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Analytical Methods

# Evaluation of the overall quality of olive oil using fluorescence spectroscopy

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ABSTRACT

The fluorescence spectra of some olive oils were examined in their natural and oxidised state, with wavelength range emissions of 300–800 nm and 300–400 nm used as excitation radiation. The fluorescence emissions were measured and an assessment was made of the relationship between them and the main quality parameters of olive oils, such as peroxide value, K232, K270 and acidity. These quality parameters (peroxide value, K232, K270 and acidity) are determined by laboratory methods, which though not too sophisticated, they are required solvents and materials as well as time consuming and sample preparation; there is a need for rapid analytical techniques and a low-cost technology for olive oil quality control. The oxidised oils studied had a strong fluorescence band at 430–450 nm. Extra virgin olive oil gave a different but interesting fluorescence spectrum, composed of three bands: one low intensity doublet at 440 and 455 nm; one strong band at 525 nm; and one of medium intensity at 681 nm. The band at 681 nm was identified as the chlorophyll band. The band at 525 nm was derived, at least partially, from vitamin E.

The results presented demonstrate the ability of the fluorescence technique, combined with multivariate analysis, to characterise olive oils on the basis of all the quality parameters studied. Prediction models were obtained using various methods, such as partial least squares (PLS), N-way PLS (N-PLS) and external validation, in order to obtain an overall evaluation of oil quality.

The best results were obtained for predicting K270 with a root mean square (RMS) prediction error of 0.08 and a correlation coefficient obtained with the external validation of 0.924. Fluorescence spectroscopy facilitates the detection of virgin olive oils obtained from defective or poorly maintained fruits (high acidity), fruits that are highly degraded in the early stages (with a high peroxide value) and oils in advanced stages of oxidation, with secondary oxidation compounds (high K232 and K270). The results indicate the potential of a spectrofluorimetric method combined with multivariate analysis to differentiate, and even quantify, the levels of oil quality. The proposed methodology could be used to accelerate analysis, is inexpensive and allows a comprehensive assessment to be made of olive oil quality.



## 1. Introduction

The demand for safe, high-quality food requires a high level of quality control and processing, which in turn requires the appropriate analytical tools. Virgin olive oil is a high-quality product in terms of its sensory and nutritional properties, and is therefore governed by strict controls on verifying its quality and purity (COI/T.15/NC No 3/REV. 6 – International Olive Council (IOC)). The comprehensive quality control of a virgin olive oil requires considerable analytical assessment with varying levels of complexity, ranging from simple analytical processes (e.g., determination of UV absorbance) to more complex ones (e.g., determination of different compounds as sterols, aliphatic alcohols and other minor compounds). Some of these procedures can be time consuming, and highlight the need to develop new analytical methods that be simpler and faster.

Spectroscopic techniques are ideal for this purpose because they are simple, cost-effective, rapid and non-destructive. Among these techniques is fluorescence spectroscopy, which has widely been applied to olive oil analysis. In a recent study, Sikorska et al. reviewed these applications in detail (Sikorska, Khmelinskii, & Sikorski, 2012). Fluorescence spectroscopy enables valuable analytical information to be obtained for different applications: (a)

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discrimination between quality grades; (b) detection of adulteration; (c) authentication of geographic origin; (d) quantification of fluorescent components; (e) monitoring of thermal and photo-oxidation; and (f) assessment of quality changes of olive oil during storage.

Fluorescence spectroscopy has several advantages. For example, it is a spectroscopic quantitative analysis method that is more sensitive and selective in terms of organic and inorganic compounds, and in general it does not require using consumable reagents and sample pre-treatment (Sikorska et al., 2004).

The fluorescence emission spectra of olive oils contain information about their polyphenol and tocopherol content (300–390 nm) Zandomeneghi, Carbonaro, & Caffarata, 2005; Giungato, Aveni, Rana, & Notarnicola, 2004. Virgin olive oils present two smooth peaks at 445 and 475 nm, a peak intensity at 525 nm and another peak at 681 nm. Kyriakidis and Skarkalis (2000) suggested that the peaks at 445 and 475 nm were related to the oxidation products of fatty acids and that the peak at 525 nm was derived from vitamin E. They also showed, however, that the addition of vitamin E acetate to virgin olive oil increases the fluorescence intensity not only at 525 nm, but also at 445 and 475 nm. They suggested that this was due to oxidised vitamin E that fluoresces at about this region. The peak at 681 nm is related to chlorophylls. The very low intensity of the peaks at 445 and 475 nm is attributable to the high content of monounsaturated fatty acids and phenolic antioxidants in virgin olive oils, which provide greater protection against oxidation.

Another important quality parameter is oil acidity (the free acids in olive oils in terms of oleic acid). Studies on this parameter have focused on the fluorescent components of the main triglycerides in oil, such as oleic, linoleic and palmitic acids. Acidity in olive oil results from hydrolytic rancidity, and the free fatty acids in olive oil derive from these acids. Oleic acid shows a band of fluorescence at 405 nm, and butyric acid (palmitic acid analogue) and linoleic acid show fluorescence bands at 273 and 325 nm. This has been reported in previous studies, which have confirmed that fluorescence intensity at 429–545 nm was due to oleic acid (Poulli, Mousdis, & Georgiou, 2005).

In recent years much research has been done on developing rapid methods that needs no preparation for assessing the quality of food. There has been an increase in the use of fluorescence spectroscopy, combined with multidimensional statistical techniques, to evaluate food quality. In most of the research conducted, the obtained fluorescence signal was assigned to specific fluorophores after fixing the excitation or emission wavelength.

The articles published on fluorescence in vegetable oils focus mainly on the characterisation of these oils. In these studies, the variation in excitation and emission wavelengths allows for the simultaneous determination of compounds that are present in the oils as polyphenols (Gracia, Zude, & León, 2009; Mújica-Ascencio, Moreno-García, Stolik Isakina, & De La Rosa-Vázquez, 2010). Guimet, Ferré, and Boqué (2005) used fluorescence and PARAFAC to detect the adulteration of extra virgin olive oil with pomace oil; other authors have also used fluorescence to study the adulteration of olive oil with other vegetable oils (Dankowska & Małecka, 2009; Poulli, Mousdis, & Georgiou, 2006, 2007; Sayago, García-González, Morales, & Aparicio, 2007).

There are several studies on the use of fluorescence spectroscopy for monitoring the deterioration of extra virgin olive oil in thermoxidized virgin olive oil (Cheikhousman et al., 2005; Poulli, Chantzos, Mousdis, & Georgiou, 2009a; Poulli, Mousdis, & Georgio, 2009b; Tena, Aparicio, & García-González, 2012; Tena, García-González, & Aparicio, 2009). There have also been studies on characterising and classifying olive oils in which the fluorescence spectra are associated with quality parameters such as peroxide value (PV) and UV absorbance at 232 nm (K232) and 270 nm (K270); this work has attempted to determine these quality parameters, but has not produced accurate prediction models (Guimet, Boqué, & Ferré, 2006; Guimet, Ferré, Boqué, Vidal, & Garcia, 2005; Sikorska, Górecki, Khmelinskii, Sikorski, & Kozioł, 2005; Zandomeneghi, Carbonaro, & Zandomeneghi, 2006).

In the light of these studies, and given that fluorophores in edible oils are chemical compounds with a very different nature, directly related to the mechanisms of oxidation, fluorescence spectroscopy is considered to be a suitable technique for evaluating the oxidative status of oils. The aim of this study, therefore, was to determine whether fluorescence spectroscopy provides enough information on quality parameters for routine control and could be used for a general characterisation of olive oils.

## 2. Material and methods

### 2.1. Samples and sample preparation

Ninety samples of fresh and heated virgin oils (seventy fresh oils and twenty lampant oils) obtained from the 2010–2011 harvest at the Venta del Llano Instituto de Investigación y Formación Agraria y Pesquera (IFAPA) in Jaén, Spain were used.

In order to obtain a wide variety of oxidised oils a rapid oxidation process of 10 samples of olive oil was carried out. Oil samples were heated in an oven at 110 °C for 96 h. Sub-samples were taken at different times to obtain different levels of oxidation (a sub-sample was taken every 5 h) and then allowed to cool to room temperature before laboratory analysis and fluorescence measurement. In this way, oils showing a wide range of oxidation were obtained (PV up to 105 meq/kg). Concerning the obtaining of oil samples with a high level of free acidity, ten samples of 30 kg were stored up to two months in closed plastic bags at room temperature to get fermentative conditions. Every week an olive lot was processed in a laboratory oil mill Abencor (Abengoa, Seville, Spain) to obtain the corresponding olive oil. In this way, oils showing a wide range of free acidity were obtained (up to 16.5°).

### 2.2. Reference chemical analysis

For all the samples, the reference values for PV, acidity and specific UV absorption at 232 nm (K232) and 270 nm (K270) were obtained following EU Regulations EEC/2568/91 and EEC/1429/92, which focus on the characteristics of olive oil and olive-residue oil and on relevant methods of analysis (EC Commission, 1992, 1991). Regulation ECC 2568/91 as amended by Regulation ECC 702/2007] June 21, 2007 (EC Commission, 2007), which defines the physical–chemical and organoleptic characteristics of olive oils and olive pomace as well as methods of assessing these characteristics. Recently, Regulation EEC 61/2011 of the Commission for amending Regulation EEC 2568/91 on the characteristics of olive oils and olive–pomace oil and on the methods of analysis included the determination of alkyl ester content as a new parameter to be considered in the category of extra virgin olive oil (EC Commission, 2011).

The samples used in this study varied widely in oxidation level, as shown in Table 1, which presents the minimum and maximum

**Table 1**
Mean and minimum–maximum values of the reference values used in the study.

| | Mean | Max | Min |
|---|---|---|---|
| PV | 22.3 | 105.5 | 1.5 |
| Acidity | 2.4 | 16.5 | 0.1 |
| K232 | 2.4 | 3.18 | 1.4 |
| K270 | 0.3 | 0.84 | 0.1 |

values, the average values and the standard deviation for each of the chemical indices.

The PV is the amount of peroxide in a sample (expressed in milliequivalents of active oxygen per kg fat) that causes the oxidation of potassium iodide. The sample was dissolved in acetic acid and chloroform, and was then treated with a potassium iodide solution. The liberated iodine was titrated with a sodium thiosulfate solution.

Acidity is defined as the free acids in olive oils in terms of oleic acid. The free fatty acid content is expressed as acidity. This is calculated using the conventional method of titration, which involves dissolving the sample in a solvent mixture and measuring the free fatty acids by volumetric analysis using an ethanolic solution of potassium hydroxide. A low level of acidity indicates that a virgin olive oil is derived from good quality olives and has been kept in peak condition throughout the production process.

The K232 and K270 values were spectrophotometric measures for quantifying the UV absorption, at 232 nm (K232) and 270 nm (K270), of a solution of the oil in cyclohexane. Spectrophotometric analysis in UV provides information about the quality of the fat, the conservation status of the oil and any deterioration that might have occurred during the technological processes. High K232 and K270 values indicate the presence of diene- and triene-conjugated systems.

### 2.3. Fluorescence spectroscopy analysis

A three-dimensional (3D) matrix for each sample was obtained, referred to as a fluorescence excitation-emission matrix (EEM). The main advantage of EEMs is that more information can be extracted from them about the fluorescent species because there is a wider area available for analysis. There are some examples in the literature of the use of EEMs for olive oils (Cheikhousman et al., 2005; Engelsen, 1997).

A spectrofluorimetric measurement of olive oil was conducted using a Cary Eclipse (Varian Ibérica, Madrid, Spain). This instrument was equipped with a continuous xenon lamp, excitation and emission monochromators, and a photomultiplier to 800 V. Poly(methyl methacrylate) disposable cells (10 × 10 mm, 4.5 mL, Deltalab, Barcelona, Spain) were used for right-angle fluorometry.

The excitation and emission slits were set to 5 nm. Eleven emission spectra were collected between 300 and 800 nm by using an excitation wavelength ranging from 300 to 400 nm (10 nm steps).

The integration time was 0.1 s, and the increasing wavelength while scanning the spectrum was 10 nm. The experimental conditions were appropriate for providing satisfactory intensity spectra, resolution and signal-to-noise ratio.

The oils were shaken vigorously before measurement in order to homogenise the samples. The oil was directly poured into a plastic cuvette which was discarded after measurement in order to improve method speed and simplify sample management.

Each sample was analysed in duplicate, and the average measurement (the value of the two scans for each sample) was measured using the original spectra, without any pre-treatment. All computations, chemometric analysis and graphics were performed using Matlab v7.4.0. (The MathWorks, Inc., Natick, MA, USA). For principal component analysis (PCA) and PLS, the PLS Toolbox v 4.11 (Eigenvector Research, Inc.) was used.

## 3. Results

Olive oil quality is usually based on chemical parameters (acidity; fatty acid composition; PV; UV absorbance, sterol content (Aparicio & Aparicio-Ruíz, 2000; Armanino, Leardi, Lanteri, & Modi, 1989; Marini et al., 2004) and sensorial analysis (Aparicio, Morales, & Alonso, 1997). Chemical analyses were performed by the laboratory, using official analytical methods [Regulation (EEC) 2568/91] with its last modification [Regulation (EEC) 702/2007] which including four quality parameters (acidity, PV, K232 and K270). Table 1 shows the parameters analysed, giving the maximum, minimum and average values.

During the first stage of the analysis of multivariate data, an initial pre-treatment of the measured signals was performed. This involved mathematical manipulation of the measuring signals prior to analysis. The pre-treatment reduced or eliminated sources of variability in signals of a random nature, such as noise. Various treatments were tested but none improved data results, finally the original data were used without any treatment. Chemometrics was aimed at finding a linear relationship between the variables of a model or a set of experimental data.

The initial studies sought to find the relationship between the fluorescence EEM parameters and acidity.

For this purpose the first step was to check that there was no correlation between oxidation parameters (PV) and acidity, as this could lead to an erroneous measurement. It was found the relationship between the values of oxidation of the samples (PV) and acidity, to confirm that some samples which presented high values of oxidation, had low acidity values too and vice versa, so that we were not measuring the same characteristic of the samples.

Fig. 1 shows the correlation between PV and acidity, confirming that there was no relationship between these variables.

The oil sample's EEM was used to conduct a multi-way PLS regression (N-PLS) to correlate the projected variable values (Bro & Multi-way calibration, 1996). This method models both the independent (X) and dependent (Y) variables simultaneously to find the latent variables in X that will best predict the latent variables in Y. The optimum number of factors calculated using the leave-one-out cross-validation procedure was seven, with an RMS error of cross-validation (RMSCEV) of 1.77 and a correlation coefficient, obtained in the validation step, of 0.931 for the acidity parameter.

The next stage involved constructing a calibration model for these parameters using a single excitation wavelength in order to accelerate the analysis and avoid the need to measure EEMs at different wavelengths. A PLS model (Massart, Vandeginste, & Buydens, 1988) was applied to each wavelength of excitation and the error values were compared in order to select the minimum value. The wavelength was selected to give the best values of correlation with the different parameters and lower error values of 380 nm. The PLS models therefore had a excitation fluorescence spectrum at 380 nm and an emission wavelength of 400–800 nm, in order to verify that performing a measurement in this wavelength could be done using the same information as EEMs.

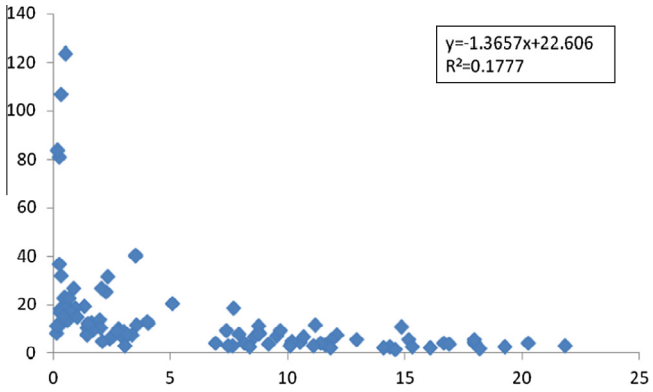


**Fig. 1.** Correlation between PV (axis *y*) and acidity (axis *x*).

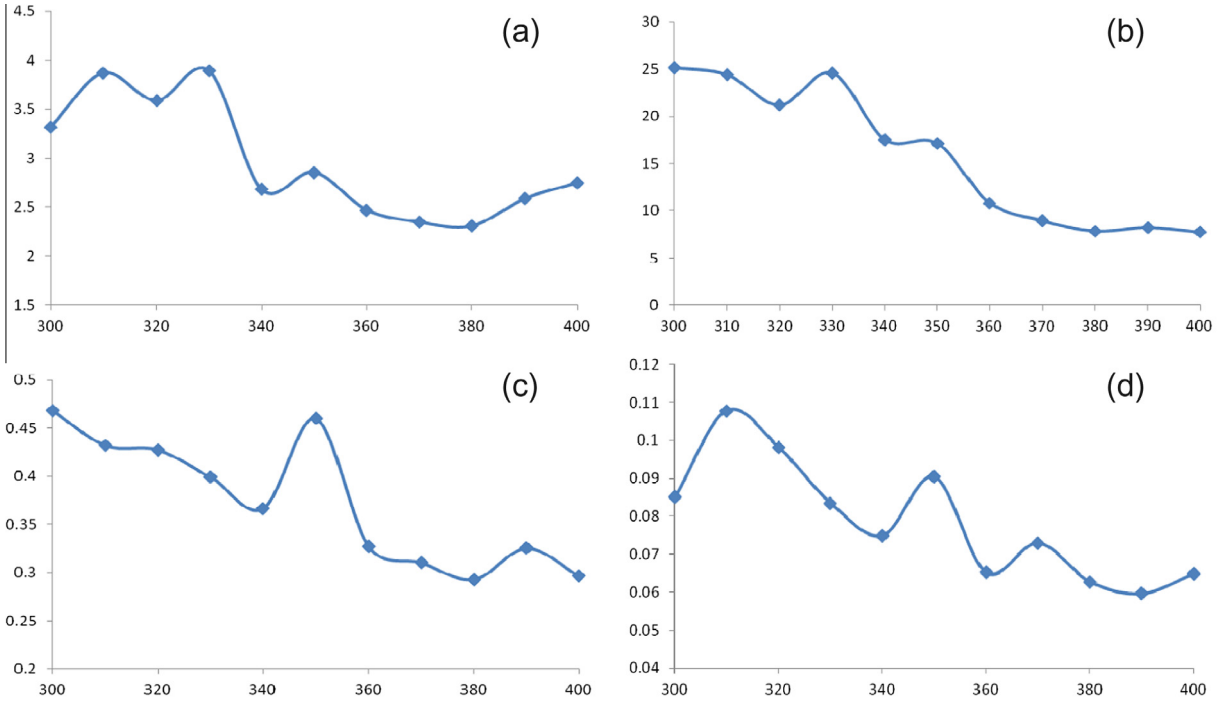


**Fig. 2.** Root mean square error of cross-validation (RMSECV) in axis *y* for (a) acidity, (b) peroxide value (PV), (c) K232 and (d) K270, respectively, for each excitation wavelength (axis *x*).

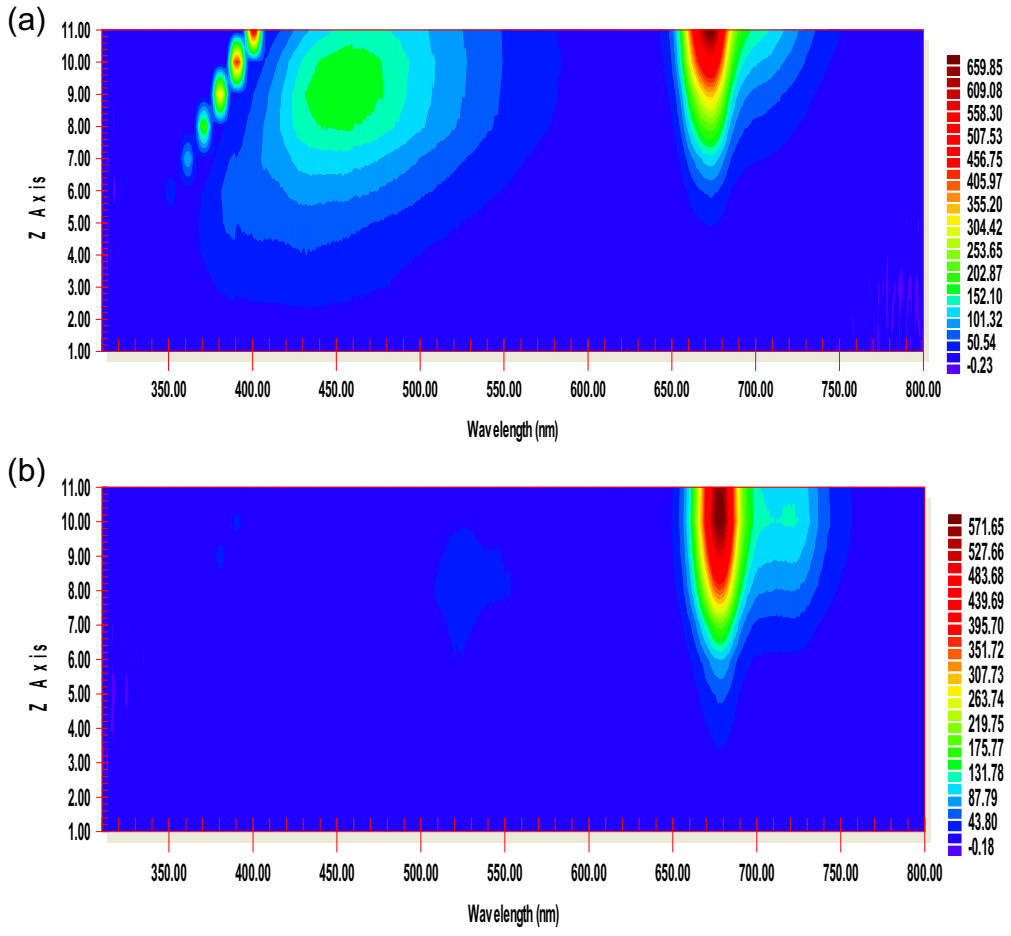


**Fig. 3.** EEMs between 300–400 nm excitation (axis *y*) and 400–800 nm emission (axis *x*) of oxidised oil (a) and fresh oil sample (b).

**Table 2**
Results obtained using the N-PLS and PLS methods and external validation for each of the parameters studied.

| | N-PLS | | PLS | | External validation | |
|---|---|---|---|---|---|---|
| | $R^2$ | Error | $R^2$ | Error | $R^2$ | Error |
| Acidity | 0.931 | 1.77 | 0.892 | 2.32 | 0.679 | 0.88 |
| PV | 0.967 | 6.74 | 0.965 | 8.17 | 0.902 | 5.81 |
| K232 | 0.908 | 0.37 | 0.881 | 0.32 | 0.907 | 0.28 |
| K270 | 0.978 | 0.06 | 0.939 | 0.06 | 0.924 | 0.08 |

The RMSECV for all the parameters for each excitation wavelength is given in Fig. 2, which shows that at 380 nm is reached the lowest values for error this error.

The number of factors used in the PLS method based on the leave-one-out cross-validation procedure was the same as that for the N-PLS method (seven); the RMSECV obtained for acidity was 2.32 and the correlation coefficient obtained in the validation step was 0.892. These results accord with those obtained Poulli et al. (2005) who found information in the fluorescence spectrum in the 429–545 nm region related with oleic acid, but did not find a good predictive model in this case.

The next step was to determine the relationship between fluorescence spectra and other quality parameters, such as those relating to the oxidation state of the oil. The PVs indicate the presence of primary oxidation products (i.e., conjugated hydroperoxides) and K232 and K270 indicate the presence of secondary oxidation products (diene- and triene-conjugated systems).

The relationship between the fluorescence EEMs of oils and these parameters was studied. The high values of the parameters indicated that oils were degraded, which was confirmed by their high fluorescence intensities.

Previous work has shown that samples with the lowest PV (low level of primary oxidation) display soft fluorescence around 415–600 nm. In contrast, the sample with the highest PV (large amounts of primary oxidation products) has its maximal fluorescence around this zone.

Samples with the lowest level of oxidation did not show fluorescence around the 415–600 nm emission wavelengths. The sample with high oxidation, however, had its maximum fluorescence around this emission wavelength (Fig. 3).

The reference method for analysing peroxides in the laboratory has an error in the analysis, as it depends largely on external factors. In addition, the PV is very variable because peroxidic compounds decompose rapidly into other oxidation compounds for contact with light and high temperatures, and this could explain the high values in the calibration error for this parameter.

The results of PLS N-quantitative analysis for predicting the PV calculated by means of the method cross-validated with an RMSECV of 6.74 and a coefficient of correlation obtained in the validation of 0.967.

A similar procedure was performed to correlate the fluorescence parameters with K232 and K270. During oil oxidation, the conversion of primary oxidation compounds produces high levels of peroxides in secondary oxidation products, which often give higher K232 and K270 values and fluorescence spectra with peaks in the 415–600 nm region, although the variation of K270 values in oils is best captured by fluorescence. Oils with the highest K270 value have a broad peak between 415 and 600 nm, with a maximum fluorescence at λem 470 nm (Guimet et al., 2005).

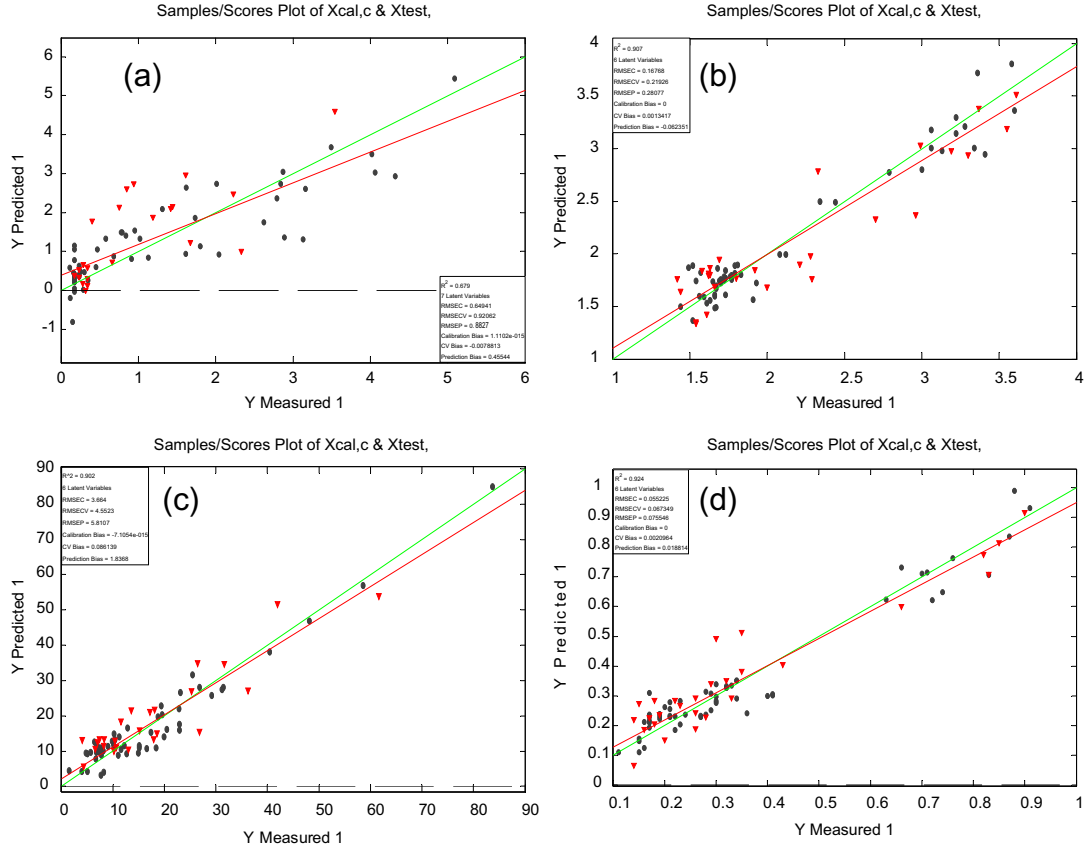


**Fig. 4.** Reference (axis *x*) vs. predicted (axis *y*) values in the independent validation set. Acidity, peroxide value, K232 and K270 were predicted by PLS external validation.

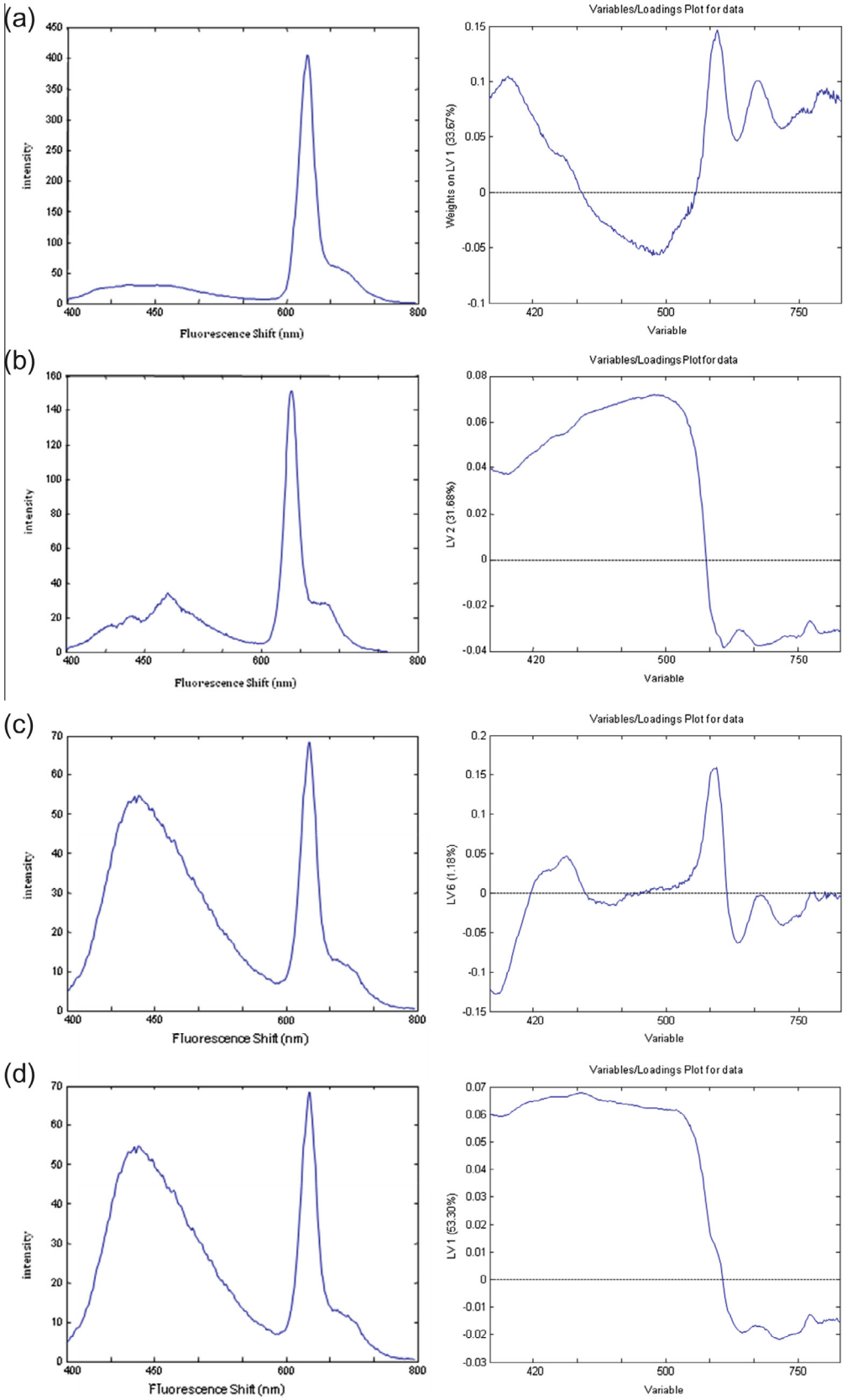


**Fig. 5.** Spectrum typical of oils and loading plots showing peroxide, acidity, K232 and K270 values: (a) high PV and low acidity, (b) low PV and high acidity, and (c) and (d) high K232 and K270 and low acidity.

The N-PLS model for K232 and K270 gave RMSECVs of 0.37 and 0.06, and correlation coefficients in the validation stage of 0.908 and 0.958, respectively.

The results obtained using the PLS model for these parameters were RMSECVs of 8.17, 0.32 and 0.06, and correlation coefficients in the validation step of 0.965, 0.881 and 0.939, respectively.

The results obtained with all methods are summarised in Table 2. A comparison of two methods shows that the results are similar and that it is possible to use the fluorescence measure at a single excitation wavelength (380 nm) to obtain enough information to provide an overview of the quality parameters of olive oil.

In order to obtain more robust results, the dataset was randomly divided into a calibration set and a validation set. The calibration set, containing 60 samples, was used to construct the model and the validation set, with 30 samples, was used to validate it. The number of factors used for the PLS method in the external validation procedure was six. The results obtained from external validation are given in Fig. 4, where the prediction error values obtained were 0.88, 5.81, 0.28 and 0.08, and correlation coefficients were 0.679, 0.902, 0.907 and 0.924, respectively.

The results obtained indicated that the models constructed for validation, using an independent group of samples, gave prediction and RSMECV values similar to those obtained using the leave-one-out procedure, indicating that these models could be used to conduct an overall assessment of oil quality.

In order to analyse the fluorescence spectrum in more detail, different areas of the spectrum were studied and related to loadings and scores of the variables. Score vectors and loading vectors were used to visualise the relationships between the spectra and the parameters in matrix X, respectively. The relationships between the spectra and parameters were investigated by using the score plots and the loading plots.

The following are the score and loading plots-plots of the parameters studied of olive oils with different levels of oxidation and acidity, so that can observe the areas that have more weight on each parameters. Fig. 5 shows the typical spectrum of oils of differing quality and their corresponding loading plot.

These results accord with those obtained by Guimet et al. (2005) using the fluorescence EEM to construct a calibration model for the peroxide parameter with an RMSECV of 1.5 and a correlation coefficient in the validation step of 0.84. This work showed poor correlation, however, between fluorescence and the K232 parameter, which could be due to the larger range of values and samples used to develop the model calibration. The values obtained for the K270 parameter were an RMSECV of 0.08 and a correlation coefficient in the validation step of 0.95, which were very similar to the values obtained with external validation. This was the best calibration model for the prediction of this parameter.

The advantage of this method, therefore, is that is possible to be determined these parameters with a spectrum at a single excitation wavelength without the need to scan the full spectrum at many excitation wavelength, thus saving considerable time.

These results are promising because they provide useful information that can be used for the rapid and easy monitoring of oil quality and show the potential use of fluorescence instruments for the overall evaluation of olive oil quality and for obtaining an overview of the fluorescence of various chemical species from the same analysis. These spectra can be measured quickly and easily, without the need for any pre-treatment of the oil sample, and could possibly be used to classify virgin olive oil. The fluorescence characteristic of oils could therefore be used both to classify oils and be useful to monitor their quality, and could provide an interesting complements to other spectroscopic methods, such as the NIR spectroscopies used widely in the oil industry.

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