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TECHNICAL NOTE

Synchronous fluorescence spectroscopy for quantitative determination of virgin olive oil adulteration with sunflower oil

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Abstract Adulteration of extra virgin olive oil with sunflower oil is a major issue for the olive oil industry. In this paper, the potential of total synchronous fluorescence (TSyF) spectra to differentiate virgin olive oil from sunflower oil and synchronous fluorescence (SyF) spectra combined with multivariate analysis to assess the adulteration of virgin olive oil are demonstrated. TSyF spectra were acquired by varying the excitation wavelength in the region 270–720 nm and the wavelength interval ($\Delta\lambda$) in the region from 20 to 120 nm. TSyF contour plots for sunflower, in contrast to virgin olive oil, show a fluorescence region in the excitation wavelength range 325–385 nm. Fifteen different virgin olive oil samples were adulterated with sunflower oil at varying levels (0.5–95%) resulting in one hundred and thirty six mixtures. The partial least-squares regression model was used for quantification of the adulteration using wavelength intervals of 20 and 80 nm. This technique is useful for detection of sunflower oil in virgin olive oil at levels down to 3.4% (w/v) in just two and a half minutes using an 80-nm wavelength interval.

Keywords Virgin olive oil · Total synchronous fluorescence spectroscopy · Adulteration · Partial least-squares (PLS) · Food quality control

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Introduction

Olive oil is an important ingredient in the Mediterranean diet. It appears to be an example of a functional food, with a variety components that may contribute to its overall therapeutic characteristics including reduction of risk factors for coronary heart disease, prevention of several forms of cancer, and modification of immune and inflammatory responses [1]. According to the International Olive Oil Council, "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. Minimal treatment such as washing, pressing, decantation, centrifugation and filtration is acceptable [2]. Virgin olive oil is expensive, so unscrupulous sellers adulterate virgin olive oil by the addition of low-grade edible oils in order to increase their profits. Sunflower oil is commonly used to adulterate virgin olive oil because of its low price and similar composition. Virgin olive oil has low levels of saturated (ca. 16%) and high levels of monounsaturated fatty acids, mainly oleic acid (ca. 64%), in contrast to seed oils that have high levels of polyunsaturated fatty acids, mainly linoleic acid [3]. Various analytical methods are used to detect adulteration of virgin olive oil. Most of the methods concerning authentication of virgin olive oils are based on gas chromatography (GC) [4] and high-performance liquid chromatography (HPLC) [5, 6]. These methods rely on quantification of fatty acids, triglycerols, sterols and hydrocarbons. They are time consuming and are not readily adaptable to use in rapid screening through portable instrumentation. Recently, spectroscopic techniques combined with multivariate analysis have been used to detect adulteration of virgin olive oil with low-quality oils. These techniques are rapid and avoid any sample preparation steps resulting in simple and easy-to-use

methods. Amongst these spectroscopic techniques are nuclear magnetic resonance (NMR) [7, 8], vibrational techniques such as near-infrared (NIR), Fourier transform infrared (FT-IR) and Fourier transform Raman (FT-Raman) [9–12]. Although advantages of vibrational techniques are well documented, they lack sensitivity [13, 14], whereas NMR requires expensive instrumentation.

Molecular fluorescence spectroscopy is a highly sensitive technique yet it is not suitable for the analysis of complex multi-component samples such as olive oil without prior separation, due to severe overlaps of excitation and emission bands. For this reason, applications of molecular fluorescence spectroscopy in the characterization of edible oils and the authentication of virgin olive oil are scarce [15–18].

In synchronous fluorescence (SyF) spectroscopy 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 ($\Delta\lambda$). By using suitable wavelength intervals, SyF reduces spectral overlaps by narrowing spectral bands and simplifies spectra [19, 20]. Consequently, SyF spectroscopy could prove beneficial for the characterization of olive oil. Recently, SyF spectroscopy has been used for the classification of edible oils [21, 22].

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. Indeed, TSyF has been used for assessing the maturity of crude petroleum oils [23] and for characterizing petroleum products [24].

The aim of this work is to assess the potential of TSyF for differentiation of virgin olive from sunflower oil and quantitative determination of olive oil adulteration using SyF spectra with multivariate analysis.

Materials and methods

Samples

Sunflower oil was purchased in a local shopping centre. Fifteen virgin olive oils were gathered by Minerva S.A. (Athens, Greece). The samples were stored in the dark at room temperature until the day of analysis. One hundred and thirty six mixtures of sunflower and virgin olive oils, with concentration of sunflower oil at varying levels (0.5–95%), were prepared. To minimize inner filter effects, samples were diluted in *n*-hexane (1% w/v) purchased from Merck. Moreover, a micro quartz cell from Hellma with an optical path length for the emitted light of just 0.5 mm was used. In these ways, the absorbance of all samples was lower than 0.1 for the whole excitation wavelength range as required in order to avoid inner filter effects for right-angle fluorescence measurements [25].

Instruments

Fluorescence spectra were acquired by using a Jobin Yvon fluorolog-3 spectrofluorometer, which 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 set at 1 nm and 0.3 s, respectively. A 950-W xenon lamp and a 1×10×45-mm quartz cell 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 270–720 nm. The region lower than 270 nm was not used to avoid inner filter effects due to the high absorbance of oil mixtures, especially those with high sunflower oil concentration. 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 TSyF spectra were plotted using the Origin software version 7.0 (OriginLab, USA, 2002).

Statistical analysis

Statistica software version 6.0 (StatSoft, USA, 2001) was used for statistical analysis. Partial least-squares regression (PLSR) is a method for relating the variations in one or several response variables (*Y* variables) to the variations of several predictors (*X* variables), with explanatory or predictive purposes. PLS models both the *X* and *Y* matrices simultaneously to find such latent variables in *X* that will best predict the latent variables in *Y* [26].

PLS was applied to produce a calibration model based on the level of sunflower oil adulteration. One hundred and one samples were used for calibration and thirty five samples for validation. No spectral pretreatment (e.g. baseline correction) was carried out. Full cross-validation was used to develop and evaluate regression models.

Results and discussion

Synchronous fluorescence spectroscopy

SyF spectra acquired from virgin olive and sunflower oils at a wavelength interval of 20 nm are depicted in Fig. 1. Virgin olive oil shows one double band around 275 and

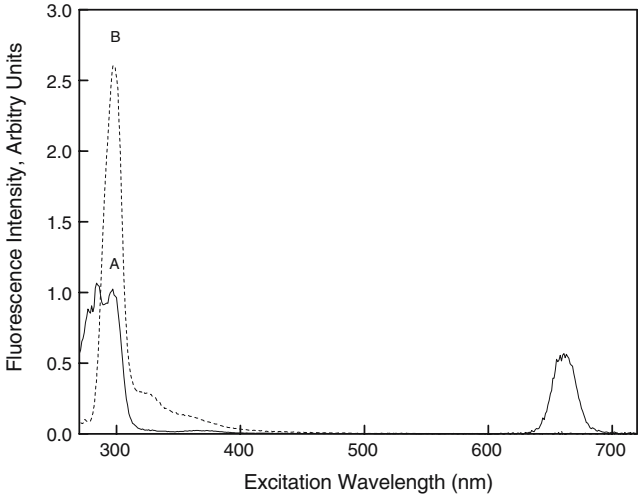

Fig. 1 Synchronous fluorescence spectra of virgin olive oil (**a**) and sunflower oil (**b**) in *n*-hexane (1% w/v) at a wavelength interval of 20 nm

297 nm and one band at 660 nm, in contrast to sunflower oil that shows a strong-intensity band around 300 nm and a weak one at 325 nm.

TSyF spectra are visualized by plotting synchronous excitation intensities at all combinations of SyF and wavelength intervals $\Delta\lambda$ in a single three-dimensional array. Two-dimensional representations as contour plots are produced by linking points of equal fluorescence intensity. Figure 2 shows the TSyF spectra of virgin olive and sunflower oils.

TSyF contour map of virgin olive oil (Fig. 2a) spreads in the excitation wavelength regions 270–325 nm, 347–365 nm and 602–685 nm and the wavelength intervals 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 lower than 56 nm.

Spectra characteristics for sunflower oil are depicted in the contour maps (Fig. 2b), spreading in the excitation wavelength region 270–385 nm. The contours are concentrated in the excitation wavelength region 290–305 nm and the wavelength interval region lower than 47 nm.

Data from the contour plots in Fig. 2 clearly indicate the potential of TSyF for the discrimination of virgin olive and sunflower oils: virgin olive oil shows a spectral region around 660 nm, which could be attributed to pigments of chlorophyll groups [27]; moreover, sunflower oil shows a region in the wavelength range 325-385 nm, attributed to linoleic acid [22]. In this region virgin olive oil gives just small signals at the wavelength interval range 30-50 nm. Presumably, differentiation of virgin olive from sunflower oil can be achieved using this area of the contour maps. It should be noted that the analytical procedure is rapid, requiring just two and a half minutes.

Quantification

Figure 3 shows the effect of sunflower concentration on SyF spectra of virgin olive oil at a wavelength interval of 20 nm. It is shown that the intensities around 295 and

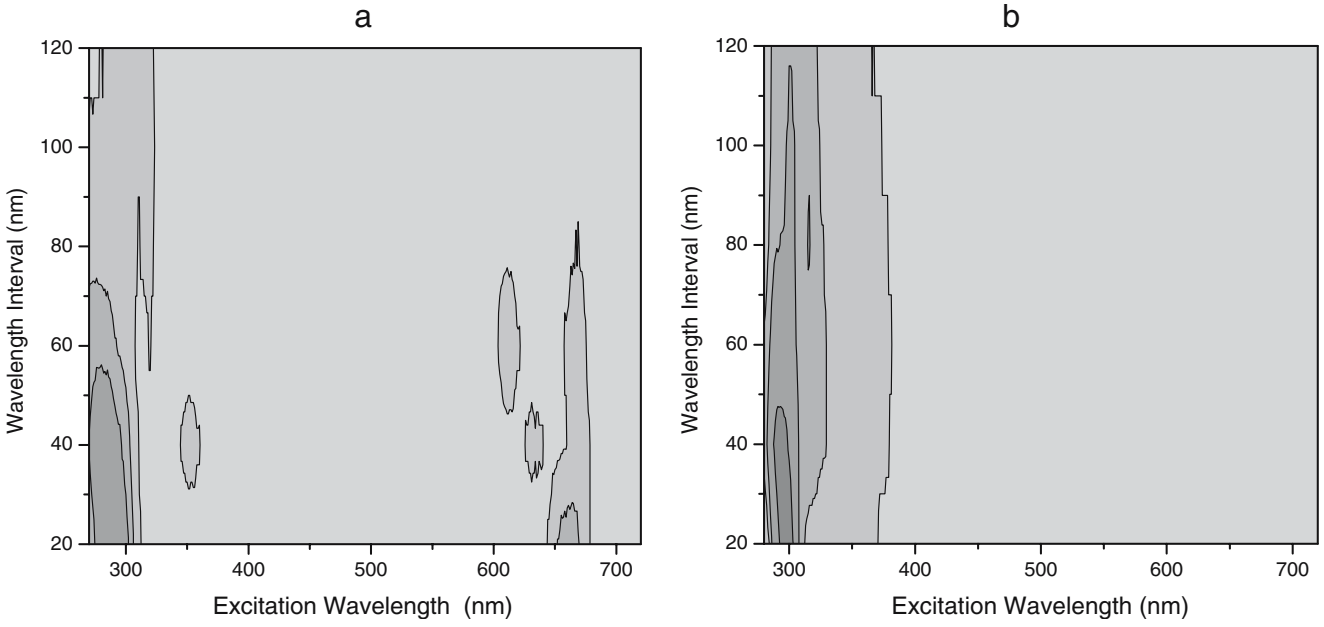

Fig. 2 Two-dimensional representation, as contour plots, of total synchronous fluorescence spectra of virgin olive (**a**) and sunflower oil (**b**). Greyscale indicates fluorescence intensities

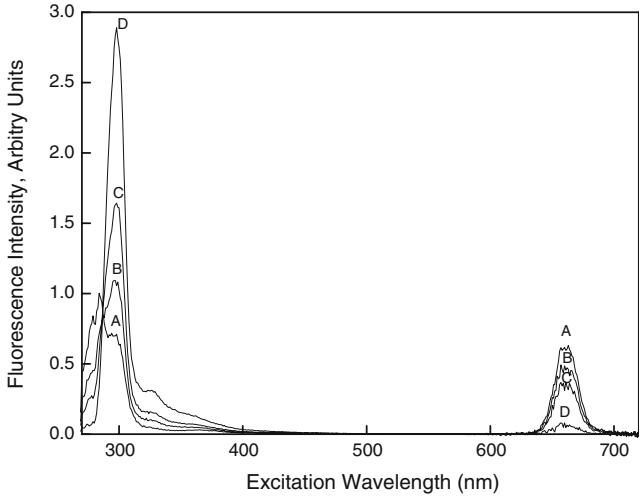

Fig. 3 Synchronous fluorescence spectra of virgin olive oil adulterated with sunflower oil at varying levels: 3% (**a**), 20% (**b**), 40% (**c**) and 90% (**d**) diluted in *n*-hexane (1% w/v) at a wavelength interval of 20 nm

325 nm are increasing presumably due to increase of unsaturation on sunflower oil addition. At the same time decreasing intensities around 275 and 660 nm are presumably due to tocopherols and chlorophylls, respectively.

For quantification one hundred and thirty six mixtures of virgin olive and sunflower oil were prepared by adulterating fifteen different virgin olive oils with sunflower oil at varying levels (0.5–95%). These levels were spaced as shown in Fig. 4.

SyF spectra were obtained by measuring in the excitation wavelength region 270–720 nm and wavelength intervals 20 and 80 nm. One hundred and one samples were used for calibration and thirty five samples for validation. Both calibration and validation sets include randomly selected mixtures from a pool containing all fifteen different virgin olive oils adulterated at different levels. To eliminate common information from the spectra we used mean centring before the analysis. Then, several regions of the spectra for both wavelength intervals were investigated for optimization of the calibration model: The region between 450 and 630 nm does not contain any bands. The region 630–690 nm resulting from chlorophylls was also excluded due to the low contribution to the calibration model. The wavelength regions 330–385 nm, 330–450 nm, 350–450 nm, 250–450 nm and 290–450 nm were also tried for building the calibration model. Best results were obtained using the excitation wavelength region 290–450 nm for both wavelength intervals. Figure 4 shows concentration values calculated from the PLS model versus the actual concentrations of sunflower in virgin olive oil for both calibration and validation sets in the excitation wavelength region 290–450 nm for a wavelength interval of 80 nm. Similar plot is obtained when using a wavelength interval of 20 nm.

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

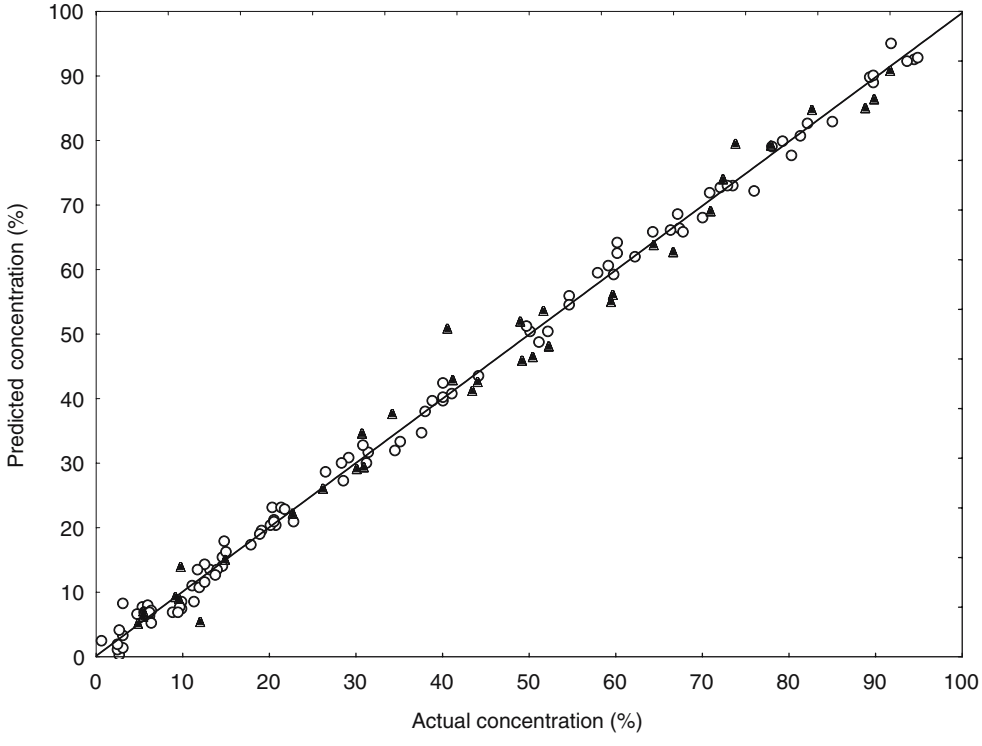

Fig. 4 Predicted versus actual concentrations of sunflower oil in virgin olive oil at a wavelength interval of 80 nm: *open circles* calibration samples, *filled triangles* validation samples

Table 1 Calibration and validation results of the PLS model using the 290–450 nm wavelength range

	Wavelength Interval ($\Delta\lambda$)	
	20 nm	80 nm
Number of PLS factors	20	23
Calibration using 101 randomly selected mixtures		
Correlation Coefficient	0.998	0.998
RMSE	1.7	1.7
Validation using 35 randomly selected mixtures		
Correlation Coefficient	0.99	0.992
RMSE	3.7	3.4

and mixtures for each wavelength interval are shown in Table 1. The detection limit, calculated as three times the standard deviation of the intercept divided by the calibration curve slope, was 3.6% and 3.4% (w/v) when using a 20-nm and 80-nm wavelength interval, respectively. According to results presented, the predictive ability for sunflower concentration in virgin olive oil is better when using a wavelength interval of 80 nm, as the 20-nm wavelength interval gives higher detection limit of validation values.

Conclusion

Synchronous fluorescence spectroscopy is a highly sensitive and simple technique. In this work, the differentiation of virgin olive and sunflower oils using total synchronous fluorescence spectra is shown. Synchronous fluorescence spectroscopy combined with multivariate techniques is successfully applied for quantitative determination of adulterant at levels higher than 3.4% (w/v) through selection of a suitable wavelength interval. It should be emphasized that the results presented in this work will allow analytical scientists in food quality control laboratories to perform rapid assessment, in just two and a half minutes, of claims concerning authenticity of virgin olive oil.

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