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Rapid Determination of Olive Oil Oxidative Stability and Its Major Quality Parameters Using Vis/NIR Transmittance Spectroscopy

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ABSTRACT: This paper reports the determination of the olive oil stability index (OSI) by multivariate models from the visible and near-infrared spectrum. The technique proposed is rapid and nondestructive and can be used as a multiparametric method. Moreover, it does not require specific instrumentation, and it is environmentally friendly. The determination of the OSI using the Rancimat instrument was used as a reference method. Predictive visible and near-infrared (vis/NIRS) models were obtained from partial least squares (PLS) for the OSI, showing satisfactory performance in independent tests as proven by the R^2 values of 0.93 and 0.94 from the calibration and the residual predictive deviation (RPD) of the external validations of 3.30 and 3.00, respectively. Predictive models for the determination of free fatty acids, peroxide value, and conjugated dienes were also developed, and their satisfactory performances were demonstrated by RPDs of 3.14, 2.84, and 2.56; hence, its multiparametric determination together with OSI would be possible.

KEYWORDS: *conjugated dienes, free fatty acids, induction time, olive oil, peroxide value, stability index, NIRS, visible*

INTRODUCTION

Lipid oxidation has been recognized as the major problem affecting edible oils, as it is the cause of important deteriorative changes in their chemical, sensory, and nutritional properties. Oxidation normally proceeds slowly at the initial stage. At any given time, which depends on the chemical composition of the oil, a sudden rise occurs in the oxidation rate. The period of time, which marks this change in the oxidation rate, is called induction period or induction time.[1,2] Virgin olive oil has a high resistance to oxidative deterioration, mainly due to two groups of compounds. The first is its fatty acid composition, characterized by a high monounsaturated-to-polyunsaturated fatty acid ratio, and the major factor providing oil oxidative stability. Second, it contains a pool of minor compounds of powerful antioxidant activity among which polyphenols stand out.[3] Most of these compounds are eliminated or drastically reduced during the refining process and, consequently, are present in much lower amounts in edible refined oils than in virgin oils. It should be highlighted that even if virgin olive oil generally has a high resistance to oxidation, some minor compounds which are also eliminated during refining, that is, free fatty acids and photosensitizers, are pro-oxidants and consequently will contribute to a high variability in the stability of virgin olive oils.[1] Moreover, an important role of chlorophylls and carotenoids in the oxidative activity of processed foodstuff, due to their antioxidant nature in the dark and pro-oxidant activity in the light, has been shown.[4]

On the other hand, extrinsic factors such as storage temperature, diluted oxygen amount, and light, greatly influence the oxidative stability of olive oil.

Olive oil can be consumed as a fruit juice called virgin olive oil (VOO); it is one of its characteristics and differentiates it from other plant oils. This oil, one of the main components of the Mediterranean diet, is recognized as a protector against cardiovascular diseases and cancer, due to its fatty acid composition and its phenolic compound content.[5] A large increase in the demand for high-quality VOO during recent years can be attributed not only to its characteristic sensory attributes but to its potential health benefits as well. The olive oil stability measurement (OSI) is necessary to gain a reasonable indication to estimate the shelf life of the product. Fatty acid composition is fairly constant, which explains why olive oil stability is correlated with its minor compound contents,[2,6] having a great influence in the stability. Thus, the OSI, which is the same as induction time or induction period, is useful to check the antioxidant role in the olive oils of its minor compounds.

The storage temperature and the concentration of diluted oxygen influence the changes in activation energies and the mechanisms of oxidation reactions. This justifies the need to consider the methods for the prediction of the olive oil resistance to oxidation under storage conditions independently from the methods for the prediction of olive oil performance at high temperatures of food processing.[1] In the first case, information related to the oil's shelf life will be obtained. Later studies in the field are focused on frying because it is the only process in which the oil can be continuously heated for a long time or in which the oil can be used many times discontinuously. The prediction of oxidative stability during oil storage is commonly carried out using several methods such as the Schaal oven test, active oxygen, OSI, or oxygen uptake.[1] The prediction of oil performance during frying is assessed by thermoxidation assays,[7] such as the Rancimat method or the procedure described by Gertz et al.[8] All of these methods are time-consuming and expensive.

The aim of improving the production process in its technical and economic aspects, as well as increasing the quality

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standards for VOO, is continuously stimulating the search for new technologies, with great interest in the olive oil industry to monitor their quality attributes using fast and reliable techniques. Among the various nondestructive techniques that can offer solutions in the olive oil sector to these needs to date, near-infrared spectroscopy (NIRS) technology, based upon multivariate models in which the spectral data are correlated to the parameter analyzed, has made major achievements. Moreover, it provides several important advantages, because it is a rapid, nondestructive, and potentially suitable multiparameter method. Additionally, no solvents or reagents are used, so it is environmentally friendly. The ability to determine the main parameters of olive oil quality, such as free acidity, peroxides, and K_{270} and K_{232} values, has been reported repeatedly.[9−13] In fact, NIRS techniques are used as a method for routine analysis of these parameters in a growing number of laboratories. Moreover, NIRS has been reported to be suitable as a method for determining the oxidation of vegetable oils by Gülgün et al.,[14] but pitifully this information is not easily accessible.

Nevertheless, the literature on olive oil oxidative stability analysis by NIRS is very scarce, but highlights a study reporting the rapid evaluation of olive oil quality by NIRS reflectance, which included induction time.[10] It is important to note that this study did not include a procedure for calibration assessment using validation exercises, with samples different from those used to develop the calibrations. Furthermore, these techniques were also applied to a study on the thermal degradation of olive oil,[15] and another one[16] has been reported using FT-NIRS with the same purpose, which mainly is a quality control of olive oil for frying. Also noteworthy is that near-infrared emission spectroscopy (NIRES) has been reported as an alternative method to measuring the oxidative stability of edible oils at frying temperatures,[17,18] in order to control oil thermal degradation as in the two previous studies. In those latter studies, oxidative stability is determined by means of the emission band at 2900 nm and its increase and broadening during heating time. Hence, information on the possibility of measuring the olive oil resistance to oxidation under storage conditions by NIRS transmittance is virtually nonexistent. Moreover, it is of great interest for the industry and laboratories working with olive oil, where we do not know whether this technique has been used to date, especially when this parameter is an important indicator of olive oil aging, their monitoring showing at present growing interest.

In this paper, the use of visible and near-infrared (vis/NIRS) multivariate models, using transmittance mode, is tested for estimating the oxidative stability of VOO. The technique we propose is truly rapid and nondestructive, it does not require the use of solvents or reagents, and it is environmentally friendly. Predictive models have been developed also for the determination of free fatty acids (FFA), peroxide value (PV), and conjugated dienes (K_{232}), parameters that are important indicators of quality deterioration of olive oils. Although not a scientific objective, because these determinations have been repeatedly reported,[9−13] our purpose is to illustrate that the possibility of measuring multiparametric together with OSI could be very interesting for laboratories working with olive oil.

MATERIALS AND METHODS

Olive Oils. The development of multivariate models was carried out from vis/NIRS spectral variables and the reference analysis of virgin olive oils. A group of 162 samples was extracted in the laboratory of the Instituto de la Grasa by using a mill MC2 (Ingeniería y Sistemas, S.L., Spain) from samples of olives provided by a research project. These were taken in different olive groves of the provinces of Seville and Huelva and were composed of the varieties Picual, Arbequina, and Manzanilla in equal quantities. Another group of 116 samples were virgin olive oils previously extracted in olive mills and sent to the laboratory, approximately equally from the provinces of Andalusia (Spain), where olive growing is more prevalent, and were composed of the varieties Picual, Arbequina, Cornicabra, and Hojiblanca in about equal quantities. There were 278 VOO samples in total, which were stored at 4 °C until spectra acquisition. The average fatty acid composition of the total set of samples used is shown in Table 1.

Table 1. Fatty Acid Composition[a]

fatty acid	%	fatty acid	%
C14:0 (myristic)	0.0	C18:2 (linoleic)	11.63
C16:0 (palmitic)	16.63	C20:0 (arachidic)	0.4
C16:1 (palmitoleic)	1.7	C18:3 (linolenic)	0.5
C17:0 (margaric)	0.0	C20:1 (eicosenoic)	0.0
C17:1 (margaroleic)	0.0	C22:0 (behenic)	0.1
C18:0 (estearic)	1.9	C24:0 (lignoceric)	0.0
C18:1 (oleic)	67.18		

[a]Average value of the total set of samples.

Instrumentation. Spectral acquisition was carried out using a vis/NIRS Labspec Pro model LSP350-2500P (Analytical Spectral Devices Inc., Boulder, CO). Labspec Pro is a vis/NIRS spectrometer equipped with three detectors. The detector for the visible range (350−1000 nm) is a fixed reflective holographic diode array with a sensitivity of 512 pixels. The portability of the equipment is possible due to the weight of the spectrometer, 8.5 kg, and size, 31.7 cm length by 11.4 cm width by 40.6 cm height. The wavelength range of 1000−1800 nm is covered by a holographic fast scanner InGaAs detector cooled at −25 °C. The same device coupled with a high-order blocking filter operates for the 1800−2500 nm interval. The instrument is equipped with internal shutters and automatic offset correction, with a scanning speed of 100 ms. The spectrometer is equipped with a spectrophotometric cuvette accessory joined by fiber optic connectors to the light source spectrometer at one side of the accessory and to the detector on the opposite side.

Spectral Acquisition. The temperature of a body has an important influence on the NIR radiation it reflects and absorbs; hence, it constitutes a decisive factor in NIRS. Therefore, the samples were taken from 4 °C storage and placed in the laboratory 18 h before processing. Prior to the recording of spectra, the 125 mL sample containers were placed in a thermostatic water bath fixed at 33 °C for 30 min, to check the stability of temperature. The spectra acquisition was performed nondestructively in transmittance mode, from each VOO sample without any other treatment. Scans were performed in a Hellma quartz spectrophotometric cuvette with 5 mm path length, and their averaged spectra were registered. The whole spectrum vis/NIRS (350−2500 nm) was acquired, each spectral variable corresponding to a 1 nm interval. Configuration for 50 spectra in continuous acquisition was used, each spectral variable corresponding to 1 nm interval. Indico Pro software (Analytical Spectral Devices Inc.) was used for this purpose. The acquisition processing time required for each sample was less than a minute, all steps included.

Reference Analysis. The OSI method using a Rancimat instrument (Metrohm, Switzerland) was applied as the reference analysis for all olive oil samples. All of the measures were made with three replicates. Briefly, a stream of purified air was passed through a sample of 5 g of oil that is held at a 110 °C constant temperature with an air flow rate of 15−20 L h^{-1}. The effluent air from the oil sample is then bubbled through a vessel containing deionized water. The conductivity of the water is continuously monitored. The effluent air contains volatile organic acids swept from the oxidizing oil, which

increase the conductivity of the water as oxidation proceeds. The OSI is defined as the time, expressed in hours, that is needed to reach the maximum change of conductivity, which is commonly called the induction time or induction period.

The reference analysis of FFA was determinated on olive oil samples according to the UNE 55070 standard.[19] The PV (mequiv O_2/kg) was determined by the iodometric assay, and conjugated dienes were determined by spectrophotometric measure by UV absorption at 232 nm (K_{232}), both according to IUPAC standard method 2.501.[20] All of these measures were made with two replicates.

Calibration Procedure and Chemometrics. Transmittance spectral data and its transformation to absorbance spectral variables were tested for model development. Mean and maximum normalization of these data and first and second Savitzky−Golay derivative treatments were also tested. The calibration sets for the development of all models were formed excluding external validation sets from the total of the olive oil samples available, constituted as indicated below. Hence, the external validation sets reserved for this purpose were not included for building the multivariate models. Two multivariate calibration models (M_1 and M_2) were independently developed for OSI prediction. A calibration model was developed for each of the parameters FFA (M_3), PV (M_4), and K_{232} (M_5). Partial least squares[21] (PLS) models were obtained for all of the parameters studied from the whole spectrum acquired (350−2500 nm) using The Unscrumbler 9.7 (CAMO Software AS, Oslo, Norway), with full cross internal validation (FCV) procedure. The models principal components (PCs) were fixed after probes using 10 PCs initially. The procedure for spectral variable selection to be included in the models consisted of performing several consecutive cycles, eliminating variables having spectral correlation coefficients with the OSI closer to zero. Variable selection ended in the last cycle that improved the statistical model R^2 and R^2_{CV}. This selection was made on the regression coefficient graph of The Unscrumbler.

Model fitness was assessed independently from the model validation procedure described below by its standard error of calibration (SEC) and proximity between R^2 and R^2_{CV}.

Model Performance Assessment. Calibration models were evaluated by external validation exercises, using them to predict the validation sets previously reserved V_1−V_5. The validation sets for the OSI models were built by selecting one of every six (V_1) and one of every four (V_2) of the total olive oil available samples, counting from the first. For the assessment of the models for FFA (V_3), PV (V_4), and K_{232} (V_5), one of every five of the total olive oil samples available for the corresponding parameter, counting from the first, was reserved. Model performance was assessed mainly according to the residual predictive deviation (RPD) from the external validation exercises above referred, which is the statistic most consensual for assessing a model's predictive accuracy.[22] RPD is described as the ratio of the SD of the reference data from the validation set to the standard error of performance (SEP). The R^2 of the simple linear regression between the analyzed and predicted values in this external validation exercise and the RMSEP were also considered. Likewise, the development of two different calibrations for the OSI, which were independently assessed by external validation exercise, has been carried out to confirm their predictive ability. For OSI models the coefficient of variation of the predictions (CV_P), defined as the ratio between the SEP and the mean from the external validation exercises ($\bar{X}_v$) V_1 and V_2 was considered and expressed as percentage. These values were compared with the repeatability coefficient of variation (CV_R) from the reference Rancimat method, calculated from the replicates and expressed as percentage.

RESULTS AND DISCUSSION

Olive Oil Spectrum. Near-infrared spectra show various overlapping bands, as a result of the first and second overtones and a combination of the fundamental vibrations, mainly carbon−hydrogen. The assignment of the major near-infrared absorption bands of agricultural products has been described by Shenk et al.,[23] among others, and with regard to olive oil and other plant oils, it has been done by Harwood and Aparicio.[24] The assignment of the major visible absorption bands of olive oil has been described by Moyano et al.[25] Olive oil spectra from the samples analyzed in this work, shown in Figure 1, agree

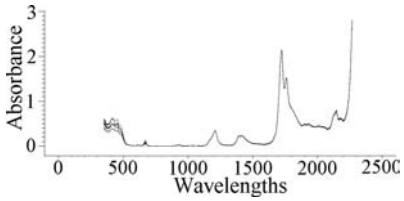

Figure 1. Olive oil vis/NIR spectrum.

with the previously indicated reports. A first minor peak occurs next to 415 nm. This area corresponds to the wavelengths of oil absorption, which is dark blue colored light, and could be due mainly to carotenoids, as well as to pheophytin *a*, pheophorbide *a*, and pyropheophytin *a*.[25] A second minor peak near 450 nm was found, corresponding to the absorption of the blue light, characteristic of carotenoids.[25] A third peak was observed approximately at 670 nm, which coincides with the absorption of chlorophylls. There are bands of high intensity, related to the strong water absorbance that exists from its first overtone at 1400−1500 nm and a combination band at 1880−2100 nm. A broad absorbance band exists around 1220 nm, probably from oil and due to second overtones of C—H and CH═CH− stretching vibrations. A high-intensity absorbance peak occurs at about 2300 nm caused by a combination of fundamental vibrations from the C—H groups.[24,26]

Population Characterization. The values from the OSI reference analysis, which correspond to the calibration and external validation sets, are included in Table 2. As can be seen,

Table 2. Statistics of Oil Stability Index

	oil stability index (h)			
	N	range	$\bar{X}$	σ
		Calibration[a]		
M_1	147	15.2−90.6	55.3	22.3
M_2	133	15.4−90.6	56.2	22.5
		Validation[b]		
V_1	30	19.7−86.8	57.1	22.0
V_2	43	15.2−88.5	54.6	22.3

[a]Set of samples used for calibration. [b]Set of samples used for validation.

wide variation ranges of the olive oils studied were integrated into the calibrations for the OSI, ranging from 15.4 to 90.6 h. The OSI average from the calibrations sets analyzed M_1 and M_2 were 55.3 and 56.2 h. Statistics from the calibration and validation sets of FFA, PV, and K_{232} are included in Table 3. The box and whiskers plots of the sample sets used for the calibrations for all determinations are shown in Figure 2. In these graphs it can be seen that sets calibration for FFA, PV, and K_{232} include some samples showing extreme values, away from the means. On the contrary, the sets calibration M_1 and M_2 for OSI are fairly centered in the plots.

Spectral Variable Analysis and Chemometry. Maximum normalization provided slightly better performance than the mean normalization treatment for all of the OSI calibrations tested. The data transformation into absorbance produced

Table 3. Statistics of Free Fatty Acids, Peroxide Value, and Conjugated Dienes

free fatty acids (oleic acid %)					peroxide value (mequiv O_2/kg)					conjugated dienes (K_{232})				
	N	range	$\bar{X}$	σ		N	range	$\bar{X}$	σ		N	range	$\bar{X}$	σ
							Calibration[a]							
M_3	222	0.1−8.7	0.5	0.9	M_4	199	5.6−43.9	17.0	10.7	M_5	223	1.0−5.0	2.0	0.8
							Validation[b]							
V_3	47	0.1−6.1	0.6	1.1	V_4	46	5.9−38.7	18.7	10.8	V_5	55	0.9−4.4	2.0	0.8

[a]Set of samples used for calibration. [b]Set of samples used for validation.

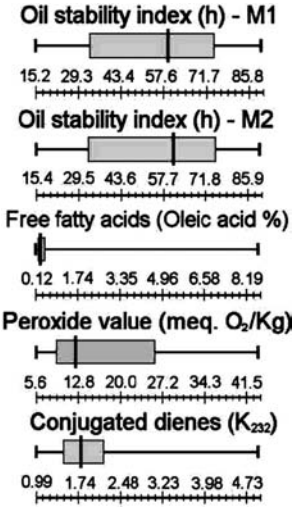

Figure 2. Box and whiskers graphs of the calibration sets.

calibration outcomes very similar to the use of transmittance, as is deduced from the R^2 and SEC values shown in Table 4. The

Table 4. Statistics of the OSI Calibration Tests

data treatment	R^{2a}	SEC[b]
none (transmittance)	0.94	5.64
absorbance	0.94	5.28
absorbance D_1SG[c]	0.91	6.71
absorbance D_2SG[d]	0.84	9.07

[a]Model squared coefficient of calibration. [b]Standard error of calibration. [c]First Savitzky−Golay derivative. [d]Second Savitzky−Golay derivative. The data were maximum normalized previously to all the treatments.

treatments with first or second Savitzky−Golay derivatives did not improve the calibration statistics in the tests, which were conducted with the M_2 set for the OSI, as is proven from the R^2 and SEC values shown in Table 4. Hence, the absorbance data maximum normalized provided the best outcomes for OSI models. The same results regarding data treatment were reached for the major parameters of the quality of olive oil included in the present study (data not shown).

The procedure for the selection of spectral variables indicated a wide range of wavelengths contributing to the models, including very diverse windows from the visible and NIR regions. However a large number of these did not contribute positively to the models, since its elimination allowed calibration's improvement. These spectral windows and single wavelengths are shown in the Figure 3, where the regression coefficients plot for M_1 and the same for M_2 are depicted. In these graphs, the horizontal line within a certain wavelength indicates zero contribution to the model. The visible spectrum can be highlighted for its contribution in both models, as well as the NIR spectral windows corresponding to 1300−1700 and 1800−2250 nm.

Oil Stability Index Models. The OSI model statistics are included in Table 5. These models correspond to spectral data

Table 5. Statistics of the Oil Stability Index Models

Model 1				
calibration (M_1)		validation (V_1)		
R^{2a}	SEC[b]	SEP[c]	CV (%)[d]	RPD[e]
0.93	6.07	6.68	11.70	3.30
Model 2				
calibration (M_2)		validation (V_2)		
R^{2a}	SEC[b]	SEP[c]	CV (%)[d]	RPD[e]
0.94	5.64	7.38	13.50	3.02

[a]Model squared coefficient of calibration. [b]Standard error of calibration. [c]Standard error of performance. [d]Repeatability coefficient of variation defined as SEP/$\bar{X}_v$, where $\bar{X}_v$ is the mean from the validation set; [e]Residual predictive deviation.

from absorbance treated with maximum normalization, which provided the best fits. The model M_1 provided R^2 = 0.93 and SEC = 6.07 (10.9%), whereas M_2 calibration statistics were R^2 =

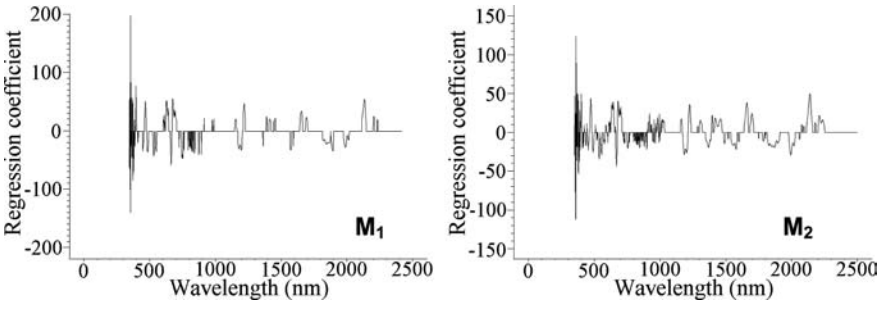

Figure 3. Wavelengths contributing to the models M_1 and M_2.

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0.94 and SEC = 5.64 (10.0%). Mailer[10] reported a correlation coefficient of 0.83 from the cross-validation procedure, in a calibration from NIR reflectance for measuring induction time, with SEC = 0.84 (19.2%), using VOO, having an OSI mean lower than that used in the present work and, hence, the smallest value of SEC. Despite the fact that this correlation value was not very high, what must be noted is this assay's lack of assessing models on the basis of external validation exercises. Moreover, although we do not know if it has been demonstrated experimentally that NIRS transmittance techniques work better in olive oil compared to the same with reflectance mode; it is very likely that the transmittance spectrum provides higher quality information, because it involves the radiation passing through the optical path in which the sample is located, thus representing a larger quantity of substance.

The dispersion plots of the prediction corresponding to the external validation exercises, carried out by using the models to determine OSI on a set of 43 samples initially reserved for this purpose, are demonstrated in Figures 4 (V_1) and 5 (V_2), and its

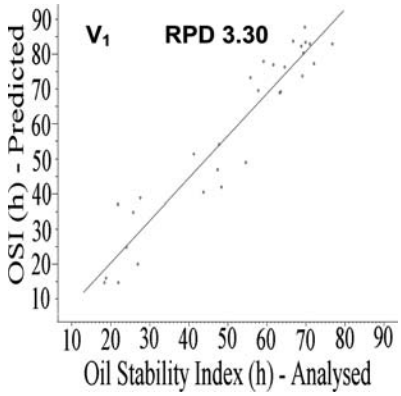

Figure 4. Validation using OSI model M_1.

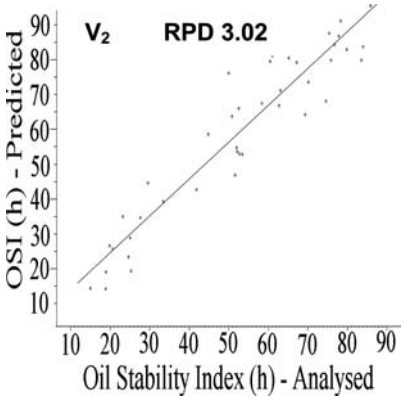

Figure 5. Validation using OSI model M_2.

statistics are shown in Table 5. The OSI model performance was satisfactory according to these data, as evidenced by the RPD values of 3.30 and 3.02 from M_1 and M_2, with its SEP being 6.68 and 7.38, respectively. The repeatability coefficients of variation of the predictions (CV_P) from the validation exercises V_1 and V_2 were 11.7 and 13.5, respectively, as is shown in Table 5. Noteworthy is the fact that calibration M_1 statistics are slightly lower than those from M_2, whereas the model external validation assessment is better for M_1. Performance of both models, however, is very similar, and these facts are explained by the different statistics from the respective calibration and external validation sample sets, as shown in Table 2.

It is mportant to consider that the reference Rancimat method for OSI in the present work showed a repeatability coefficient of variation CV_R = 11.3, hence very similar to the CV_P from V_1 indicated before. The standard for OSI official method[27] refers to an interlaboratory reproducibility coefficient of variation (RSD_R) of 9.1% obtained in a collaborative study on the stability of rapeseed oil and palm oil determined at 100 °C, with an instrumental CV_R = 3.3%. The same standard[27] reports a value for RSD_R = 10.2% from another study implying 15 laboratories.

The above data demonstrate the feasibility of estimating the OSI through vis/NIRS spectroscopy for measuring the oxidative stability of olive oils. The goodness of the models' statistics and their assessment satisfactory altogether shows this technique can be an advantageous alternative to other methods of analysis of oxidative stability of olive oils.

Olive Oil Major Quality Parameters. The statistics of the FFA (M_3), PV (M_4), and K_{232} (M_5) models are detailed in Table 6, showing R^2 = 0.86, 0.87, and 0.82, respectively. Their

Table 6. Statistics of the Free Fatty Acids, Peroxide Value, and Conjugated Diene Models

Free Fatty Acids (FFA)			
calibration (M_3)		validation (V_3)	
R^{2a}	SEC[b]	SEP[c]	RPD[d]
0.86	0.34	0.35	3.14
Peroxide Value (PV)			
calibration (M_4)		validation (V_4)	
R^{2a}	SEC[b]	SEP[c]	RPD[d]
0.87	3.81	3.82	2.84
Conjugated Dienes (K_{232})			
calibration (M_5)		validation (V_5)	
R^{2a}	SEC[b]	SEP[c]	RPD[d]
0.82	0.32	0.32	2.56

[a]Model squared coefficient of calibration. [b]Standard error of calibration. [c]Standard error of performance. [d]Residual predictive deviation.

fair performances have been proven by the RPD values of 3.14, 2.84, and 2.56 from the external validations, which are plotted in Figures 6 (V_3), 7 (V_4), and 8 (V_5). These outcomes are

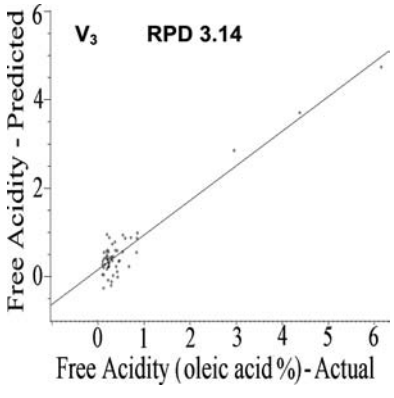

Figure 6. Validation using the free fatty acids model (M_3).

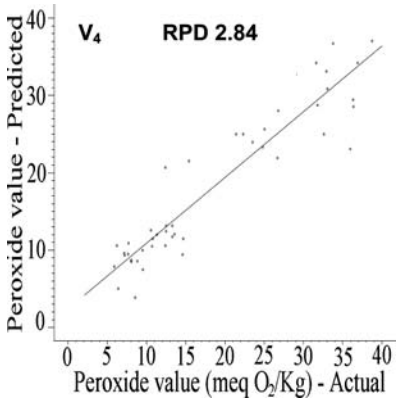

Figure 7. Validation using the peroxide value model (M_4).

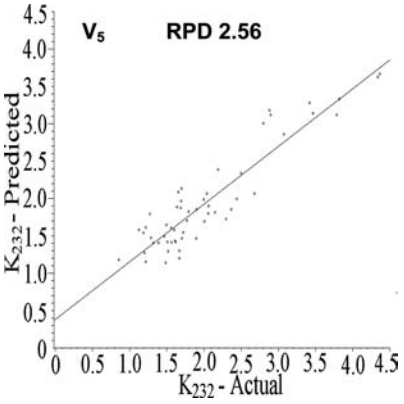

Figure 8. Validation using the conjugated dienes model (M_5).

similar to others found in the literature. Some examples are the correlation coefficients of cross validation (1-VR) of 0.94 for FFA and 0.85 for PV reported by Mailer[10] for olive oil, with which no external validations were made. Conte et al.[9] reported $R = 0.74$ for PV, as well the ability to correctly classify the extra virgin olive oil with 90% probability, with a margin of error of 0.3%, regarding a calibration for FFA, for which R was not mentioned. Armenta et al.[11] analyzed the chemometric methodology to develop predictive models for FFA and PV, reporting values $R^2 = 0.99$ for both FFA and PV using calibration, validation, and prediction sets that were limited to 14, 10, and 9 samples in olive oils. It is possible to estimate RPD at about 3.2 and 1.1 for FFA and PV, respectively, from the data of this last study, although these statistics were not specified.

As was said, the development and validation of the calibrations carried out in the present work allow illustration of the interest of using NIRS as a multiparametric technique. This could be very interesting for laboratories working with olive oil. It is important to note such application in routine determinations requires the use of software specifically developed for this purpose.

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Notes

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