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Classification of edible oils using synchronous scanning fluorescence spectroscopy

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Abstract

Total luminescence and synchronous scanning fluorescence spectroscopy techniques were tested as regards their ability to characterize and differentiate edible oils, including soybean, sunflower, rapeseed, peanut, olive, grapeseed, linseed and corn oils. Total luminescence spectra of all oils studied as *n*-hexane solutions exhibit an intense peak, which appears at 290 nm in excitation and 320 nm in emission, attributed to tocopherols. Some of the oils exhibit a second long-wavelength peak, appearing at 405 nm in excitation and 670 nm in emission, belonging to pigments of the chlorophyll group. Additional bands were present in the intermediate range of excitation and emission wavelengths in some oils, arising from unidentified compounds. Similarly, bands attributed to tocopherols, chlorophylls and unidentified fluorescent components were detected in the synchronous-scanning fluorescence spectra. Classification of oils based on their synchronous fluorescence spectra was performed using a non-parametrical k nearest neighbours method and linear discriminant analysis. Both methods provided very good discrimination between the oil classes with low classification error. The results presented demonstrate the capability of the fluorescence techniques for characterizing and differentiating vegetable oils.

1. Introduction

Fats and oils constitute one of the major categories of food products, as they contain many nutrients. There has been great interest in studying the chemical composition of oils, since such information is valuable for the assessment of oil quality. As well as the major components, tri-fatty acid esters of glycerol, vegetable oils contain about 2–5% of minor compounds in a wide range of chemical classes. These compounds have a marked influence on the oil quality. For instance, tocopherols and carotenoids affect the oxidative stability of oils, whereas chlorophylls are responsible for oil photooxidation (deMan, 1999).

Gas and liquid chromatography are the techniques that have been widely used for oil analysis (Cert, Moreda, & Perez-Camino, 2000). Chromatographic techniques are highly selective and have low detection limits for many relevant compounds; however, they often involve extraction and preconcentration, which make the overall analysis time-consuming. Therefore, chromatographic methods cannot be used for real-time in situ analysis. Moreover, chromatographic methods are expensive, as they require solvents of high purity for analytic separation and sample extraction. Spectroscopic methods present an alternative to chromatography, which can be applied quickly and inexpensively. The main disadvantage of spectroscopic methods is their lack of selectivity; hence, the data on qualitative and quantitative composition often have to be extracted using advanced multivariate procedures. Pattern recognition routines are used for qualitative analysis, based

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either on statistical methods or on artificial neural networks (Bro et al., 2002).

A number of applications of spectroscopic methods in food analysis have been reported. Infrared spectroscopic techniques, such as near infrared spectroscopy and mid IR spectroscopy, have been used for testing authenticity of foods, such as vegetable oils (Lai, Kemsley, & Wilson, 1994), olive oil (Bertran et al., 1999; Downey, McIntyre, & Davies, 2003), coffee (Downey, Briandet, Wilson, & Kemsley, 1997), honey (Qiu, Ding, Tang, & Xu, 1999), orange juice (Towmey, Downey, & McNulty, 1995), milk (Woo et al., 2002), meat (Yang & Irudayaraj, 2001) and some others (Tzouros & Arvanitoyannis, 2001).

Fluorescence spectroscopy is one of the most promising techniques of increasing importance for complex food analysis. Among the benefits of fluorescence spectroscopy is its enhanced selectivity as compared to others spectroscopic methods, the high sensitivity to a wide array of potential analytes and, in general, the avoidance of consumable reagents and of extensive sample pre-treatment (Oldham, McCarroll, McGown, & Warner, 2000). However, conventional fluorescence techniques, relying on measurements of single emission or excitation spectra, are often insufficient in the analysis of complex systems. In such cases, total luminescence or synchronous scanning fluorescence techniques may improve the analytic potential of fluorescence measurements (Ndou & Warner, 1991).

Total luminescence spectroscopy (TLS) involves simultaneous acquisition of multiple excitation and emission wavelengths in order to increase the method selectivity. The resulting emission-excitation data matrix (EEM) provides a total intensity profile of the sample over the range of excitation and emission wavelengths scanned (Ndou & Warner, 1991). Synchronous fluorescence spectrometry takes advantage of the ability to vary both the excitation and emission wavelengths during the analysis. In this method, excitation and emission monochromators are scanned simultaneously, synchronized so that a constant wavelength difference is maintained between the two (Ndou & Warner, 1991). Both these techniques have been successfully used in the analysis of crude oils, pharmaceuticals and polycyclic aromatic hydrocarbons, to characterize motor oils, and to characterize, differentiate and classify natural organic matter, such as humic matter (Guiteras, Beltran, & Ferrer, 1998; Patra & Mishra, 2002; Persson & Wedborg, 2001; Ndou & Warner, 1991). Several papers have reported the potential of fluorescence in the analysis of food products. Fluorescence spectroscopy was used for monitoring coloured precursors in the sugar industry (Baunsgaard, Andersson, Arndal, & Munck, 2000; Baunsgaard, Norgaard, & Godshall, 2000; Bro, 1999; Bro et al., 2002), analysis of fish oil (Pedersen, Munck, & Engelsen, 2002), flour, soap and frying oil (Engelsen, 1997). A few articles have explored the possibility of application of fluorescence methods to the analysis of vegetable oils (Engelsen, 1997; Kyriakidis & Skarkalis, 2000; Giungato, Notarnicola, & Colucci, 2002).

We have recently applied total fluorescence spectroscopy and synchronous fluorescence spectroscopy for characterization of various kinds of edible oils (Sikorska et al., 2003). The present paper continues our effort to explore the possibility of application of fluorescence methods in analysis of vegetable oils. Total fluorescence and synchronous scanning fluorescence techniques were used for characterization of commercially available vegetable oils. The objective of the present study was to investigate intrinsic fluorophores of edible oils in order to discriminate between eight different types of oils. The samples were discriminated by applying statistical methods to the synchronous fluorescence spectra of oils.

2. Materials and methods

2.1. Materials

The studies were performed on eight commercially available edible oils, including soybean, sunflower, rapeseed, peanut, olive, grapeseed, linseed and corn oils. The linseed oil was obtained at a local oil manufacturer; other oils were acquired in a supermarket and had expiry dates exceeding the maximum duration of the experiments.

α-Tocopherol (97%), *n*-hexane and acetone were purchased from Aldrich. Bacteriopheophytin *c* was a gift from Prof. D. Frąckowiak, Poznań University of Technology.

2.2. Fluorescence measurements

Fluorescence spectra were obtained on a Fluorolog 3-11 spectrofluorometer, Spex-Jobin Yvon S.A. A Xenon lamp source was used for excitation. Excitation and emission slit widths were 2 nm. The acquisition interval and the integration time were maintained at 1 nm and 0.1 s, respectively. A reference photodiode detector, at the excitation monochromator stage, compensated for the source intensity fluctuations. Individual spectra were corrected for the wavelength response of the system. Right-angle geometry was used for oil samples diluted in *n*-hexane (1% v/v) in a 10 mm fused-quartz cuvette.

Three-dimensional spectra were obtained by measuring the emission spectra, in the range 290–700 nm, repeatedly, at excitation wavelengths from 250 to 450 nm, spaced by 5 nm intervals in the excitation domain. Fully corrected spectra were then concatenated into an excitation-emission matrix.

Three-dimensional plots and contour maps of total luminescence spectra were produced using the DataMax

Grams/32 programme. All contour maps were plotted using the same scale range of fluorescence intensities (0–2×10^7 intensity units) and number of contours. The data along the Z-axis were interpolated to improve appearance.

The synchronous fluorescence spectra were collected by simultaneously scanning the excitation and emission monochromator in the 250–700 nm range, with constant wavelength differences, $\Delta\lambda$, between them. Four spectra were recorded for each sample, with $\Delta\lambda$ of 10, 30, 60, 80 nm. Fluorescence intensities were plotted as function of the excitation wavelength.

2.3. Statistical methods

Samples from four bottles were analyzed for each of the oils in triplicate, giving twelve independent measurements. Four synchronous fluorescence spectra with different $\Delta\lambda$ (10, 30, 60, 80 nm) were recorded. As a result, a total of 96 synchronous fluorescence spectra were recorded at each of the $\Delta\lambda$ values. Two methods of discriminant analysis were used for the purpose of multiple group classification: nearest neighbours method (kNN) (Wu & Massart, 1997) and linear discriminant analysis (LDA) (Kemsley, 1996; Roggo, Duponchel, Ruckebusch, & Huvenne, 2003).

The k-nearest neighbours is a well-known non-parametric classification method (Wu & Massart, 1997). The principle of this method is that the test object is assigned to the cluster which is the most represented in the set of k nearest training objects. For each data point, we search for the closest data points, called "nearest neighbours" and decide, according to the values of these neighbours. kNN is one of the simplest learning techniques – the learner only needs to store the examples, while the classifier does its work, by observing the examples most similar to the one to be classified. The k values were chosen in the range of $k = 1, \ldots, 10$ for classification purposes, due to the size of our sample set, which was too small for larger values of k to be meaningful. This non-parametrical method was chosen because popular parametrical methods, like LDA and quadratic discriminant analysis, are often unsuitable for datasets with number of variables higher than the number of objects, due to either the observation matrix singularity, or non-normality of the data set. The nearest neighbours method allows us to perform the analysis using entire spectra, without any reduction of the data sets.

Additionally, LDA was performed on simplified data sets. For this purpose, six wavelengths were extracted from the synchronous spectrum recorded at a particular $\Delta\lambda$ and analyzed. The LDA method provided good results, while being very straightforward in calculation and interpretation. Discriminant coordinates were found for the purpose of graphical presentation, with the two principal discriminant coordinates used in the plots.

The bootstrap method was used to estimate the classification error. In this method the data set was randomly split in two independent sets: training and test. The training set was used to construct the rule and the test set to test it. This procedure was repeated many times. The version 0.632+ of this method was applied, which has small bias and variance values, and 50 bootstrap replications were performed, which turned out to be sufficient; additional bootstrap replications caused no further change of the classification error estimate (Efron, 1983; Efron & Tibshirani, 1997).

All the statistical procedures were implemented in Matlab 6.5.

3. Results and discussion

3.1. Total luminescence spectra

Total luminescence spectra were recorded for oils dissolved in n-hexane. A low concentration of samples, 1% v/v, was chosen to avoid spectral distortions, which may occur in concentrated oils. Fig. 1 shows contour maps of their luminescence, constructed in such a way that one axis represents the emission and another the excitation wavelength, while the contours are plotted by linking points of equal fluorescence intensity. Such a two-dimensional representation often facilitates the qualitative analysis of the fluorescence patterns.

The spectra shown in Fig. 1, are in general very similar to those obtained previously for a different set of oils. Assignment of emission bands to the specific chemical components, based on comparison of three-dimensional and single excitation and emission spectra with the spectra of the respective reference compounds, was described in detail previously (Sikorska et al., 2003).

The relatively intense band, observed for each of the oils studied, with excitation in the range of about 270–310 nm and emission in the range of about 300–350 nm, has been ascribed to the emission of tocopherols and tocotrienols (Sikorska et al., 2003). The long-wavelength band, at 350–420 nm in excitation and 660–700 nm in emission, present in olive oil (very low intensity) and linseed oil, is characteristic of the fluorescence of pigments of the chlorophyll group, which includes chlorophylls a and b and pheophytins a and b (Sikorska et al., 2003). Tocopherols (α, β, γ and δ) are present in oils in variable amounts, from 70 to 1900 mg/kg, depending on the type of oil (deMan, 1999). Pigments of the chlorophyll group occur mainly in crude oils obtained directly by the extraction of oilseed, and are subsequently removed during processing (deMan, 1999).

The spectra of the oils reveal the presence of an additional emission band in the intermediate range, at about

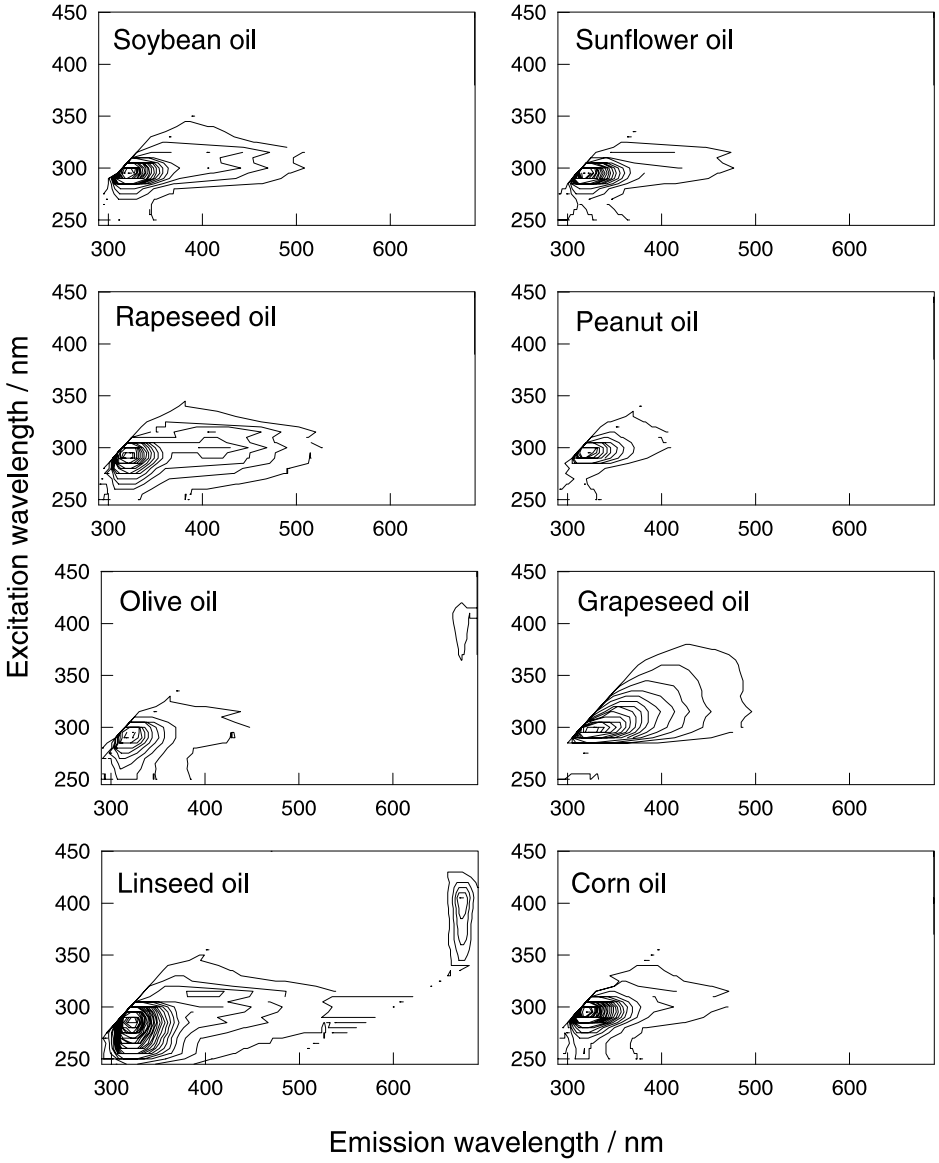

Fig. 1. Contour maps of total luminescence of edible oils diluted in *n*-hexane, 1% v/v.

400–450 nm. The shape and intensity of these intermediate emissions varies from one oil to another; hence further studies are needed to identify the respective fluorophores. The single excitation spectra measured at the emission wavelength of 410 nm for different oils, despite their similarity, exhibit variability (Fig. 2). The analysis of these spectra shows that chemical compounds responsible for this emission in different oils may belong to the same class, although their chemical structure may vary slightly to cause the variations observed.

Apart from qualitative distinctions, the oils studied also vary in fluorescence intensities of the particular components. For instance, a relatively intense tocopherol emission is observed for corn, soybean, sunflower and linseed oils, an intermediately intense one – for rapeseed oil and a weak one for grapeseed, olive

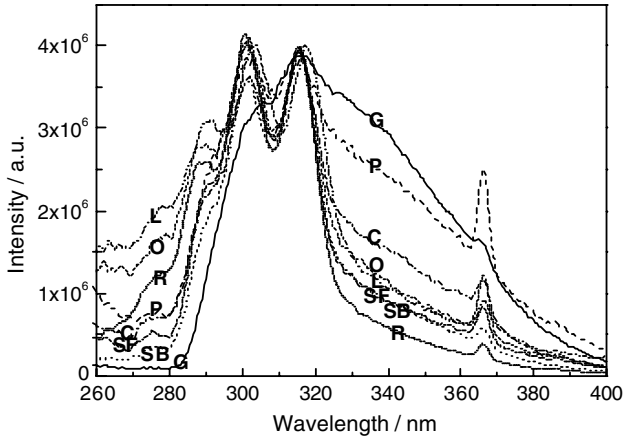

Fig. 2. Excitation spectra of edible oils, $\lambda_{em} = 410$ nm, diluted in *n*-hexane, 1% v/v: SB – soybean, SF – sunflower, R – rapeseed, P – peanut, O – olive, G – grapeseed, L – linseed and C – corn oils.

and peanut oils. These differences may correlate with the variation in tocopherol contents of the respective oils; however, due to the complexity of the system studied, any quantitative predictions require further investigation.

The total luminescence spectra of oils recorded presently and in our previous study (Sikorska et al., 2003) are in fact very similar to those reported for virgin and refined olive oils in isooctane by Giungato et al. (2002). They found an emission band with the excitation maximum at 285 nm and the emission maximum at about 315 nm for virgin olive oils, attributed to tocopherols, and a second band with the excitation at 410 nm and emission at 669 nm, ascribed to the chlorophyll emission. For refined oils, the tocopherol band was observed with excitation and emission maxima at 295 and 331 nm, respectively, while the chlorophyll emission was undetectable. Another emission band was observed for this oil with two excitation maxima at 300 and 315 nm and an emission maximum at 406 nm, the origin of this emission remaining uncertain.

Characterization and identification of individual fluorescent components, although desirable, is dispensable for the purpose of overall characterization and differentiation of oil samples based on their fluorescence properties. Evaluation of differences in total spectral characteristics of the samples is a viable alternative to the sample discrimination based on qualitative and quantitative analysis of individual components. This total spectral characterization approach will be used in the present paper for the oil sample discrimination.

Total luminescence spectroscopy can be used for fingerprinting of the respective oils, and may, for example, allow oil identification and quality monitoring; however, it is a time-consuming method. Acquisition of contour maps of sufficient resolution, using conventional spectrofluorometers, requires a large number of emission scans for each sample. The analysis may be speeded up with CCD or video-spectrofluorometers; however, such instruments are not widely accessible in laboratories (Guilbault, 1999). Thus, for analytical purposes, the synchronous fluorescence techniques may be used instead of TLS.

3.2. Synchronous fluorescence scan

Another techniques, of great potential in analysis of mixtures of fluorescent compounds, is synchronous scanning fluorescence spectroscopy. In this method both excitation and emission characteristics are included into the spectrum by simultaneous scanning excitation and emission wavelength at a constant difference between them. As a result, the selectivity for individual components is considerably improved; additionally, much more information on mixtures of fluorescent compounds is gained. The synchronous fluorescence scanning method is a very simple and effective means of obtaining data for several compounds present in a mixture in a single scan (Guilbault, 1999; Ndou & Warner, 1991).

As we have already seen, the main fluorescent components identified in the present and previous studies of oils are compounds of the tocopherol and chlorophyll groups. Fig. 3 shows the synchronous fluorescence spectra of the representatives of these two groups: bacteriopheophytin *c* and for α-tocopherol, along with its excitation and emission spectra.

As is evident from Fig. 3(a), the shape and intensity of synchronous spectra depend on the difference between the excitation and emission wavelengths $\Delta\lambda$, which defines the overlap of the absorption and emission bands. For the lowest $\Delta\lambda = 10$ nm, an effective bandwidth reduction is observed as compared to the emission band, with the synchronous spectrum consisting of a single narrow band with a maximum at 301 nm. At higher $\Delta\lambda$ values, the maximum of the synchronous spectrum is shifted to the blue and broadened, with

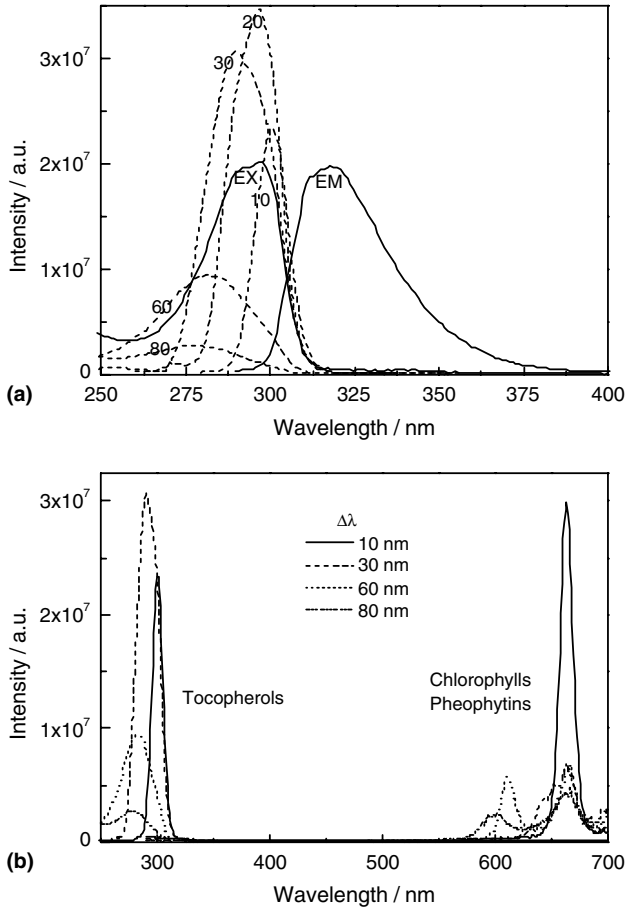

Fig. 3. (a) Excitation ($\lambda_{em} = 325$ nm), emission ($\lambda_{ex} = 295$ nm) and synchronous fluorescence spectra ($\Delta\lambda = 10$, 20, 30, 60, and 80 nm) of α-tocopherol in *n*-hexane. (b) Comparison of synchronous scanning fluorescence spectra of α-tocopherol in *n*-hexane and bacteriopheophytin *c* in acetone, ($\Delta\lambda = 10$, 30, 60, and 80 nm).

additional fluorescence intensity changes. The highest intensity is observed for $\Delta\lambda = 20$ nm, which corresponds to the Stokes shift for α-tocopherol in *n*-hexane. A further increase of $\Delta\lambda$ results in a lower band intensity. A single band was present in the synchronous spectrum of α-tocopherol for each of the $\Delta\lambda$ values tested.

Fig. 3(b) presents the synchronous fluorescence spectra of bacteriopheophytin *c* compared to those of α-tocopherol. The bacteriopheophytin *c* differs in structure from pheophytins and chlorophylls *a* and *b*, in that the pyrrole ring IV is not reduced, and the position 17 is esterified by an acrylic residue – instead of a propionic group, the terminal carboxylic group being generally not esterified (Schoefs, 2003). However, the absorption and emission spectra of the pigments of chlorophylls group are very similar; thus the respective spectra of bacteriopheophytin *c* are shown for the sake of comparison. For $\Delta\lambda = 10$ nm, a sharp intense band exists in the synchronous spectrum with a maximum at 664 nm. The band intensity decreases at higher $\Delta\lambda$. The spectra measured for $\Delta\lambda = 60$ and 80 nm exhibit two bands with relatively low intensities at 665, 610 nm and 665, 603 nm, respectively.

The synchronous fluorescence spectra of oils diluted in *n*-hexane, for eight oils studied, are shown in Fig. 4.

The synchronous fluorescence spectra obtained, with a small $\Delta\lambda = 10$ nm, show an effective bandwidth reduction, resulting in spectral simplification. These spectra have a major band with a maximum at around 300 nm for all the oils.

For $\Delta\lambda = 30$ nm, the short-wavelength emission band is broadened and its maximum is shifted to the blue by about 5 nm, for all the oils studied. Simultaneously, an increase of fluorescence intensity is observed. The fluorescence intensity is again reduced at still higher $\Delta\lambda$ values (60, 80 nm), accompanied by further broadening of the emission band. Based on similarity of position, shape and intensity of synchronous fluorescence scans observed for oils and for pure α-tocopherol in *n*-hexane, the short-wavelength emission band can be attributed to fluorescence of tocopherols, supporting the previous identification. However, for α-tocopherol a single band

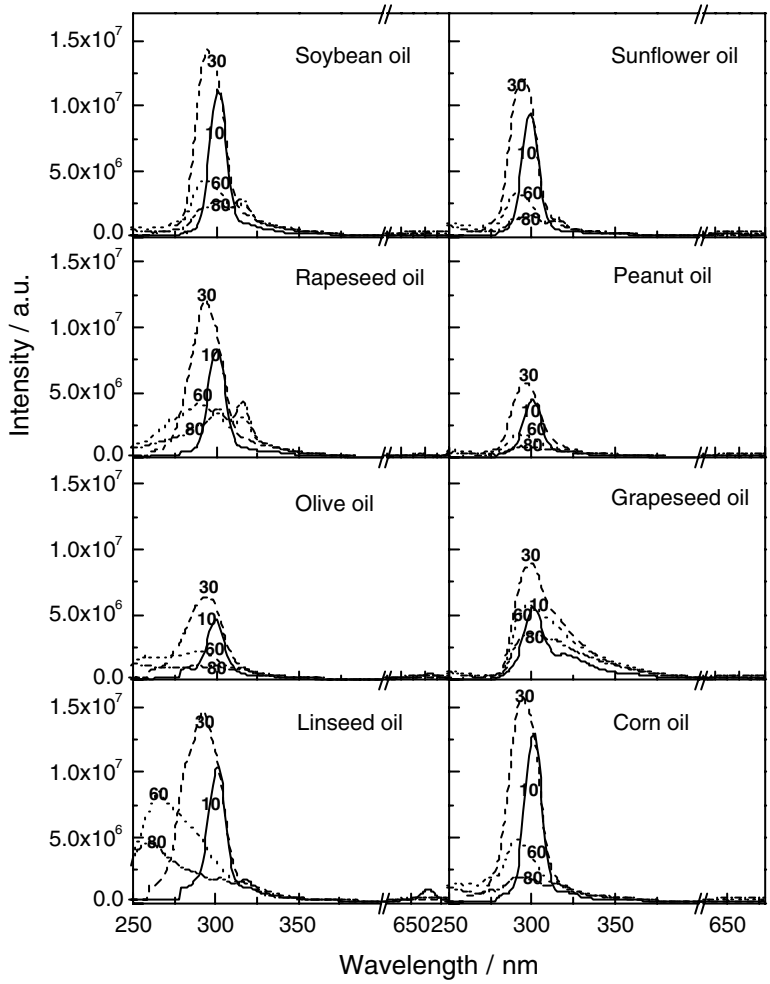

Fig. 4. Synchronous scan fluorescence spectra of edible oils in *n*-hexane, dilution 1%, v/v; the spectral curves are marked by the values of $\Delta\lambda = 10$, 30, 60, and 80 nm.

is present even at the largest $\Delta\lambda = 80$ nm, while in the synchronous fluorescence spectra of oils a new weak band appears at higher $\Delta\lambda$ with the maximum at about 317–319 nm, depending on the oil. This observation supports an earlier conclusion that fluorescent compounds different from tocopherols are present in vegetable oils. Moreover, a comparison of synchronous spectra, particularly of those recorded at larger $\Delta\lambda$ values, reveals some differences, which may indicate the presence of other fluorophores or at least of a different tocopherol species in the studied oils.

An additional weak, long-wavelength band with a maximum at 666 nm is observed for linseed oil, ascribed to the pigments of the chlorophyll group. The presence of chlorophylls or pheophytins in this oil is also evident from their three-dimensional spectra. This band is practically absent in synchronous fluorescence spectra recorded at larger $\Delta\lambda$, due to successive intensity reduction, as may be concluded by comparison with the bacteriopheophytin *c* synchronous spectra.

The spectral profiles of synchronous fluorescence spectra of different oils vary significantly between different oils samples. The spectral pattern, for each of the oils, depends on excitation and emission profiles of fluorescent components, and thus is unique for each sample (mixture). As a result, a synchronous spectrum contains more information than an ordinary excitation or emission spectrum. The synchronous fluorescence spectrum thereby becomes a signature or spectral fingerprint of the particular oil sample. Such spectra may be used, for example, in qualitative analysis, for oil identification or authentication purposes. However, for such applications a visual comparison of spectra could be insufficient and pattern recognition methods should be used.

3.3. Classification of oils using synchronous fluorescence spectra

In order to compare the set of synchronous fluorescence spectra of different oils, statistical analysis was performed. The ability of the fluorescence data to discriminate different kinds of oils was investigated by applying two statistical methods. The *k* nearest neighbours (*k*NN) method was applied using the entire spectra as input, while the linear discrimination method used selected excitation/emission wavelength pairs as input.

Table 1 gives results of the *k*NN method. The discrimination between different oils was very good, with a very low classification error of 1–2% and low SD values. The best discrimination was achieved using $k = 3$ for the spectra recorded at $\Delta\lambda = 30$, 60 or 80 nm.

Next, LDA was applied for selected wavelengths from synchronous spectra. Such an analysis, although simplified and limited to only six excitation/emission wavelength pairs, gave a satisfactory separation between different oils. The low error and SD values presented in Table 2 illustrate that all the oils were clearly discriminated by this method. The best separation was achieved at $\Delta\lambda = 30$ nm. The fraction of correct classification was in the range of 99%. These results show that complete synchronous spectra may not be needed to discriminate between the oil classes. Instead, fluorescence intensity could be measured at selected excitation/emission wavelengths, and then be subject to discriminant analysis.

The results of the LDA analysis can be visualized by plotting them on a plane, see Fig. 5. The maps defined by the two discriminant coordinates show that different $\Delta\lambda$ values gave different separations of the oil classes. At $\Delta\lambda = 10$ nm, various classes are located close to one to another, which could result from the simplification of

Table 1
Classification of edible oils based on entire synchronous scan fluorescence spectra using the *k* nearest neighbour method

k		$\Delta\lambda$			
		10 nm (%)	30 nm (%)	60 nm (%)	80 nm (%)
1	Error	1.00	0.89	1.11	1.04
	SD	1.38	1.27	1.39	1.40
2	Error	0.96	0.95	1.08	1.19
	SD	1.36	1.35	1.39	1.51
3	Error	1.09	0.85	0.95	0.91
	SD	1.39	1.23	1.34	1.36
4	Error	1.08	1.12	1.10	0.97
	SD	2.79	1.41	1.36	1.93
5	Error	1.27	1.09	1.03	1.33
	SD	1.43	1.45	1.35	1.57
6	Error	1.34	1.02	1.44	1.73
	SD	2.50	1.46	2.88	4.38
7	Error	1.43	1.35	1.12	1.40
	SD	3.83	3.16	2.54	4.47
8	Error	1.48	2.13	1.71	2.17
	SD	4.13	6.03	4.56	5.32
9	Error	1.27	2.40	1.17	1.56
	SD	2.92	6.93	1.48	3.85
10	Error	2.62	3.44	3.05	2.32
	SD	6.33	7.95	8.08	6.13

Table 2
Classification of edible oils using LDA for six selected excitation wavelengths[a]

	$\Delta\lambda$			
	10 nm (%)	30 nm (%)	60 nm (%)	80 nm (%)
Error	0.96	0.64	1.07	0.93
SD	1.36	1.07	1.37	1.45

[a] Excitation wavelengths from synchronous scanning fluorescence spectra used for LDA:
$\Delta\lambda = 10$ nm: 290, 301, 315, 322, 360 and 666 nm.
$\Delta\lambda = 30$ nm: 290, 295, 300, 315, 330 and 666 nm.
$\Delta\lambda = 60$ nm: 270, 285, 295, 310, 315 and 666 nm.
$\Delta\lambda = 80$ nm: 260, 290, 300, 310, 315 and 666 nm.

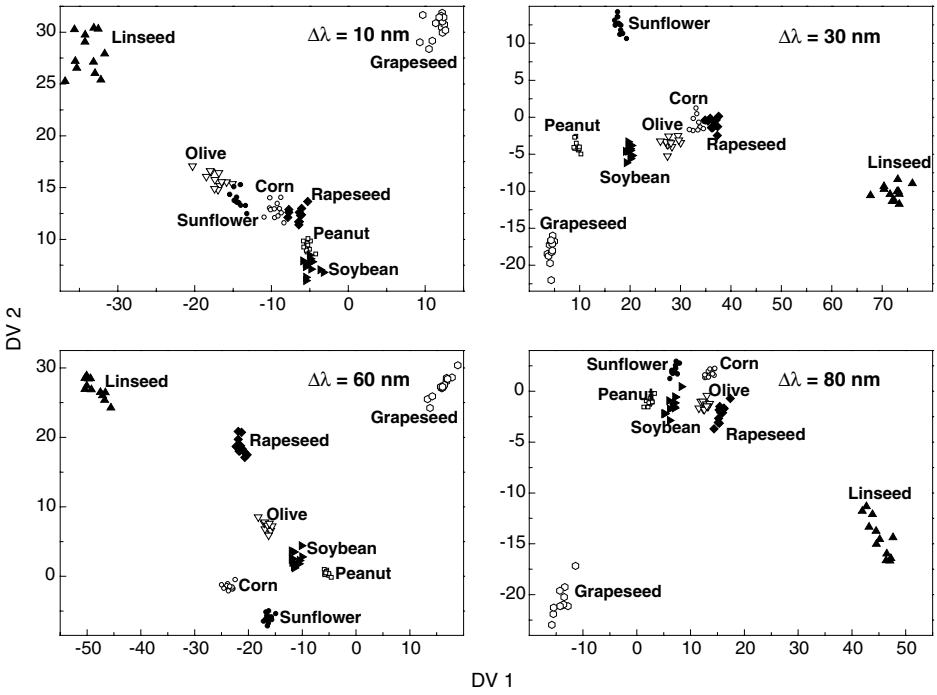

Fig. 5. Classification of edible oils using LDA.

the synchronous spectra recorded at this small difference between the excitation and emission wavelengths. The separation is improved at $\Delta\lambda = 30$ nm, with only corn and linseed oils still poorly spaced. For the $\Delta\lambda$ values of 60 and 80 nm the separation of all oils classes is good, the best being at $\Delta\lambda = 60$ nm. These changes are a consequence of changes in spectral characteristics at larger $\Delta\lambda$ values. At larger difference between the excitation and emission wavelengths, the synchronous spectra become more informative and vary more strongly between individual samples. Interestingly, the grapeseed and linseed oils are distinctly separated from the others, regardless of the $\Delta\lambda$ value.

Thus, we conclude that synchronous fluorescence spectra are efficient fingerprints, allowing identification of the edible oils. The synchronous spectra, complemented by appropriate statistical tools, may be used as an efficient method for edible oil discrimination. The present results demonstrate that oil discrimination is possible, at least in terms of the oil type. The potential sensitivity of the method for the brand and other origin indicators will be investigated in future.

4. Conclusions

Fluorescence emission spectroscopy was successfully used to characterize and discriminate the edible oil samples. Fluorescence measurements are simple and can be used to classify different types of oils. Several distinct spectral ranges, such as those corresponding to tocopherols and chloprophylls, could be identified in the spectra and used as markers for differentiation of oils with a high degree of accuracy.

It was proved that the synchronous fluorescence method gives a possibility of classifying different classes of edible oils using a single scan. It is quite possible that this approach can also be used for quantitative evaluation of fluorescent constituents after an appropriate calibration. Such quantitative assay of some fluorescent pigments may be of interest for analytical purposes, as their concentration gives useful indications about cultivars.

The present method, which analyzes a complex mixture without separation, is extremely useful from the practical point of view. Fluorescence provides high sensitivity, simplicity and selectivity and may serve as a complement to other spectroscopic techniques used in edible oil analysis.

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References

Baunsgaard, D., Andersson, C. A., Arndal, A., & Munck, L. (2000). Multi-way chemometics for mathematical separation of fluorescent

colorants and colour precursors from spectrofluorimetry of beet sugar and beet sugar thick juice as validated by HPLC analysis. *Food Chemistry, 70*, 113–121.

Baunsgaard, D., Norgaard, L., & Godshall, M. A. (2000). Fluorescence of raw cane sugars evaluated by chemometrics. *Journal of Agricultural and Food Chemistry, 48*, 4955–4962.

Bertran, E., Blanco, M., Coello, J., Iturriaga, H., Maspoch, S., & Montoliu, I. R. (1999). Determination of olive oil free fatty acid by Fourier transform infrared spectroscopy. *Journal of the American Oil Chemists Society, 76*, 611–616.

Bro, R. (1999). Exploratory study of sugar production using fluorescence spectroscopy and multi-way analysis. *Chemometrics and Intelligent Laboratory Systems, 46*, 133–147.

Bro, R., van den Berg, F., Thybo, A., Andersen, C. M., Jorgensen, B. M., & Andersen, H. (2002). Multivariate data analysis as a tool in advanced quality monitoring in the food production chain. *Trends in Food Science and Technology, 13*, 235–244.

Cert, A., Moreda, W., & Perez-Camino, M. C. (2000). Chromatographic analysis of minor constituents in vegetable oils. *Journal of Chromatography, 881*, 131–148.

deMan, J. M. (1999). *Principles of food chemistry*. New York: Kluwer Academic/Plenum Publishers.

Downey, G., Briandet, R., Wilson, R. H., & Kemsley, E. K. (1997). Near and mid-infrared spectroscopy in food authentication: Coffee varietal identification. *Journal of Agricultural and Food Chemistry, 45*, 4357–4361.

Downey, G., McIntyre, P., & Davies, A. N. (2003). Geographic classification of extra virgin olive oils from the eastern Mediterranean by chemometric analysis of visible and near-infrared spectroscopic data. *Applied Spectroscopy, 57*, 158–163.

Efron, B. (1983). Estimating the error rate of a prediction rule – Improvement on cross-validation. *Journal of the American Statistical Association, 78*, 316–331.

Efron, B., & Tibshirani, R. (1997). Improvements on cross-validation: The 632+ bootstrap method. *Journal of the American Statistical Association, 92*, 548–560.

Engelsen, S. B. (1997). Explorative spectrometric evaluations of frying oil deterioration. *Journal of the American Oil Chemists Society, 12*, 1495–1508.

Giungato, P., Notarnicola, L., & Colucci, L. (2002). Evaluation of fluorescence spectroscopy potential in edible oil analysis. In R. Zielinski (Ed.), *Current trends in commodity science* (pp. 513–518). Poznań: Poznań University of Economics Press.

Guilbault, G. G. (1999). *Practical fluorescence*. New York: Marcel Dekker.

Guiteras, J., Beltran, J. L., & Ferrer, R. (1998). Quantitative multicomponent analysis of polycyclic aromatic hydrocarbons in water samples. *Analytica Chimica Acta, 361*, 233–240.

Kemsley, E. K. (1996). Discriminant analysis of high-dimensional data: A comparison of principal components analysis and partial least squares data reduction methods. *Chemometrics and Intelligent Laboratory Systems, 33*, 47–61.

Kyriakidis, N. B., & Skarkalis, P. (2000). Fluorescence spectra measurement of olive oil and other vegetable oils. *Journal of AOAC International, 83*, 1435–1439.

Lai, Y. W., Kemsley, E. K., & Wilson, R. H. (1994). Potential of Fourier transform infrared spectroscopy for the authentication of vegetable oils. *Journal of Agricultural and Food Chemistry, 42*, 1159.

Ndou, T. T., & Warner, I. M. (1991). Applications of multidimensional absorption and luminescence spectroscopies in analytical-chemistry. *Chemical Reviews, 91*, 493–507.

Oldham, P. B., McCarroll, M. E., McGown, L. B., & Warner, I. M. (2000). Molecular fluorescence, phosphorescence, and chemiluminescence spectrometry. *Analytical Chemistry, 72*, 197R–209R.

Patra, D., & Mishra, A. K. (2002). Study of diesel fuel contamination by excitation emission matrix spectral substraction fluorescence. *Analytica Chimica Acta, 454*, 209–215.

Pedersen, D. K., Munck, L., & Engelsen, S. B. (2002). Screening for dioxin contamination in fish oil by PARAFAC and N-PLSR analysis of fluorescence landscapes. *Journal of Chemometrics, 16*, 451–460.

Persson, T., & Wedborg, M. (2001). Multivariate evaluation of the fluorescence of aquatic organic matter. *Analytica Chimica Acta, 434*, 179–192.

Qiu, P. U., Ding, H. B., Tang, Y. K., & Xu, R. J. (1999). Determination of chemical composition of commercial honey by near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry, 47*, 2760–2765.

Roggo, Y., Duponchel, L., Ruckebusch, C., & Huvenne, J. P. (2003). Statistical tests for comparison of quantitative and qualitative models developed with near infrared spectral data. *Journal of Molecular Structure, 654*, 253–262.

Schoefs, B. (2003). Chlorophyll and carotenoid analysis in food products. Properties of the pigments and methods of analysis. *Trends in Food Science and Technology, 13*, 361–371.

Sikorska, E., Romaniuk, A., Khmelinskii, I. V., Herance, R., Bourdelande, J. L., Sikorski, M., & Kozioł, J. (2003). Characterization of edible oils using total luminescence spectroscopy. *Journal of Fluorescence, 14*, 25–35.

Towmey, M., Downey, G., & McNulty, B. (1995). The potential of NIR spectroscopy for the detection of adulteration of orange juice. *Journal of the Science of Food and Agriculture, 67*, 77–84.

Tzouros, N. E., & Arvanitoyannis, I. S. (2001). Agricultural produces: Synopsis of employed quality control methods for the authentication of foods and application of chemometrics for the classification of foods according to their variety or geographical origin. *Critical Reviews in Food Science and Nutrition, 41*, 287–319.

Woo, Y. A., Terazawa, Y., Chen, J. Y., Iyo, C., Terada, F., & Kawano, S. (2002). Development of a new measurement unit (MilkSpec-1) for rapid determination of fat, lactose, and protein in raw milk using near-infrared transmittance spectroscopy. *Applied Spectroscopy, 56*, 599–604.

Wu, W., & Massart, D. L. (1997). Regularised nearest neighbour classification method for pattern recognition of near infrared spectra. *Analytica Chimica Acta, 349*, 253–261.

Yang, T., & Irudayaraj, J. (2001). Characterization of beef and pork using Fourier-transform infrared photoacoustic spectroscopy. *Food Science and Technology, 34*, 402–409.