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Analytica Chimica Acta 542 (2005) 151–156

ANALYTICA CHIMICA ACTA

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Classification of edible and lampante virgin olive oil based on synchronous fluorescence and total luminescence spectroscopy

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Received 3 December 2004; received in revised form 16 March 2005; accepted 24 March 2005

Abstract

Total luminescence and synchronous fluorescence spectroscopies were tested as regards their ability to differentiate edible from lampante virgin olive oils. Total luminescence spectra were recorded by measuring the emission spectra in the range 350–720 nm at excitation wavelengths from 320 to 535 nm. The synchronous fluorescence spectra of 41 edible and 32 lampante virgin olive oils were acquired by synchronous scanning the excitation and emission monochromator maintained at an offset value of 80 nm. Classification of virgin olive oils based on their synchronous fluorescence spectra was performed by hierarchical cluster analysis and principal component analysis using the spectral range of 429–545 nm. Principal component analysis provided better discrimination between the two classes, without any classification error, while hierarchical cluster analysis allowed 97.3% correct classification. These results indicate the capability of fluorescence techniques to differentiate virgin olive oils according to their quality.

Keywords: Synchronous fluorescence spectroscopy; Total luminescence spectroscopy; Principal component analysis; Hierarchical cluster analysis; Virgin olive oil

1. Introduction

Virgin olive oils are classified and priced according to acidity. According to the International Olive Oil Council, ‘virgin olive oil’ is the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal that do not lead to any chemical change [1]. Unit operations involve just washing, pressing, decantation, centrifugation and filtration. Virgin olive oil that has acidities lower than 3.3 degrees (% (w/w) free fatty acid content calculated as oleic acid) is suitable for consumption without any treatment [2]. Virgin olive oils of higher acidities, referred to as lampante olive oils, are refined in order to become edible [3]. The most important quality parameter for virgin olive oil is the acidity that is related to organoleptic characteristics and therefore to price. Virgin olive oil pricing depends on acidity: Price increases in proportion to every 0.1% decrease of acidity.

Spectroscopic techniques are gaining popularity as they can facilitate rapid analytical methods for various products [4,5]. The commonly used vibrational spectroscopic techniques such as Fourier transform infrared and Raman (FT-IR and FT-Raman) are not suitable for the assessment of minor components as they lack sensitivity [4,6]. On the other hand, molecular fluorescence spectroscopy is a well-established analytical technique, which provides sensitivities [7] that normal modes of vibrational techniques cannot compete with. However, methods based on fluorescence spectroscopy are not well suited for the analysis of complex multi-component samples without prior separation, due to severe overlaps of excitation and emission bands. In such cases, synchronous fluorescence (SyF) techniques or total luminescence (TL) spectroscopy improve the analytical potential of fluorescence spectroscopy [8,9].

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doi:10.1016/j.aca.2005.03.061

In SyF the excitation and emission monochromators are scanned simultaneously in such a manner that a constant wavelength interval is kept between emission and excitation wavelengths. Using suitable offset values ($\Delta\lambda$), SyF reduces spectral overlaps by narrowing spectral bands and simplifies spectra by preferentially amplifying strong fluorescence bands [8,10,11].

TL spectra are obtained by the excitation–emission data matrix. That is by plotting fluorescence intensity as a combined function of excitation and emission wavelengths.

Total synchronous fluorescence (TSyF) spectra are obtained by plotting fluorescence intensity as a combined function of the wavelength and $\Delta\lambda$. TSyF spectra combine the advantages of TL and SyF spectra.

Beyond determinations of single analytes, SyF has been recently used for the determination of proprieties such as the age and origin of high voltage transformer insulation oils [12] and classification of different edible oils [13]. This was possible by chemometric evaluation of SyF spectra using techniques such as principal component analysis, linear discriminant analysis and k nearest neighbour method. Another interesting application of SyF along this line is the diagnostic monitoring of urine [14]. Although methodologies were not developed, SyF spectra were characteristic of various health condition states and the course of disease.

In this work, a method based on SyF spectroscopy combined with multivariate data analysis is presented for the discrimination of lampante from edible virgin olive oil without any sample pretreatment.

2. Experimental

2.1. Material

Seventy-three samples were collected in the period October–November 2002. They included 41 edible and 32 lampante virgin olive oils. Oleic, linoleic and *n*-butyric acid were purchased from Riedel-de Haën, Acros Organics and Sigma, respectively.

2.2. Spectra acquisition

Measurements were carried out by the Jobin Yvon fluorolog-3 spectrofluorometer. This is a fully computer-controlled instrument using a double-grating monochromator for excitation and a single-grating emission monochromator. Excitation and emission slit widths were set at 2 nm. The acquisition interval and integration time were maintained at 1 nm and 0.3 s, respectively. Right-angle geometry was used for spectral acquisition of virgin olive oil samples in a 10 mm × 10 mm × 45 mm quartz cell. Spectra were acquired from olive oils as received, nine months after their extraction.

TL spectra were obtained by measuring the emission spectra, in the range 350–720 nm, repeatedly, at excitation wavelengths from 320 to 535 nm, spaced by 5 nm intervals in the excitation domain. Fully corrected for the photomultiplier detector spectral response and emission grating were then concatenated into an excitation-emission matrix.

SyF spectra were collected by simultaneously scanning the excitation and emission monochromator in the 250–750 nm range. The standard scan used for olive oil classification was a constant wavelength synchronous scan with an offset value of 80 nm.

TSyF spectra were obtained by measuring the emission spectra in the range 250–720 nm and varying the wavelength interval from 20 to 180 nm in 20 nm intervals. The spectra were fully corrected for the excitation lamp, the photomultiplier detector spectral response and emission, excitation gratings.

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

2.3. Data analysis

Data analysis was performed using the Statistica software version 6.0 (StatSoft, USA, 2001). For data analysis the spectral range 429–545 nm was used. Raw spectra were used as acquired without any pretreatment.

Chemometrics is an approach to analytical and measurement science that uses mathematical, statistical and other methods of formal logic to determine the properties of substances that otherwise would be difficult to measure directly [15].

Principal component analysis (PCA) is a statistical tool commonly used for classification of data. The main aim of PCA is to reduce a large number of variables to a much smaller number of principal components (PCs) that capture the vast majority of variance in the data. This reduces the dimensionality of the data considerably, enabling effective visualization, regression and classification of multivariate data [16,17].

Hierarchical cluster analysis (HCA) is an unsupervised classification procedure that involves the assessment of similarity between objects based on their measured properties (variables). Objects are grouped in clusters in terms of their nearness in the multidimensional space. In this work, the distance matrix was calculated using Manhattan distances. From the distance matrices the dendrograms were created using the Ward algorithm. This agglomeration method is generally recommended for large data sets. The elements or clusters are joined with the criterion that the sum of heterogeneities of all clusters should increase as little as possible [17,18].

3. Results and discussion

3.1. Total luminescence spectra

TL spectra were recorded for lampante and edible virgin olive oils. Fig. 1 shows contour maps of their luminescence,