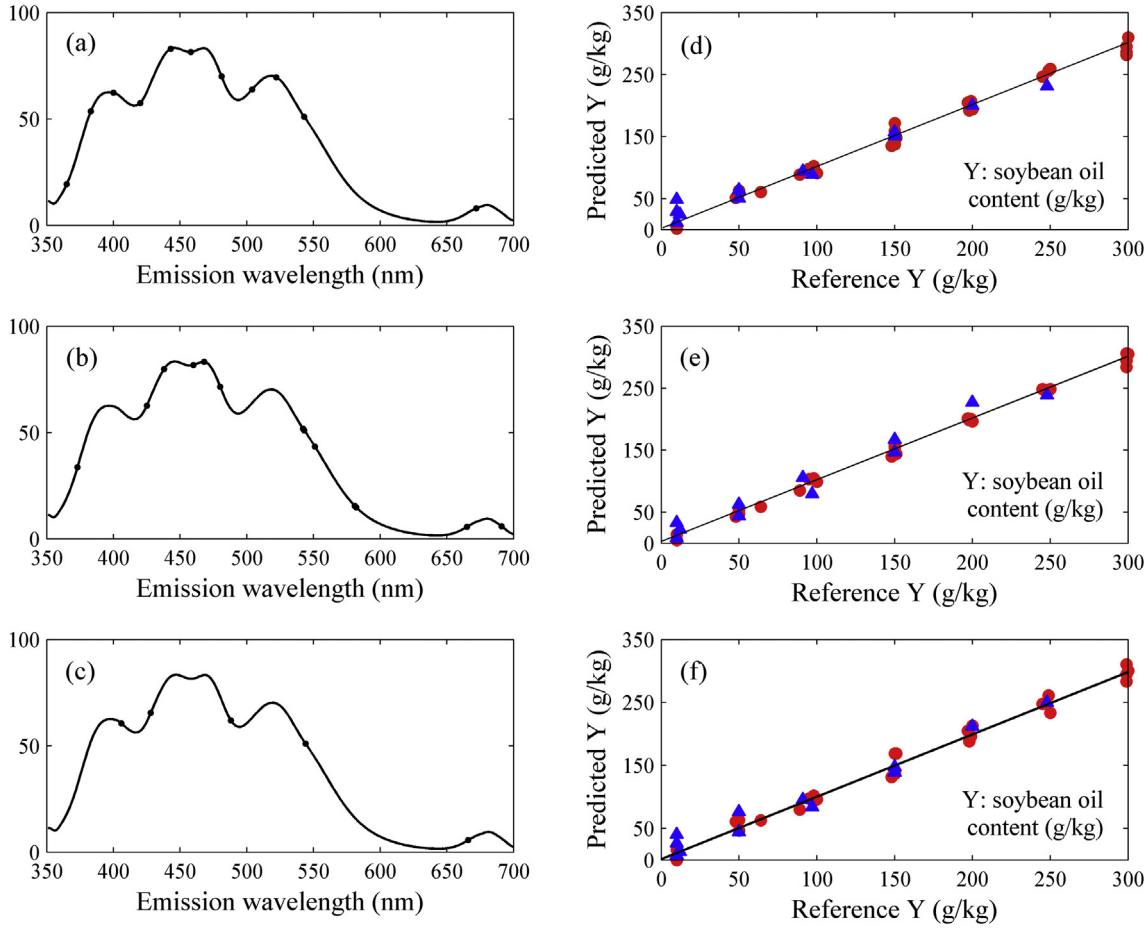


**Fig. 2.** Average spectra of EVOOs with indication of variable selected (•) by (a) SPA, (b) GA and (c) SW algorithms. Predicted versus reference plots obtained with (d) SPA-MLR, (e) GA-MLR and (f) SW-MLR models for determination of EVOO adulteration in the (●) calibration and (▲) prediction sets.

Fig. 3 shows the predicted versus reference plots for the PLS and PLS-JK models.

As well as the results obtained from the fluorescence data, the models developed with data from UV-VIS spectroscopy provided similar results, where samples were well distributed along the bisectrix line, indicating the absence of systematic errors in the data.

Table 2 shows the paired *t*-test, at a 95% confidence level, for UV–Vis data. As can be seen, the *t*-test did not identify statistically significant differences between the predicted and reference values, except for the GA-MLR model.

**Table 3**
Final prediction results in terms of RMSEC, RMSEP, determination ($R^2$) coefficients of level of adulteration (g/kg) and number of variables (for MLR models) or latent variables (for PLS models). *N* is the number of prediction samples analyzed by UV–Vis spectroscopy.

| Statistical methods | *N* | Fluorescence Measurement | | | |
|---|---|---|---|---|---|
| | | RMSEC (g/kg) | RMSEP (g/kg) | $R^2$ | Variables or latent variables |
| PLS | 12 | 28.3 | 13.3 | 0.98 | 3 |
| PLS-JK | 12 | 28.2 | 13.4 | 0.98 | 3 |
| SPA-MLR | 12 | 30.2 | 23.6 | 0.97 | 9 |
| GA-MLR | 12 | 14.5 | 24.2 | 0.98 | 14 |
| SW-MLR | 12 | 22.6 | 30.4 | 0.94 | 5 |

Calibration range: 10–300 g/kg of soybean oil in EVOO samples.
PLS: Partial Least Squares.
PLS-JK: Partial Least Squares – Jack-Knife.
MLR: Multiple Linear Regression.
SPA-MLR: Successive Projections Algorithm - Multiple Linear Regression.
GA-MLR: Genetic Algorithm - Multiple Linear Regression.
SW-MLR: Stepwise - Multiple Linear Regression.
EVOO: Extra Virgin Olive Oil.
RMSEC: Root Mean Square Error of Calibration.
RMSEP: Root Mean Square Error of Prediction.

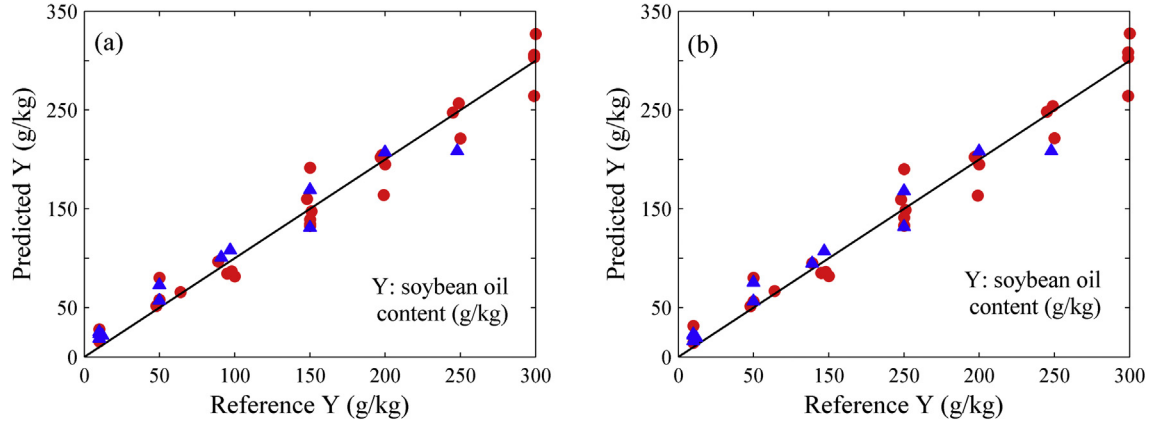


**Fig. 3.** Predicted versus reference plots obtained with (a) PLS and (b) PLS-JK models for determination of EVOO adulteration in the (●) calibration and (▲) prediction sets.

## 4. Conclusion

This study compared different chemometric models for the quantification of adulteration in EVOO samples, using fluorescence and UV–Vis spectroscopy. PLS, PLS-JK, SPA-MLR, GA-MLR and SW-MLR regression models were developed and their predictive ability was assessed based on values of RMSEC, RMSEP and determination ($R^2$) coefficients.

For fluorescence spectroscopy, the *F*-test did not show significant difference between the models developed. The paired *t*-test also found no significant differences between the predicted values from the models and reference values.

When the regression methods were evaluated for UV–Vis spectroscopy, higher RMSEP values were found. Moreover, in the *F*-test, there was difference between the PLS, PLS-JK methods and MLR methods developed with previous selection of variables.

The results indicate that the two spectroscopic techniques have similar performance with respect to predictive ability of the regression models. With this, the choice of which one of the techniques to use should consider aspects such as lower cost, simplicity, speed and greater analytical sensitivity. In addition, when compared to other analytical techniques, both techniques are non-destructive and do not require pretreatment of samples. These preliminary results are indicative that the screening approach is promising and can support official analytical methods for identifying adulteration of EVOOs with vegetable oils of lower quality. However, these results need to be confirmed by analyzing a larger set of samples.

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