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CHARACTERIZATION OF EDIBLE OILS USING SYNCHRONOUS SCANNING FLUORESCENCE SPECTROSCOPY

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Synchronous scanning fluorescent spectroscopy is explored as a tool for edible oil quality control and identification. Characteristic bands existing in the respective spectra and significant changes of these bands resulting from thermal oxidation and photo-oxidation of the oils confirm the potential of the method for the edible oil analysis. The method consists in monitoring and quantification of minor luminescent constituents, such as tocopherols, chlorophylls and polyphenols, and may be used for quality control, identification and manufacturing technology authentication of edible oils. Analysis of mixtures should be possible with suitable chemometric tools and appropriate calibration. High sensitivity of the method to oxidation is guaranteed by the inherent high sensitivity of luminescent techniques, and the anti-oxidant properties of tocopherols. The method does not require any separation steps.

INTRODUCTION

Current methodology for food analysis includes conventional separation methods, like gas and liquid chromatography [Cert *et al*., 2000]. These techniques enable qualitative and quantitative analysis of food components, however, they are expensive, time-consuming, and often require an appropriate sample pre-treatment step before the analysis. In recent years there has been a lot of research activity in developing new rapid methods for quality assessment of food products, which do not require any separations steps. Spectroscopic techniques are ideally suited for this purpose. The spectroscopic methods applied for food analysis include: mass spectrometry, infrared and near--infrared spectroscopy, Raman and nuclear magnetic resonance spectroscopy [Osborne, 2003]. Infrared spectroscopy in near- and mid-IR region has been used for exploring authenticity of foods such as vegetable oils [Lai *et al*., 1994], olive oil [Bertran *et al*., 1999], coffee [Downey *et al*., 1997], honey [Qiu *et al*., 1999], and orange juice [Towmey *et al*., 1995]. Spectroscopic methods are simple, cost-effective, rapid and non-destructive and could serve as a tool for detecting adulteration or for routine analysis, provided proper calibration and validation procedures with data acquisition protocols were established.

Several papers have discussed the potential of fluorescence in the analysis of food products [Baunsgaard *et al*., 2000a, b; Bro, 1999; Bro *et al*., 2002; Engelsen, 1997]. Fluorescence spectroscopy is a rapid, selective and relatively simple method for characterizing molecular environments, much more sensitive than most other spectroscopic methods, and may be performed directly on a food product. Multidimensional emission techniques, like total luminescence and synchronous scanning fluorescence spectroscopy, are particularly suitable for studies of complex food systems. Total luminescence spectroscopy implies collection of an excitation-emission matrix, giving total fluorescence characteristics of a sample [Ndou & Warner, 1991].

Synchronous fluorescence spectrometry has been described as a method to improve the selectivity of conventional luminescence spectrometry by taking full advantage of the ability to vary both the excitation and the emission wavelength during analysis. In this method, excitation and emission monochromators are scanned simultaneously, being synchronized so that a constant wavelength difference is maintained between the two [Gutierrez *et al*., 1987; Patra & Mishra, 2002]. Since its introduction by Lloyd [Lloyd, 1971], synchronous luminescence has been used in analysis of crude oils, pharmaceuticals and polycyclic aromatic hydrocarbons. Synchronous fluorescence scanning has been applied extensively to characterise, differentiate and classify natural organic matter, such as humic matter [Oldham *et al*., 2000; Warner *et al*., 1996].

Recently, we have shown the possibilities of application of total luminescence spectroscopy for characterization of vegetable oils and monitoring their changes during photo- and auto oxidation [Sikorska *et al*., 2003]. Vegetable oils are constituted predominantly by triacylglycerols (95-98%), containing other compounds of a wide range of chemical classes, among them tocopherols and chlorophylls, which due to their molecular structure (conjugated double bounds) are excellent fluorescent probes.

This article explores the possibility of application of the synchronous scanning fluorescence methods in the analysis of vegetable oils. To our best knowledge, there are no reports on the application of synchronous scanning technique to the analysis and fingerprinting of food samples.

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MATERIAL AND METHODS

Oils. The studies were performed on six commercially available edible oils, including: rapeseed 1 (refined), rapeseed 2 (produced by mechanical means), soybean, corn, olive and linseed oil. The linseed and rapeseed 2 oils were obtained at a local oil manufacturer; other oils were purchased in a supermarket and had expiry dates exceeding the maximum duration of the experiments.

Fluorescence measurements. Fluorescence spectra were obtained on a Fluorolog 3-11 Spex-Jobin Yvon spectrofluorometer. Xenon lamp source was used for excitation. Excitation and emission slit widths of 2 nm were used. 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 from 290 to 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.

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. Six spectra were recorded for each sample, with $\Delta\lambda$ changing from 10 nm to 60 nm with 10 nm intervals.

Oxidation of oils. In photo-oxidation experiments the samples of neat oils (20 cm^3) were placed in open glass vessels, and exposed directly to light from two tungsten lamps, 100 W each, for 176 h at 20°C.

In thermal oxidation experiments, the neat oil samples were kept at 200°C in darkness for 20 h.

RESULTS AND DISCUSSION

Total luminescence spectroscopy

Three-dimensional spectra, known as excitation-emission matrix or total luminescence spectra, in which one axis represents the excitation wavelength, the second – the emission wavelength and the third – the intensity, were measured for oil samples diluted in n-hexane. Typical spectra are shown in Figure 1.

These spectra give a complete description of the fluorescent components of the mixture and exhibit different features for various oils. Assignment of emission bands to the specific chemical components was based on an analysis of the respective excitation and emission fluorescence spectra. A relatively intense band, observed in every oil studied, with excitation in the region of about 270–310 nm and emission in the region of about 300–350 nm was ascribed to tocopherols and tocotrienols. A long-wavelength band, at 350–420 nm in excitation and 660–700 nm in emission, present in olive oil and linseed oil, is characteristic for fluorescence of the chlorophyll group pigments. This group includes chlorophylls *a* and *b* and pheophytins *a* and *b*. The spectra of oils reveal an additional emission band in the intermediate region, at about 400–450 nm. The shape and intensity of this intermediate emission vary for different oils.

The total luminescence spectrum may be used as a fingerprint of the respective oil, and may for example allow oil identification and quality control. However, acquisition of contour maps of sufficient resolution, using conventional spectrofluorometers, is time-consuming, as it requires a large number of conventional emission scans for each sample. An alternative, much faster way to measure the total luminescence is by using CCD cameras or video fluorometers.

Synchronous scanning fluorescence spectroscopy

Another way to analyze the complex mixture of fluorophores is to apply the synchronous scanning fluorescence spectroscopy. The idea of synchronous scanning may be visualized on a three-dimensional spectrum. Synchronous spectrum represents an intensity profile along a 45° section through the excitation-emis-

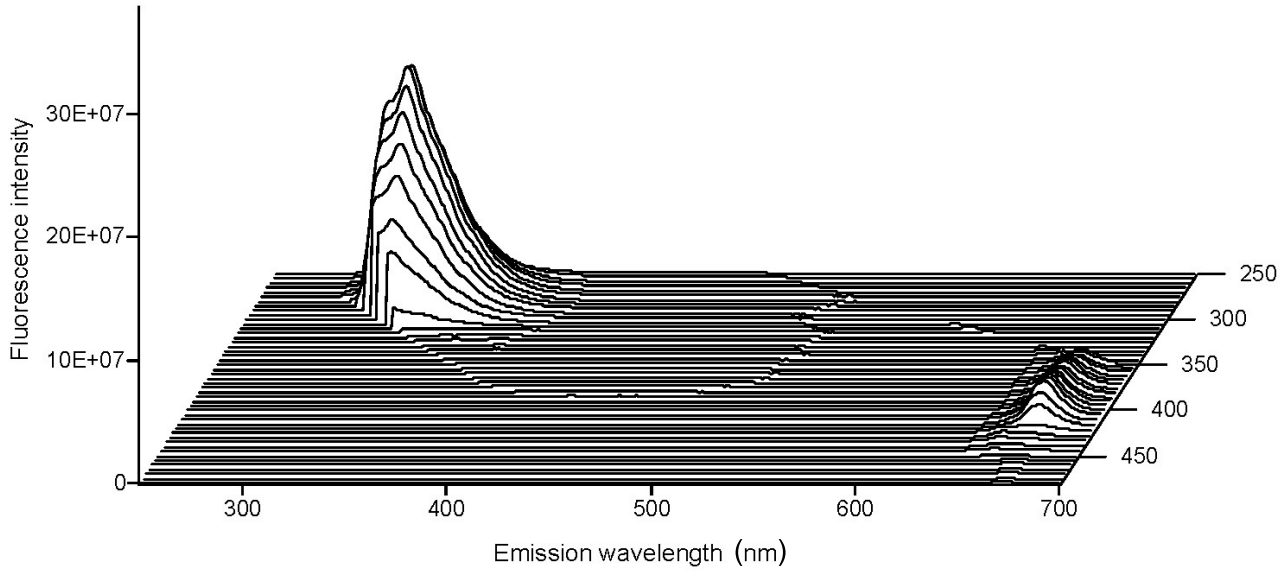

FIGURE 1. The three-dimensional total luminescence spectra of rapeseed oil 2 in n-hexane, 1% v/v.

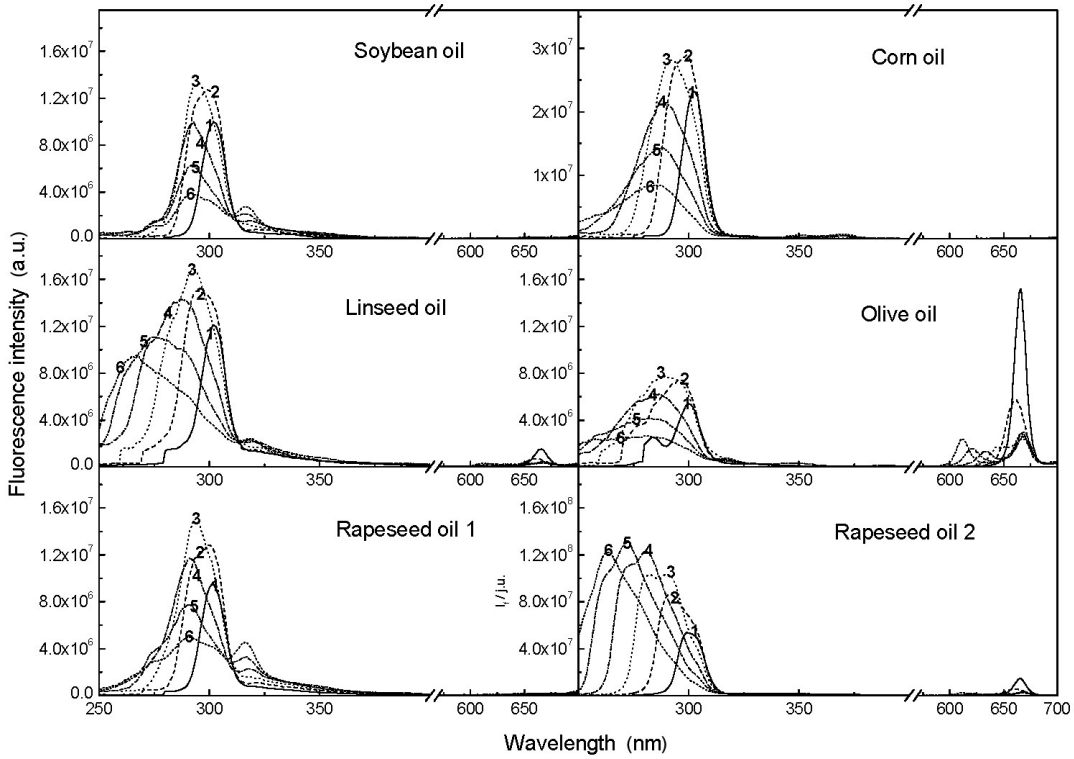

FIGURE 2. Synchronous fluorescence spectra of oils diluted in n–hexane, 1% v/v, recorded at various Δλ, 1=10 nm, 2–20 nm, 3–30 nm, 4–40 nm, 5–50 nm, 6– 0 nm.

sion matrix. Although the synchronous fluorescence scanning discards the total luminescence information outside the scanning line, it remains a very simple and effective means of obtaining emission data for several compounds in a single scan.

In synchronous scanning spectrofluorometry, the choice of an appropriate synchronized wavelength difference is critical for success. The selection of Δλ is usually empirical. Generally, the recommended value provides the best spectral resolution and the minimal band-width, while avoiding interference from the Rayleigh scattering. Presently, we recorded synchronous fluorescence spectra using Δλ values from 10 nm to 60 nm with a 10 nm step. The resulting sets of synchronous fluorescence spectra of oils studied are shown in Figure 2.

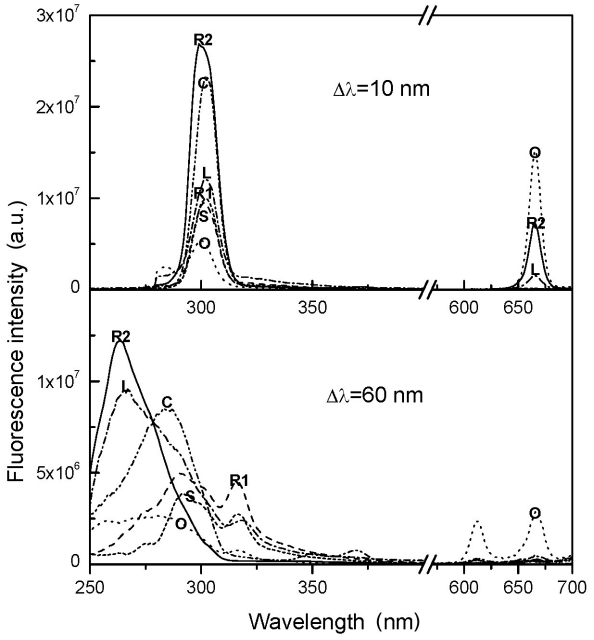

FIGURE 3. Comparison of synchronous fluorescence spectra of oils recorded at Δλ=10 nm and Δλ=60 nm: S – soybean oil, O – olive oil, C – corn oil, L –linseed oil, R1 and R2 – rapeseed oils.

Synchronous fluorescence spectra obtained with small Δλ (Δλ=10 nm) show an effectively decreased bandwidth, resulting in spectral simplification. These spectra have a major band with a maximum at around 300 nm for all oils, attributed to tocopherols. An additional long-wavelength band with a maximum at 666 nm is observed in linseed, olive and rapeseed oil 2, ascribed to chlorophylls. For soybean, linseed and rapeseed oil 1, a low-intensity band is observed at about 320 nm. Another very weak fluorescence is present in the spectra of corn oil, with the maximum at about 380 nm.

The tocopherol band becomes broader at increasing Δλ (20 nm and 30 nm), while its maximum is shifted to shorter wavelengths and the intensity grows. A further increase of Δλ (40-60 nm) causes an intensity reduction accompanied by additional broadening. The only exception is the rapeseed oil 2, for which the highest intensity is observed at Δλ = 50 nm. Moreover, in the spectra of soybean, linseed and rapeseed oil 1 at larger Δλ values, the weak band with the maximum at about 320 nm has an increased intensity. Similar changes in positions, shapes and intensities of synchronous fluorescence scans are observed for pure α-tocopherol in n-hexane (not shown). However, a single emission band is present for this compound even for the largest Δλ=60 nm, while the synchronous fluorescence spectra of oils contain several bands. This confirms the presence of other fluorescent compounds, different from α-tocopherol, also evident from the analysis of total luminescence spectra. Compari-

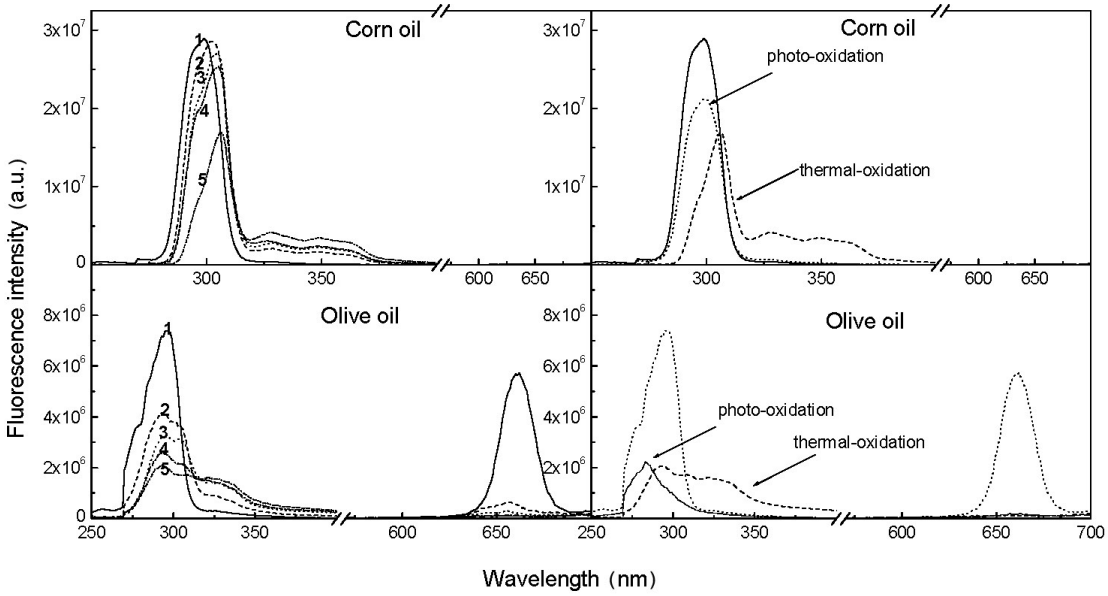

FIGURE 4. Left: Changes in synchronous fluorescence spectra of corn oil (top) and olive oil (bottom) during thermal oxidation, spectra after 1–0, 2–5, 3–10, 4–15, 5–20 h of heating (200°C). Right: Effect of photo- and thermal oxidation on synchronous fluorescence spectra of corn oil (top) and olive oil (bottom).

son of synchronous fluorescence spectra of various oils at a given Δλ allows differentiation between samples, Figure 3. The spectra recorded at larger Δλ are more characteristic, and thus more useful for oil fingerprinting. An interesting possibility is the usage of a complete set of spectra recorded at various Δλ values for the purpose of oil identification and analysis.

Oxidation of oils

Recently, we have shown that total luminescence spectra may be used to control the degree of photo- and thermal-oxidation of vegetable oils, in particular, of the tocopherol and chlorophyll loss. In the current studies we checked the possibility of using the synchronous fluorescence scanning for this purpose.

As a result of thermal oxidation, characteristic changes in synchronous fluorescence spectra are observed. Figure 4 shows typical spectral changes in corn and olive oils. Namely, the tocopherol (and chlorophyll) band intensity decreases, and simultaneously a new emission band appears in the 320–380 nm wavelength range. In contrast, the photo-oxidation of corn and olive oils leads to an intensity reduction of the tocopherol and chlorophyll bands, without any new bands appearing. As we have concluded previously from the analysis of total luminescence spectra, here once again the comparison of synchronous spectra of oils exposed to high temperatures and light indicates that different products are formed, resulting from different mechanisms of thermal- and photo-oxidation.

The individual spectral pattern characteristic for an oil depends on excitation and emission profiles of fluorescent components, being unique for each sample or mixture. Thus, a synchronous spectrum contains more information than a conventional excitation or emission spectrum. The synchronous fluorescence spectrum thereby becomes a signature or spectral fingerprint of the particular oil sample.

CONCLUSIONS

The results presented show that synchronous scanning fluorescence spectroscopy can be successfully used for characterization of edible oil samples. Synchronous fluorescence scan gives a possibility to characterize various edible oils by a single scan. These measurements are simple and can serve as rapid screening techniques in the oil analysis. Distinct regions, such as those pertaining to tocopherols and chloprophylls, identified in the spectra, can be used as markers for differentiation of oils and monitoring their quality with a high degree of accuracy.

Such an analysis method of complex mixtures, requiring no separation, is extremely useful for the industry. Moreover, fluorescence provides high sensitivity, simplicity and selectivity, which are important in chemical analysis. The methods discussed can also be used for quantitative analysis of fluorescent constituents after appropriate calibration.

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