Fluorescence Spectroscopy for Monitoring Rapeseed Oil Upon Heating

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Abstract: The aim of this work was to determine the heating effect on the thermo-degradation process of rapeseed oil (RO) by rapid fluorescence method. Reference measurements were carried out by chromatographic methods in order to compare results with those obtained by fluorescence spectroscopy. The main compounds which were monitored are polar compounds and tocopherols. During heating process, two temperatures were used: 171°C and 189°C for 4.5 h. The results have showed that the tocopherol content decreases, especially at the highest temperature, and the polar content increases of 20% at the end of heating. These results are in agreement with literature. PLS model was built in order to predict polar and tocopherol contents. The results obtained from this method were satisfactory.

Keywords: fluorescence; rapeseed oil; polar compounds; tocopherol; eating process; PLS regression

INTRODUCTION

Because of its high content in linolenic acid, rapeseed oil (RO) consumption could allow to rebalance the ω6/ω3 ratio of occidental diets. But on the other hand, a high unsaturation level induces a greater susceptibility to oxidation. Di- and especially tri-unsaturated fatty acids are prone to thermo-oxidation upon heating, giving rise to various oxidation products such as aldehydes, ketones, oxygen-containing dimers, some of which presenting mutagenic activity [1]. These oxidation products are globally measured by extracting and weighing the polar fraction of the oil. Oils with more than 25% of polar contents are considered as inconsumable. For this reason, the French law recommends vegetable oils with C18:3 higher than 2% to be used exclusively for dressing. For the case of high tocopherol and oleic acid contents which allow to stabilize the oil during cooking, a revision of the regulation is under consideration. In this context, the present study was carried out to determine the heating effect on the thermo-degradation process of RO. For this approach a rapid and simple fluorimetric method to measure the oil quality is developed which could be used for routine control purposes.

EXPERIMENTAL

Rapeseed oil was purchased at a local supermarket. Two liters of oil were heated at 171°C and 189°C for 4.5 hours in a commercial deep fryer (Oleoclean, SEB, France). Samples (10 ml) were taken each 15 minutes during the heating process. After each sampling, the oil was rapidly cooled in ice and analyzed twice. Fatty acid profiles were determined at t = 0 and t = 4.5. Tocopherols, and polar compounds were measured as indicators of the thermo-oxidative stress. Concomitantly, fluorescence spectra were recorded in a front face mode directly on the oil without any preparation.

Fatty acid profiles were determined after transesterification by NaOH/MeOH and HCL/MeOH. The resulting fatty acid methyl esters were quantified by GC on a BPX-70 capillary column using a gaz chromatograph HP 5890 serie II.

Vitamin E was determined on the oil samples diluted 50 fold in propan-1-ol by reverse phase HPLC with fluorimetric detection at $\lambda_{ex}/\lambda_{em}$ = 290 nm/330 nm as previously reported [3]. The mobile phase was composed of 684 ml acetonitrile, 220 ml THF, 68 ml methanol and 28 ml ammonium acetate (1 g/l). Analyses were done twice and results are expressed as mg/kg.

Polar compounds were determined twice according to the AOCS method cd 20-91, slightly modified. The polar and apolar fraction were separated on a silica gel column. The apolar faction were eluted with a mixture of light petroleum and diethyl ether 87/13 (v/v) and polar fraction was later eluted with dietyl ether. Fraction were evaporated and weighted.

Front face fluorescence spectra were recorded on a spectrofluorimeter (Xenius, SAFAS, Monaco). This fluorimeter allows measuring fluorescence excitation spectra as well as emission spectra. Readings of tocopherol fluorescence excitation where carried out by setting the emission wavelength at 330 nm. The molecules appearing during the heating process were detected by fluorescence emission spectra with an excitation wavelength fixed at 298 nm.

Data processing methods. Calibration models were built using fluorescence spectra and corresponding molecule of interest. For this purpose partial least square (PLS) regression was applied (Cats, Chemometrics Analysis ToolS developed by Institut National Agronomique & University of Aveiro).

The number of latent variables (LV) was chosen regarding the first minimum of the root mean square error of cross-validation (RMSECV). Calibration statistics are given in the figures accounting for the models dimensionality, coefficient of determination (R^2), and standard error of prediction (SEP).

RESULTS AND DISCUSSION

Fatty acid profile

The present oil had the following fatty acid profile: C16:0, 3.9%; C18:0, 1.4% ; C18:1, 64.4%; C18:2, 22.3% ; C18:3, 7.9%. There was no significant variation during heating, except a low decrease in the c18:3/c18 ratio (2 at t = 0 to 1.45 at t = 4.5 h). These results confirm previous data showing that much higher temperature (> 220°C) are needed to significantly degrade c18:3.

Vitamin E: HPLC and fluorescence analysis

The mean levels of α- and γ-tocopherols are in agreement with those reported previously for RO: 408 and 339 mg/kg; 347 and 320 mg/kg, respectively [4]. After 4.5 hours, theses levels fell to 236 (68.2%) and 226 (70.6%) mg/kg at 189°C and 318 (78%) and 269 (79.3%) mg/kg at 171°C. At the end of heating process, for each temperature, α-tocopherols contents were much smaller than those of γ-tocopherol. At 171°C the remaining content of tocopherol in the oil after heating is 10% higher than the one at 189°C.

When recording the maximal intensity of fluorescence spectra at 290 nm (at exc 330 nm) (Figure 1), an exponential decrease was observed and when heating at 189°C and 171°C (Figure 2). These results are in agreement with the α- and γ-tocopherol content chromatographic data: the total tocopherol decrease was comparable between HPLC and fluorescence data. A PLS model (partial least squares) was built allowing to predict the tocopherol content by fluorescence.

RMSECV = 0.01, SEP = 0.01, and R^2 = 0.9847

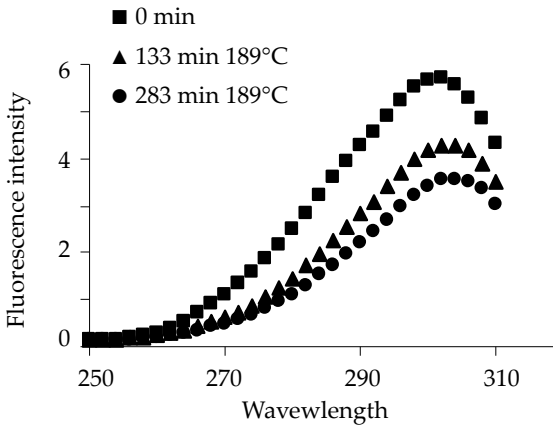

Figure 1. Fluorescence spectra of emission wavelength at 330 nm

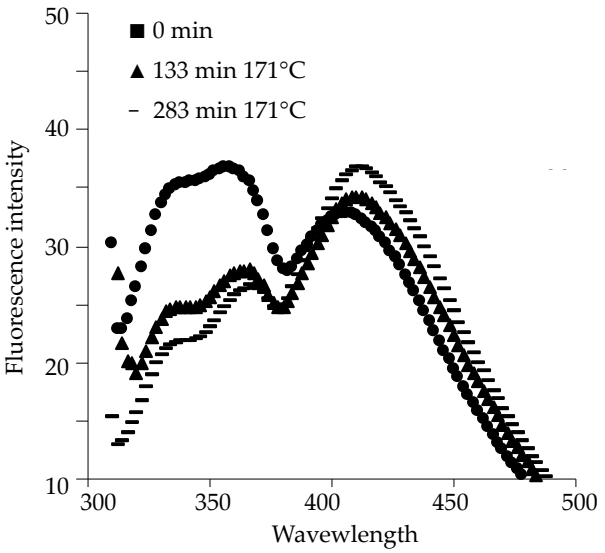

Figure 2. Fluorescence spectra of wavelength excitation at 298 nm

Polar compounds/fluorescence of excitation spectra at 298 nm

The formation of polar compounds was linear dependent with the heating time and correlated respectively with zero rate order of 0.0695% by minutes (R^2 = 0.95) at 189°C and 0.0639% by minutes (R^2 = 0.79) at 171°C.

When recording the fluorescence emission spectrum at excitation 298 nm, three main peaks of fluorescence intensity appeared with maxima at 330 nm (tocopherols), 360 nm and 420 nm (Figure 3). The fluorescence at 330 nm decreases due to the tocopherol evolution during heating; whereas the two other peaks slightly increased. The data collected from the fluorescence spectra were well correlated to the formation of the polar compounds. A PLS regression was obtained:

RMSECV = 3.2, SEP = 1.8, and R^2 = 0.9571

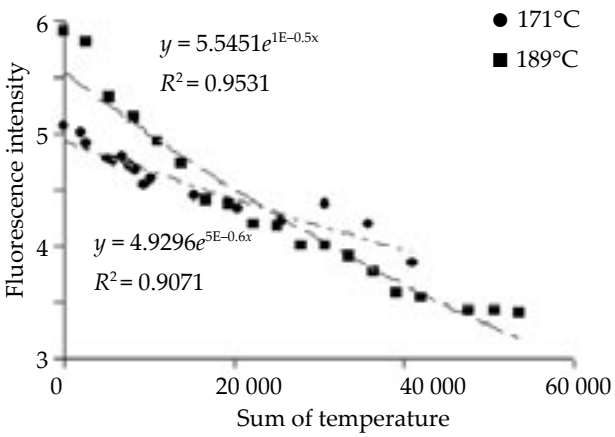

Figure 3. Fluorescence values at 300 nm

CONCLUSION

This study was carried out to evaluate the stability of rapeseed oil upon heating at two different temperatures, 171°C and 189°C. After 4.5 hours, 20% and 30% of the tocopherols were degraded in function of the temperature. α-Tocopherol was more affected than γ-tocopherol. At the same time, 30% of polar compounds were formed at the end of the heating time, i.e., a higher level than recommended. These results confirm that rapeseed oil is unstable; even if the high thermo-degradation in the present oil is (perhaps) related to its bad quality (10% polar compounds were found in the commercial bottle). In other hand, temperature has a small effect on the polar compounds accumulation rate.

We show that fluorescence spectroscopy is a powerful and simple method to predict tocopherols and polar compounds, indicators of the oil stability. PLS regression was calculated between fluorescence spectra at 290/330 nm and tocopherols chromatographic values, then, between fluorescence spectra at 298/360–420 nm and polar compounds. It is still ambiguous what types of polar fractions compounds are fluorescing at this wavelength range. We only confirmed that the polar fraction exhibited a similar fluorescence spectral shape as the heated oil, whereas the apolar fraction had similar fluorescence spectra of the unheated oil. Further work is needed to determine the oxidized fatty acids responsible for the observed shape of the fluorescence spectra. Nevertheless, the fluorescence spectroscopy is a simple, easy to run and rapid method to monitor the oil quality and the degradation upon heating.

References

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