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Rapid synchronous fluorescence method for virgin olive oil adulteration assessment

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Abstract

This paper describes the differentiation of virgin olive from olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils using total synchronous fluorescence (TSyF) spectra. TSyF spectra are acquired by varying the excitation wavelength in the region 250–720 nm and the wavelength interval ($\Delta\lambda$) in the region from 20 to 120 nm. It is shown that adulterants can be discriminated from virgin olive oil using a wavelength interval of 20 nm and excitation wavelength region 315–400, 315–392, 315–375, 315–365, 315–375 and 315–360 for olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils, respectively. Thirty one virgin olive oil mixtures with each potential adulterant were prepared at varying levels with emphasis at low concentrations. The partial least-squares regression model was used to quantify adulteration. This technique is useful for detection of olive-pomace, corn, sunflower, soybean, rapeseed and walnut oil in virgin olive oil at levels of 2.6%, 3.8%, 4.3%, 4.2%, 3.6%, and 13.8% (w/w), respectively.

Keywords: Olive oil; Synchronous fluorescence spectroscopy; Adulteration; Seed oils; Food quality control

1. Introduction

Virgin olive oil is highly appreciated because of its fine aroma, pleasant taste and health benefits. According to the International Olive Oil Council (IOOC, 2003) "virgin olive oil" is the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alterations in the oil. Virgin olive oil, due to its high price, is a target for adulteration with low price/quality oils. Seed oils including corn, sunflower, rapeseed, soybean and walnut oils as well as low quality olive oil such as olive-pomace are commonly used for olive oil adulteration. In comparison to seed oils, virgin olive oil has low levels of saturated (~16%) and high levels of monounsaturated fatty acids, mainly oleic acid (~64%) (Allen & Hamilton, 1983). Olive-pomace oil is produced through extraction of olive-pomace with toxic organic solvents and has significantly lower nutritive and price value in comparison to virgin olive oil.

Adulteration is a major issue in the olive oil market. Detection is important for the protection of wealth and health of consumers. It should be noted here that olive oil is the major edible oil used in the Mediterranean coast by European Union consumers. Italians, Spaniards and Greeks are consuming 29, 32 and 47 g, per person per day, respectively (McEvoy & Gomez, 1999). Various methods are used to detect adulteration spanning from classic wet chemistry (Harwood & Aparicio, 2000) to chromatographic methods relying on quantification of fatty acids, triglycerols, sterols and hydrocarbons (Andrikopoulos, Giannakis, & Tzamtzis, 2001; Antoniosi, Carrilho, & Lanças, 1993; Flor, Hecking, & Martin, 1993). Recently, spectroscopic techniques, which are rapid and easy to use, combined with multivariate analysis have also been used to detect adulteration. Amongst these spectroscopic techniques are nuclear magnetic resonance (NMR) (Fragaki,

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Spyros, Siragakis, Salivaras, & Dais, 2005; Mavromoustakos, Zervou, Bonas, Kolocouris, & Petrakis, 2000) and vibrational techniques (Christy, Kasemsumran, Du, & Ozaki, 2004; Lai, Kemsley, & Wilson, 1995; López-Dŕez, Bianchi, & Goodacre, 2003; Ozen & Mauer, 2002). Molecular fluorescence spectroscopy is a highly sensitive technique, yet largely ignored for the characterization of edible oils. Very early work pointed out good prospects for characterization of edible oils through fluorimetry (Wolfbeis & Leiner, 1984). Recently, characterization of edible oils using total luminescence spectroscopy has been described (Sikorska et al., 2004) along a spectrofluorimetric method for hazelnut oil detection in olive oil (Sayago, Morales, & Aparicio, 2004). Molecular fluorescence is not suitable for the analysis of complex multi-component samples without prior separation, due to severe overlaps of excitation and emission bands. In such cases, synchronous fluorescence (SyF) could prove beneficial as both the excitation and emission monochromators are scanned simultaneously in such a manner that a constant wavelength interval is kept between emission and excitation wavelengths ($\Delta\lambda$). Using suitable $\Delta\lambda$, SyF reduces spectral overlaps by narrowing spectral bands and simplifies spectra (Patra & Mishra, 2002a; Vo-Dinh, 1978).

Total synchronous fluorescence (TSyF) spectra are obtained by plotting fluorescence intensity as a combined function of the excitation wavelength and the wavelength interval. In this way, spectra selectivity is increased and multi-component samples are better characterized. In this respect, TSyF has the potential to evolve as a valuable analytical tool.

TSyF has been used for assessing the maturity of crude petroleum oils (Ryder, 2004) and for characterizing petroleum products (Patra & Mishra, 2002b). Recently, we described a TSyF method for the classification of edible and lampante olive oil (Poulli, Mousdis, & Georgiou, 2005).

Although analytical methodologies used in commercial and government laboratories cover almost all olive oil adulterants, they are time consuming and require sample transportation to the lab. This leaves space for rapid adulteration detection methods that could be implemented through portable instruments. Such methods, based on TSyF, are presented and evaluated in this article.

2. Materials and methods

2.1. Materials

Olive-pomace, corn, sunflower, rapeseed, soybean and walnut oil were purchased in a local shopping center. Samples were stored in the dark at room temperature until the day of analysis. Olive oil was spiked with the aforementioned oils at thirty one different levels from 0.5% to 95% w/w, with emphasis in concentration levels bellow 30% w/w. Samples thus prepared were diluted 1% w/v in *n*-hexane purchased from Merck to minimize inner filter effects (Lakowicz, 1999).

2.2. Fluorescence measurements

Fluorescence spectra were acquired by the Jobin Yvon fluorolog-3 spectrofluorometer. This is a fully computer controlled instrument using a double-grating monochromator for excitation and a single-grating emission monochromator. Excitation and emission slit widths were set at 2 nm. The acquisition interval and integration time were maintained at 1 nm and 0.3 s, respectively. A xenon lamp 950 W and a quartz cell $1 \times 10 \times 45$ mm were used. Right-angle geometry was used for spectral acquisition.

SyF spectra were collected by simultaneously scanning the excitation and emission monochromator in the excitation wavelength range 250–720 nm. TSyF spectra were obtained by measuring the excitation wavelength in the same spectral region and varying the wavelength interval from 20 to 120 nm in 20 nm intervals. The spectra were corrected for the excitation lamp, the photomultiplier detector spectral response and emission and excitation gratings.

Contour maps of total synchronous scan fluorescence spectra were plotted using the Origin software version 7.0 (OriginLab, USA, 2002).

2.2.1. Adulteration quantification

The excitation wavelength regions 315–400, 315–392, 315–375, 315–365, 315–375 and 315–360 nm using 20 nm as a wavelength interval were used for building the calibration model for olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils, respectively.

2.3. Statistical analysis

Microsoft Excel 2000 and Statistica software version 6.0 (StatSoft, USA, 2001) were used for statistical analysis. Partial least-squares regression (PLSR) was applied to produce a calibration model based on the level of adulteration. Twenty one samples were used for calibration and ten samples for validation per adulterant. No spectral pretreatment, such as baseline correction was carried out. Full cross validation was used to develop and evaluate regression models.

3. Results and discussion

3.1. Synchronous fluorescence spectroscopy

Fig. 1 shows the simplification and amplification of SyF spectra of virgin olive oil by using different wavelength intervals. As shown, the number of fluorescence bands increases along wavelength interval, while most fluorescence intensities decrease. Virgin olive oil in the spectral region higher than 315 nm features a flat spectral response when using 20 nm or less wavelength interval.

SyF spectra acquired from virgin olive, olive-pomace, corn, sunflower, rapeseed, soybean and walnut oils at 20 nm wavelength interval are depicted in Fig. 2. Virgin

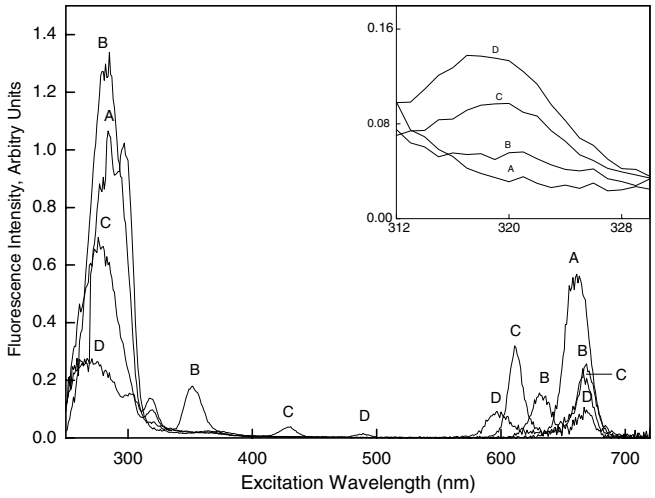

Fig. 1. Synchronous fluorescence spectra of virgin olive oil at different wavelength intervals: 20 nm (A), 40 nm (B), 60 nm (C) and 80 nm (D) diluted in *n*-hexane (1% w/v).

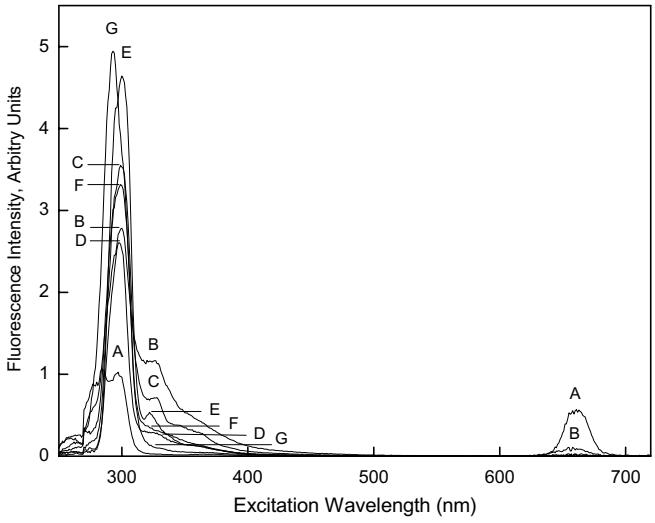

Fig. 2. Synchronous fluorescence spectra of virgin olive oil (A), olive-pomace oil (B), corn oil (C), sunflower oil (D), soybean oil (E), rapeseed oil (F) and walnut oil (G) diluted in *n*-hexane (1% w/v) at wavelength interval 20 nm.

olive oil shows a double band in the 275–297 nm region plus a band at 660 nm, in contrast to other oils that show a strong intensity band around 300 nm and a weak to moderate strong band near 325 nm. These are summarized in Table 1 where the % intensities of edible oils relative to the 292 nm band of walnut oil are shown in parentheses.

TSyF spectrum is a single three-dimensional array of SyF spectra and wavelength intervals. Two dimensional representations as contour plots are produced by linking points of equal fluorescence intensity. Fig. 3 shows contour plots of TSyF spectra of virgin olive, olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils. TSyF contour map of virgin olive oil spreads in the excitation wavelength region 250–325 nm, 347–365 nm and 602–685 nm and the wavelength interval region 20–120 nm, 30–50 nm and 20–76 nm, respectively. The contours are concentrated in the excitation wavelength region 270–300 nm and in the wavelength interval region lower than 56 nm.

Table 1
Synchronous scan fluorescence bands of edible oils acquired at $\Delta\lambda = 20$ nm

Oil	Wavelength (nm)
Walnut	292 (100)
Soybean	300 (94), 325 (11)
Corn	300 (72), 325 (15)
Rapeseed	298 (67), 325 (7)
Olive-pomace	298 (57), 325 (23), 660 (3)
Sunflower	297 (53), 325 (5)
Virgin olive oil	276 (18), 297 (21), 660 (12)

% Relative intensities to the 292 nm band of walnut oil are shown in parentheses.

Olive-pomace oil TSyF contour map spreads in the excitation wavelength 250–445 nm and 656–665 nm and the wavelength interval region 20–120 nm and 20–25 nm, respectively. The contours are concentrated in the excitation wavelength region 284–318 in the wavelength interval region lower than 85 nm.

Spectra characteristics for corn, sunflower and soybean oils depicted in the contour maps spread in the excitation wavelength region 250–410 nm, 250–385 nm and 255–382 nm, respectively. The contours for corn oil are concentrated in the excitation wavelength region 285–308 nm and the wavelength interval region lower than 68 nm in contrast to sunflower oil where the contours are concentrated in the excitation wavelength region 290–305 nm and the wavelength interval region lower than 47 nm. The contours of soybean oil are in the excitation wavelength range 285–310 nm and the wavelength interval region lower than 62 nm.

TSyF contour maps of rapeseed and walnut oils spread in the excitation wavelength region 250–380 nm and 250–365 nm, respectively. The contours of rapeseed oil are concentrated in the excitation wavelength region 275–305 nm and the wavelength interval region 20–67 nm while the contours of walnut oil are concentrated in the excitation wavelength region 272–305 and the wavelength interval region lower than 72 nm.

Data presented in the contour plots of Fig. 3 clearly indicate the potential of TSyF for edible oil discrimination. It is shown that only olive oils show a spectral region around 660 nm, which could be attributed to pigments of chlorophyll groups (Kyriakidis & Skarkalis, 2000). Moreover, all studied oils beyond virgin olive oil show a band in the wavelength region higher than 315 nm when using wavelength interval 20 nm. This band could be attributed to linoleic acid (Poulli et al., 2005). It is clearly indicated that differentiation of virgin olive oil from low quality oils can be achieved using this wavelength region.

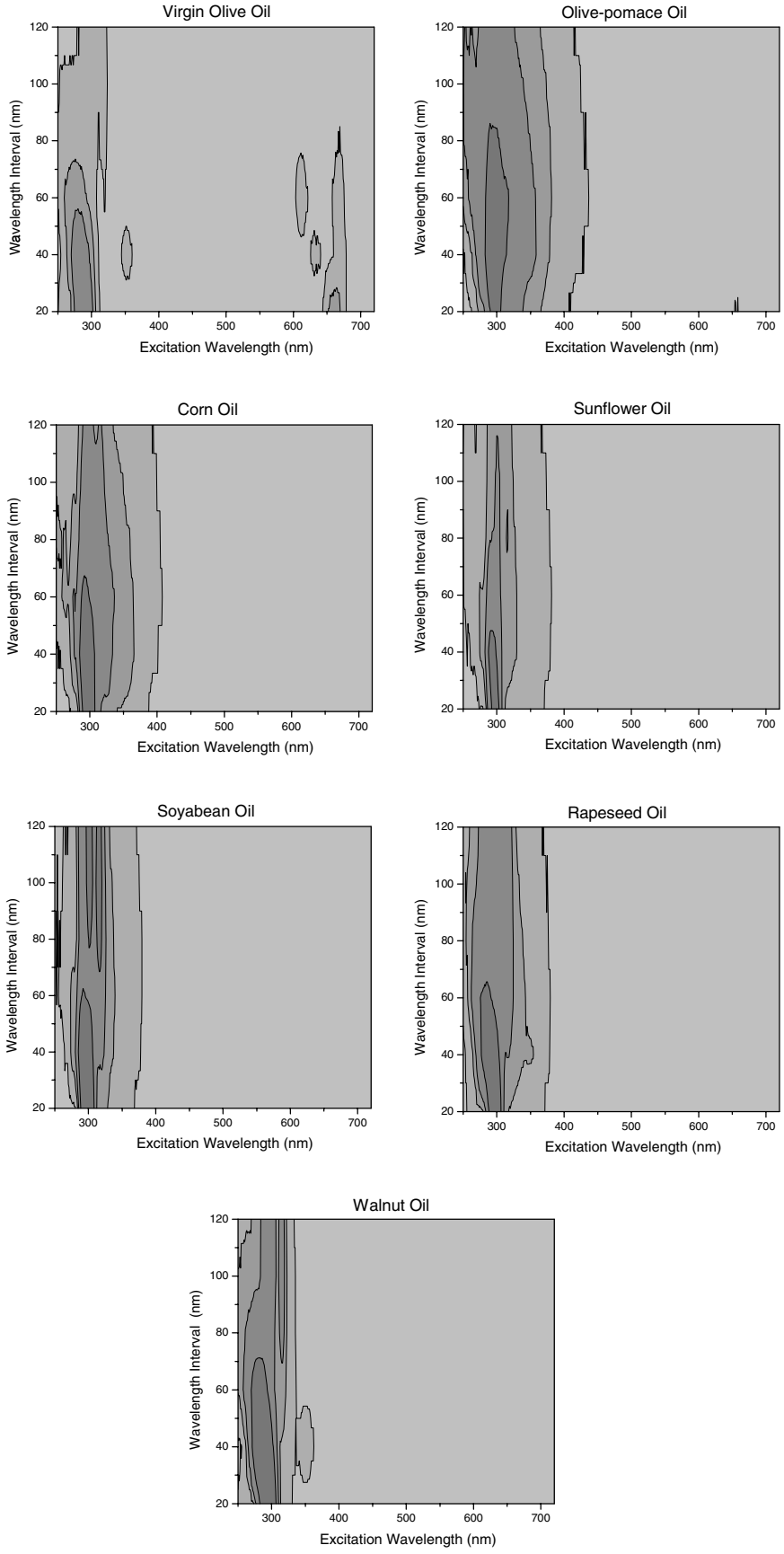

Fig. 3. Two-dimensional representation, as contour plots, of total synchronous fluorescence spectra of virgin olive oil and potential adulterants. Grayscale indicates fluorescence intensities.

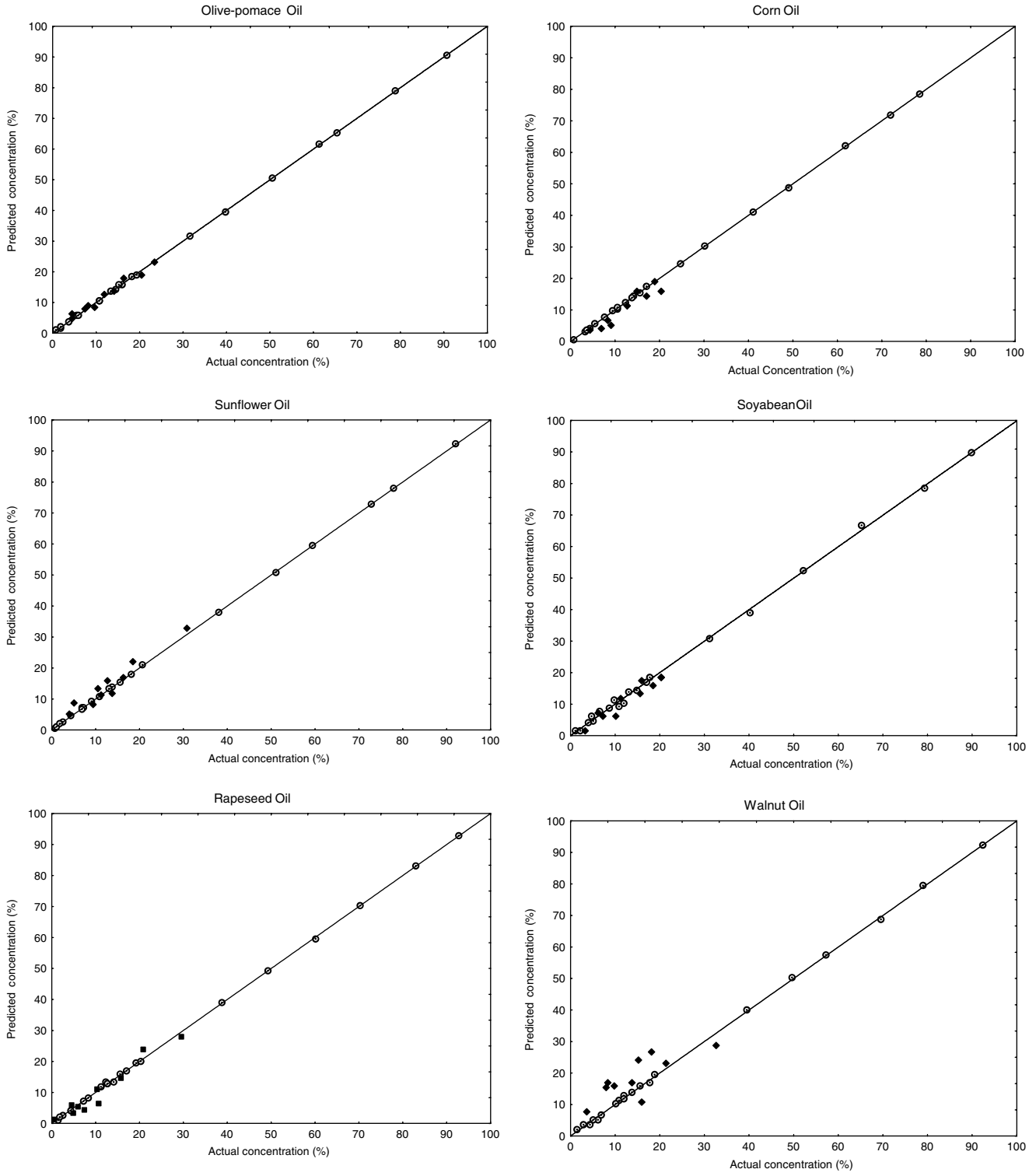

Fig. 4. Predicted versus actual concentration of adulterants in virgin olive oil at a wavelength interval of 20 nm: (open circles) calibration samples, (filled squares) validation samples.

3.2. Quantification

Thirty one mixtures of virgin olive and its adulterant were prepared at varying levels (0.5–95% w/w) spaced as shown in Fig. 4. SyF spectra were obtained by scanning the excitation wavelength region 250–400 nm at the wavelength interval 20 nm. Twenty one samples were used for calibration and ten samples for validation. Detection of low adulteration levels is an analytical challenge: Mixtures from all concentration levels were used as the calibration

Table 2
Calibration and validation results of the PLS model using 20 nm wavelength interval

	Adulterant					
	Olive-pomace	Corn	Sunflower	Soybean	Rapeseed	Walnut
Wavelength range (nm)	315–400	315–392	315–375	315–365	315–375	315–360
Number of PLS factors	8	14	9	5	9	11
Calibration using 21 randomly selected mixtures spanning from 0.5% to 95% w/w adulterant level						
Correlation coefficient	0.999	1.0	0.999	0.999	0.999	0.999
RMSE	0.2	0.02	0.2	1.1	0.3	0.6
Validation using 10 randomly selected mixtures of lower than 30% w/w adulterant level						
Correlation coefficient	0.98	0.96	0.97	0.96	0.97	0.8
RMSE	1.2	1.9	2.2	1.7	2.3	4.5

set, while the validation set includes virgin olive oils adulterated at lower than 30% w/w levels. Data in Fig. 3 at 20 nm wavelength interval shows that virgin olive oil features signals from excitation wavelength regions lower than 315. Therefore, differentiation of adulterants is achieved using a wavelength region higher than 315. Different wavelength regions were used for each adulterant: According to data in Fig. 3, olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils for 20 nm wavelength interval show signals up to 400, 392, 375, 365, 375 and 332 nm excitation wavelengths, respectively. According to these observations, the wavelength regions 315–400, 315–392, 315–375, 315–365, 315–375 and 315–332 were used for building the calibration model for olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils, respectively. As the spectral range used for walnut oil was narrow, it was difficult to build a calibration model. This was dealt with by extending the region up to 360 nm.

Fig. 4 shows concentration values calculated from the PLS model versus the actual concentrations of adulterants in virgin olive oil for both calibration and validation sets.

The root mean square error (RMSE) of calibration and validation sets, the correlation coefficient between actual and predicted value for both sets and the number of factors and mixtures for each adulterant are shown in Table 2. The detection limit, calculated as three times the standard deviation of the intercept divided by the calibration curve slope, was 2.6%, 3.8%, 4.3%, 4.2%, 3.6%, and 13.8% (w/w) for olive-pomace, corn, sunflower, soybean, rapeseed and walnut oil, respectively. Detection limits calculated are in accordance with data shown in Fig. 2 where olive-pomace oil gives the strongest and walnut oil the lowest signals in this wavelength region.

4. Conclusion

This work presents total synchronous scan fluorescence spectra of different edible oils and a rapid method for adulteration detection of virgin olive oil with them. The developed method, beyond laboratories, can be also be implemented through portable instruments for rapid assessment of claims concerning virgin olive oil authenticity. The method combined with multivariate chemometric techniques is successfully applied for quantitative determination of adulterants at levels down to 2.6% (w/w).

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