Food Research International 45 (2012) 103–108

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Chemical changes of thermoxidized virgin olive oil determined by excitation–emission fluorescence spectroscopy (EEFS)

Noelia Tena, Ramón Aparicio, Diego L. García-González *

Instituto de la Grasa (CSIC), Padre García Tejero 4, E-41012 Sevilla, Spain

ARTICLE INFO

Article history:
Received 5 August 2011
Accepted 12 October 2011

Keywords:
Excitation–emission matrix (EEM)
PARAFAC
Thermoxidation
Virgin olive oil
Phenols
Oxidation products

ABSTRACT

The fluorophores present in virgin olive oil evolve during any thermoxidation process and thereby fluorescence spectroscopy can be used as a straightforward approach to study the oxidative degree of oils. Samples of heated virgin olive oils collected from a fryer every two hours up to 94 h were analyzed to study their excitation–emission matrix (EEM) and the evolution of the fluorescent composition during the thermoxidation process. The potential usefulness of EEM to study thermoxidized virgin olive oils has been explored. For this purpose, parallel factor analysis (PARAFAC) was used to interpret the results. The emission profile obtained from three-factor PARAFAC model calculated for 47 samples of thermoxidized oil in the range of $\lambda_{ex} = 250–298$ nm and $\lambda_{em} = 300–700$ nm allows the decomposition of the fluorescence landscape in specific information about phenols, pigments and oxidation products respectively. The chemical interpretation from the PARAFAC model was checked with chemical analysis of phenols, tocopherols and chlorophyll pigments.

1. Introduction

The ability of virgin olive oil to emit fluorescent radiation is due to the presence of fluorescent compounds as chlorophylls, pheophytins, tocopherols, Vitamin E and oxidative compounds (Galano, Durán, Correa, Roldán, & Rodriguez, 2003; Sikorska et al., 2004). Previous studies based on fluorescence spectroscopy of olive oil have associated several emission spectrum regions with these compounds present in olive oil. The region between 300 and 400 nm is attributed to the tocopherols and phenols (Sikorska, Górecki, Khmelinskii, Sokorski, & Koziol, 2005; Zandomeneghi, Carbonaro, & Caffarata, 2005), the region of 440–445 nm shows good correlation with the K232 and K270 (Kyriakidis & Skarkalis, 2000), the derivatives of vitamin E are associated with the region 400–600 nm (Dupuy et al., 2005), and the bands in the region of 600–700 nm are associated with the chlorophyll and pheophytin pigments (Dupuy et al., 2005).

Different approaches based on fluorescence spectroscopy have been implemented for the characterization of edible oils. The differences between the fluorescence emission spectra of olive oil and other edible oils (Sikorska et al., 2005) and between different categories of olive oil (Nicoletti, 1990) have been previously described. The fluorescence spectra have also been used to detect adulterations, as the presence of hazelnut oil in virgin olive oil (Sayago, Morales, & Aparicio, 2004), and even to determine the degree of oil oxidation and thermal deterioration in virgin olive oils (Poulli, Chantzos, Mousdis, & Georgiou, 2009; Tena, García-González, & Aparicio, 2009). On the other hand, the fluorescence excitation spectra with emission at 330 and 450 nm have been used to control the evolution of phenols, vitamin E and degradation of hydroperoxides during heating (Cheikhousman et al., 2005). Furthermore, applications with total synchronous fluorescence spectroscopy and excitation–emission fluorescence spectroscopy (EEFS) have been developed to monitor changes in the olive oil during storage in different conditions (Sikorska et al., 2008).

A technique selected to ensure the safety and quality of any food product should be rapid and easy to use (Belton et al., 1995). Fluorescence spectroscopy, being a rapid technique, provides valuable information on thermolabile compounds (e.g. phenols, vitamin E, pigments), and therefore, this technique has the advantage over other spectroscopic methodologies of providing specific information on oxidation. In consequence, it can be proposed as a suitable tool for monitoring the deterioration degree of frying olive oils. Synchronous fluorescence and excitation–emission fluorescence spectroscopies are preferable for the analysis of complex multicomponent samples such as edible oils, compared with conventional fluorescence spectroscopy (Poulli, Chantzos, Mousdis, & Georgiou, 2009; Poulli, Mousdis, & Georgiou, 2009). In particular the excitation–emission fluorescence spectroscopy (EEFS) involves the simultaneous acquisition of a range of excitation wavelengths (λ_{ex}) and emission wavelengths (λ_{em}). As a result, a total fluorescence profile of the sample over the scanned range is obtained. This fluorescence landscape is called the excitation–emission matrix (EEM) and it has the ability of obtaining

* Corresponding author. Tel.: +34 954 61 15 50; fax: +34 954 61 67 90.
E-mail address: dluisg@cica.es (D.L. García-González).

0963-9969/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2011.10.015

simultaneous information of different compounds in the sample. This procedure improves the selectivity of fluorescence spectroscopy and it has proved to be useful in discerning olive oils by slight differences in their composition.

The statistical tools to interpret the EEFS results require dividing the information provided by the three-dimensional matrix (EEM) into two-dimensional arrays. The parallel factor analysis (PARAFAC) has the advantage of working with three-dimensional arrays providing a limited number of factors that represent the whole matrix. The model obtained with PARAFAC simplifies the interpretation of the excitation–emission profiles and it provides a unique solution, regardless of the type of rotation of the factors (Harshman, 1970).

The objective of this work is to propose the excitation–emission fluorescence spectroscopy combined with PARAFAC algorithm as a tool to monitor the changes in the fluorescence composition of virgin olive oil during thermoxidation. For this purpose, the information obtained from the EEM was processed to determine when the oil had to be discarded for human consumption by correlating the most significative spectral bands with the percentage of total polar compounds. The chemical assignment of the spectral bands was done according to the work reported by other authors and supported by the chemical analysis of phenols, tocopherols, and chlorophyll pigments.

2. Materials and methods

2.1. Sample preparation

The study was carried out with forty-seven samples of thermoxidized virgin olive oils. These samples were obtained through a thermoxidation process carried out in a 4 L domestic fryer with automatic temperature controller (Heidolph EKT 3001, Schwabach, Germany). The fryer was set at 190 °C for 94 h in cycles of 8 h per day. A sample of 40 mL was collected every 2 h until the end of the heating process. Samples were kept in brown glass vials at 4 °C until further chemical and spectroscopic analyses.

2.2. Analysis of total polar compounds

The percentage of total polar compounds (% PC) was determined gravimetrically according to the IUPAC Standard Method No. 2.507. Nonpolar and polar fractions were separated from 1 g of oil by silica gel column chromatography (20 g silica adjusted to a water content of 5%, w/w) hexane/diethyl ether (90:10, v/v) and 150 mL diethyl ether as elution systems, respectively. The nonpolar fraction was eluted with 150 mL of n-hexane/diethyl ether (90:10, v/v), while the polar fraction was eluted with 150 mL of diethyl ether. Efficiency of the separation was confirmed by TLC using n-hexane/diethyl ether/acetic acid (80:20:1, v/v/v) and visualized with iodine vapor. The percentage of polar fraction was calculated by weighing both fractions after the evaporation of the solvents.

2.3. Excitation–emission fluorescence spectroscopy (EEFS)

Spectrofluorimetric measurements were performed with a Cary Eclipse (Varian Ibérica, Madrid, Spain). This instrument was equipped with a continuous xenon lamp, excitation and emission monochromators, and a photomultiplier. A 10 mm × 10 mm quartz cell (3 mL) was used for "right-angle" RA fluorometry. The wavelength range for the emission monochromator was 300–700 nm, and for the excitation monochromator was 250 nm at 298 nm, intervals for each dimension being 1 and 4 nm respectively. The polarizer operated in automatic mode. The excitation and emission slits were both of 5 nm. The photomultiplier voltage was set at 600 V to avoid saturation. The time taken to acquire a spectrum was of 10 min (intermediate speed). All data were exported to an Excel spreadsheet and were subsequently treated with the softwares Matlab v7.8 (The MathWorks, Natick, MA) and Statistica v7.0 (Statsoft, Tulsa, OK). The experimental conditions allowed getting spectra of satisfactory intensity, resolution and signal-to-noise ratio. After each sample the cell was cleaned using detergent, followed by Milli-Q hot water and acetone to dry it and eliminate possible rests of samples. Each sample was analyzed in duplicated.

2.4. Determination of tocopherols

Tocopherols were analyzed by following the IUPAC Standard Method No. 2.432. A solution of oil in hexane (10 mg/mL) was analyzed by HPLC (Agilent Technologies 1200) on a silica gel column (Merck, Superspher Si60, particle size 4 μm, 250 mm × 4 mm i.d.), eluting with hexane/2-propanol (99:1, v/v) at a flow rate of 1 mL/min. The injection volume was of 20 μL. A fluorescence detector (Agilent Technologies 1100) with the excitation wavelength set at 290 nm and emission wavelength at 330 nm was used. The quantification was carried out by using an external calibration with a solution of α-tocopherol/Hexane (0.5–5.0 μg/mL) as standard.

2.5. Determination of phenols

A standard solution (0.5 mL) made of *p*-hydroxyphenylacetic (0.12 mg/mL) and *o*-coumaric acids (0.01 mg/mL) in methanol was added to a sample of filtered virgin olive oil (2.5 g). A rotary evaporator at 40 °C under vacuum was used to evaporate the solvent and the oily residue was dissolved in 6 mL of hexane.

The diol-bonded phase cartridge was conditioned according to Mateos et al. (2001). After the sample loading, the final residue was extracted with 500 μL of methanol–water (1:1 v/v) at 40 °C and a filtrated aliquot (20 μL) of the final colorless solution was injected onto the HPLC system (LaChrom Elite Tokio, Japan), equipped with a diode array detector. The column was a Lichrospher 100RP-18 column (4.0 mm i.d. × 250 mm; 5 μm, particle size) (Darmstadt, Germany) maintained at 30 °C. The gradient elution, at a flow rate of 1.0 mL/min, was achieved by using the following mobile phases: a mixture of water/phosphoric acid (95.5:0.5 v/v) (solvent A) and methanol–acetonitrile (50:50 v/v) (solvent B). The change of solvent gradient was programmed as follows: from 95% (A)-5% (B) to 70% (A)-30% (B) in 25 min; 62% (A)-38% (B) in 10 min; 62% (A)-38% (B) in 5 min; 55% (A)-45% (B) in 5 min; 47.5% (A)-52.5%(B) in 5 min and 100% (B) in 5 min, followed by 5 min of maintenance. The chromatographic signals were obtained at 235, 280, and 335 nm.

Quantification of phenols cinnamic acid and lignans was carried out at 280 nm by using *p*-hydroxyphenylacetic acid as internal standard. Quantification of flavones was done at 335 nm by using *o*-coumaric acid as internal standard. The response factors and recoveries were based on the procedure carried out by Mateos et al. (2001).

2.6. Determination of chlorophyll pigments

The determination of chlorophyll pigments was carried out by implementing the method proposed by Gertz and Fiebig (2006) to quantify the pirofeofitina a. A sample of 300 mL was diluted in 1 mL of hexane. Separation of the pigments (pheophytins, pyropheophytin a and chlorophylls) was carried out by using a miniaturized column chromatography on a silica gel column. After loading the sample, the eluate of chlorophyll pigments were collected by passing 5 mL of acetone and it was analyzed by HPLC (Agilent technologies 1100, Santa Clara, California) equipped with a C18-RF Spherisorb ODS-2 (25 cm × 4 mm id; 3 μm particle size) (Waters, Saint Quentin-Yvelines, France) and a diode array detector. It worked in isocratic regime, using H_2O/MeOH/acetone as mobile phase (4:36:60 v/v/v) with a flow of 1 mL/min. The chromatographic signals were obtained at 410 nm. The compounds identified and quantified were: pheophorbide, chlorophyll, pheophytins a, a′, b, and b′, and pyropheophytin a.