Fluorescence spectroscopy for monitoring extra virgin olive oil deterioration upon heating

Rana Cheikhousman, Manuela Zude, Delphine Jouan-Rimbaud Bouveresse, Douglas N. Rutledge, Ines Birlouez-Aragon

Fluorescence Spectroscopy for Monitoring Extra Virgin Olive Oil Deterioration Upon Heating

*R. CHEIKHOUSMAN, M. ZUDE, D. J.-R. BOUVERESSE, D. N. RUTLEDGE and I. BIRLOUEZ-ARAGON**

*Laboratoire de Chimie Analytique, Institut National Agronomique Paris-Grignon, Paris, France, *E-mail: birlouez@inapg.fr*

Abstract: Fluorescence spectroscopy is a reliable and fast method for determining the deterioration of extra virgin olive oil (EVOO). EVOO was analysed by HPLC for determining antioxidant changes in EVOO due to heating at 170°C for 3 h. This thermal oxidation caused a significant exponential decrease in hydroxytyrosol (OH-Tyr) and vitamin E (vitE), while the reduction in the tyrosol content was relatively small. Hydroperoxydes were analysed by an indirect colorimetric method. Their content decreased exponentially during the heating process. Fluorescence excitation spectra with emission wavelength at 330 nm were recorded to monitor the evolution of polyphenols and vitE, while fluorescence excitation spectra with emission at 450 nm were used to monitor the evolution of hydroperoxydes. Results of partial least-square calibration models (PLS) show that the degradation of polyphenols, vitE and hydroperoxide, as well as formation of fluorescent components derivatives can be quantified with fluorescence spectroscopy. Application of chemometrical data analysis allows to optimise the extraction of the information contained in the data. Fluorescence can therefore be used as a rapid technique for evaluating the quality of heat-treated EVOO.

Keywords: fluorescence; EVOO; polyphenols; vitamin E; hydroperoxide; chemometrics

INTRODUCTION

Extra virgin olive oil (EVOO) is considered as a particularly stable vegetable oil upon frying [1]. This high stability can be explained by (i) the fatty acid composition high in oleic acid (55–88%) and low in PUFA (2–21%), and *(ii)* the presence of highly antioxidative polyphenols (117–231 mg Eq gallic acid/kg) [2]. The latter is concluded from comparison of the stability between EVOO and refined olive oil containing only traces of polyphenols [3]. Vitamin E (vitE), the main tocopherol of EVOO, is not particularly high (251–369 mg/kg). Furthermore, phenolic compounds seem more effective than vitE in protecting olive oil against oxidation [2, 3]. Controlling the polyphenol and vitE content of oils is of high interest, since they condition both the nutritional quality and the stability upon heating [2, 4, 5]. Presently labourious and time-expensive analytical methods such as GC-MS and HPLC are applied to assess oil quality. Faster analytical methods would be useful for applications in production processes and quality assessment [6]. Fluorescence spectroscopy can provide a sensitive method for determining oil quality as recently shown by Sikorska *et al*. (2003) [7] on soybean, corn, sunflower and olive oils. Application of this method on EVOO has a high potential, since the main interesting components, tyrosol derived polyphenols and vitE are naturally fluorescent. The present study aims at evaluating the potential of fluorescence spectroscopy for determining the evolution of EVOO quality during heating. For this purpose, EVOO was heated and subsequently analysed in different spectral regions by means of fluorescence emission and excitation spectra. The spectral data were analysed and compared to conventional phenol, vitE and hydroperoxides quantification by means of multivariate analysis.

EXPERIMENTAL

Sample preparation. Heat treatment was carried out on commercial extra virgin olive oils (EVOO) purchased at a local supermarket. It was heated at 170°C for 3 hours in a commercial deep fryer (Oleoclean, SEB, France). Samples (10 ml) were taken every 15 minutes during the heating process for subsequent composition analysis. Three repetitions were measured for each EVOO. Hydroperoxides, vitE and phenols were analysed as indicators of the oxidative stress. Concomitantly, fluorescence spectra were recorded.

Analytical determinations. Tyrosol and hydroxytyrosol were analysed by HPLC using a reversed-phase C_{18} (EQUISIL ODS 5 µm, 250 × 4.6 mm) C18 column (CLUZEAU, France) eluted with 2% acetic acid and detected by fluorescence at $\lambda_{ex}/\lambda_{em}$ = 280 nm/330 nm. Forty µl of a 1 g/l DHAP obtained from Fluka-Sigma-Aldrich (Steinheim, Germany) phenols were quantified using DHAP as internal standard.

VitE was determined on oil samples diluted 20 fold in n-hexane containing BHT 1% by the HPLC with fluorimetric detection at $\lambda_{ex}/\lambda_{em}$ = 290 nm/330 nm as reported by [8]. The mobile phase was composed of 684 ml acetonitrile, 220 ml tetrahydrofuran, 68 ml methanol and 28 ml ammonium acetate (1 g/l).

Hydroperoxides were analysed by an indirect colorimetric method. The oil sample was diluted (100 to 400 fold according to the degree of oxidation) in propanol-1 before reacting 30 min at room temperature with the methanolic reaction mixture containing xylenol orange and iron II. The solution was filtered on nylon filters before reading the absorbance at 560 nm.

Fluorescence spectra of the diluted oil (1000 folds with propanol-1) were recorded on a Spex fluorimeter (Jobin-Yvon, France). Two excitation spectra were recorded: one at emission at 330 nm, corresponding to phenols and vitE, and the second at emission at 450 nm that is associated to the newly formed molecules. The fluorescence was recorded in counts per second.

RESULTS AND DISCUSSION

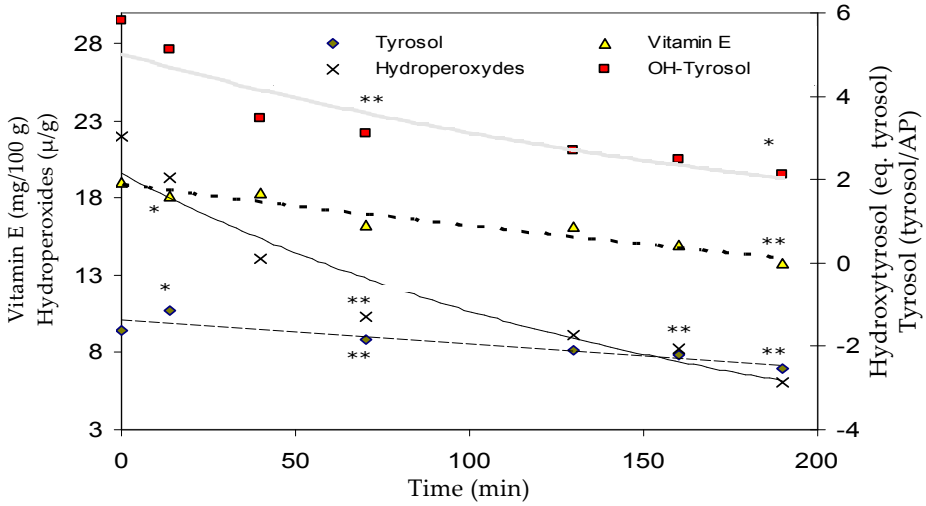
Evolution of hydroxytyrosol, tyrosol, vitE, and hydroperoxides in EVOO during heating

During the heating process, all components decreased.

The hydroxytyrosol especially in EVOO rapidly lost after 60 min of heating 46.8% of its initial content and 36.2% after 190 min remained. Tyrosol decreased more slowly in comparison with hydroxytyrosol. 92.3% and 73.9% of their initial value remained after 60 and 190 min of treatment, respectively.

Vitamin E decreased exponentially after a time lag of 60 min of heating. This can be explained by the consequence of the protective effect of the hydroxytyrosol. At the end of heating process, 27.79% of the initial level of vitE content had decreased (Figure 1).

The hydroperoxide content also, possibly producing secondary oxidation product, decreased



**P = 0.05, *P = 0.1

Figure 1. Evolution of EVOO composition during heating: vitamin E (vitE), hydroperoxydes, tyrosol and hydroxytyrosol (OH-Tyrosol)

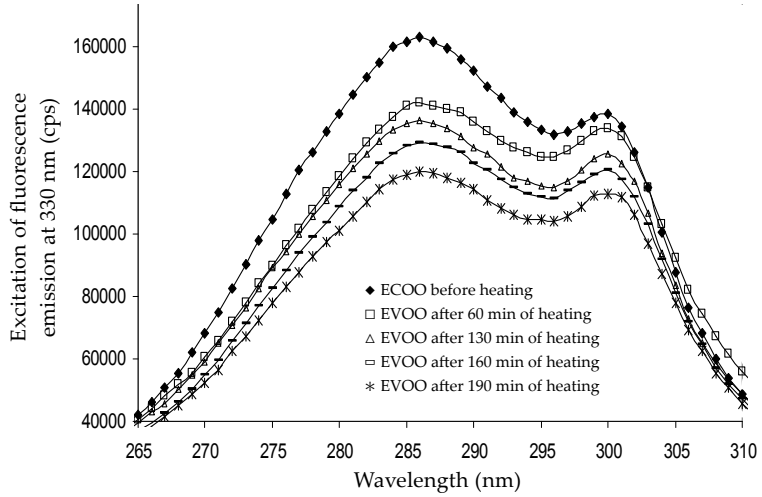


Figure 2. Excitation spectra with emission at 330 nm of EVOO before, after 60, 130, 160 and 190 min of heating at 170°C during 3 h

exponentially during the heating process, 26.74% of their initial level remaining after 120 min. Such a decrease can be explained by the degradation of hydroperoxides, while at the same time other compounds are newly formed by oxidation. A less rapid diminution was measured after 60 min. At the end of the heating impact 27.65% of its initial content remained.

Evolution of phenols, vitE, and hydroperoxides in EVOO measured by means of fluorescence spectroscopy analysis

Oil fluorescence spectra were measured at different heating times to evaluate oil degradation due to the heat impact. Fluorescence excitation spectra with emission wavelength at 330 nm were recorded (Figure 2), to monitor the evolution of vitE and polyphenols. The fluorescence intensity slowly decreased during the heating process. Fluorescence excitation spectra with emission wavelength at 450 nm were recorded, to monitor the evolution of hydroperoxide by means of fluorescence spectroscopy (Figure 3). During the heat treatment, the fluorescence intensity increased and was inversely correlated with the hydroperoxide content of the oil. Particularly in the wavelength range between 250 nm and 370 nm, development of new fluorescence occurred. These fluorophores should be related to some degradation products of hydroperoxides.

These components derivatives may be the aldehydes or polymers of certain components which fluoresce at this wavelength of excitation.

Calibration results

EVOO compounds were modelled with PLS model using fluorescence spectra with emission at 330 nm for prediction of polyphenols and vitE,

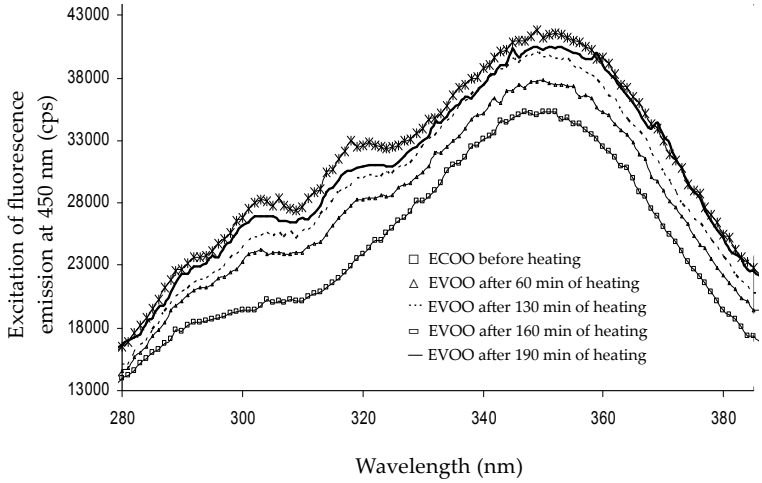


Figure 3. Excitation spectra with emission at 450 nm of EVOO before, after 60, 130, 160 and 190 min of heating at 170°C during 3 h

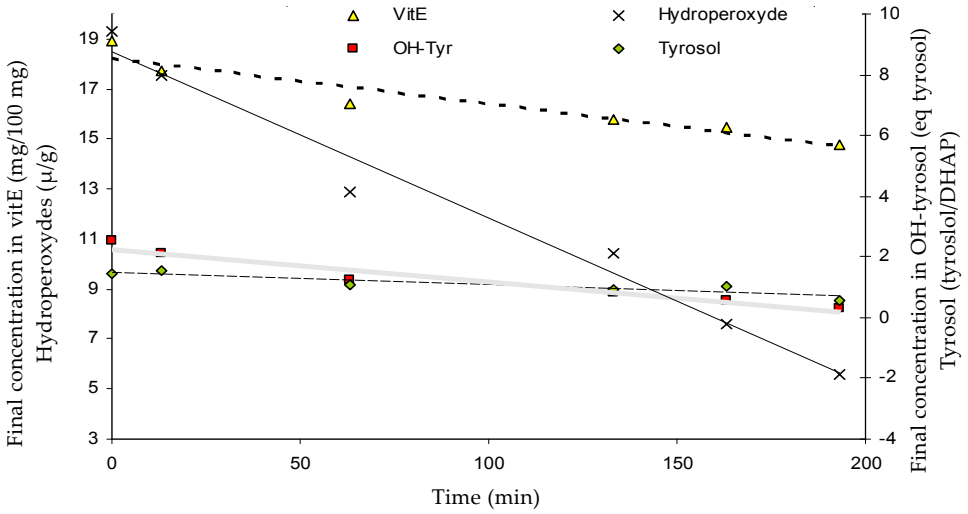


Figure 4. PLS models relating fluorescence spectra to vitamin E (vitE), tyrosol, hydroperoxydes, and hydroxytyrosol (OH-Tyr) in EVOO

while fluorescence with emission at 450 nm was used for predicting hydroperoxides. This was done for evaluating the correlation between the variation in the oil fluorescence spectra and the chromatographic data as shown in Figure 4. It was shown that a strong correlation exists (R^2 = 0.94, 0.86, 0.82, 0.96 for the hydroxytyrosol, tyrosol, vitE and hydroperoxides, respectively), with the root mean square error of cross-validation (RMSECV) equal to 0.45, 9.82, 1.12, 3.27 for the hydroxytyrosol, tyrosol, vitE and hydroperoxides, respectively.

CONCLUSION

The hydroxytyrosol is the major antioxidant in EVOO, which had a rapid and exponential loss of its concentration in EVOO. It protects tyrosol and vitamin E, which remained practically stable after 3 h of heating at 170°C. However, hydroxytyrosol was not effective to protect the hydroxyperoxydes of degradation. This component decreased exponentially during the heat treatment. It is assumed that aldehydes and polymerised components were formed during the degradation of hydroperoxides. This phenomenon explains the physical modifications of this oil during the heating.

Fluorescence excitation spectra with emission at 330 nm were indicative for the vitE and polyphenol contents. These spectra slowly diminished during the heating process showing a logarithmic trend comparable to the contents changes measured by means of HPLC. The degradation of polyphenols and vitE can be quantified with fluorescence spectroscopy. The fluorescence excitation spectra with emission at 450 nm were used to monitor the evolution of hydroperoxides. Prediction results of PLS calibration models based on oil fluorescence spectra and HPLC data of polyphenols, vitE and hydroperoxides were highly correlated. The fluorescence excitation spectra with emission wavelength at 450 nm contained high information on hydroperoxides, which can be explained by the degradation of hydroperoxide and formation of components derivatives which fluoresce.

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