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Excitation−Emission Fluorescence Spectroscopy Combined with Three-Way Methods of Analysis as a Complementary Technique for Olive Oil Characterization

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This paper shows the potential of excitation−emission fluorescence spectroscopy (EEFS) and three-way methods of analysis [parallel factor analysis (PARAFAC) and multiway partial least-squares (N-PLS) regression] as a complementary technique for olive oil characterization. The fluorescence excitation−emission matrices of a set of Spanish extra virgin, virgin, pure, and olive pomace oils were measured, and the relationship between them and some of the quality parameters of olive oils (peroxide value, K_{232}, and K_{270}) was studied. N-PLS was found to be more suitable than PARAFAC combined with multiple linear regression for correlating fluorescence and quality parameters, yielding better fits and lower prediction errors. The best results were obtained for predicting K_{270}. EEFS allowed detection of extra virgin olive oils highly degraded at early stages (with high peroxide value) and little oxidized pure olive oils (with low K_{270}). The proposed methodology may be used as an aid to analyze doubtful samples.

KEYWORDS: Olive oils; characterization; fluorescence; three-way methods

INTRODUCTION

Olive oil is obtained from the fruit of the olive tree (*Olea europaea* L.). There are different grades of olive oils [e.g., extra virgin (EV), virgin (V), pure (or simply olive oil) (P), and olive pomace (OP) oil, among others]. Each of these grades must fulfill some specifications. Due to its nutritional and economic importance, olive oil authentication is an issue of great interest in the manufacturing countries. Authenticity covers many aspects, including adulteration, mislabeling, characterization, and misleading origin (*1*). Olive oil authentication is usually based on chemical parameters [acidity, major fatty acids composition, peroxide value (PV), ultraviolet absorbance, trinolein content, and sterol composition] (*1*−*3*) and sensory analysis (*4*).

Olive oils are oxidized in the dark in contact with oxygen. As a result, essential fatty acids are destroyed and the fat-soluble vitamins E (tocopherols) and A (β-carotene) disappear (oxidation). Oxidation products have an unpleasant flavor and odor and may affect the nutritional value of the oil. Nevertheless, the low content on polyunsaturated fatty acids and the natural antioxidants (phenolic compounds, tocopherols, and β-carotene) present in olive oils protect them against oxidation. The four pigments contained in olive oils (chlorophylls *a* and *b* and their derivates pheophytins *a* and *b*) also act as antioxidants in the dark, but have an oxidizing effect in the presence of light (photo-oxidation) (*5*). As a result of the oxidation of polyunsaturated fatty acids, conjugated hydroperoxides are formed (primary oxidation products). These compounds have high absorbance in the ultraviolet (UV) region at 232 nm (K_{232}), and they are also detected by measuring the peroxide value (PV) of the oils. Due to their low stability, hydroperoxides decompose rapidly into aldehydes, ketones, and low molecular weight acids (secondary oxidation products). These compounds have high absorbance at 270 nm (K_{270}) (*5*). Due to the role of chlorophylls as sensitizers in the photo-oxidation mechanism, the longer the oils are exposed to light, the more rapidly will be the conversion of conjugated hydroperoxides into secondary oxidation products. This implies an increase in K_{270}. Evaluation of the oxidation state of oils should not be done only on the basis of the peroxide value. This is because the oxidation products present in greatly degraded oils are not detected by measuring the peroxide value and this parameter may actually give normal values. Thus, other parameters must be considered, especially sensory analysis. The processes involved in olive oil production also influence their stability. Thus, refining processes remove almost totally phenolic compounds. As a result, P and OP oils, which undergo refining processes during their manufacturing, are more prone to degradation than EV or V olive oils. In addition, refining

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processes produce conjugated dienes and trienes. These compounds increase K_{232} and K_{270} values, respectively.

Although the determination of quality and purity parameters of olive oils is done according to official methods of analysis, samples that do not fulfill the requirements are usually analyzed again to ensure the results. Sometimes this implies much work, because some of the determinations are tedious and time-consuming, as in the case of the PV, which involves several steps. For this reason, a complementary technique capable of providing rapid information for doubtful samples would be very helpful.

Fluorescence spectroscopy has been used in the past for determining olive oil authenticity (*6*). The advantages of this technique are its speed of analysis, lack of solvents and reagents, and requirement of only small amounts of sample. In addition, it is a noninvasive technique. Kyriakidis and Skarkalis (*7*) showed that useful information can be extracted from the fluorescence spectra of native vegetable oils. They showed that the fluorescence spectra of virgin olive oils between 400 and 700 nm measured at an excitation wavelength (λ_{ex}) of 365 nm have clear differences compared to the spectra of other vegetable oils. Virgin olive oils present two low peaks at 445 and 475 nm, one intense peak at 525 nm, and another peak at 681 nm. Kyriakidis and Skarkalis suggested that the peaks at 445 and 475 nm were related to fatty acid oxidation products and that the one at 525 nm was derived from vitamin E. However, they also showed that addition of vitamin E acetate to a virgin olive oil increased fluorescence intensity not only at 525 nm but also at 445 and 475 nm. They stated that this was due to oxidized vitamin E, which emits fluorescence approximately in this region. Finally, the peak at 681 nm was related to the chlorophylls. The very low intensity of the peaks at 445 and 475 nm of virgin olive oils is due to their large content of monounsaturated fatty acids and phenolic antioxidants, which provide them more stability against oxidation. All refined oils show only one intense and wide peak at around 400−560 nm, which is due to a larger oxidation state of these oils as a result of their large content of polyunsaturated fatty acids. Fluorescence of native olive oils has also been used for detecting adulterations (*8*).

Besides measuring one fluorescence spectrum at one λ_{ex}, a set of fluorescence spectra at different λ_{ex} can also be recorded. Thus, for each sample, a three-dimensional landscape is obtained, the so-called fluorescence excitation−emission matrix (EEM). The main advantage of EEMs is that more information about the fluorescent species can be extracted, because the bands arising in a wider area are considered. There are some examples in the literature of the application of excitation−emission fluorescence spectroscopy (EEFS) to native olive oils (*9*−*12*).

The aim of this paper is to study the potential of EEFS combined with three-way methods of analysis [parallel factor analysis (PARAFAC) and multiway partial least-squares (N-PLS) regression)] as a complementary technique for olive oil characterization. The relationship between the fluorescence EEMs of EV, V, P, and OP oils and some of the quality parameters of oils (peroxide value, K_{232}, and K_{270}) is studied. In addition, the PARAFAC factors provide fingerprints for the different oil types.

MATERIALS AND METHODS

Samples and Reagents. A set of 33 olive oil samples, consisting of 13 EV, 2 V, 16 P, and 2 OP oils, were kindly supplied by the official laboratory of the Catalan government in Spain. All oils came from Spanish cultivars and were obtained during the same harvesting year (final 2003−2004). The chemical analyses were performed by this laboratory, according to official methods of analysis [Regulation (EEC) 2568/91] and included quality parameters (acidity, peroxide value, K_{232}, K_{270}, ΔK) and purity parameters (individual fatty acids, trans isomers, sterols, stigmastadienes, erythrodiol, and uvaol). **Table 1** shows some of the parameters analyzed. The peroxide value and K_{232} of some of the samples were not available. They are left blank in **Table 1**. Oils were stored in amber glass bottles. The fluorescence EEMs were measured directly from the samples, without any prior treatment. All samples were measured in duplicate, and the mean value of each sample was always used.

Table 1. Analytical Parameters of Oils[a]

sample	peroxide value (mequiv of O_2/kg)	K_{232}	K_{270}
EV1	10	1.74	0.15
EV2	9	1.81	0.18
EV3	6	2.05	0.11
EV4	8	2.21	0.11
EV5	7	1.80	0.09
EV6	11	1.79	0.14
EV7	9	1.96	0.10
EV8	12	2.11	0.15
EV9	7	1.93	0.23
EV10		2.30	0.16
EV11		2.30	0.14
EV12	19	3.21	0.13
EV13	9	1.97	0.21
V1	11	2.29	0.19
V2	11	2.22	0.21
P1	5		0.20
P2	6		0.24
P3	5		0.29
P3	9		0.28
P5	4		0.27
P6	5		0.32
P7	3		0.32
P8	3		0.35
P9	5		0.26
P10	6		0.59
P11	5		0.41
P12	6	1.78	0.15
P13	6	1.91	0.18
P14	3		0.34
P15	6		0.42
P16	7		0.34
OP1	2		1.34
OP2	3		1.23

[a] Legal limits [Regulation (EEC) 2568/91]: PV (mequiv of O_2/kg), 20 (EV and V), 15 (P and OP); K_{232}, 2.50 (EV), 2.60 (V); K_{270}, 0.22 (EV), 0.25(V), 0.90 (P), 1.70 (OP).

(±)-α-Tocopherol acetate was purchased from Sigma-Aldrich Chimie (Alcobendas, Spain) and was stored at 7 °C.

Instrumentation and Software. EEMs were measured with an Aminco Bowman series 2 luminescence spectrometer equipped with a 150 W xenon lamp and 10 mm quartz cells. The instrument detector was operated using the EmL/Ref channel and applying a 600 V voltage. Excitation and emission ranges were $\lambda_{ex} = 300-390$ nm and $\lambda_{em} = 415-600$ nm, with 5 nm intervals in both dimensions. The band-pass of both monochromators was set at 4 nm. The scan rate was 30 nm s^{-1}. The instrument software was used to correct the EEMs for deviations in the ideality of the lamp, monochromators and detector (*13*, *14*).

Data were exported to ASCII code and processed with Matlab software (version 6.5) (*15*). The chemometric models were calculated with the PLS-Toolbox (*16*).

RESULTS AND DISCUSSION

Exploratory Analysis. *Fluorescence EEMs of Oils.* **Figure 1** shows the EEMs in the range between $\lambda_{ex} = 300-390$ nm and $\lambda_{em} = 415-600$ nm of one sample of each type studied

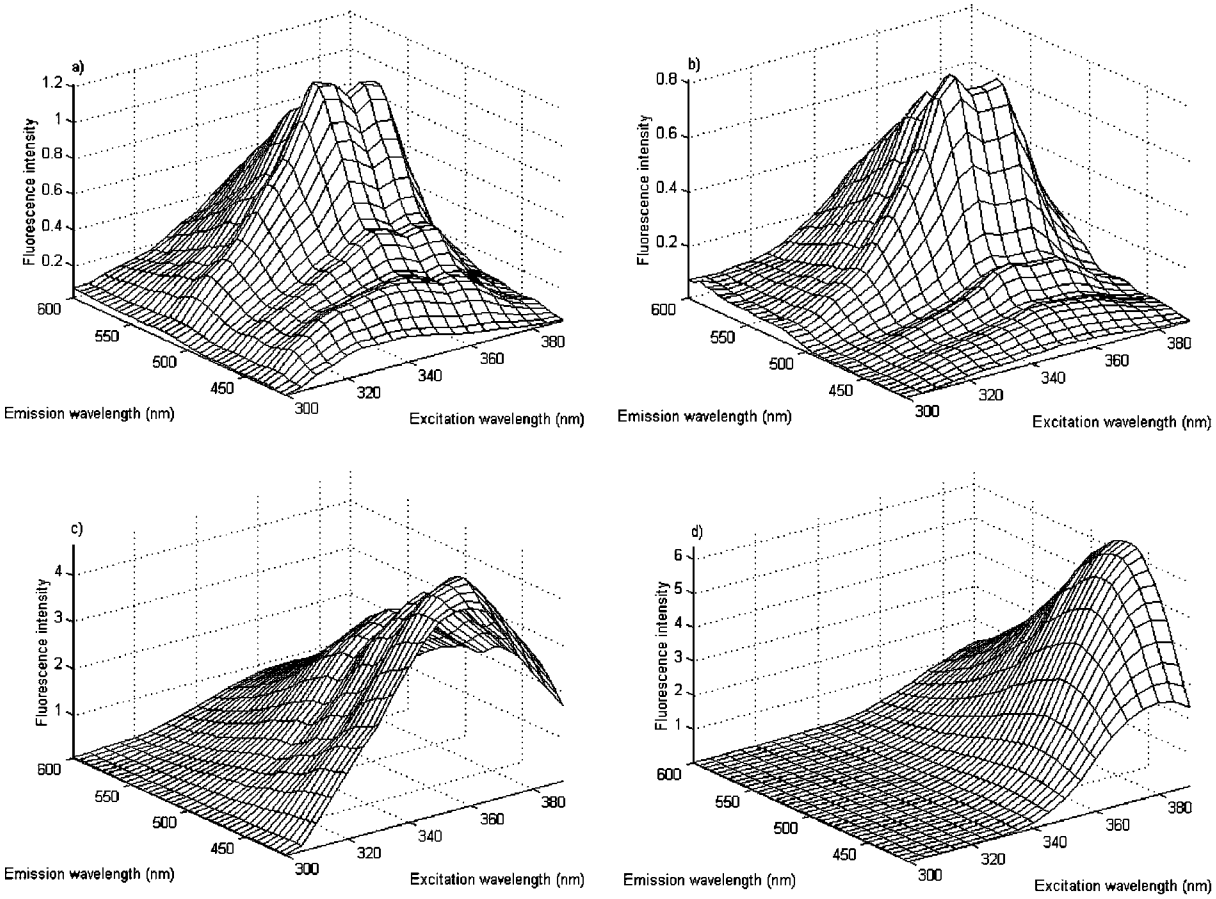

Figure 1. EEMs between λ_{ex} = 300–390 nm and λ_{em} = 415–600 nm of an EV (**a**), a V (**b**), a P (**c**), and an OP (**d**) oil.

(EV, V, P, and OP). Most of the samples analyzed displayed the same pattern and thus, in general, the types of oils can be differentiated from their fluorescence landscapes. EV and V oils present their maximum fluorescence intensity at emissions above 500 nm (**Figure 1a**,**b**). On the contrary, P and OP oils exhibit much more fluorescence intensity below 500 nm (**Figure 1c**,**d**). The main difference between these two types of oils is that EEMs of OP oils have very little fluorescence when exciting below λ_{ex} = 340 nm.

Despite the general trend, there are some samples (EV12, P12, and P13) with special fluorescence landscapes. The EEM of sample EV12 is very different from those of the other EV olive oils. It exhibits strong fluorescence at emissions below 500 nm (**Figure 2a**). On the other hand, the EEMs of samples P12 and P13 are very similar to those of EV oils. **Figure 2b** shows the EEM of sample P12. It is well-known that P oils consist of a blend of EV and refined oils. We suggest that the similarity between P12, P13, and EV oils is due to a high ratio of EV/refined oils in P12 and P13, which would explain the shape of their EEMs.

PARAFAC. To look into the whole set of fluorescence data, the EEMs of the 33 samples were arranged in a three-dimensional structure of size 33 × 38 × 19 (samples × number of λ_{em} × number of λ_{ex}). The array was decomposed by PARAFAC (*17*) using different numbers of factors. In all cases, non-negativity constraints for the resolved profiles in all modes were applied. This was done to obtain a realistic solution, because the concentrations and the spectra should be positive. Residual analysis indicated that the optimal number of factors was three (98.65% of explained variance).

Figures 3 and **4** show the spectral profiles and the sample projection plots obtained from the PARAFAC model. The emission profile of factor 1 (**Figure 3a**) is very similar to the fluorescence spectra of EV olive oils, whereas that of factor 2 is very similar to the fluorescence spectra of refined oils (*7*). As mentioned above, the peak at λ_{em} = 525 nm is thought to be related to vitamin E (**Figure 3a**, factor 1) and the peak between λ_{em} = 415−560 nm to oxidation products (**Figure 3a**, factor 2). The sample projection plots (**Figure 4**) show that the oil types are quite differentiated on the basis of the PARAFAC factors. OP oils are very different from the rest of samples, having the highest values on factor 2 and the lowest on factor 1. This indicates that factor 2 describes mainly the oxidation products contained in OP oils and that OP oils are the ones having the lowest content on vitamin E. EV and V oils have the lowest values on factor 2 because of their stability against oxidation. These two types of oils cluster together because acidity, which is the parameter that distinguishes the two grades, is not captured by fluorescence measurement. EV and P oils have similar values on factor 1. This means that these two types of oils have similar vitamin E contents. However, P oils have larger values on factor 3. This suggests that factor 3 may be related to the presence of degradation products of oils produced during the manufacturing processes. Note that EV12 is very similar to P oils as far as factor 3 is concerned and that P12 and P13 cluster with EV oils in all of the plots. The special characteristics of these three samples have been commented above.

Figure 5 shows the three-dimensional structure of the PARAFAC factors obtained after multiplying each pair of excitation and the emission profiles plotted in **Figure 3**. Note the resemblance between the PARAFAC factors and the EEMs plotted in **Figure 1**. Factor 1 (**Figure 5a**) describes well EV and V oils (**Figure 1a**), factor 2 (**Figure 5b**) describes OP oils

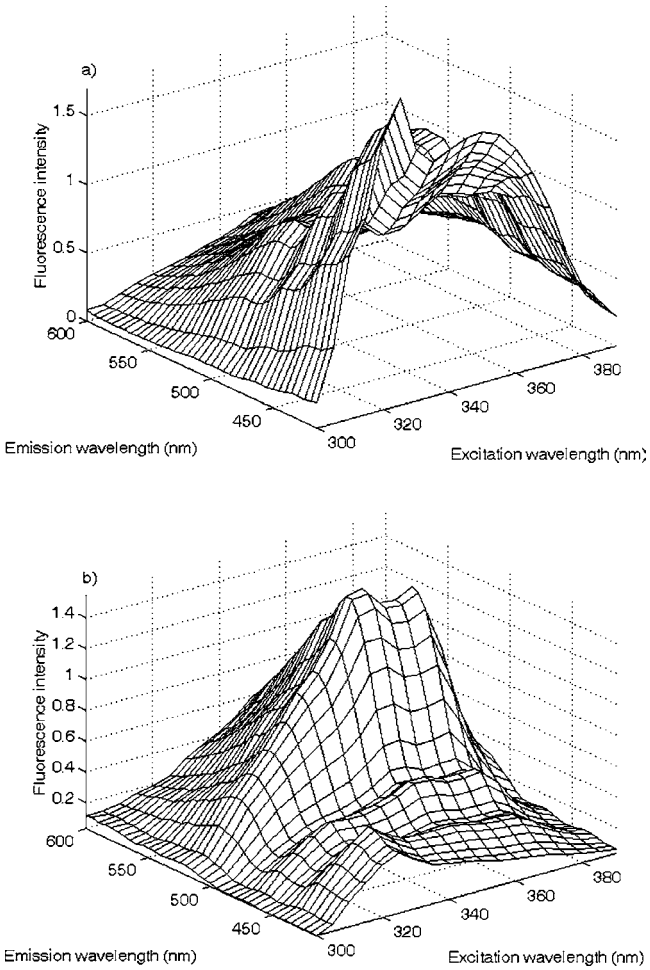

Figure 2. EEM between λ_{ex} = 300–390 nm and λ_{em} = 415–600 nm of EV12 (**a**) and P12 (**b**).

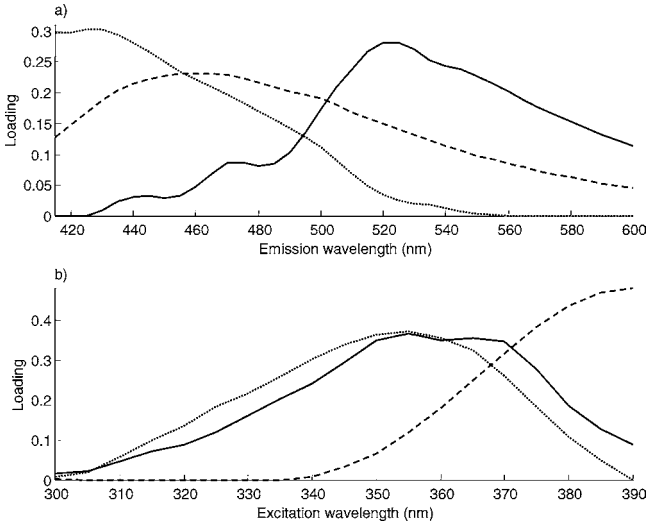

Figure 3. Emission (**a**) and excitation (**b**) profiles obtained from the three-factor PARAFAC model calculated on the EEMs of the 33 oils in the range λ_{ex} = 300–390 nm and λ_{em} = 415–600 nm: factor 1 (—), factor 2 (- - -), factor 3 (•••).

(**Figure 1c**), and factor 3 (**Figure 5c**) describes P oils (**Figure 1b**). Hence, the PARAFAC factors may be used as a fingerprint of the types of olive oils studied.

Vitamin E and Fluorescence. To confirm the hypothesis that the fluorescence peak at λ_{em} = 525 nm corresponds to vitamin E, we added vitamin E acetate [(±)-α-tocopherol acetate] to an EV olive oil sample. We used vitamin E acetate and not pure vitamin E because the latter is more unstable and is quickly oxidized by atmospheric oxygen (*7*). Vitamin E acetate was added directly to the oil so as to avoid solvent interferences and to obtain spectra directly comparable to those of the raw oil. The addition was made at two concentration levels. The first one consisted of adding 160 ppm of vitamin E acetate, which is equivalent to 146 ppm of pure vitamin E. The second addition consisted of 320 ppm of vitamin E acetate, which is equivalent to 292 ppm of pure vitamin E. Then, the EEMs of the spiked samples and the raw oil were recorded at the same range as for the previous oils. To avoid detector saturation, its sensitivity was set to 60% of full scale using the more concentrated sample. Each sample was measured in duplicate, and the mean value of each pair of EEMs was calculated. For a better visualization of the changes produced after the addition of vitamin E acetate, we extracted the fluorescence spectra at λ_{ex} = 350 nm from the entire EEMs (**Figure 6**). This λ_{ex} was selected because it provides the most intense fluorescence spectra. The plot confirms that the addition of vitamin E to an EV olive oil increases fluorescence intensity at 525 nm. However, the peaks at 445 and 475 nm also increase. This was already observed by Kyriakidis and Skarkalis (*7*), who explained that this may be due to the fluorescence that oxidized vitamin E emits near this region. The hypothesis that fatty acid oxidation products are mainly responsible for the peaks at 445 and 475 nm in the fluorescence spectra of EV oils could not be confirmed at this stage.

Relationship between Fluorescence and Primary Oxidation Products. *Fluorescence, PV, and K_{232}.* PV and K_{232} indicate the presence of primary oxidation products (i.e., conjugated hydroperoxides) in olive oils. We studied the relationship between fluorescence EEMs of oils and these parameters. All samples have values within the limits established by Regulation (EEC) 2568/91 (**Table 1**), with the exception of EV12, which has a K_{232} above the limit (2.50). This sample also has a PV very superior to those of the rest of EV oils and very close to the maximum allowed (20 mequiv of O_2/kg). The high values of these parameters indicated that this sample has been much degraded and, thus, presents rancidity. This may explain its special fluorescence. However, further analysis confirmed that this sample had not been adulterated, because the content of stigmastadienes was below the maximum allowed (0.15 mg/kg).

Figure 7 shows a chart of the fluorescence EEMs arranged in increasing order of PV. The general trend is that OP oils have the lowest PV, whereas EV and V oils have the highest ones. We suggest that the low content of hydroperoxides in OP oils is due to the fact that they have been further oxidized into carbonyl compounds (secondary oxidation products), which do not contribute to PV. As was commented in the Introduction, most of the natural antioxidants in OP oils are removed during the manufacturing processes. This makes these oils very prone to oxidation. Therefore, conversion of primary oxidation products into secondary oxidation products is more probable in these oils. This means that most of the oxidation products present in OP oils are not detectable by indicators of primary oxidation products, such as PV or K_{232}, but they should be detected by indicators of secondary oxidation products, such as K_{270}. The samples with the lowest PV display strong fluorescence around λ_{ex} = 340−390 nm and λ_{em} = 415−600 nm (**Figure 1c**). On the contrary, the sample with the highest PV (EV12) has its maximal fluorescence around λ_{ex} = 315−

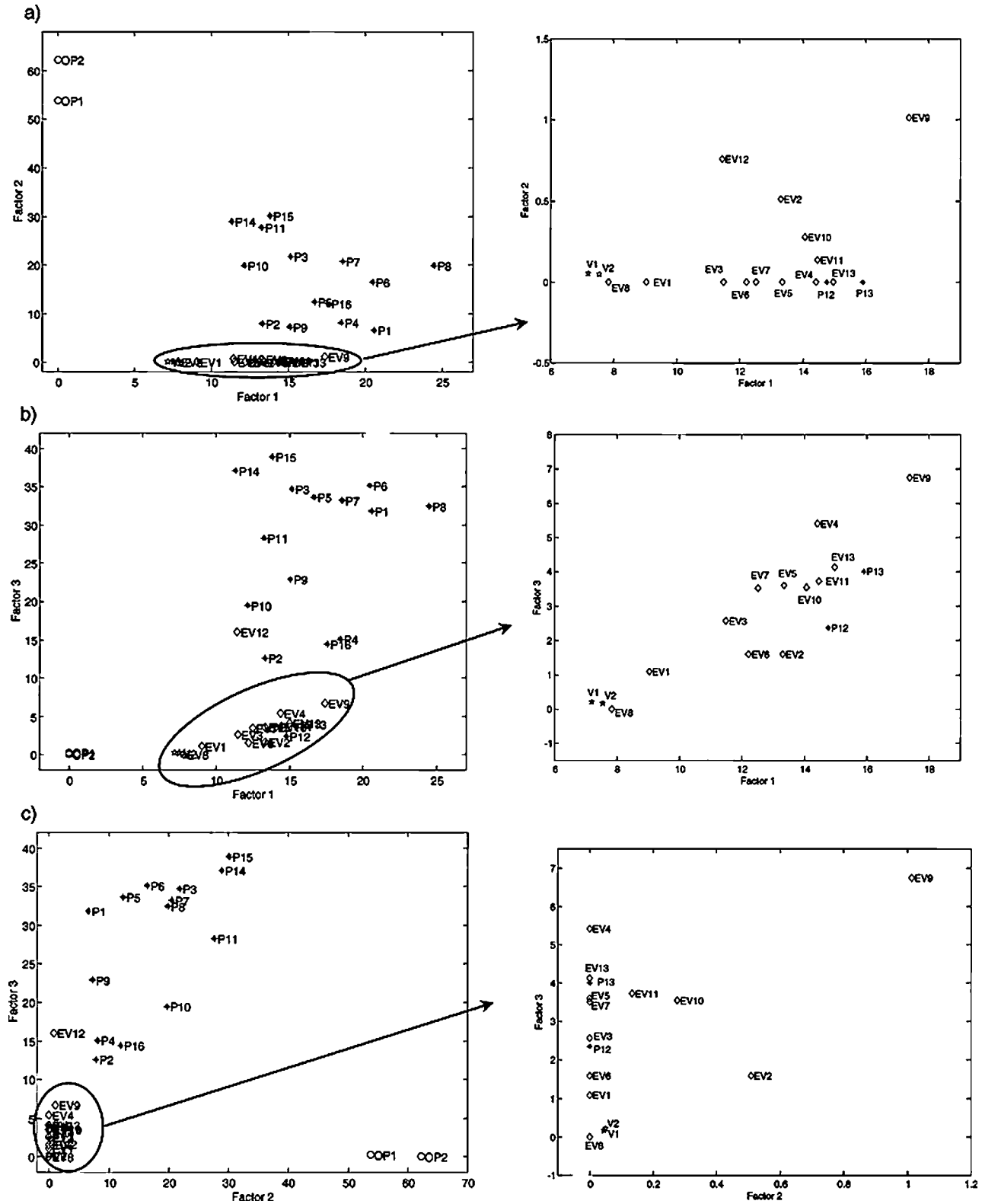

Figure 4. Sample projection plots of the three-factor PARAFAC model calculated on the EEMs of the 33 oils in the range λ_{ex} = 300–390 nm and λ_{em} = 415–600 nm. Oil types: EV (◇); V (☆); P (∗); OP (○). The region containing EV and V oils has been enlarged (plots on the right).

370 nm and $\lambda_{em} = 415-460$ nm (**Figure 2a**). Therefore, oils at early degradation stages (i.e., with large amounts of primary oxidation products) can be detected by fluorescence, because they exhibit strong fluorescence between $\lambda_{ex} = 315-370$ nm and $\lambda_{em} = 415-460$ nm, which does not occur for well-conserved samples.

Correlation between Fluorescence and PV. The EEMs of samples with known PV (**Table 1**) were arranged in a three-dimensional array of size 30 × 38 × 19 (samples × number of λ_{em} × number of λ_{ex}). EV12 was excluded for its high PV.

A three-factor PARAFAC model (98.76% explained variance) was calculated on the array, applying again non-negativity constraints on all modes. The spectral profiles and sample projection plots were very similar to those plotted in **Figures 3** and **4**. A multiple linear regression (MLR) model was then applied to correlate the values of the projected samples (i.e.,

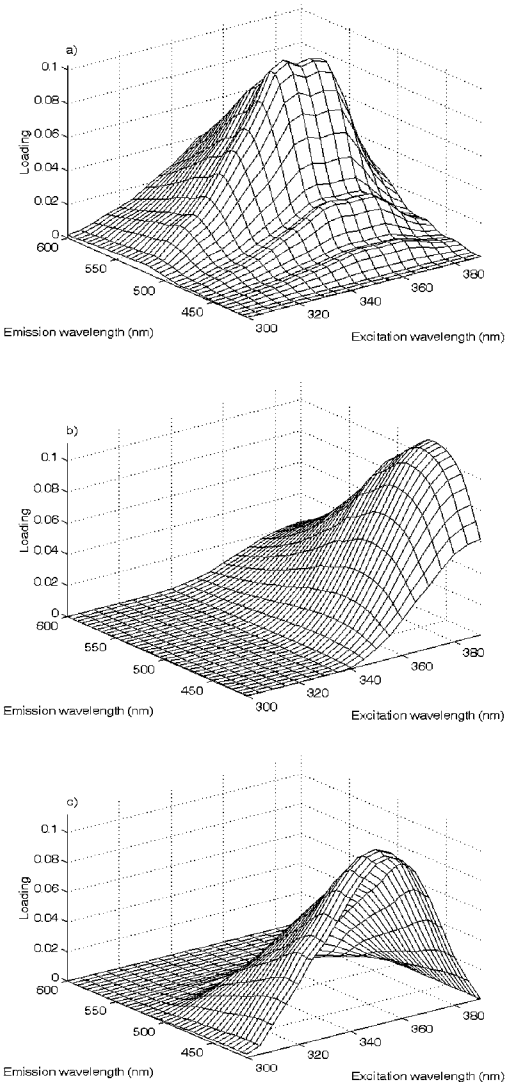

Figure 5. Three-dimensional structures of factor 1 (**a**), factor 2 (**b**), and factor 3 (**c**) from PARAFAC.

scores) obtained from PARAFAC with the PV. The prediction error calculated by means of the leave-one-out cross-validation procedure was 1.7 mequiv of O_2/kg, and the correlation coefficient of the MLR model obtained in the validation step was $r_{val} = 0.78$. Despite the global correlation observed, no good predictions were obtained for some samples. For example, EV3, P12, P13, P2, P10, and P15 have similar measured PV, but their predicted values differ considerably. This may indicate that the model is sensitive to some variations in fluorescence that occur between these samples that are not captured when the PV is measured.

We checked if correlation between the fluorescence EEMs and PV could be improved by using the N-PLS regression method. N-PLS is a generalization of PLS to multiway data (*18*). This method has some nice properties, because it models both the independent (*X*) and the dependent (*Y*) variables simultaneously to find the latent variables in *X* that will best predict the latent variables in *Y*. The model was applied on the centered data (across the sample mode). The optimum number of factors, selected by leave-one-out cross-validation, was nine [99.92% of explained variance (*X*), 88.15% of explained variance (*Y*)]. The high number of factors obtained is probably due to the presence of some samples that are not very well fitted by the model. This would force the model to require more factors so as to reduce the error. However, as the number of samples available for doing this study was not very high, we decided not to remove any sample so as not to lose robustness. **Figures 8** and **9** show the spectral profiles and sample projection plots of the first two factors. For a better visualization, the region containing EV and V oils in **Figure 9** has been enlarged. The types of oils appear quite separated on the basis of the N-PLS factors (**Figure 9**). OP oils have the highest values on factor 2. This factor is related to fluorescence at λ_{em} around 430 nm and λ_{ex} around 320 and 350 nm. P oils have the lowest values on factor 2, and EV and V oils have the highest values on factor 1. This factor is related to a wide fluorescence peak around $\lambda_{em} = 460$ nm and $\lambda_{ex} = 370$ nm. Again, P12 and P13 are grouped with EV and V oils.

Figure 10 shows the predicted versus measured PV obtained from the nine-factor N-PLS model. Using N-PLS, correlation between fluorescence and PV was improved compared to MLR on the PARAFAC scores. In addition, the prediction errors were lower. A similar procedure was carried out to correlate fluorescence and K_{232} (not shown). However, a poor correlation was observed between these two parameters. This may indicate that some of the species that contribute to K_{232} do not emit fluorescence in the range studied.

From the results obtained, we can state that EEFS is capable of detecting samples highly degraded at early stages, because they emit strong fluorescence around $\lambda_{ex} = 315-370$ nm and $\lambda_{em} = 415-460$ nm. Thus, samples having high PV (such as EV12) can be detected rapidly by recording their EEM. Hence, EEFS is proposed as a rapid complementary technique for samples with high PV.

Relationship between Fluorescence and Secondary Oxidation Products. *Fluorescence and K_{270}.* As has been commented previously, K_{270} is also an indicator of the oxidation state of oil, because secondary oxidation products (aldehydes, ketones, and other carbonyl compounds) absorb at 270 nm. **Table 1** shows the K_{270} of the 33 samples. As can be seen, in general there is a relationship between the oil type and its K_{270}. OP oils have the highest K_{270}, whereas EV oils tend to have the lowest values of this parameter. This indicates that OP oils are the most deteriorated, and thus they contain more secondary oxidation products, whereas EV oils are the most preserved, which was expected due to their larger content on natural antioxidants. Note that, as a general trend, the higher the K_{270}, the lower the PV is, so there is an inverse relationship between the amount of primary and secondary oxidation products in oils. As was explained above, this is due to the conversion of primary oxidation products into secondary oxidation products. The variation of K_{270} values in oils is also captured by the fluorescence EEMs. The oils having the highest K_{270} (OP) exhibit a wide peak between $\lambda_{ex} = 340-390$ nm and $\lambda_{em} = 415-600$ nm, with a maximum fluorescence at $\lambda_{ex} = 390$ nm and $\lambda_{em} = 470$ nm (**Figure 1c**). For oils having the lowest K_{270} (EV) the main fluorescence peaks appear above $\lambda_{em} = 500$ nm (**Figure 1a**), with the exception of EV12.

Correlation between Fluorescence and K_{270}. We applied MLR to correlate the PARAFAC scores of the 33 samples with K_{270}. The PARAFAC model chosen was that depicted in **Figures 3** and **4**. In this case, sample EV12 was included because it was not an outlier with regard to K_{270}. Validation was performed by leave-one-out cross-validation following the same procedure as above. The correlation coefficients were $r_{cal} = 0.97$ and $r_{val} = 0.95$, and the calibration and prediction errors were root-mean-square error of calibration (RMSEC) = 0.07 and root-mean-square error of cross-validation (RMSECV) = 0.08.

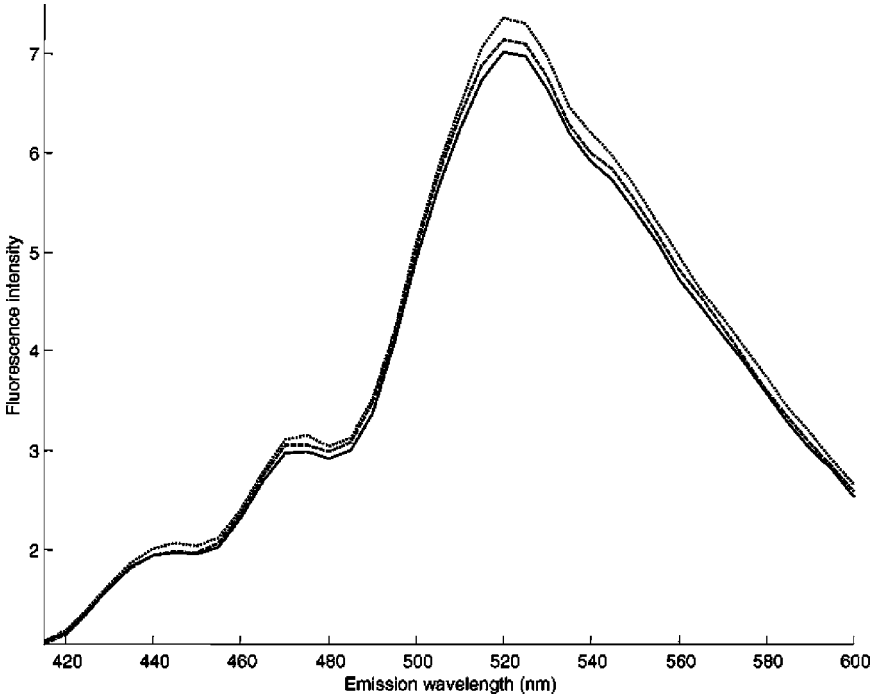

Figure 6. Mean fluorescence spectra at λ_{em} = 415–600 nm and λ_{ex} = 350 nm: raw EV olive oil (—); EV olive oil with 146 ppm of vitamin E added (- - -); EV olive oil with 292 ppm of vitamin E added (•••).

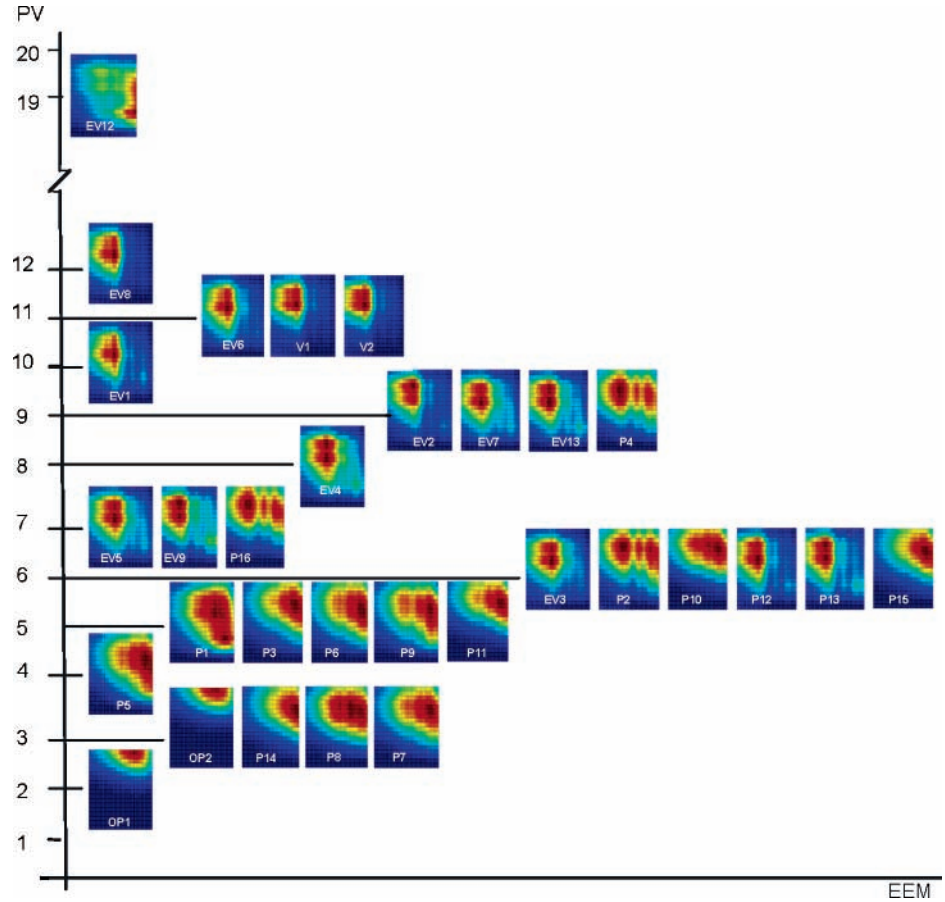

Figure 7. EEMs of oils arranged in increasing order of PV (in mequiv of O_2/kg): horizontal axis, λ_{em} = 415–600 nm (from right to left); vertical axis, λ_{ex} = 300–390 nm (from bottom to top).

Despite the high correlation coefficients obtained for the whole set of samples, little correlation was observed for EV oils.

We tried to improve the correlation between fluorescence and K_{270} by applying N-PLS. Data were centered across the first mode (i.e., a matrix where each row consisted of all the emission spectra of one sample concatenated was created; then each column of this matrix was centered by subtracting its mean value). The optimum number of factors was selected by leave-

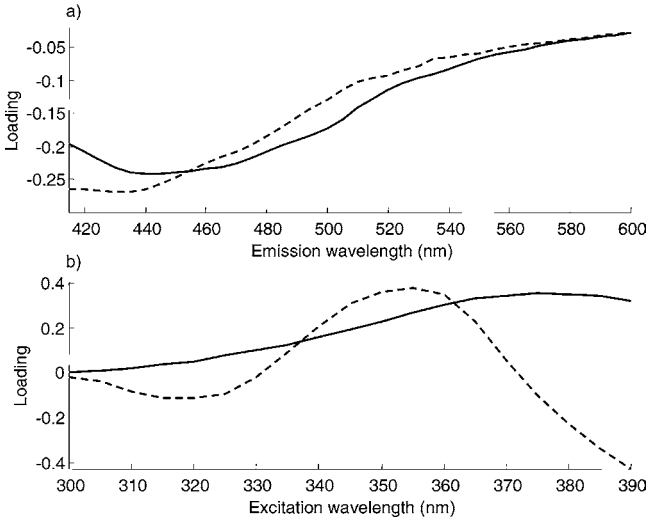

Figure 8. Emission (**a**) and excitation (**b**) profiles of the first two factors of the nine-factor N-PLS model calculated to correlate the fluorescence EEMs with the PV: factor 1 (—); factor 2 (- - -).

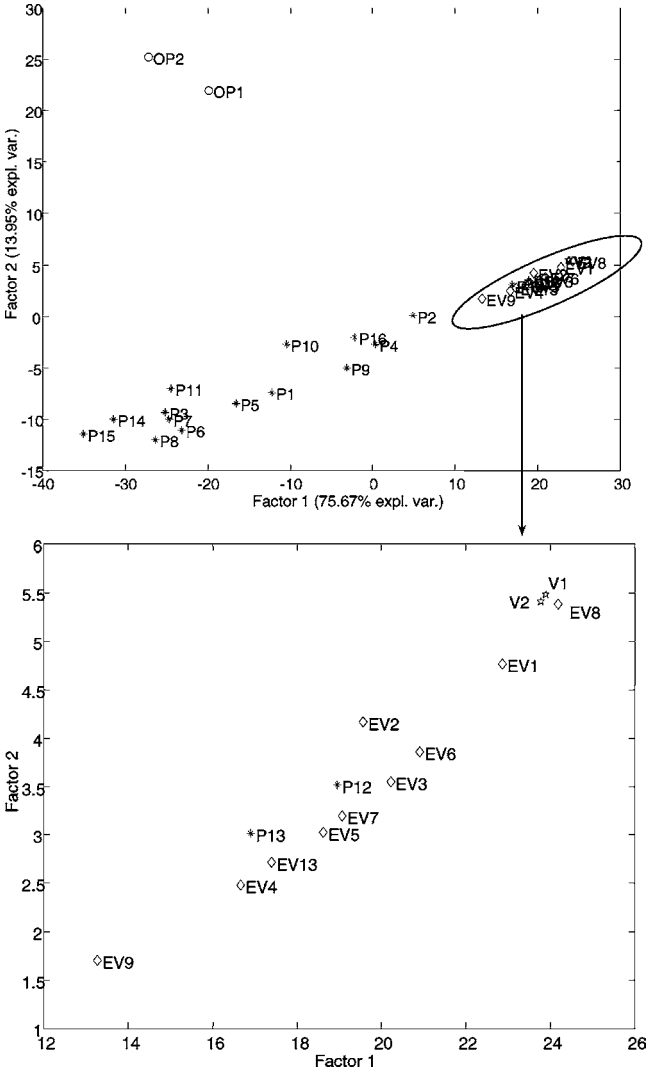

Figure 9. Sample projection plot of the first two factors of the nine-factor N-PLS model calculated to correlate the fluorescence EEMs with the PV: EV (◇); V (☆); P (∗); OP (○).

one-out cross-validation. Six factors were found to be significant [99.75% of explained variance (*X*), 97.32% of explained

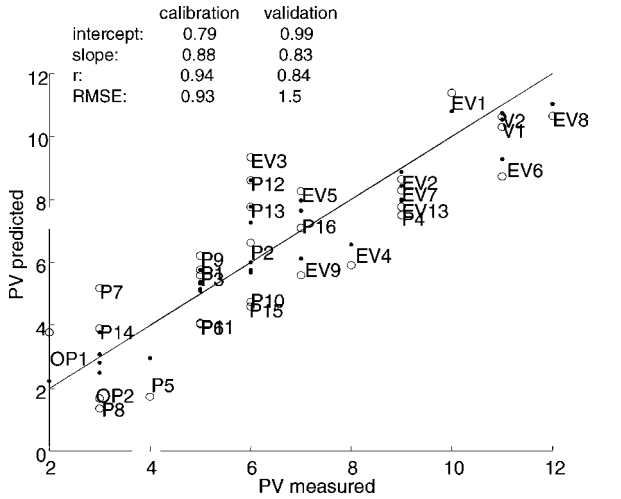

Figure 10. Predicted versus measured PV from the nine-factor N-PLS model in the range between λ_{ex} = 300–390 nm and λ_{em} = 415–600 nm: calibration (●); validation (○). RMSE (calibration) is the root-mean-square error of calibration, and RMSE (validation) is the root-mean-square error of cross-validation.

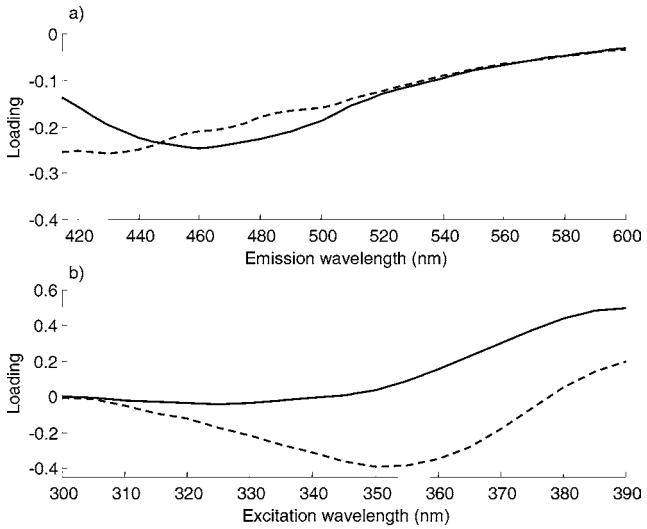

Figure 11. Emission (**a**) and excitation (**b**) profiles of the first two factors of the six-factor N-PLS model calculated to correlate the fluorescence EEMs with K_{270}: factor 1 (—); factor 2 (- - -).

variance (*Y*)]. **Figures 11** and **12** show the spectral profiles and sample projection plots of the first two factors. In **Figure 12**, the region containing EV and V oils has been enlarged to make the visualization easier. Note that the profiles of factor 1 (**Figure 11**) are very similar to those of **Figure 8**. On the contrary, factor 2 presented more differences. When N-PLS is applied to correlate fluorescence and K_{270}, the excitation profile has only one wide peak with a minimum at 350 nm. OP oils have the lowest values on factor 1, whereas EV and V oils tend to have the highest (**Figure 12**). P oils have the highest values on factor 2.

Figure 13 shows the predicted versus measured K_{270} values obtained from the six-factor N-PLS model. The group of samples not including OP oils has been enlarged. Using N-PLS enabled a better fit compared to that obtained from MLR applied to the PARAFAC scores, especially for EV samples. As can be seen from the plot, the N-PLS factors are highly correlated with K_{270} (r_{cal} = 0.99, r_{val} = 0.96), and the prediction errors are lower compared to MLR on the PARAFAC scores.

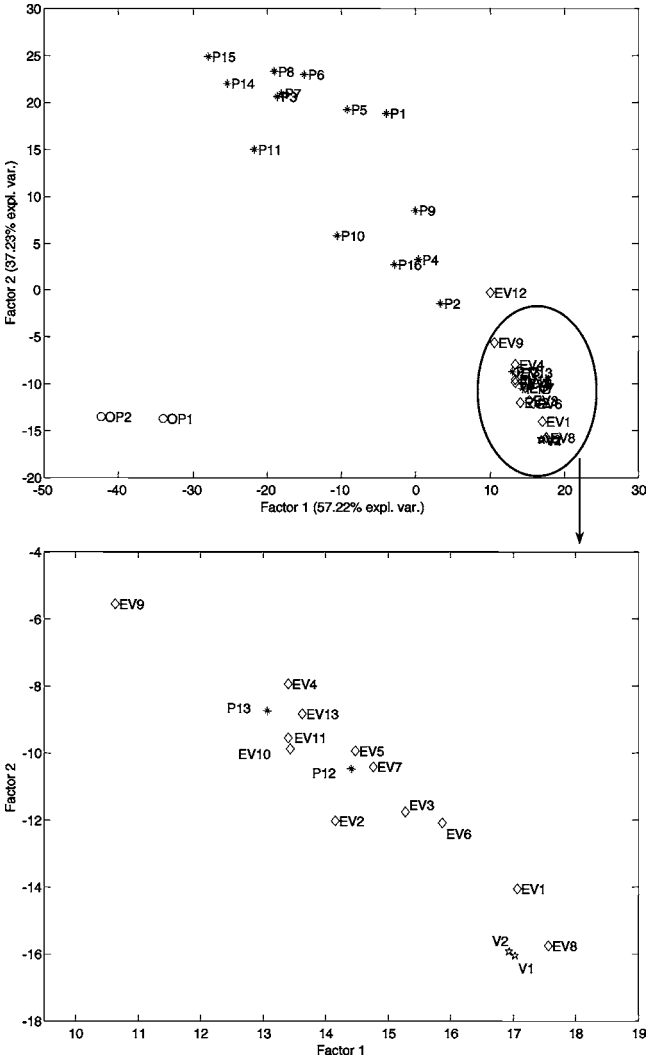

Figure 12. Sample projection plot of the first two factors of the six-factor N-PLS model calculated to correlate the fluorescence EEMs with K_{270}: EV (◇); V (☆); P (∗); OP (○).

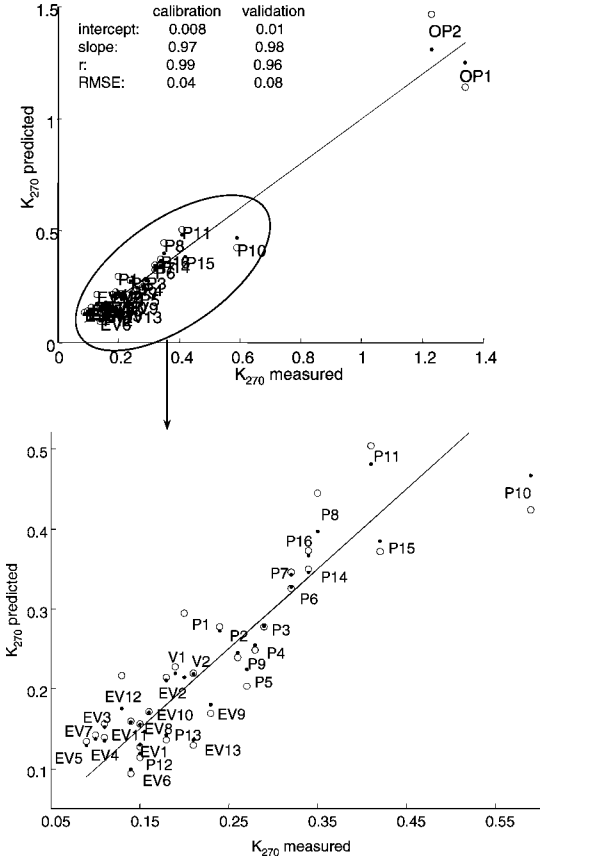

Figure 13. Predicted versus measured K_{270} from the six-factor N-PLS model in the range between λ_{ex} = 300–390 nm and λ_{em} = 415–600 nm: calibration (●); validation (○). RMSE (calibration) is the root-mean-square error of calibration, and RMSE (validation) is the root-mean-square error of cross-validation.

Throughout this study, we have shown that the fluorescence data of samples P12 and P13 are very similar to those of EV oils. Nevertheless, the analytical parameters shown in **Table 1** confirm that these samples belong to the P grade, because all of the parameters are within the limits established. As far as K_{270} is concerned, P12 and P13 have the lowest values among the P group (**Table 1** and **Figure 13**). K_{270} is an indicator of the quality of oils. Low K_{270} values indicate low content of secondary oxidation products, which is due to a high stability of oils. As was commented above, the appearance of the EEMs of P12 and P13 seems to indicate a high ratio of EV/refined oils in these samples. This would imply having a high level of natural antioxidants such as phenolic compounds, which would explain their stability. Hence, EEFS may be useful for studying the quality of P oils.

Conclusions. This paper has shown the potential of EEFS and three-way methods of analysis (PARAFAC and N-PLS) as a complementary technique for olive oil characterization. This methodology is proposed as an aid to determine the quality of olive oils and may be especially helpful for doubtful samples. Concretely, it has been shown that EEFS enables the detection of EV oils highly deteriorated at early stages (with high PV) and little oxidized P oils (with low K_{270}). However, the results reported have to be interpreted by taking into account the low number of samples analyzed. In further studies we aim to enlarge the data set and include more doubtful samples to confirm these results.

The methodology presented here is somewhat innovative. Previous studies on olive oils had already reported the study of the correlation between fluorescence intensity and quality parameters. However, none of them used EEFS and three-way chemometric methods. The latter has some additional advantages. For instance, EEFS enables one to obtain an overview of the fluorescence of various chemical species from the same analysis, which may be of interest for finding trends or patterns in the data. In addition, the chemometric analysis of these data enables one to extract the spectral profiles related to the fluorescent species of oils. These profiles may be later used to make a comparative study of the contribution of the fluorescent species in the oil samples.

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