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Analysis of Olive Oils by Fluorescence Spectroscopy: Methods and Applications

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1. Introduction

Fluorescence spectroscopy is a well established and extensively used research and analytical tool in many disciplines. In recent years, a remarkable growth in the use of fluorescence in food analysis has been observed (Christensen et al., 2006; Sadecka & Tothova, 2007; Karoui & Blecker, 2011). Vegetable oils including olive oil constitute an important group of food products for which fluorescence was successfully applied. Fluorescence is a type of photoluminescence, a process in which a molecule, promoted to an electronically excited state by absorption of UV, VIS or NIR radiation, decays back to its ground state by emission of a photon. Fluorescence is emission from an excited state, in which the electronic spin is equal to that in the ground state, and typically equal to zero. Such transitions are spin allowed, and occur at relatively high rates, typically 10^8 s^{-1} (Lakowicz, 2006).

A unique feature of fluorescence, distinguishing it from other spectroscopic techniques, is its inherently multidimensional character (Christensen et al., 2006). Excitation of molecules results from absorption of radiation at the energy corresponding to the energy difference between the ground and excited states of a given fluorophore. Subsequently, radiation at a lower energy characteristic for the specific molecule is emitted during its deactivation. Thus, fluorescence properties of every compound are characterized by two types of spectra: excitation and emission. This feature and the fact that not all of the absorbing molecules are fluorescent both contribute to higher selectivity of fluorescence as opposed to absorption spectra.

Another important advantage of fluorescence is its higher sensitivity. In contrast to absorption measurements, the emitted photons are detected against a low background, making fluorescence spectroscopy a very sensitive method. The sensitivity of fluorescence is 100-1000 times higher than that of the absorption techniques, enabling to measure concentrations down to parts per billion levels (Guilbault, 1999).

The fluorescent analysis of olive oils takes advantage of the presence of natural fluorescent components, including phenolic compounds, tocopherols and pheophytins, and their oxidation products. Oils are complex systems and therefore conventional fluorescent

techniques, relying on recording of single emission or excitation spectra, are often insufficient if directly applied. In such cases, total luminescence or synchronous scanning fluorescence techniques are used, improving the analytic potential of the fluorescence measurements. With contributions from numerous analytes, the autofluorescence of olive oil exhibits numerous overlapping bands. Such complex spectra should be analyzed using multivariate and multiway methods.

Analytical applications of fluorescence to olive oils include discrimination between the different quality grades, adulteration detection, authentication of virgin oils, quantification of fluorescent components, monitoring thermal and photo-oxidation and quality changes during storage.

In this chapter the application of fluorescence spectroscopy to qualitative and quantitative analysis of olive oils is reviewed. Methodological aspects of fluorescence measurements and analysis of fluorescence spectra are also discussed.

2. Fluorescence of olive oils

2.1 Fluorescence characteristics of olive oil and its components

Conventionally, two basic types of spectra characterize the fluorescent properties: excitation and emission spectra. For a system containing a single fluorophore, the shape and location of the excitation and emission spectra are independent of respective chosen emission and excitation wavelengths. However, for a system containing several fluorescent components, the excitation and emission spectra depend on particular emission and excitation wavelength used for measurements. Therefore, in systems containing several fluorophores, single-wavelength spectra are insufficient for a comprehensive description of fluorescent properties, thus multidimensional measurement methods should be used.

The most comprehensive characterization of a multicomponent fluorescent system is obtained by measurement of an excitation-emission matrix, known also as a total luminescence spectrum or fluorescence landscape. This technique was first introduced by Weber (1961). After the first application to edible oils by Wolfbeis & Leiner (1984), it has been intensively used for exploring oil fluorescence. Total luminescence spectra are usually obtained by measurement of emission spectra at several excitation wavelengths. They may be presented as a three dimensional plot, with the fluorescence intensity plotted in function of the excitation and the emission wavelengths (Ndou and Warner, 1991; Guilbault, 1999). Another representation of the total luminescence is obtained using two-dimensional contour maps, in which one axis represents the emission and another – the excitation wavelength, and the contours are plotted by linking points of equal fluorescence intensity, Fig.1. The total luminescence spectrum gives a comprehensive description of the fluorescent components of the mixture and may serve as a unique fingerprint for identification and characterization of the sample studied. The acquisition of contour maps at sufficient resolution (determined by the number of individual emission spectra recorded) on conventional spectrofluorometers is time-consuming, requiring a large number of scans for each sample (Guilbault, 1999).

Alternatively, multicomponent fluorescent systems may be investigated by the synchronous fluorescence techniques, proposed by Lloyd, (1971). This technique involves simultaneous

scanning of both excitation and emission wavelengths, keeping a constant difference between them. Synchronous scanning fluorescence spectroscopy is very useful for the analysis of mixtures of fluorescent compounds, because both excitation and emission characteristics are included into a single spectrum. Although it provides less information than the excitation-emission matrix, it may still present a viable alternative to the total luminescence measurements due to its inherent simplicity and rapidity. A set of synchronous spectra recorded at different wavelength intervals may be concatenated into a total synchronous fluorescence spectrum. In such spectra fluorescence intensity is plotted as a function of the excitation wavelength and the wavelength interval. Both single wavelength interval and total synchronous fluorescence spectra were used for studies of olive oils (Sikorska et al. 2005a; Poulli et al. 2005). The relation between various kinds of fluorescence spectra of a virgin olive oil is presented in Fig. 1.

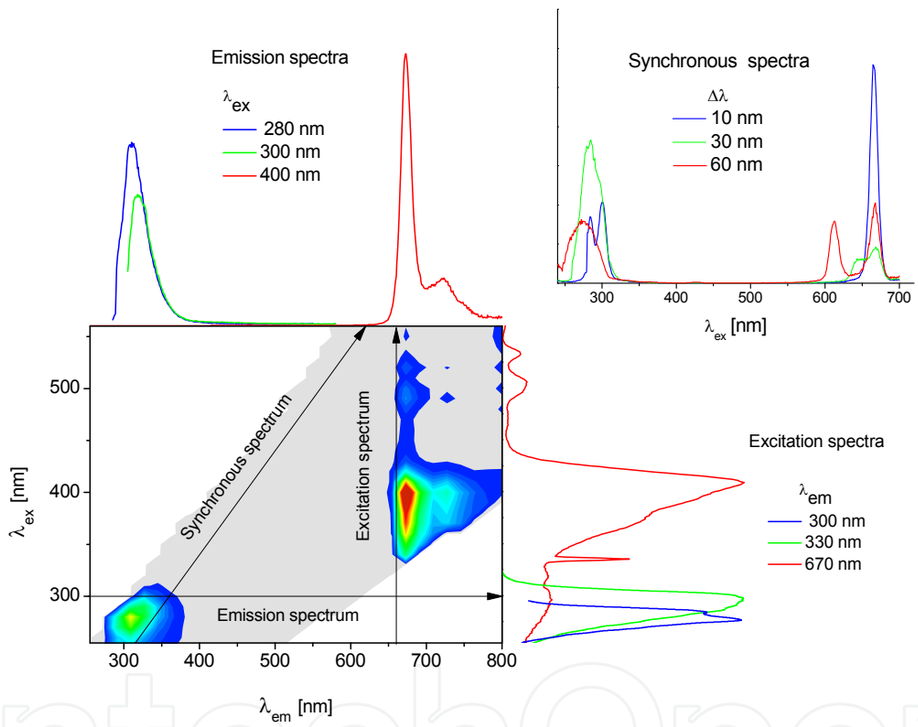


Fig. 1. Different types of fluorescence spectra; fluorescence spectra of a diluted sample of extra virgin olive oil (1%, v/v, in n-hexane) are shown as an example.

Numerous factors affect measured fluorescence intensity and spectral distribution. These factors are related to the nature and the concentration of fluorophores, their molecular environment, and scattering and absorption effects. They may be immeasurably important in complex natural systems, such as oils, and have to be taken into account when measuring and interpreting the fluorescence spectra. Fluorescence intensities are proportional to the concentration over only a limited range of optical densities (Lakowicz, 2006). To obtain proportionality between the fluorescence intensity and the fluorophore concentration, the absorbance at the excitation wavelength should be below 0.05 and close to zero in the emission spectral region. At higher concentrations, the inner filter effects have to be taken into account. These effects may decrease the observed fluorescence intensity by either

reducing the intensity of the excitation or by absorbing the emitted radiation. To avoid the inner filter effects due to the high optical densities or sample turbidity, appropriate geometry of sample illumination should be used. The most common geometry using right-angle observation of the center of a centrally illuminated sample is only appropriate for diluted solutions with low optical densities. For opaque samples, front-face illumination is achieved using either triangular or square cuvettes oriented at 30 or 60° relative to the incident beam.

The effects of concentration and sample geometry on oil spectra were addressed by several authors. Zandomeneghi et al. (2005) in the very detailed studies compared the emission fluorescence spectra of undiluted extra virgin olive oil obtained with the traditional setup (right-angle fluorescence) and using front face fluorescence. The absorption of undiluted olive oil samples was from 2 up to 12 absorbance units, on passing from 325 to 260 nm, and therefore the inner filter phenomena affected the right angle spectra considerably. Excluding the long-wavelength chlorophyll region, significant differences in the number, shape, intensity, and position of the bands in spectra of the same undiluted oil obtained with right angle and front face geometry were observed, Fig. 2. The right angle fluorescence spectra showed considerable distortions, even after the mathematical corrections for the inner filter effects due to the absorption of both the excitation and emission radiation were applied. The front-face fluorescence spectra were less affected by self-absorption and thus provided reliable information about type of fluoropores and their concentration. It was also demonstrated that analysis of spectra affected by inner filter effects may lead to spectral misinterpretation and invalid assignments of origin of some fluorescent bands (M. Zandomeneghi & G. Zandomeneghi, 2005; Zandomeneghi et al., 2006).

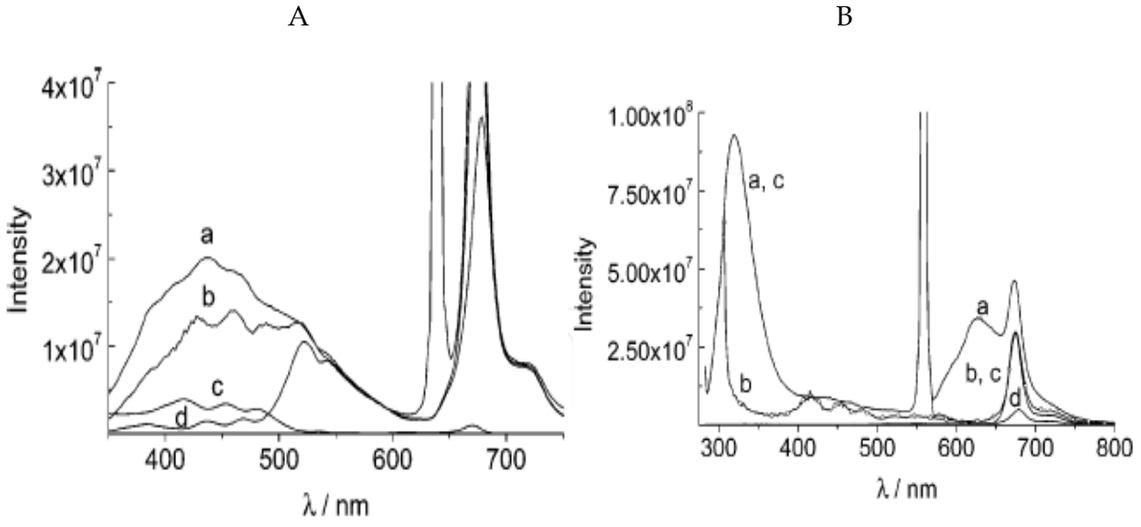


Fig. 2. A. Emission spectra of olive oil with λ_{ex} = 320 nm. (a) front face spectrum; (b) right angle spectrum with the absorption correction; (c) absorption spectrum of the oil multiplied by 10^6; (d) right angle spectrum without the absorption correction. B. Emission spectra of olive oil with λ_{ex} = 280 nm. (a) front face spectrum; (b) right angle spectrum with the absorption correction and multiplied by 3.7; (c) front face spectrum, second-order corrected; (d) right angle spectrum. Reprinted with permission from Zandomeneghi et al. 2005. Copyright 2005 American Chemical Society.

Typical fluorescence spectra of extra virgin and refined olive oils are shown in Fig. 3, (Sikorska et al., 2011). The fluorescence depends on sample concentration; therefore spectra for diluted and intact samples are shown. Both total fluorescence and total synchronous spectra are presented for the same oils, to enable comparison.

Based on the published data, one may conclude that the fluorescence properties depend on the quality grade of olive oils (Kyriakidis&Skarkalis, 2000; Poulli et al., 2006; Guimet et al., 2004a). For a selected quality category, the spectra may show minor differences between samples, however, the general features remain similar, permitting identification and authentication of oil samples.

The total fluorescence spectrum of diluted extra virgin olive oils, measured with the use of right angle geometry, exhibits two intense bands, one with excitation at about 270–330 nm and emission at about 295–360 nm and the second with excitation at about 330–440 nm and emission at about 660–700 nm, Fig. 3. An additional band appears in spectra of refined olive oil, located in the intermediate range, with excitation at 280-330 nm and emission at 372-480 nm. The long-wavelength band has a lower intensity in refined as compared to virgin olive oil (Sikorska et al., 2011).

The spectra of the same undiluted oils measured with the front face geometry method show a clearly different fluorescence pattern. The spectra are not affected by the inner filter effect, because front face geometry was used for measurement of undiluted samples. Additional bands are observed in the spectra of extra virgin olive oil at about 310–390 nm in excitation and 440–580 nm in emission. The ratio of fluorescence intensity of short- and long-wavelength bands is lower as compared to the spectrum of the diluted sample. The spectrum of undiluted refined olive oil exhibits a broad band with emission at 350-600 nm, two maxima at 320/420 nm and 365/450 nm in excitation/emission, and a long wavelength emission at 650-700 nm. Only a trace of the short-wavelength emission is observed with the maximum at 300/331 nm (Sikorska et al., 2011).

The differences in the spectra between diluted and undiluted samples may result from the high fluorophore concentrations in the intact oil samples and a variety of molecular interactions, such as quenching and energy transfer, which alter fluorescence characteristics. The effect of concentration on the total and synchronous fluorescence spectra of vegetable oils including olive oils was reported by Sikorska et al. (2004; 2005b).

The total synchronous fluorescence spectra of undiluted oils showed dependence of spectral shape and intensity on the wavelength interval ($\Delta\lambda$) used in the measurements, with the presence of particular bands dependent on $\Delta\lambda$. At lower values of $\Delta\lambda$ the bandwidths are reduced and the spectrum is simplified as compared to the total fluorescence spectra. Appearance of new bands or splitting of existing bands is typically observed with increasing $\Delta\lambda$. Emission bands are present in the excitation region below 310 nm, 310-350 nm, 350-380 nm, and above 550 nm in spectra of virgin olive oils (Sikorska et al., 2011). Similar spectral characteristics for virgin olive oil were reported by Poulli et al., (2006). The bands in total synchronous fluorescence spectra were observed in the 270–325, 347–365 and 602–685 nm excitation wavelength ranges with the respective wavelength intervals of 20–120, 30–50 and 20–76 nm.

Refined oils are characterized by a relatively weak band between 290-320 nm, a very broad band spreading to about 500 nm, and a band above 550 nm. All of these bands equally appear in the total fluorescence spectra (Sikorska et al., 2011).

The identification of origin of the particular emission bands relies mainly on comparison to the spectra of chemically pure fluorescent components. The fluorescence properties of compounds occurring in oils or suggested to contribute to their emission are listed in Table 1. The short wavelength band in total fluorescence spectra, which covers the region of 270–330 nm in excitation and 295–360 nm in emission, corresponds to the band at 280–310 nm in the total synchronous fluorescence spectra and is assigned to tocopherols and phenols. This assignment has been confirmed by several observations. Firstly, it was shown that a similar band appears in various vegetable oils, either cold-pressed or refined, and not only in olive oils (Sikorska et al., 2004). Olive oils contain considerable amounts of phenolic compounds, with their concentrations significantly reduced in refined oils. This observation seems to confirm that tocopherols also contribute to the emission observed in this wavelength range. In fact, tocopherols are present in most vegetable oils in widely variable amounts, from 70 to 1900 mg/kg (Cert et al., 2000). The vitamin E group includes four natural tocopherols (α-, β-, γ-, δ-) and four tocotrienols (αT3, βT3, γT3, δT3), all — in the R-configuration at the three double bonds in the side-chain of tocotrienols. Due to their structural similarity, all of these compounds exhibit very similar UV-absorption spectra and have similar fluorescence properties, Table 1. Of all tocopherols, α-tocopherol is predominant in olive oils. Indeed, the band in olive oil spectra being discussed is similar to the one in the total luminescence spectrum of α-tocopherol dissolved in *n*-hexane. Moreover, conventional excitation and emission spectra of the olive oils in the wavelength range mentioned are also similar to those of α-tocopherol, and the excitation spectra are in good agreement with the absorption spectrum of α-tocopherol in *n*-hexane (Sikorska et al. 2004). Still, the detailed analysis of excitation and emission spectra suggests contributions from several other fluorophores.

There still remain some inconsistencies concerning the assignment of vitamin E (tocopherol) bands in olive oil spectra. In one of the pioneering papers, where the emission spectra of various oils were reported, it has been suggested that the bands in the emission spectrum (λ_{ex}=365 nm) with the maximum at 525 nm may partly originate from compounds of the vitamin E group, or their derivatives formed upon oxidation (Kyriakidis & Skarkalis, 2000). However, this interpretation is based on spectra of undiluted olive oils measured using right angle geometry, and therefore strongly affected by inner filter effects, and in some cases referring to the spectral region where no emission of tocopherols is present (Zandomeneghi et al. 2005). It should be underlined that the emission of vitamin E in *n*-hexane has its maximum at about 320 nm, with a similar maximum appearing in the spectra of oils. Moreover, it has been stated (Zandomeneghi et al. 2006) that the known products of oxidation of R-α-, β-, γ-, δ-tocopherols, the R-α-, β-, γ-, δ-tocopherolquinones, are all nonfluorescent substances (Pollok & Melchert, 2004).

Note that a considerable number of minor components belonging to different classes of phenolic compounds such as phenolic acids, phenolic alcohols, hydroxyisochromans, secoiridoids, lignans, and flavonoids are present in virgin olive oils (Servili et al., 2004). Most of polyphenols are fluorescent substances, absorbing in the 260-310 nm range and emitting in the near-UV range, with their bands centered at 310-370 nm (M. Zandomeneghi & G. Zandomeneghi, 2005). These phenolic compounds can be detected by fluorescence after separation by HPLC, using excitation/emission wavelengths of 264/354, 310/430 or 280/320 nm (Dupuy et al., 2005). Fluorescence typical for phenolic components of olive oils was reported recently by Tena et al., (2009), using excitation at 270 nm with the fluorescence maxima appearing in the 362-420 nm range, Table 1.

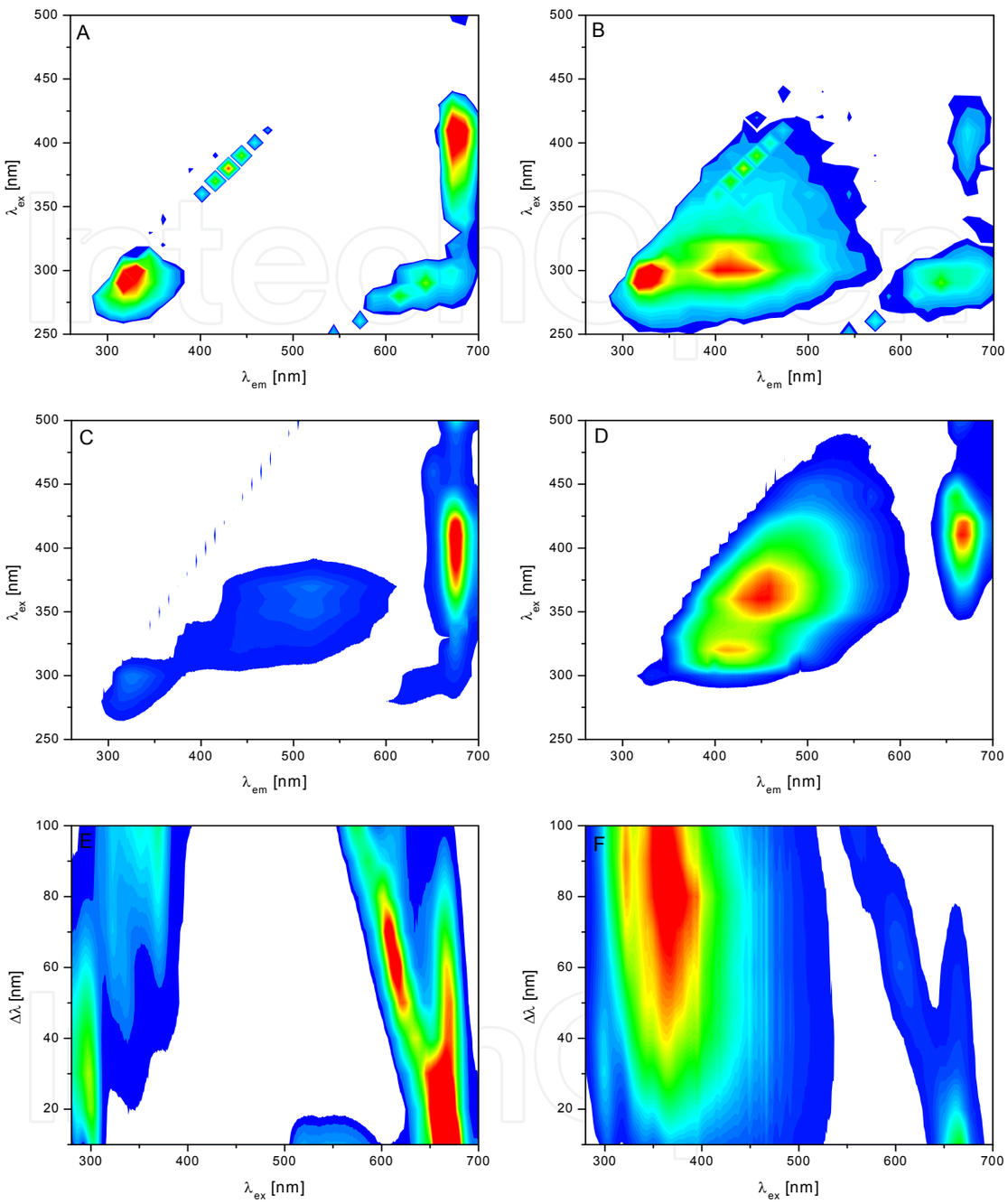


Fig. 3. Fluorescence spectra of extra virgin and refined olive oil: A. Total fluorescence spectrum of diluted olive oil, 1% in n-hexane, B. Total fluorescence spectrum of diluted refined olive oil, 1% in n-hexane, C. Total fluorescence spectrum of undiluted extra virgin olive oil, front face geometry, D. Total fluorescence spectrum of undiluted refined olive oil, front face geometry, E. Total synchronous fluorescence spectrum of undiluted extra virgin olive oil, front face geometry, F. Total synchronous fluorescence spectrum of undiluted refined olive oil, front face geometry (Sikorska et al., 2011).

	Solvent	λ_{abs} [nm]	ε [dm^3 mol^{-1} cm^{-1}]	Solvent	λ_{ex} [nm]	λ_{em} [nm]
Vitamin E						
α-Tocopherol [a]	ethanol	292	3265	n-hexane	295	320
β- Tocopherol [a]	ethanol	296	3725	n-hexane	297	322
δ- Tocopherol [a]	ethanol	298	3515	n-hexane	297	322
γ- Tocopherol [a]	ethanol	298	3809	n-hexane	297	322
α-Tocotrienol [a]	ethanol	292	3652	n-hexane	290	323
β- Tocotrienol [a]	ethanol	292	3540	n-hexane	290	323
δ- Tocotrienol [a]	ethanol	297	3403	n-hexane	292	324
γ- Tocotrienol [a]	ethanol	297	3737	n-hexane	290	324
Chlorophylls						
Chlorophyll *a* [b,c,d]	acetone	430	94700	ether	436	668
		663	75000	acetone	405	669
				9:1 acetone/water	430[e]	669
Chlorophyll *b* [b,c,d]	acetone	455	131000	ether	436	648
		645	47100	acetone	405	652
				9:1 acetone/water	458 [e]	653
Pheophytin *a* [b,c,d]	acetone	409	101800	ether	436	673
		666	44500	9:1 acetone/water	406 [e]	671
Pheophytin *b* [b,c,d]	acetone	434	145000	ether	436	661
		654	27800	9:1 acetone/water	435[e]	658
				-	-	
Pheophorbide *a* [b,c,d]	acetone	409	119200	-	-	-
		667	55200	-	-	-
Phenolic compounds						
Oleuropein	ethanol/n-hexane	282	-	ethanol/n-hexane	270	310
Vanillic acid [e]				methanol	270	349
Syringic acid [e]					270	361
Gallic acid [e]					270	382
p-Coumaric acid [e]						416
o-Coumaric [e]					270	426
Cinnamic acid [e]					270	420
Tyrosol [e]					270	420
Caffeic acid [e]					270	457

λ_{abs}, λ_{exc}, λ_{em} – absorption, excitation and emission maxima, ε - molar absorption coefficient, [a] (Eitenmiller et al., 2008), [b] (Ward et al., 1994), [c] (Undenfriend, 1962), [d](Diaz et al., 2003), [e](Tena et al., 2009).

Table 1. Fluorescence properties of olive oil components.

The fluorescence spectra of a vitamin E standard in hexane and an oil polyphenol extract in methanol–water at the same concentration as found in the extra virgin olive oil were investigated separately (Cheikhousman et al., 2005). The maximums in the fluorescence excitation spectrum measured at the 330 nm emission wavelength of oil polyphenol extract and vitamin E were observed at 284 and 290 nm, respectively. The spectral contribution of both the tocopherols and phenolic compounds to the fluorescence of extra virgin olive oil was confirmed by the similarity between the reconstructed spectrum of the mixture and the spectrum of extra virgin oil (Cheikhousman et al., 2005). Recently the fluorescence intensity at 280/320 nm in excitation/emission was successfully used to determine phenol contents in methanol/water extracts of olive oils (Papoti & Tsimidou, 2009).

Thus, both tocopherols and phenolic compounds contribute to the short-wavelength emission of the olive oils, with the tocopherol contribution dominating in refined oils. The exact positions of the maxima of the short-wavelength emission vary slightly between various oils, which may result from differences in the respective tocopherol composition. Note that in oils obtained by physical methods (cold pressing), including olive, linseed and rapeseed oils, this fluorescence maximum was blue-shifted as compared to refined oils, pointing out the difference between fluorescence of refined and cold-pressed oils in this spectral region (Sikorska et. al., 2004).

Due to the similar fluorescence properties of tocopherols and some phenolic compounds (Table 1), their emission appears as a single broad band, therefore a detailed study of excitation and emission spectra in this region is required to reveal presence of various species (Sikorska et al., 2008a). The excitation and emission spectra of virgin olive oil measured respectively at λ_{em}=330 nm and λ_{ex}=295 nm agree very well with the respective spectra of α-tocopherol, Fig. 4. On the other hand, the excitation and emission spectra measured respectively at λ_{em}=300 nm and λ_{ex}=280 nm are blue shifted as compared to α-tocopherol, being attributed to the phenolic compounds, according to Cheikhousman et al. (2005).

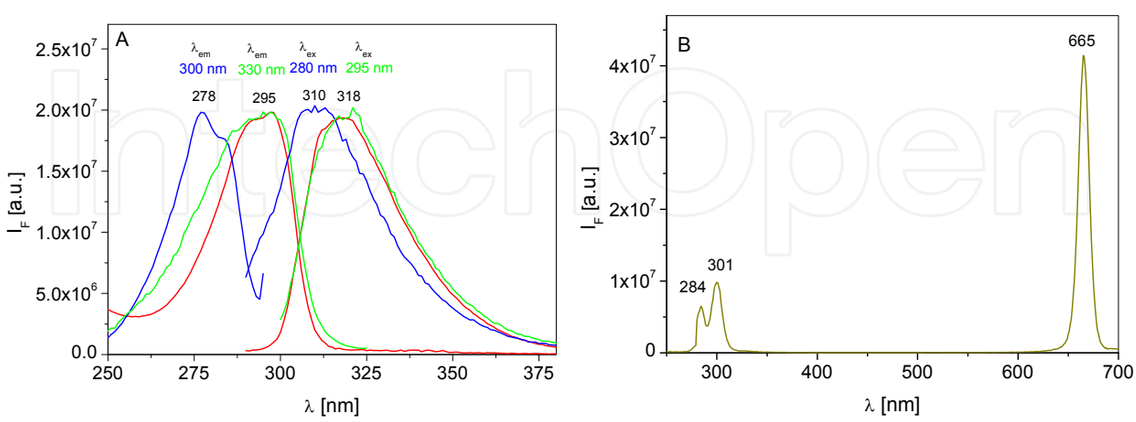


Fig. 4. (A) Excitation spectra (λ_{em}=300 and 330 nm) and emission spectra (λ_{ex}=280 and 295 nm) of extra virgin olive oil (green and blue) and tocopherol (red); the respective excitation and emission wavelengths are shown in the picture; (B) synchronous fluorescence spectrum (Δλ = 10 nm) of extra virgin olive oil, (Sikorska et al., 2011).

Synchronous fluorescence spectroscopy offers a superior solution to the problem of the overlapping spectral bands, by reducing their spectral widths. The synchronous fluorescence spectrum of virgin olive oil measured at $\Delta\lambda$=10 nm shows two separate bands, with the band at 301 nm attributed to tocopherol. Linear correlation was found between the fluorescence intensity at 301 nm and the total tocopherol concentration as determined chromatographically, for a set of different diluted vegetable oils (1% v/v, in n-hexane) (Sikorska et. al., 2005b).

The band at 284 nm could originate from phenolic compounds such as phenolic aglycons, based on the molecules of tyrosol and hydroxytyrosol, derived from phenolic glycosides present in the olives. Oleuropein aglycons are present in high amounts in virgin olive oil. The synchronous spectrum ($\Delta\lambda$=10 nm) of pure oleuropein exhibits a maximum at about 289 nm and is shifted by about 5 nm as compared to the fluorescence band observed in the virgin olive oil. This shift may result from the solvent, as the phenolic compounds are poorly soluble in nonpolar solvents, the spectrum was recorded in the n-hexane – ethanol mixture. On the other hand, the emission observed in the oil may originate from oleuropein derivatives, with slightly different emission properties (Sikorska et al., 2008).

The band splitting, observed in synchronous spectra only at small $\Delta\lambda$ values, seems to be characteristic for virgin olive oils and has been not observed for refined oils (Sikorska et. al., 2005a). Synchronous fluorescence spectra acquired for virgin olive and sunflower oils at $\Delta\lambda$=20 nm were reported to have one double band at around 275 and 297 nm, and one more band at 660 nm, in contrast to sunflower oil that shows an intense band at around 300 nm and a weak one at 325 nm (Poulli et al., 2006).

The synchronous fluorescence spectra of phenolic compounds (tyrosol, p-coumaric and caffeic acids) present in virgin olive oils were measured at $\Delta\lambda$=30 nm (Dupuy et al., 2005). The spectra present a fluorescence band in the 275-350 nm spectral range, with the maxima for tyrosol and tocopherol observed respectively at 276 and 295 nm. Interestingly, it was shown that the mathematically calculated emission spectrum from a mixture of tyrosol, p-coumaric and caffeic acids, and α-tocopherol was very similar to the experimentally obtained spectrum of olive oil. Therefore, we once more conclude that the fluorescence emission between 275 and 400 nm has contributions from both tocopherols and phenolic compounds (Dupuy et al., 2005).

A long-wavelength band is observed in the olive oil spectra, with excitation at about 350–420 nm and emission at about 660–700 nm, corresponding to the band above 550 nm in total synchronous fluorescence spectra. This band was attributed to pigments of chlorophyll group, based on its excitation and emission characteristics (Zandomeneghi et al., 2005; Diaz et al., 2003). This group includes chlorophylls *a* and *b*, and pheophytins *a* and *b*, derived from chlorophylls by loss of magnesium. The emission spectra of these four chlorophyll derivatives are very similar, with their maxima in the range of 653 to 671 nm in 9:1 acetone/water (Diaz et al., 2003). Their presence is characteristic for virgin olive oils, being reduced to trace amounts in refined oils.

The origin of the emission in the intermediate region for both virgin and refined olive oils is unclear. It seems that various components may be responsible for bands appearing in this region. Wolfbeis & Leiner (1984), suggested that in addition to tocopherols and chlorophylls, parinaric acid, a conjugated 18:4 (n-3) acid, may also contribute to vegetable oil emission.

Recently, fluorescence from conjugated fatty acids including α-eleostearic acid, *cis*-parinaric acid and 8,10,12,14,16-octadecapentaenoic acid, was reported for Borage oil (Smyk et al., 2009). The fluorescence spectrum of methyl ester of the *cis*-parinaric acid has the maximum at 416 nm. It was also suggested that the low intensity emission with the maximum at 524 nm may originate from riboflavin (vitamin B_2) in virgin olive oils. Riboflavin is a polar, water soluble compound and no quantitative data regarding the presence of riboflavin in olive oils are available. This vitamin was found in olive pulp (Zandomeneghi et al., 2005). Additional emission in the intermediate region has been detected as a result of oxidation (Cheikhousman et al., 2005; Poulli et al., 2009a, 2009b; Tena et al., 2009; Sikorska et al., 2008).

2.2 Methods of analysis of fluorescence data

In past decades improvements in both spectroscopic instruments and computers contributed to the extensive application of fluorescence spectroscopy in food analysis, including olive oils. Although even the simple conventional analysis of fluorescence spectra may produce valuable data, most of the successful applications rely on multivariate methods for extracting useful analytical information from the measured fluorescence signals.

According to Christensen et al. (2006), several conditions should be met in an ideal system for fluorescence measurements: 1) the concentration of the fluorophores must be sufficiently low, to be approximately linearly related to the fluorescence intensity, 2) signals from each of the fluorescent components must beindependent of each other, 3) the signal contribution from interfering species must be insignificant compared to the target fluorophore signal. Most of these conditions, however, are routinely violated in intact food samples. Therefore, due to the complex character of the spectra, they are rarely used for direct analysis, being rather used as spectral patterns or fingerprints of particular samples. The vast amount of spectral information contained in such fingerprints could be used in qualitative and quantitative analysis.

Multivariate and multiway methods are specifically suited for treatment of such complex spectral data. The multivariate analysis has several advantages over the univariate approach. It enables analysis of nonselective signals in the presence of spectral interferences, providing diagnostic tools for detection of the outliers. Their application to spectral data has provided important tools for food analysis, where they can be used for exploration, classification and calibration purposes (Christensen et al., 2006).

Traditional multivariate analysis of fluorescence data is performed on a series of emission, excitation or synchronous spectra arranged into a matrix. It starts usually with data exploration that is aimed at discovering structures in the data set, clustering of objects and outlier detection. This analysis does not require any prior knowledge of the explored data, employing unsupervised pattern recognition methods, including principal component analysis (PCA). Other methods used to explore the food fluorescence data include hierarchical cluster analysis, non-negative matrix factorization, common components and specific weights analysis, and canonical correlation analysis (Sadecka & Tothova, 2007).

Three-way models are used for analysis of sets of fluorescence excitation-emission matrices, including parallel factor analysis (PARAFAC) and the Tucker model. The PARAFAC model decomposes the fluorescence data into a number of components. These components correspond to the distinct fluorophores present in the samples. The analysis provides relative concentrations of each of the fluorophores in the mixture, accompanied by the

respective excitation and emission loadings, which correspond to the respective excitation and emission spectra, facilitating identification of the fluorescent constituents. This approach is called mathematical chromatography, enabling qualitative and quantitative analysis of the individual mixture components (Bro, 2003; Christensen et al., 2006).

The analytical problem of the food quality assessment often involves assignment of a particular product to a specific category. To perform such classification, supervised pattern recognition methods are used. In these methods the information about the class membership of the samples in a certain category is used to derive classification rules, which are next applied to classify new samples into correct categories on the basis of patterns present in their measurements (Berrueta et al., 2007). A number of classification techniques were used for analysis of food fluorescence data in the supervised mode: linear discriminate analysis, factorial discriminate analysis, k - nearest neighbors, discriminate partial least squares regression (DPLS), soft independent modeling of class analogy, and artificial neural networks.

Multivariate calibration is the most important and successful combination of chemometrics with spectral data used in analytical chemistry. The calibration consists of building a relationship between a desired chemical, biological or physical property of a sample, and its spectrum. The advantage of such approach is the replacement of the wet chemical measurements of a concentration, which are usually slow and expensive, by the spectral measurements, which are nondestructive and fast, requiring little or no sample preparation and producing no waste chemicals. The multivariate regression methods most frequently used in fluorescence analysis are partial least-squares regression (PLS) and principal component regression (PCR). N-way partial least-squares regression (N-PLS) is used for calibration analysis of fluorescence excitation-emission matrices (Geladi, 2003).

3. Application of fluorescence in olive oil analysis

Application of fluorescence to quality assessment of olive oils was proposed already in the beginning of the 20th century. From 1925, when mercury lamp with the Wood's filter became commercially available, visual observation of oil fluorescence induced by UV light was utilized to detect adulteration of extra virgin olive oils. It was shown that extra virgin olive oils exhibit characteristic yellow fluorescence, due to chlorophylls, while fluorescence of refined oils was blue due to the changes in chlorophyll content during the refining process. This method allowed detecting adulteration of extra virgin olive oils at the level of 5% with refined oils (Sidney & Willoughby, 1929; Glantz, 1930). The use of Wood's lamp was accepted as the U.S. official method for detection of olive oil adulteration. (Kyriakidis & Skarkalis, 2000). The authors of the first papers that reported spectral properties of fluorescence of vegetable oils also point out practical applications of fluorescence spectra as fingerprints in oil analysis (Wolfbeis & Leiner, 1984; Kyriakidis & Skarkalis, 2000).

3.1 Discrimination between quality grades of olive oil

Olive oil is an economically important product and its quality control and detection of possible fraud are of great interest. Olive oils are classified and priced according to acidity. The most expensive is the high-quality extra-virgin olive oil. This oil may be subject of both mislabeling and adulteration. Refined olive oil is obtained from virgin olive oil using

refining methods that do not alter the initial glyceridic structure; pure olive oil (or simply olive oil) consists of a blend of virgin and refined olive oil. The potential of fluorescence to discriminate olive oils of different quality was the subject of several studies. Both total luminescence and synchronous fluorescence spectra combined with various chemometric approaches were successfully used for this purpose.

Scott et al. (2003) used total luminescence spectra of four different types of edible oils: extra virgin olive, non-virgin olive, sunflower and rapeseed oils. The spectra of undiluted oil samples were measured in the excitation range from 350 to 450 nm with 10 nm intervals and in the emission range from 400 to 720 nm with 5 nm interval. Three supervised neural network algorithms were used for sample classification: simplified fuzzy adaptive resonance theory mapping, traditional back propagation and radial basis function. The 100% correct classification was obtained using all methods.

Guimet et al. (2004a) in a series of studies investigated possibility of application of total fluorescence spectra for discrimination between various quality grades of olive oils. The excitation-emission matrices of undiluted oils were measured using right angle geometry. The hierarchical agglomerative clustering method with the Euclidean distance as a similarity measure and the average linkage method were applied to discriminate between three classes of commercial Spanish olive oils (virgin olive oils, pure olive oils, and olive-pomace oils). To optimize the sample grouping into clusters, different preprocessing methods and two spectral ranges were tested, which either included or not the fluorescence peak of chlorophylls. The oils were distinguished using the unfolded excitation–emission fluorescence matrices in the 300-400 nm excitation range and 400-600 nm emission range, thus excluding the chlorophyll band, Fig. 5. The large variations in the chlorophyll band intensity, even between samples of the same type, tend to deteriorate oil discrimination. The optimal preprocessing included normalization of the unfolded spectral excitation–emission fluorescence matrices, followed by column autoscaling. The comparison of the results obtained from the excitation–emission fluorescence matrices to those from a single emission (λ_{ex}=345, 360, 390 nm) and excitation (λ_{em}=410 nm) fluorescence spectrum analysis showed the advantage of the total fluorescence data, which result in a significantly better discrimination.

Other studies used unfold PCA and PARAFAC to explore the excitation–emission fluorescence matrices of virgin and pure olive oils (Guimet, 2004b). The spectral ranges studied were λ_{ex}=300–400 nm, λ_{em}=400–695 nm and λ_{ex}=300–400 nm, λ_{em}=400–600 nm. The first range included chlorophylls, whose peak was much more intense than those of the other components. The second range did not include the chlorophyll peak, being limited to the fluorescence spectra of the oxidation products and vitamin E. The three-component PARAFAC model on the second range (chlorophylls excluded) was found to produce the most useful results. With this model, it was possible to distinguish well between the two groups of oils and to calculate the underlying fluorescent spectra of the three families of compounds. Both unfold PCA and PARAFAC applied to the excitation–emission matrices showed clear differences between fluorescence of the two main groups of olive oils (virgin and pure). Chlorophylls had a strong influence on the models because of their high fluorescence intensity and high variability. Differentiation between the two types of oils was better when the chlorophyll fluorescence region was excluded from the models. The oxidation products are the species that most contribute to the separation between the two

groups. PCA was calculated from the emission spectra of oils between λ_{em}=400 and 695 nm measured at λ_{ex}=365 nm (Guimet, 2004b).

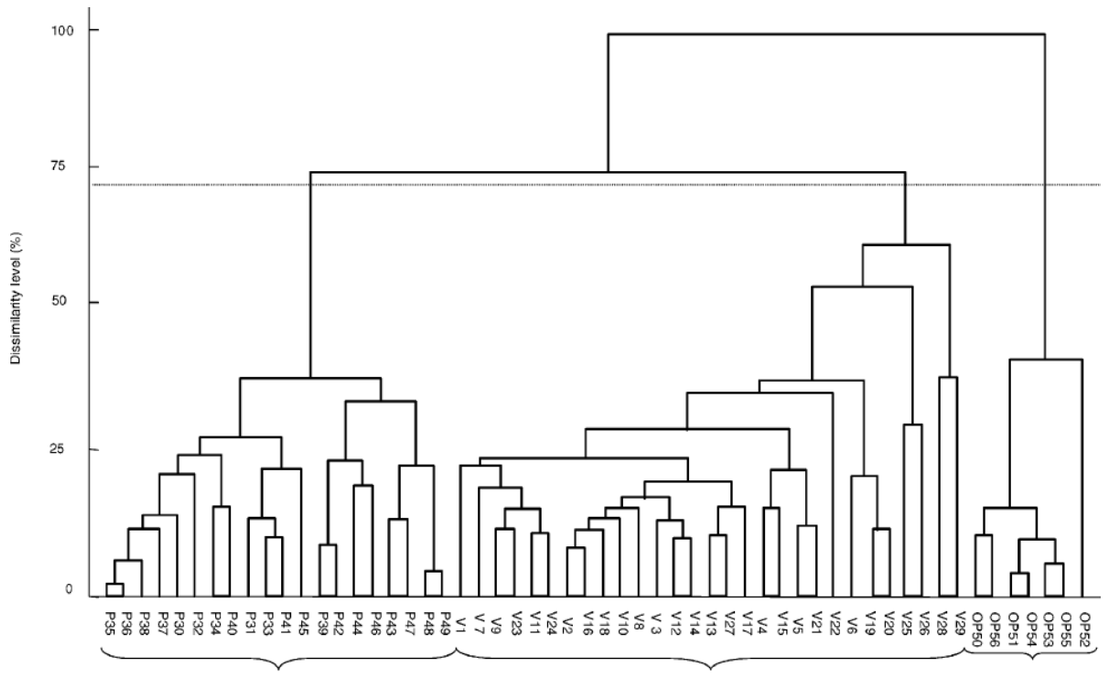


Fig. 5. Dendrogram of the 56 x 861 unfolded matrix (λ_{ex} =300-400 nm, λ_{em} = 400-600 nm) using the Euclidean distance as similarity measure and the average linkage method. The distance is expressed as a percentage of dissimilarity (normalized and autoscaled spectra): virgin V, pure, P; and olive-pomace, OP oils. Reprinted with permission from Guimet et al., 2004a. Copyright 2004, American Chemical Society.

Non-negative matrix factorization with Fisher's linear discriminant analysis were applied for discriminating between different types of olive oils: 1) discrimination between commercial Spanish olive oils of different quality (virgin, pure, and olive-pomace oil); 2) discrimination between virgin oils from two ''Siurana'' (Protected Denomination of Origin) regions; 3) discrimination between the original ''Siurana'' virgin olive oils and oils adulterated with olive-pomace oil at 5% (w/w) level (Guimet et al., 2006). In all cases, classifications at above 90% confidence were achieved. The proposed method was also compared to PARAFAC and discriminant N-PLS regression. The classification results were better with non-negative matrix factorization than PARAFAC for two data sets out of three. Non-negative matrix factorization combined with Fisher's linear discriminant analysis was also comparable with discriminant N-PLS regression, giving better classifications for the second data set, but slightly worse results for the other two. The main advantage of non-negative matrix factorization with respect to discriminant N-PLS regression is that its basis functions are more interpretable than the regression loadings, because they are positive and correspond to parts of the spectra than can be directly related to the fluorescent components of oils.

The fluorescence excitation-emission matrices (λ_{ex}=300-390 nm and λ_{em}=415-600 nm) were used in studies of the Spanish extra virgin, virgin, pure, and olive pomace oils, to investigate the relationship between oil fluorescence and the conventional quality parameters,

including peroxide value, K_{232}, and K_{270} (Guimet et al., 2005c). Multiway methods were applied to the data analysis: PARAFAC with multi-linear regression and N-PLS regression. Better regression fits and lower prediction errors were obtained using N-PLS. The best results were obtained for prediction of K_{270}. The detection of extra virgin olive oils was highly degraded at early stages (with high peroxide value) and little oxidized pure olive oils (with low K_{270}).

Synchronous fluorescence spectroscopy combined with multi-dimensional chemometric techniques was applied to the classification of virgin olive oils according to their quality by Poulli et al. (2005). They studied the fluorescence of virgin olive oils and lampante oils, using total fluorescence, synchronous and total synchronous fluorescence spectra. Total luminescence spectra recorded in the 350–720 nm range while exciting in the 320 to 535 nm range showed different shapes and intensities for the two classes of oils. Lampante olive oil had a broad emission with its maximum at 450-500 nm in addition to the 685-690 nm peak. Total synchronous fluorescence spectra measured at $\Delta\lambda$=20-180 nm had emission peaks between 500-700 nm, depending on $\Delta\lambda$, for both classes of oils. However, lampante oil had additional fluorescence in the of 360–500 nm range, which is not observed for edible virgin olive oil. Classification of virgin olive oils based on their synchronous fluorescence spectra ($\Delta\lambda$=80 nm) was performed by hierarchical cluster analysis and PCA using the 429–545 nm spectral range. The authors conclude that the fluorescence in the 429–545 nm range, which they used for data analysis, originates from oleic acid. PCA provided 100% correct discrimination between the two classes, while hierarchical cluster analysis allowed 97.3% correct classification.

3.2 Adulteration detection of olive oils

A few papers were published in recent years on the use of fluorescence to assess adulteration detection of virgin olive oils. Adulteration of virgin olive oils has been a common fraud practice that involves addition of cheaper oils, including olive oils of lower quality or other plant oils. The most common adulterants found in virgin olive oil are refined olive oil, pomace oil, residue oil, synthetic olive oil–glycerol products, seed oils, and nut oils. The current analytical standards for olive oil enable detection of the presence of almost all of the possible adulterants; however, they require the measurement of several parameters established by the EU Regulations: (EEC) No 2568/91 and (EC) No 796/2002.. Thus, rapid methods to detect olive oil adulteration are important for quality control purposes (Karoui & Blecker, 2011).

Hazelnut oil is chemically similar to virgin olive oil; its presence is difficult to detect at low concentration levels using standard methods. A different approach was tested to detect this type of adulteration using fluorescence (Sayago et al., 2004; Sayago et al., 2007). The emission spectra of undiluted olive oil mixtures with virgin and refined hazelnut oils with excitation at 350 nm were measured (Sayago et al., 2004). The spectra were subjected to mathematical treatment by calculation of the first derivative. One-way analysis of variance was used for the selection of suitable wavelengths to differentiate oil samples. The response to the addition of adulterant, as evaluated by multiple linear regression, was linear for virgin olive and refined hazelnut oil mixtures (R^2=0.99), and for virgin olive and virgin hazelnut oil mixtures (R^2=0.98). Stepwise linear discriminant analysis used to discriminate genuine from adulterated olive oil samples allowed 100% correct classifications for each

mixture separately, and also for the entire set of samples. Another work explored application of the fluorescence spectroscopy to differentiate between refined hazelnut and refined olive oils (Sayago et al., 2007). Classification of these oils based on their excitation (in 300-500 nm spectral range, using λ_{em}=655 nm) and emission spectra (in the 650-900 nm range, using λ_{ex}=350 nm) was performed, using PCA and artificial neural networks. Both methods provided good discrimination between the refined hazelnut and olive oils. Using the artificial neural networks model, the presence of refined hazelnut oils in refined olive oils was robustly detected at levels exceeding 9%.

Several studies devoted to the detection of of adulteration of virgin olive oil with sunflower oil. Poulli et al. (2006) applied total synchronous fluorescence to differentiate virgin olive from sunflower oil and synchronous fluorescence combined with PLS regression for quantitative determination of olive oil adulteration. Total synchronous fluorescence spectra were acquired in the 270–720 nm range, using the wavelength interval variable from $\Delta\lambda$=20 to 120 nm. The emission band at around 660 nm was only observed in virgin olive oil, attributed to pigments of the chlorophyll group. For sunflower, in contrast to virgin olive oil, a fluorescence band in the 325–385 nm excitation range is observed. This band was attributed to linoleic acid, however, there are no published data on fluorescence of this compound. In contrast, virgin olive oil has only small signals in this range if scanned at 30 to 50 nm wavelength interval. Synchronous fluorescence spectra of virgin olive oil recorded at $\Delta\lambda$=20 nm show a double band at 275 and 297 nm and a single band at 660 nm, in stark contrast to sunflower oil that has an intense band at around 300 nm and a weak one at 325 nm. For quantification of the adulteration, the PLS regression model was used for analysis of synchronous fluorescence spectra of mixtures of virgin olive oil and sunflower oil at $\Delta\lambda$=20 and 80 nm, Fig. 6. The detection limits were 3.6% and 3.4% (w/v) when using the 20 and 80 nm wavelength intervals, respectively.

The potential of fluorescence spectroscopy for detecting adulteration of extra virgin olive oil with olive oil has been investigated recently (Dankowska & Małecka, 2009). Synchronous fluorescence spectra were collected in the 240–700 nm range, using $\Delta\lambda$=10, 30, 60 and 80 nm. A narrow band at around 300 nm appeared in the synchronous fluorescence spectrum at $\Delta\lambda$=10 nm, attributed to tocopherols, and an intense band with a peak at around 665 nm, attributed to compounds of the chlorophyll group. The raw spectra were subject to calculation of the first and second derivatives to find the maximum or the intersection point. Five wavelengths at each of the wavelength intervals were chosen for further analysis. Multiple regression analysis was applied separately to the data acquired at each of the wavelength intervals. The ability to detect olive oil in extra virgin olive oil was better at the wavelength interval of 60 or 80 nm, rather than 10 or 30 nm. Using the spectra acquired at 60 and 80 nm wavelength intervals, the lowest detection limits of adulteration were 8.9% and 8.4% at 350 and 302 nm, respectively.

Fluorescence was used to detect adulteration of virgin olive with others oils (Poulli et al., 2007). Synchronous fluorescence spectra of virgin olive, olive-pomace, corn, sunflower, rapeseed, soybean and walnut oils at 20 nm wavelength interval were used for analysis. Virgin olive oil shows a double band in the 275–297 nm range and a single band at 660 nm, in contrast to other oils that show a strong band around 300 nm and a weak to moderate band near 325 nm. Total synchronous fluorescence spectra were acquired for the excitation wavelength in the 250–720 nm range and the wavelength interval $\Delta\lambda$ in the 20 to 120 nm range. Total synchronous

fluorescence spectra for olive oils show a spectral band at around 660 nm, attributable to pigments of chlorophyll group. Moreover, all studied oils save the virgin olive oil show a band at above 315 nm when using $\Delta\lambda$=20 nm. This band could be attributed to linoleic acid. It has been suggested that differentiation of virgin olive oil from low quality oils can be achieved using this wavelength region. The PLS regression model was used to quantify adulteration using 20 nm synchronous fluorescence spectra. This technique enabled 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 (Poulli et al., 2007).

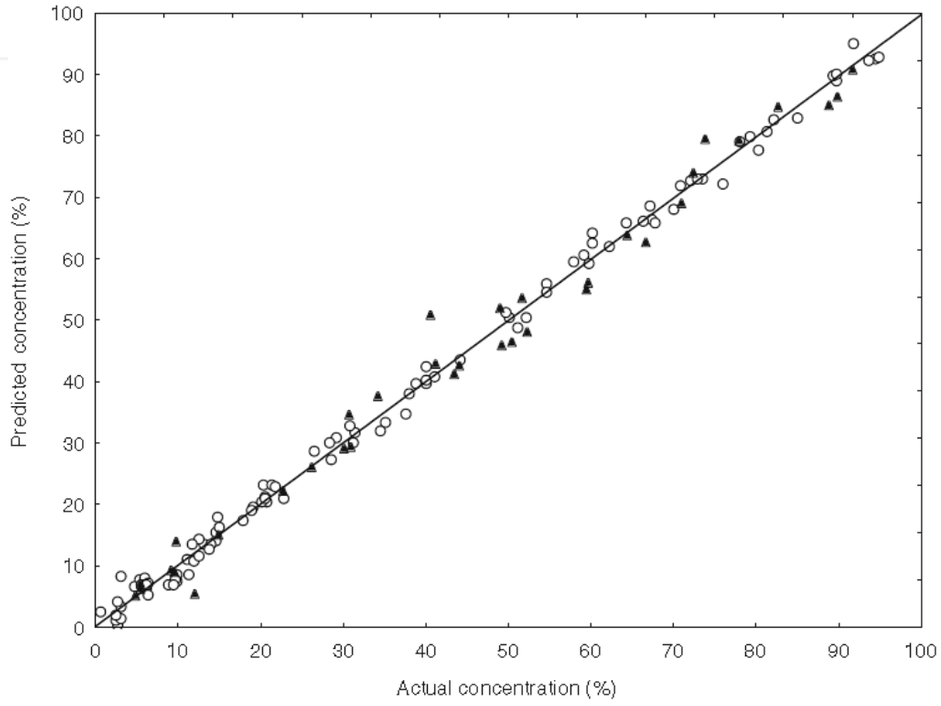


Fig. 6. Predicted versus actual concentrations of sunflower oil in virgin olive oil at a wavelength interval of 80 nm: open circles - calibration samples, filled triangles - validation samples. Reprinted with permission from Poulli et al., 2006. Copyright 2006, Springer.

For adulteration detection of extra virgin olive oil with rapeseed oil a neural network method – a simplified fuzzy adaptive resonance theory mapping - was found to be very efficient, resulting in a total of 99.375% correctly classified oil samples at the 5% v/v adulteration level (Scott et al., 2003). It was shown for extra virgin olive oil adulterated by rapeseed oil that the percentage of adulteration may be described by either a radial basis functional network (2.435% RMSE) or a simple Euclidean distance relationship of the PCA scores (2.977% RMSE).

3.3 Authentication of virgin olive oils geographical origin

Classification of virgin olive oils according to variety and/or geographical origin is of great importance for the producers, importers, and consumers. Dupuy et al. (2005) developed a method to discriminate olive oils according to their geographic origin. Samples of virgin olive oil from five French registered designations of origin (RDOs), namely, Nyons, Vallé des Baux, Aix-en-Provence, Haute-Provence, and Nice, were discriminated by applying multivariate

regression methods to synchronous fluorescence spectra of oils. The synchronous fluorescence spectra were collected in the 250-700 nm range at a constant wavelength difference of 30 nm between the excitation and emission wavelengths. The PLS regression analysis of synchronous fluorescence spectra allowed to determine the origin of the oils with satisfactory results, despite the similarity between two denominations of origin (Baux and Aix) that are composed by some common cultivars (Aglandau and Salonenque). The interpretation of the regression coefficients shows that RDOs are correlated to chlorophylls, pheophytins, tocopherols, and phenolic compounds, present in different amount for each origin (Dupuy et al., 2005).

Guimet et al. (2005a) developed a method based on excitation-emission matrices and three-way methods for detecting adulterations of pure olive oils in extra virgin olive oil from the protected denomination of origin (PDO) "Siurana", which is a prestigious distinction given to the extra virgin olive oils produced in a specific area in the south of Catalonia, Spain. Excitation and emission ranges were λ_{ex}=300–390 nm and λ_{em}=415–600 nm, respectively. Unfold PCA and PARAFAC were used for exploratory analysis. Discrimination between non-adulterated and adulterated samples was performed using Fisher's linear discriminant analysis and discriminant N-PLS regression. Using discriminant N-PLS regression, 100% correct classification was obtained. Adulteration at around 5% level was quantified, with a prediction error of 1.2% (Guimet et al., 2005a). In another study, Guimet et al. (2005b) achieved discrimination between oils from the two PDO "Siurana" regions by means of discriminant unfold PLS regression, giving correct classification for 94% of samples for "Siurana-Camp" and 100% for "Siurana-Montsant" oils.

3.4 Quantification of fluorescent components in olive oils

The olive oil autofluorescence is attributed to minor components, species such as tocopherols, phenols and chlorophylls, thus fluorescence spectroscopy has been used to analyze these compounds in olive oils. Diaz et al. (2003) used fluorescence for determination of chlorophylls *a* and *b* and pheophytins *a* and *b* in olive oil samples. The analysis was accomplished by PLS multivariate calibration using the three types of spectra (excitation, emission, and synchronous spectra of these solutions). The best results were obtained for the excitation spectra. The optimum wavelength range to record the excitation spectra (λ_{em}=662 nm) was selected to minimize the contribution of pheophytin *a* and to maximize the contribution of the other pigments, which are the minor constituents in olive oil. To perform the PLS calibration, a set of samples with final concentration ranges varying from 140 to 560 ng mL^{-1} for pheophytin *a*, from 10 to 40 ng mL^{-1} for chlorophyll *a* and pheophytin *b*, and from 20 to 80 ng mL^{-1} for chlorophyll *b* was used as the calibration matrix. The oil samples were diluted in acetone. Recovery values from olive oil, spiked with chlorophylls *a* and *b* and pheophytins *a* and *b*, were in the ranges of 70-112, 71-111, 76-105, and 82-109%, respectively.

Fluorescence was proposed as an alternative to the Folin-Ciocalteu assay for estimation of the total phenol content in virgin olive oil, olive fruit and leaf polar extracts (Papoti & Tsimidou, 2009). Phenol content in olive oils was determined by measuring the fluorescence intensity of methanol/water extract, with the excitation/emission wavelengths set at 280/320 nm. The method was shown to be more sensitive (limit of detection and limit of quantification values 10-fold lower) and three times faster then the Folin-Ciocalteu assay. Good correlation was found with the results of colorimetric assay (r = 0.69, n = 65) for virgin olive oil extracts.

Fluorescence combined with PLS regression was used to determine tocopherol homologues (α-, β-, γ-, and δ- tocopherol) in the quaternary mixture and the oils (Diaz et al., 2006). The calibration set that included mixtures of tocopherols dissolved in hexane: diethyl ether (70:30 v/v) was constructed based on the central composite plus a full factorial plus a fractionated factorial design. PLS regression was applied to analyze matrices of fluorescence excitation and emission spectra and with fluorescence excitation, emission, and synchronous spectra. For analysis of synthetic samples, recoveries around 100% were obtained. For the analysis of the oils, the samples were diluted in hexane, cleaned in silica cartridges and then tocopherols were eluted with hexane:diethyl ether (90:10 v/v). The method was applied to different edible oils giving satisfactory results for α-, β-, and γ-, but not for δ- tocopherol.

PLS regression was utilized to develop calibration models between front face and right angle synchronous fluorescence spectroscopy for the characterization of edible oils and total tocopherol content as determined by HPLC (Sikorska et al., 2005b). The studies were performed on commercially available edible oils: olive, grapeseed, rapeseed, soybean, sunflower, peanut, and corn oils were analyzed. The regression models showed a good ability to predict tocopherol content. The best fitting results were obtained for 1% v/v diluted oils and for bulk samples using the entire spectrum, yielding the regression coefficient of 0.991, and the root mean square error of cross-validation of 8%.

3.5 Monitoring thermal and photo-oxidation of olive oils

The studies of thermal deterioration of oils are important because changes during oxidation involve degradation of oil constituents and formation of new products that alter quality attributes and nutritional profile, as the oxidation products are potentially toxic. Fluorophores in olive oils are compounds that can participate in oxidation, thus fluorescence spectroscopy can serve as a tool for better understanding of oil oxidation. The fluorescence was compared to other spectroscopic techniques (NIR/VIS, FT-IR and FT-Raman) and chemical and physical methods in determining the deterioration of frying oils, collected from a commercial Chinese spring roll plant, (Engelsen, 1997). Fluorescence has been measured by using five selected excitation wavelengths varying from 395 to 530 nm. Data analysis was performed using PCA and PLS regression. Overal, fluorescence provided the best models for the anisidine value, oligomers, iodine value, and vitamin E concentration, among the spectroscopic techniques used.

Fluorescence spectrometry and PLS regression were used as a rapid technique for evaluating the quality of heat-treated extra virgin olive (Cheikhousman et al., 2005). Two commercial extra virgin olive oils were heated at 170°C for 3 h. Changes in excitation spectra were correlated with changes in concentrations determined by other methods. The fluorescence excitation band emitting at 330 nm was attributed to vitamin E and some fluorescent polyphenols. This fluorescence decreased during the heating process, with the exponential decay constant similar to that obtained chromatographically. Fluorescence excitation spectra with the emission wavelength at 450 nm were inversely correlated with the hydroperoxide content of oil. Indeed, the degradation products generated during heating, particularly the compounds formed by reaction between amino-phospholipids and aldehydes, fluoresce in this wavelength range.

Thermal deterioration of extra virgin olive oils was studied by Tena et al. (2009). The sample of virgin olive oil was heated at 190°C for 94 h in cycles of 8 h per day. The fluorescence intensity in the spectral region between 290 and 400 nm decreased during the oxidation and

a bathochromic shift of the maximum from 350-360 to around 420-440 nm was observed. The fluorescence observed in the 300-390 nm range was assigned to tocopherols together with polyphenols; the information collected from the spectra was compared to the results of the HPLC analysis of these compounds. The observed changes in the spectral profile were explained by the decrease of the tocopherols and phenols and the increase of the oxidation products of vitamin E homologues correlated to K_{232} and K_{270}, and hydrolysis products. The intensity of the band between 630 and 750 nm, associated with chlorophylls and pheophytins, decreased exponentially with the thermal oxidation time.

The fluorescence intensity recorded at 350 nm and at the wavelength of the spectral maximum occurring in the range of 390-630 nm allowed to explain the increase of the percentage of polar compounds during the experiment. It was stated that the spectra of the undiluted heated oils with maxima at 490 nm or higher correspond to polar compounds exceeding 25%, which is the maximum percentage acceptable for edible oils used in frying.

Poulli et al. (2009b) studied the effect of heating to 100, 150 and 190° C on extra virgin olive, olive pomace, sesame, corn, sunflower, soybean, and a commercial blend of oils. The changes in fluorescence were assessed by measuring total synchronous fluorescence spectra, in the 250-720 nm excitation range, with the wavelength interval, $\Delta\lambda$, from 20 to 120 nm at 20 nm step. The synchronous fluorescence intensities below 315 nm recorded at $\Delta\lambda$=80 nm decreased during heating, presumably due to the consumption of phenolic antioxidants by the lipid radicals generated. The decrease of the fluorescence bands in the 250-350 and 350-400 nm ranges for extra virgin olive and olive pomace oil, respectively, was in accordance with the percentage of trolox equivalent antioxidant capacity reduction. The bands in the total synchronous fluorescence spectra at below 350 nm disappeared during heating, with those at 600-700 nm also decreasing, probably due to the decay of antioxidant compounds and chlorophyll, respectively. The bands in the 400-450 nm range increased, probably due to the formation of secondary oxidation products. PCA of synchronous fluorescence spectra obtained at $\Delta\lambda$=80 nm allowed oil discrimination according to the degree of oxidation. For extra virgin olive, olive pomace, and sesame oil the spectral range of 300-500 nm was used for classification, while the 320-520 nm range was more appropriate for corn, soybean, and sunflower oil, and a commercial blend of oils. Spectroscopic changes are indicative of oxidative deterioration as measured through wet chemistry methods: peroxide value, p-anisidine value, totox value, and radical-scavenging capacity (Poulli, 2009a).

Extra virgin olive oil is very stable in the dark; it is susceptible to oxidation under UV light. An accelerated thermal and photooxidation under UV light was studied by Poulli et al. (2009a, 2009b) on samples of extra-virgin, regular-quality and pomace olive oils. Synchronous fluorescence spectra were collected using the 250–720 nm excitation range at $\Delta\lambda$=80 nm. Extra virgin olive oil bands in the 300–330 nm range decreased during oxidation, while the fluorescence in the 350–550 nm range increased during the initial 8 h and then remained almost constant for up to 12 h. Regular quality olive oil exhibited fluorescence in the 300–550 nm range. The bands in the 300–370 nm range decreased during oxidation, whereas fluorescence bands in the 370–550 nm range increased during the initial period and remained almost constant afterwards. Also, the fluorescence bands of pomace oil in the 350–550 nm range decreased during the initial period of the experiment and then a small additional increase was observed. All olive oils show fluorescence bands in the 550–700 nm range, attributed to chlorophyll pigments, intensive in extra virgin olive oil and with very low intensity in olive-pomace oil. These bands decreased significantly due to deterioration

of the chlorophyll pigments involved in photo-oxidation. Total synchronous fluorescence spectra were obtained by scanning the excitation wavelength in the same spectral range and changing the wavelength interval from 20 to 120 nm at 20 nm steps. These spectra showed considerable changes during oxidation for all of the oils studied, Fig. 7. Fluorescence intensity in the 600–720 nm range, attributed to chlorophylls, decreased significantly. In contrast, the fluorescence bands in the low-wavelength range expanded up to 590 nm. PCA applied to the synchronous fluorescence spectra recorded at $\Delta\lambda$=80 nm in the 300–500 nm range reveals five different classes of oils depending on their oxidation degree.

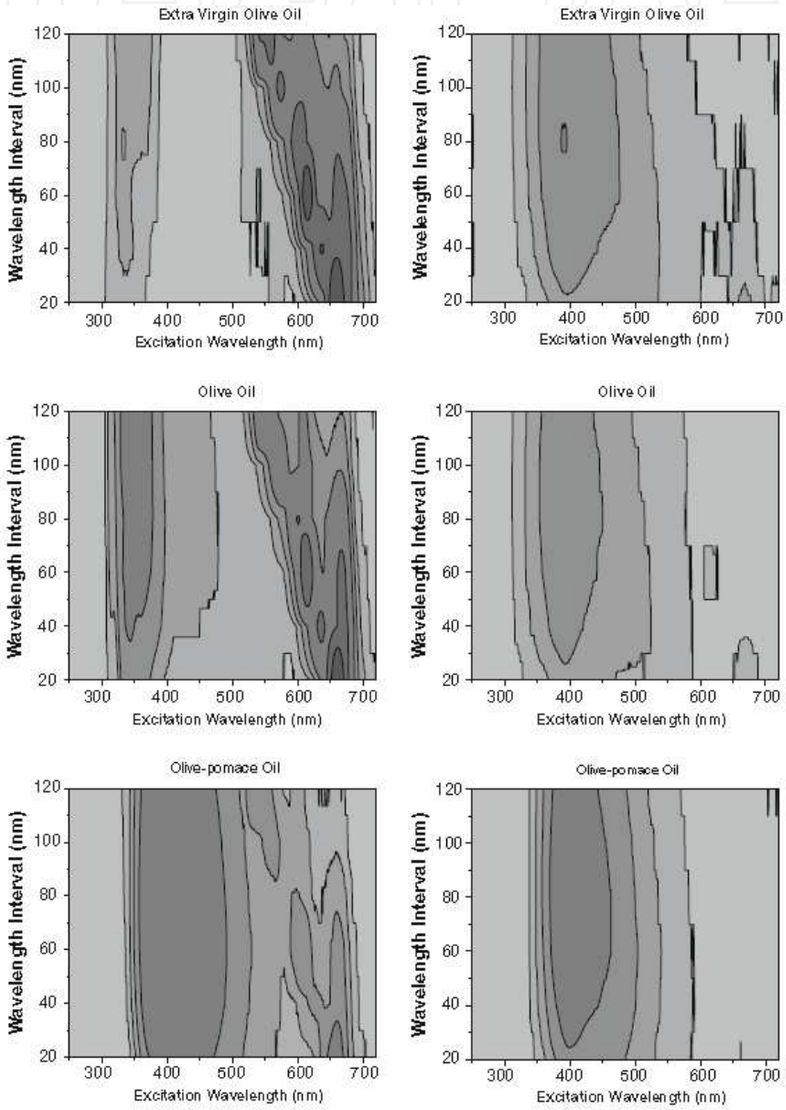


Fig. 7. Total synchronous fluorescence spectra: contour plots of olive oils before (left) and after 12 h (right) exposure to UV light at 80°C. Grayscale indicates fluorescence intensities. Reprinted from *Food Chemistry*, (2009) Vol.117, No.3, Poulli K. I.; Mousdis G. A. & Georgiou C. A., "Monitoring Olive Oil Oxidation Under Thermal and UV Stress Through Synchronous Fluorescence Spectroscopy and Classical Assays", pp. 499-503, Copyright (2009), with permission from Elsevier.

An interesting study on thermal oxidation of extra virgin olive oils has been published recently (Navarra et al., 2011). Among different experimental techniques (including FTIR and rheology) time-resolved luminescence was used to investigate early steps of the thermally induced oxidative process. The oxidation process was followed at three different heating temperatures (30, 60 and 90°C) as a function of time for up to 35 days. The chlorophyll fluorescence lifetime increased from 6.0 ± 0.1 ns, measured before, to 6.3 ± 0.1 ns, measured after 35 days of experiment. These changes were in agreement with the observed viscosity rise, resulting from formation of polar molecules with propensity to form hydrogen bonds. The viscosity increase reduced the frequency of collisions between the chromophore and its environment, consequently lowering the non-radiative contribution to the luminescence decay.

3.6 Assessing quality changes of olive oil during storage

Fluorescence spectroscopy was applied to monitoring changes in virgin olive oil during storage (Sikorska et al. 2008b). The extra virgin olive oil samples were stored for the period of 12 month in different conditions: in clear and green glass bottles exposed to light, and in darkness. Changes occurring in olive oil during storage were assessed by total fluorescence and synchronous scanning fluorescence spectroscopy techniques. In the total fluorescence spectra the intensity of emissions ascribed to tocopherols and chlorophyll pigments decreased during storage, depending on the storage conditions. Additional bands appeared in oils exposed to light in the intermediate range of excitation and emission wavelengths. Bands attributed to tocopherols, chlorophylls and those tentatively ascribed to phenolic compounds were observed in the synchronous scanning fluorescence spectra, allowing monitoring of the storage effects on these constituents. PCA of the synchronous fluorescence spectra revealed systematic changes in the overall emission characteristics dependent on the storage conditions, such as exposure to light, and packaging, Fig. 8.

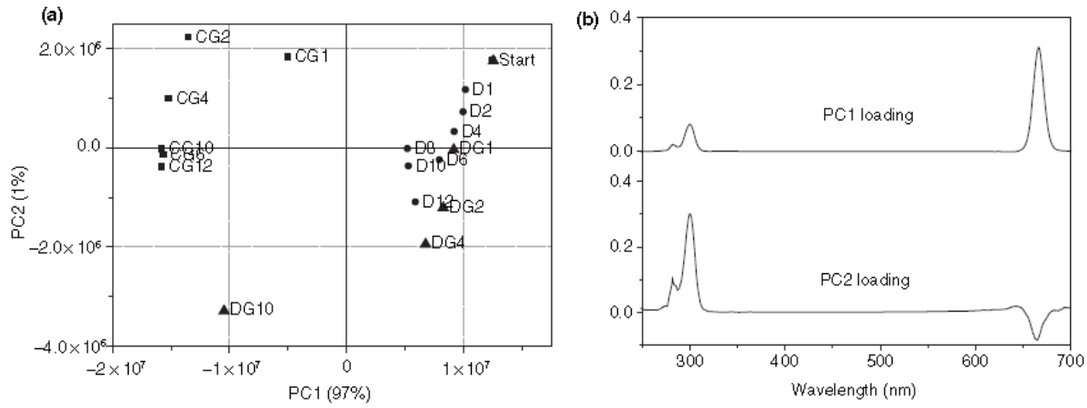


Fig. 8. (a) Scores plot for the two most significant principal components, PC1 vs. PC2, of a PCA of the synchronous scanning fluorescence (SSF) spectra (Δλ = 10 nm) of virgin olive oil samples. A nonstored sample (Start), and samples stored in different conditions: in darkness (D), in green glass bottles (DG) and in clear glass bottles (CG). The samples are numbered according to the months of storage. The values in brackets describe the fraction of the total variation explained by each of the PC. Each point represents an average of the spectra obtained from three replicates . (b) One-vector loading plots for the PC1 and PC2 principal components. Reprinted with permission from Sikorska et al., 2008b. Copyright 2008, John Wiley and Sons.

4. Conclusions

Fluorescence spectra of olive oils contain information about fluorophores (tocopherols, phenolic compounds, and chlorophylls) that are important for oil quality. The spectra may be used to monitor either selected constituents or to determine overall sample characteristics, which may serve as the spectral fingerprint. The analytic potential of fluorescence is enhanced by application of multivariate data analysis methods for the analysis of spectra.

The review of literature data demonstrates that fluorescence measurements conducted directly on olive oil samples with subsequent multivariate data analysis can be efficiently used for qualitative and quantitative analysis as a valid alternative to conventional, chemical methods of quality assessment. These methods can be used for oil discrimination and for quantitative determination of fluorescent components after an appropriate calibration. Further studies are needed to resolve various issues that are important for practical application of the fluorescence techniques, among which are the method verification for specific kinds of oil and identification and quantification of other fluorescent oil constituents.

5. Acknowledgment

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6. References

Berrueta L. A.; Alonso-Salces R. M. & Heberger K. (2007). Supervised Pattern Recognition in Food Analysis. *Journal of Chromatography* A, Vol.1158, No.1-2, pp. 196-214, ISSN 0021-9673

Bro R. (2003). Multivariate Calibration. What is in Chemometrics for the Analytical Chemist?. *Analytica Chimica Acta*, Vol.500, No.1-2, pp. 185-194, ISSN 0003-2670

Cert A.; Moreda W. & Perez-Camino M. C. (2000). Chromatographic Analysis of Minor Constituents in Vegetable Oils. *Journal of Chromatography* A, Vol.881, No.1-2, pp. 131-148, ISSN 0021-9673

Cheikhousman R.; Zude M.; Bouveresse D. J. R.; Leger C. L.; Rutledge D. N. & Birlouez-Aragon I. (2005). Fluorescence Spectroscopy for Monitoring Deterioration of Extra Virgin Olive Oil During Heating. *Analytical and Bioanalytical Chemistry*, Vol.382, No.6, pp. 1438-1443, ISSN 1618-2650

Christensen J.; Norgaard L.; Bro R. & Engelsen S. B. (2006). Multivariate Autofluorescence of Intact Food Systems. *Chemical Reviews*, Vol.106, No.6, pp. 1979-1994, ISSN 1520-6890

Dankowska A. & Małecka M. (2009). Application of Synchronous Fluorescence Spectroscopy for Determination of Extra Virgin Olive Oil Adulteration. *European Journal of Lipid Science and Technology*, Vol.111, No.12, pp. 1233-1239, ISSN 1438-9312

Díaz T. G.; Merás I. D.; Correa C. A.; Roldan B. & Cáceres M. I. R. (2003). Simultaneous Fluorometric Determination of Chlorophylls A and B and Pheophytins A and B in Olive Oil by Partial Least-Squares Calibration. *Journal of Agricultural and Food Chemistry*, Vol.51, No.24, pp. 6934-6940, ISSN 1618-2650

Díaz, T. G.; Durán-Merás, I.; Cáceres, M. I. R.; Murillo, B. R. (2006). Comparison of Different Fluorimetric Signals for the Simultaneous Multivariate Determination of Tocopherols in Vegetable Oils. *Applied Spectroscopy*, Vol.60, No.2, pp. 194-202, ISSN 1943-3530

Dupuy N.; Le Dreau Y.; Ollivier D.; Artaud J.; Pinatel C. & Kister J. (2005). Origin of French Virgin Olive Oil Registered Designation of Origins Predicted by Chemometric Analysis of Synchronous Excitation-Emission Fluorescence Spectra. *Journal of Agricultural and Food Chemistry*, Vol.53, No.24, pp. 9361-9368, ISSN 1618-2650

Eitenmiller R. R.; Ye L. & Landen W. O. (2008). Vitamin E: Tocopherols and Tocotrienols. In *Vitamin Analysis for the Health and Food Sciences*, 2 edn CRC Press, New York, ISBN 0849326680

Engelsen S. B. (1997). Explorative Spectrometric Evaluations of Frying Oil Deterioration. *Journal of the American Oil Chemists Society*, Vol.74, No.12, pp. 1495-1508, ISSN 1558-9331

Geladi P. (2003). Chemometrics in Spectroscopy. Part 1. Classical Chemometrics. *Spectrochimica Acta Part B*, Vol.58, No.5, pp. 767-782, ISSN 0584-8547

Glantz A. L. (1930). Fluorescence of Olive Oil Under Ultra-Violet Light. *Industrial & Engineering Chemistry Analytical Edition*, Vol.2, No.3, pp. 256-258, ISSN 0096-4484

Guilbault G. G. (1999). *Practical Fluorescence*, Marcel Dekker, ISBN 0824712633, New York

Guimet F.; Boque R. & Ferre J. (2004a). Cluster Analysis Applied to the Exploratory Analysis of Commercial Spanish Olive Oils by Means of Excitation-Emission Fluorescence Spectroscopy. *Journal of Agricultural and Food Chemistry*, Vol.52, No.22, pp. 6673-6679, ISSN 1618-2650

Guimet F.; Ferre J.; Boque R. & Rius F. X. (2004b). Application of Unfold Principal Component Analysis and Parallel Factor Analysis to the Exploratory Analysis of Olive Oils by Means of Excitation-Emission Matrix Fluorescence Spectroscopy. *Analytica Chimica Acta*, Vol.515, No.1, pp. 75-85, ISSN 0003-2670

Guimet F.; Ferre J. & Boque R. (2005a). Rapid Detection of Olive-Pomace Oil Adulteration in Extra Virgin Olive Oils From the Protected Denomination of Origin "Siurana" Using Excitation-Emission Fluorescence Spectroscopy and Three-Way Methods of Analysis. *Analytica Chimica Acta*, Vol.544, No.1-2, pp. 143-152, ISSN 0003-2670

Guimet F.; Boque R. & Ferre J. (2005b). Study of Oils From the Protected Denomination of Origin "Siurana" Using Excitation-Emission Fluorescence Spectroscopy and Three-Way Methods Of Analysis. *Grasas y Aceites*, Vol.56, No.4, pp. 292-297, ISSN 1988-4214

Guimet F.; Boque R. & Ferre J. (2006). Application of Non-Negative Matrix Factorization Combined With Fisher's Linear Discriminant Analysis for Classification of Olive Oil Excitation-Emission Fluorescence Spectra. *Chemometrics and Intelligent Laboratory Systems*, Vol.81, No.4, pp. 94-106, ISSN 0169-7439

Guimet F.; Ferre J.; Boque R.; Vidal M. & Garcia J. (2005c). Excitation-Emission Fluorescence Spectroscopy Combined with Three-Way Methods of Analysis as a Complementary Technique for Olive Oil Characterization. *Journal of Agricultural and Food Chemistry*, Vol.53, No.24, pp. 9319-9328, ISSN 1618-2650

Karoui R. & Blecker Ch. (2011). Fluorescence Spectroscopy Measurement for Quality Assessment of Food Systems—A Review. *Food and Bioprocess Technology*. Vol.4, No.3, pp. 364-386, ISSN 1935-5149

Kyriakidis N. B. & Skarkalis P. (2000). Fluorescence Spectra Measurement of Olive Oil and Other Vegetable Oils. *Journal of AOAC International*, Vol.83, No.24, pp. 1435-1439, ISSN 1944-7922

Lakowicz J. R. (2006). *Principles of Fluorescence Spectroscopy*, Third edn. Kluwer Academic/Plenum Publishers, ISBN 0387312781, New York

Lloyd J. B. F. (1971). Synchronized Excitation of Fluorescence Emission Spectra. *Nature (London) Physical Science*, Vol.231, pp. 64-65, ISSN 1476-4687

Navarra G.; Cannas M.; D'Amico M.; Giacomazza D.; Militello V.; Vaccaro L. & Leon M. (2011). Thermal Oxidative Process in Extra-Virgin Olive Oils Studied by FTIR, Rheology and Time-Resolved Luminescence, *Food Chemistry*, Vol.126, No.3, pp. 1226-1231, ISSN 0308-8146

Ndou T. & Warner I. M. (1991). Applications of Multidimensional Absorption and Luminescence Spectroscopies in Analytical Chemistry. *Chemical Reviews*, Vol.91, No.4, pp. 493-507, ISSN 1520-6890

Papoti V. T. & Tsimidou M. Z. (2009). Looking Through the Qualities of a Fluorimetric Assay for the Total Phenol Content Estimation in Virgin Olive Oil, Olive Fruit or Leaf Polar Extract, *Food Chemistry*, Vol.112, No.1, pp. 246-252, ISSN 0308-8146

Pollok, D. & Melchert, H. U. (2004). Determination of alpha-Tocopherolquinone in Human Serum Samples by Liquid Chromatography with Fluorescence Detection and On-Line Post-Column Derivatization. *Journal of Chromatography* A, Vol.1056, No. 1-2, pp. 257-262, ISSN 0021-9673

Poulli K. I.; Mousdis G. A. & Georgiou C. A. (2005). Classification of Edible and Lampante Virgin Olive Oil Based on Synchronous Fluorescence and Total Luminescence Spectroscopy. *Analytica Chimica Acta*, Vol.542, No.2, pp. 151-156, ISSN 0003-2670

Poulli K.I.; Mousdis G. A. & Georgiou C. A. (2006). Synchronous Fluorescence Spectroscopy for Quantitative Determination of Virgin Olive Oil Adulteration with Sunflower Oil. *Analytical and Bioanalytical Chemistry*. Vol.386, No.5, pp. 1571-1575, ISSN 1618-2650

Poulli K. I.; Mousdis G. A. & Georgiou C. A. (2007). Rapid Synchronous Fluorescence Method for Virgin Olive Oil Adulteration Assessment. *Food Chemistry*, Vol.105, No.1, pp. 369-375, ISSN 0308-8146

Poulli K. I.; Chantzos N. V.; Mousdis G. A. & Georgiou C. A. (2009a). Synchronous Fluorescence Spectroscopy: Tool for Monitoring Thermally Stressed Edible Oils. *Journal of Agricultural and Food Chemistry*, Vol.57, No.18, pp. 8194-8201, ISSN 1520-5118

Poulli K. I.; Mousdis G. A. & Georgiou C. A. (2009b). Monitoring Olive Oil Oxidation Under Thermal and UV Stress Through Synchronous Fluorescence Spectroscopy and Classical Assays, *Food Chemistry*, Vol.117, No.3, pp. 499-503, ISSN 0308-8146

Sadecka J. & Tothova J. (2007). Fluorescence Spectroscopy and Chemometrics in the Food Classification - A Review. *Czech Journal of Food Sciences*, Vol.25, No.4, pp. 159-173, ISSN 1212-1800

Sayago A.; Morales M.T. & Aparicio R. (2004). Detection of Hazelnut Oil in Virgin Olive Oil by a Spectrofluorimetric Method. *European Food Research and Technology*, Vol.218, No.5, pp. 480-483, ISSN 1438-2385

Sayago A.; Garcia-Gonzalez D. L.; Morales M.T. & Aparicio R. (2007). Detection of the Presence of Refined Hazelnut Oil in Refined Olive Oil by Fluorescence Spectroscopy. *Journal of Agricultural and Food Chemistry*, Vol.55, No.6, pp. 2068-2071, ISSN 1520-5118

Scott S. M.; James D.; Ali Z.; O'Hare W. T. & Rowell F. J. (2003). Total Luminescence Spectroscopy With Pattern Recognition for Classification of Edible Oils. *Analyst*, Vol.128, No.7, pp. 966-973, ISSN 0003-2654

Servili M.; Selvaggini R.; Esposto S.; Taticchi A.; Montedoro G. & Morozzi G. (2004). Health and Sensory Properties of Virgin Olive Oil Hydrophilic Phenols: Agronomic and Technological Aspects of Production That Affect Their Occurrence in the Oil. *Journal of Chromatography* A, Vol.1054, No.1-2, pp. 113-127, ISSN 0021-9673

Sidney M. S. & Willoughby C. E. (1929). Olive Oil Analytical Method. Part II The Use of the Ultraviolet Ray in the Detection of Refined in Virgin Olive Oil, *Journal of the American Oil Chemists' Society*, Vol.6, No.8, pp. 15-16, ISSN 0003-021X

Sikorska E.; Romaniuk A.; Khmelinskii I. V.; Herance R.; Bourdelande J. L.; Sikorski M. & Kozioł J. (2004). Characterization of Edible Oils Using Total Luminescence Spectroscopy. *Journal of Fluorescence*, Vol.14, No.1, pp. 25-35, ISSN 1573-4994

Sikorska E.; Górecki T.; Khmelinskii I. V.; Sikorski M. & Kozioł J. (2005a). Classification of Edible Oils Using Synchronous Scanning Fluorescence Spectroscopy. *Food Chemistry*, Vol.89, No.2, pp. 217-225, ISSN 0308-8146

Sikorska E.; Gliszczyńska-Świgło A.; Khmelinskii I. & Sikorski M. (2005b). Synchronous Fluorescence Spectroscopy of Edible Vegetable Oils. Quantification of Tocopherols. *Journal of Agricultural and Food Chemistry*, Vol.53, No.18, pp. 6988-6994, ISSN 1520-5118

Sikorska E. (2008a). Metody fluorescencyjne w badniach żywności, AE Poznań. ISBN 978-83-7417-360-5, Poznań, Poland

Sikorska E.; Khmelinskii I. V.; Sikorski M.; Caponio F.; Bilancia M. T.; Pasqualone A. & Gomes T. (2008b). Fluorescence Spectroscopy in Monitoring of Extra Virgin Olive Oil During Storage. *International Journal of Food Science and Technology*, Vol.43, No.1-2, pp. 52-61, ISSN 1365-2621

Sikorska E.; Khmelinskii I. V. & Sikorski M. (2011). Unpublished results

Smyk B.; Amarowicz R.; Szabelski M.; Gryczynski I. & Gryczynski Z. (2009). Steady-State and Time-Resolved Fluorescence Studies of Stripped Borage Oil, *Analytica Chimica Acta*, Vol.646, No.1-2, pp. 85-89, ISSN 1520-5118

Tena N.; Garcia-Gonzalez D. L. & Aparicio R. (2009). Evaluation of Virgin Olive Oil Thermal Deterioration by Fluorescence Spectroscopy. *Journal of Agricultural and Food Chemistry*, Vol.57, No.22, pp. 10505-10511, ISSN 1520-5118

Undenfriend S. (1962). *Fluorescence Assay in Biology and Medicine*. Academic Press, New York

Ward K.; Scarth R.; Daun J. K. & Thorsteinson C. T. (1994). Comparison of High-Performance Liquid Chromatography and Spectrophotometry to Measure Chlorophyll in Canola Seed and Oil. *Journal of the American Oil Chemists' Society*, Vol.71, No.9, pp. 931-934, ISSN 0003-021X

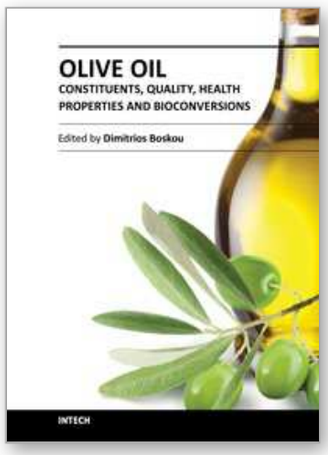
Weber G. (1961). Enumeration of Components in Complex Systems by Fluorescence Spectrophotometry. *Nature*, Vol.190, No.4770, pp. 27-29, ISSN 1476-4687

Wolfbeis O. S. & Leiner M. (1984). Characterization of Edible Oils via 2D-Fluorescence, *Mikrochimica Acta*, Vol.1, pp. 221-233, ISSN 0026-3672

Zandomeneghi M.; Carbonaro L. & Caffarata C. (2005). Fluorescence of Vegetable Oils: Olive Oils. *Journal of Agricultural and Food Chemistry*, Vol.53, No.3, pp. 759-766, ISSN 1520-5118

Zandomeneghi, M. & Zandomeneghi, G. (2005). Comment on Cluster Analysis Applied to the Exploratory Analysis of Commercial Spanish Olive Oils by Means of Excitation-Emission Fluorescence Spectroscopy. *Journal of Agricultural and Food Chemistry*, Vol.53, No.14, pp. 5829-5830, ISSN 1520-5118

Zandomeneghi M.; Carbonaro L. & Zandomeneghi G. (2006). Comment on Excitation-Emission Fluorescence Spectroscopy Combined with Three-Way Methods of Analysis as a Complementary Technique for Olive Oil Characterization. *Journal of Agricultural and Food Chemistry*, Vol.54, No.14, pp. 5214-5215, ISSN 1520-5118



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The health-promoting effects attributed to olive oil, and the development of the olive oil industry have intensified the quest for new information, stimulating wide areas of research. This book is a source of recently accumulated information. It covers a broad range of topics from chemistry, technology, and quality assessment, to bioavailability and function of important molecules, recovery of bioactive compounds, preparation of olive oil-based functional products, and identification of novel pharmacological targets for the prevention and treatment of certain diseases.

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