Analytical Methods

A spectroscopic and chemometric study of virgin olive oils subjected to thermal stress

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ABSTRACT

The paper describes a study of thermal stress of three different samples of virgin olive oil in terms of oxidative stability. Fatty acid composition, evaluation of oxidative stability under forced conditions (OSI), determination of UV-spectrophotometric oxidation indexes (k_{232} and k_{270}) and spectral properties were explored along the thermal treatment. The samples were subjected to heating treatment at 180 °C and evaluated after 0, 30, 60, 90, 120, 150 and 180 min. Middle infrared (MIR) and visible–near infrared (Vis–NIR) spectra were elaborated by partial least squares modelling to individualise regions and bands where critical variations were present. Two bands were found as principal influential ones (1245–1180 cm^{-1} and 1150–1030 cm^{-1}) on MIR while one primary region was identified on Vis–NIR (2200–1325 cm^{-1}).

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1. Introduction

Extra virgin olive oil (EVOO) is widely known because of its high health benefits and sensory quality in comparison to other oils and fats (Bendini et al., 2007), especially due to the presence of a high ratio between monounsaturated and polyunsaturated fatty acids and its antioxidant fraction (principally lipophilic and hydrophilic phenolic compounds) (Aparicio, Roda, Albi, & Gutiérrez, 1999).

Virgin olive oil is a principal ingredient in the Mediterranean diet and it is consumed in different ways: raw in salads, on traditional food (i.e. as breakfast in "tostada" in the South of Spain or as meal in pasta or "bruschetta" in the South of Italy), toasts and other foodstuffs (Cerretani, Biasini, Bonoli-Carbognin, & Bendini, 2007), but often it is also consumed after domestic heating, such as fried, boiled or after conventional and microwave heating (Brenes, García, Dobarganes, Velasco, & Romero, 2002). These thermal treatments are commonly utilised for home-cooking, food catering and industrial processes (Brenes et al., 2002; Carrasco-Pancorbo et al., 2007; Cheikhousman et al., 2005). Several studies published in literature up to now have compared the effects of conventional and microwave heating on the physical and chemical parameters of extra virgin olive oil (Albi, Lanzón, Guinda, Pérez-Camino, & León, 1997; Brenes et al., 2002; Caponio & Gomes, 2001; Vieira & Regitano-D'Arce, 1999; Valli et al., 2010).

It has been observed that heating can affect the phenolic fraction and the oxidation stability and degradability of oil. In fact, Cerretani, Bendini, Rodriguez-Estrada, Vittadini, and Chiavaro (2009) have studied the effect of microwave heating treatments on phenols compared to the effects produce by oxidation or heating by conventional oven; particularly lignans have shown the highest stability to the thermal treatments due to their strong antioxidant properties (Carrasco-Pancorbo et al., 2007). The parameters that have been proven to influence the extent of oxidation and the degradation of oils in a highest extension during heating are oil composition, time and temperature of heating, food (in the case that some food is in contact with the oil), ratio between surface and volume of the oil (Andrikopoulos, Kalogeropoulos, Falirea, & Barbagianni, 2002; Christie, Brechany, Sebedio, & Le Quere, 1993). However, strong interactions exist among these variables and because of that, they are difficult to control and define (Jorge, Márquez-Ruiz, Martín-Polvillo, Ruiz-Méndez, & Dobarganes, 1996).

More recently, Valli et al. (2010) have studied the phenolic fraction of two EVOO and their admixtures after thermal treatments using microwave and conventional oven by HPLC-DAD/MSD and NMR spectroscopy. In this work authors have observed an increase of the dialdehydic forms of secoiridoids (dialdehydic form of elenolic acid lacking a carboxymethyl group, EDA; p-hydroxyphenylethanol linked to the dialdehydic form of elenolic acid,

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p-HPEA-EDA) after microwave and conventional heat treatments. It is likely that reasonable chemical conversions from elenolic acid (EA), p-hydroxyphenylethanol linked to elenolic acid (p-HPEA-EA), and 3,4-dihydroxyphenylethanol linked to elenolic acid (3,4-DHPEA-EA) to their respective dialdehydic forms (EDA; p-HPEA-EDA; 3,4-dihydroxyphenylethanol linked to the dialdehydic form of elenolic acid, 3,4-DHPEA-EDA) were induced by heating.

Other analytical techniques as FT-IR have been recently applied to the analysis of phenolic fraction of EVOO (Cerretani et al., 2010). Spectroscopic FT-IR coupled with chemometrics methods have been successfully used to detect olive oil adulteration (Lerma-García, Ramis-Ramos, Herrero-Martínez, & Simó-Alfonso, 2009; Maggio, Cerretani, Chiavaro, Kaufman, & Bendini, 2010; Ozen & Mauer, 2002) and freshness (Sinelli, Cosio, Gigliotti, & Casiraghi, 2007). The chemometric algorithm partial least square (PLS) has been repeatedly and extensively used to obtain different quality parameters of edible oils (Al-Alawi, Van de Voort, & Sedman, 2004; Iñón, Garrigues, Garrigues, Molina, & De la Guardia, 2003; Li, Van de Voort, Ismail, & Cox, 2000b; Li et al., 2000a).

In this work three different EVOOs have been subjected to heating treatment by conventional oven with the following aims:

(1) to study the changes in the oxidative stability (k_{232}, k_{270} and OSI time) and in the spectroscopic properties (Vis–NIR and FT-MIR) occurring in this process;

(2) to find, by spectroscopic analysis, the compounds or the families of compounds that are most important in this oxidation pathway;

(3) to explore the opportunity of adopting some rapid spectroscopic methods (Vis–NIR and FT-MIR) for the control of cooked olive oils.

2. Materials and methods

2.1. Apparatus

Fatty acid (FA) analyses were performed using an Autosystem XL Perkin Elmer (Shelton, CT, USA) gas chromatograph equipped with a flame ionisation detector (FID). The determination of k_{232} and k_{270} was carried out using an UV–vis 1800 instrument (Shimadzu Co., Kyoto, Japan), which had a six slot shuttle and a system for temperature control of working conditions. The oxidative stability of samples was evaluated using an eight-channel oxidative stability instrument (Omnion, Decatur, IL, USA). The FT-MIR spectra were acquired on a Tensor 27™ FTIR spectrometer system (Bruker Optics, Milan, Italy), fitted with a Rocksolid™ interferometer and a DigiTect™ detector system coupled to an attenuated total reflectance (ATR) accessory. NIR analysis was carried out by NIR Lab Near Infra Red by transmittance instrument (SACMI Imola S.C., Imola, Bologna, Italy).

2.2. Materials, reagents and standards

Potassium hydroxide, methanol, n-hexane, isooctane and acetone were purchased from Merck (Darmstadt, Germany). The standard mixture of FA methyl esters (GLC 463) was supplied by Nu-Chek (Elysian, MN, USA).

2.3. Samples and thermal treatment

Three different samples of extra virgin olive oil (named A, B and C) from different Italian regions (Abruzzo, Marche and Puglia) harvested in the fall of 2009, were analysed in this experimental study. The oils were different in terms of cultivar and ripening degree.

For analytical purposes, six aliquots (50 g) of each sample were inserted in 250 mL opened glass beakers (7.2 cm i.d.) and subjected to conventional heating at 180 °C in a oven (model M20-VN, Instruments s.r.l, Bernareggio, Milan, Italy). The beakers were removed from the oven at fixed intervals of 30 min, obtaining samples with different heating treatments (30, 60, 90, 120, 150, 180 min) to be analysed. All heated samples were cooled at room temperature (23 ± 1 °C) for 30 min and stored in bottles without headspace at 12 °C before chemical analysis.

2.4. Fatty acid composition

The FA composition of oil samples was determined as fatty acid methyl esters (FAMEs) after alkaline treatment, obtained by mixing 0.05 g of oil dissolved in 2 mL of n-hexane with 1 mL of 2 N potassium hydroxide in methanol, and subsequent gas chromatographic analysis, according to Bendini, Cerretani, Vecchi, Carrasco-Pancorbo, and Lercker (2006), with slight modifications. Analytes were separated on a RTX-2330 capillary column (30 m × 0.25 mm i.d., 0.2 μm film thickness) from Restek (Bellefonte, PA, USA). Column temperature was held at 140 °C for 5 min and then it was increased at 2.5 °C min^{-1} until 240 °C. The FID and the injector temperatures were both set at 250 °C. Peak identification was accomplished by comparing the peak retention times with those of the GLC 463 FAME standard mixture, injected under the same gas chromatographic conditions. The GC response factor of each FA was also calculated by using the GLC 463 FAME standard mixture.

2.5. Determination of k_{232} and k_{270}

The UV-spectrophotometric indexes (k_{232} and k_{270}) were determined according to the European Communities official methods and the following amendments (European Union Commission, 1991). To calculate the k_{270} and k_{232} values, the oil samples were diluted in isooctane (1:100 v/v for k_{270} and 1:1000 v/v for k_{232}), placed into a 1 cm quartz cuvette, and analysed at the wavelengths of 270 and 232 nm, against a blank of isooctane. Three replicates were prepared and analysed per sample.

2.6. Evaluation of oxidative stability under forced conditions

An eight-channel oxidative stability instrument (OSI) (Omnion) was used. To obtain the OSI induction time, a stream of purified air

Table 1
FA composition, OSI values, k_{232} and k_{270} values of samples before the thermal treatment.[a]

	A	B	C
C16:0	15.43 ± 0.01	10.52 ± 0.02	12.79 ± 0.12
C16:1 A	0.13 ± 0.00	0.11 ± 0.00	0.12 ± 0.01
C16:1 B	1.27 ± 0.00	0.58 ± 0.00	1.07 ± 0.01
C17:0	0.06 ± 0.00	0.16 ± 0.00	0.04 ± 0.01
C17:1	0.10 ± 0.00	0.23 ± 0.00	0.08 ± 0.00
C18:0	2.01 ± 0.01	2.73 ± 0.02	1.78 ± 0.01
C18:1 n−9	65.64 ± 0.01	76.01 ± 0.07	72.81 ± 0.13
C18:1 n−7	3.26 ± 0.01	1.68 ± 0.03	2.79 ± 0.02
C18:2	10.34 ± 0.01	6.40 ± 0.01	7.10 ± 0.03
C20:0	0.38 ± 0.00	0.40 ± 0.00	0.30 ± 0.00
C18:3 n−3	0.95 ± 0.00	0.77 ± 0.00	0.76 ± 0.01
C20:1	0.29 ± 0.01	0.27 ± 0.00	0.26 ± 0.01
C22:0	0.12 ± 0.00	0.13 ± 0.00	0.09 ± 0.00
OSI time (h)	11.35	32.15	20.20
k_{232}	2.59 ± 0.10	2.29 ± 0.35	3.23 ± 0.40
k_{270}	0.10 ± 0.01	0.15 ± 0.01	0.26 ± 0.00

[a] Data are expressed as mean of three determinations, with the standard deviations (except for OSI time). Fatty acid composition, determined by gas chromatography analysis and expressed as percentages; OSI time, oxidative stability index expressed as hours and hundredths of hours; k_{232} and k_{270} values determined by spectroscopic analysis.
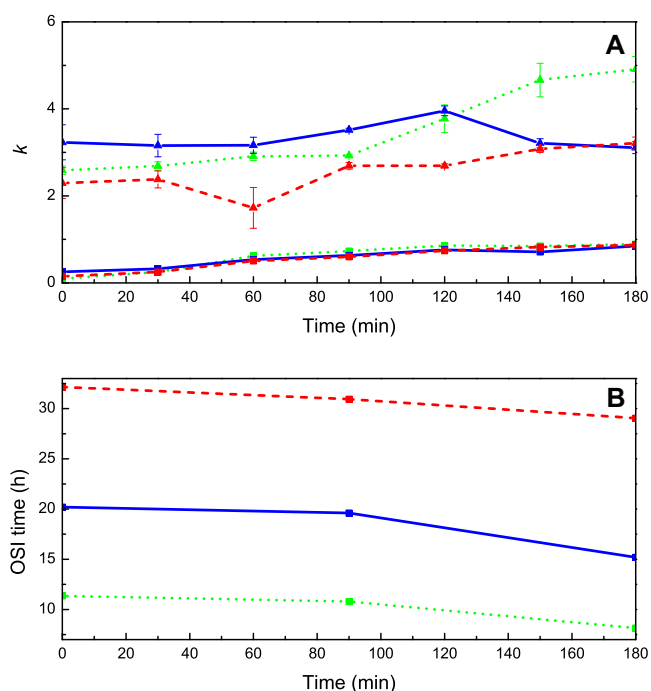
Fig. 1. (A) Plot of k_{232} (▲) and k_{270} (■) measured at heating times 0, 30, 60, 90, 120, 150 and 180 min for samples A (···), B (--) and C (—). (B) OSI time values (h), of samples A (···), B (--) and C (—) monitored at heating times 0, 90 and 180 min.

(120 mL min⁻¹ air flow rate) was passed through 5.0 ± 0.1 g oil sample heated at 110 ± 0.1 °C, under atmospheric pressure. The effluent air contains especially short chain acids as formic acid and other volatile compounds formed during thermal oxidation of the oil; these substances were recovered and measured in deion-ised water, as an increase of conductivity. The OSI time was expressed in hours and hundredths of hours, which was defined as a measure of the oxidative stability of oil.

2.7. FT-MIR spectroscopy

A small amount of the oil samples (about 1 g) was uniformly deposited on the crystal surface of ATR accessory (Specac Inc., Woodstock, GA, USA), equipped with a ZnSe 11 reflection crystal. Analyses were carried out at room temperature. Spectra were acquired (32 scans/sample or background) in the wavenumber range of 4000–700 cm⁻¹ at a resolution of 4 cm⁻¹, and the data exported by OPUS r. 6.0 (Bruker Optics) software in ASCII compatible format. For each sample, the absorbance spectrum was collected against a background, obtained with a dry and empty ATR cell. Three spectra per sample were recorded. After acquiring each spectrum, the ATR crystal was cleaned with a cellulose tissue soaked in *n*-hexane and then rinsed with acetone.

2.8. Vis–NIR spectroscopy

The samples were inserted in cuvettes (optical glass; light path: 20 mm; Hellma, Jena, Germany). Spectra were acquired using the halogen lamps as light source and an optical filter of 200 FN, a measuring time of 6 ms, within a wavenumber range of 33,000–9000 cm⁻¹. Three spectra per sample were recorded in ASCII compatible format. After acquiring each spectrum, the glass container was cleaned with *n*-hexane, soap and then rinsed with acetone.

2.9. Chemometrics methods

PLS regression aims to find the relationship between a set of predictor (independent) data, X ($m \times n$), and a set of responses (dependent), Y ($m \times l$). Here, n and l are the independent and dependent variables, respectively, and m is the observation vector.
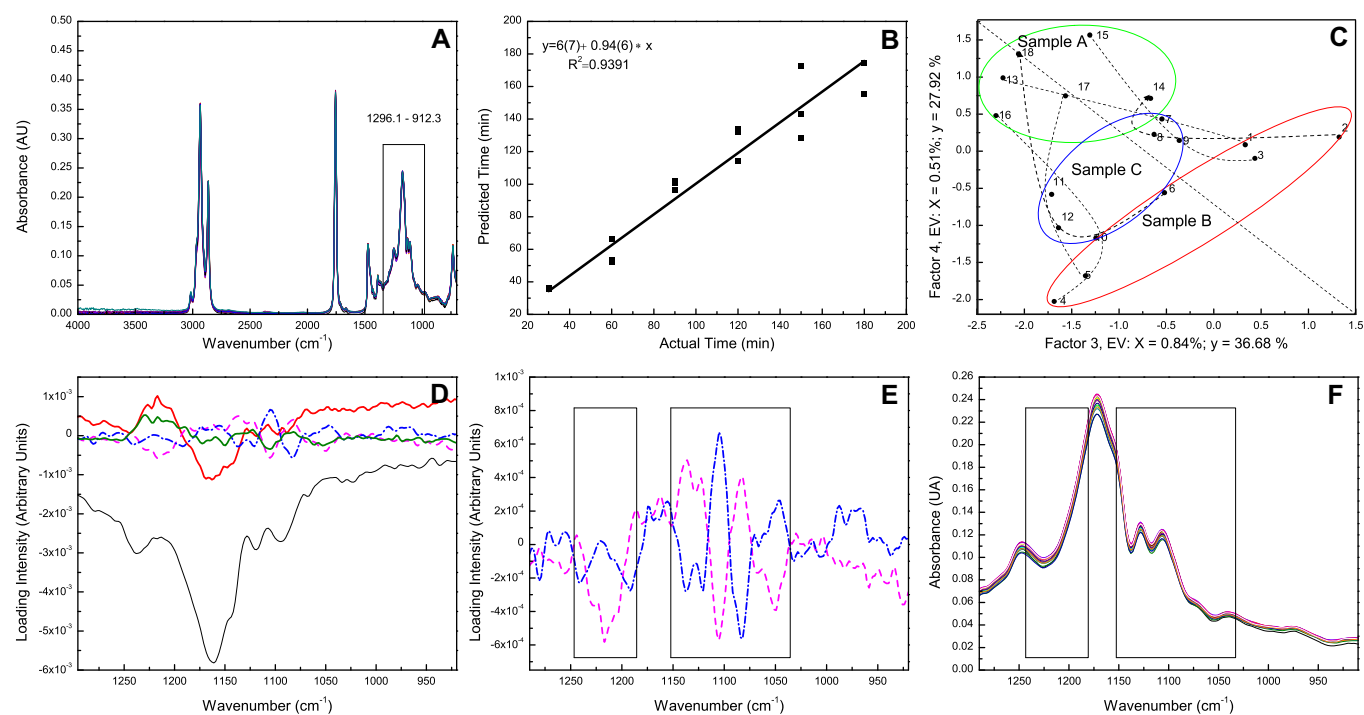

Fig. 2. (A) Full FT-MIR spectra (4000–700 cm⁻¹). (B) Actual vs. predicted heating time values in the calibration set in the selected spectral range (1296.1–912.3 cm⁻¹). (C) Third and 4th PLS factor score plot, see reference number in Table 2. (D) First 5 PLS loadings vectors. (E) Third and 4th PLS loadings vectors. (F) FT-MIR spectra in the selected wavenumber range (1296.1–912.3 cm⁻¹).

Table 2
Code Numbers used for each sample in PLS score plot of MIR and PLS score plot of NIR.

Code	PLS-FT-MIR	Time (min)	Code	PLS-NIR	Time (min)
1	B	120	1	B	120
2	B	150	2	B	150
3	B	180	3	B	180
4	B	30	4	B	30
5	B	60	5	B	60
6	B	90	6	B	90
7	C	120	7	C	120
8	C	150	–	–	–
9	C	180	8	C	180
10	C	30	9	C	30
11	C	60	10	C	60
12	C	90	11	C	90
13	A	120	12	A	120
14	A	150	13	A	150
15	A	180	14	A	180
16	A	30	15	A	30
17	A	60	16	A	60
18	A	90	17	A	90

However, it differs from the multiple linear regression technique (MLR) mainly that PLS is able to give stable predictions even when *X* contains highly correlated variables. Detailed description of PLS method and its algorithms could be found elsewhere (Haaland & Thomas, 1988).

The chemometrics computations were performed in Matlab 7.0 (Mathworks, Inc., Natwick, MA, USA). Variable selection was implemented using the graphical interface MVC1 provided by Olivieri, Goicoechea, and Iñón (2004). A PLS Score Plots was performed using Tomcat Toobox (Daszykowski et al., 2007). PLS was run on mean-centred data.

All programs were run on an ACER-Aspire 5050 computer with an AMD Turion™ 64 Mobile, 2.20 GHz microprocessor and 2.00 Gb of RAM.

3. Results and discussion

3.1. Compositional analysis

Table 1 groups the fatty acid composition, the OSI time, and the k_{232} and k_{270} coefficients for the three olive oils studied. Before thermal stress only sample B showed k_{232} and k_{270} values that were both below the limits established by the EC Regulation for EVOO category (European Union Commission, 2007), which corresponded to 2.50 and 0.22, respectively. Sample C showed both values over these limits, while sample A exceeded the limit only for k_{232} (Table 1).

Oxidative status of the samples were evaluated by monitoring the trend of conjugated dienes (k_{232}) and trienes (k_{270}) during the thermal stress (Hrncirik & Fritsche, 2005; Lerma-García, Simó-Alfonso, et al., 2009; Mancebo-Campos, Fregapane, & Desamparados Salvador, 2008) and analysing the samples heated at different times of treatment (Fig. 1, part A). The three samples underwent a significant increase of k_{270} with heating (Allouche, Jiménez, Gaforio, Uceda, & Beltràn, 2007; Bendini, Valli, Cerretani, Chiavaro, & Lercker, 2009), reaching values higher than the legal limit after only 30 min of thermal treatment (see Fig. 1, part A). During heating, a common trend for k_{232} among samples was not evidenced (Fig. 1, part A). As reported in Fig. 1, part B, the OSI time (h) was also checked halfway through the treatment (90 min) and at the end (180 min). Before the thermal treatment, the three samples showed high differences among OSI values: such a difference in oxidative stability could be easily related to the FA composition of the oils (Table 1), as previously reported in literature (Aparicio et al., 1999; Lerma-García, Simó-Alfonso, et al., 2009). In fact, sample B showed the highest OSI time value (32.15 h), the highest oleic acid content and the lowest amounts of polyunsaturated FA (linoleic and linolenic acids). Moreover, at the end of the thermal treatment sample B was the one that registered the lowest decrease (9.64%) in terms of OSI time, confirming its remarkable oxidative
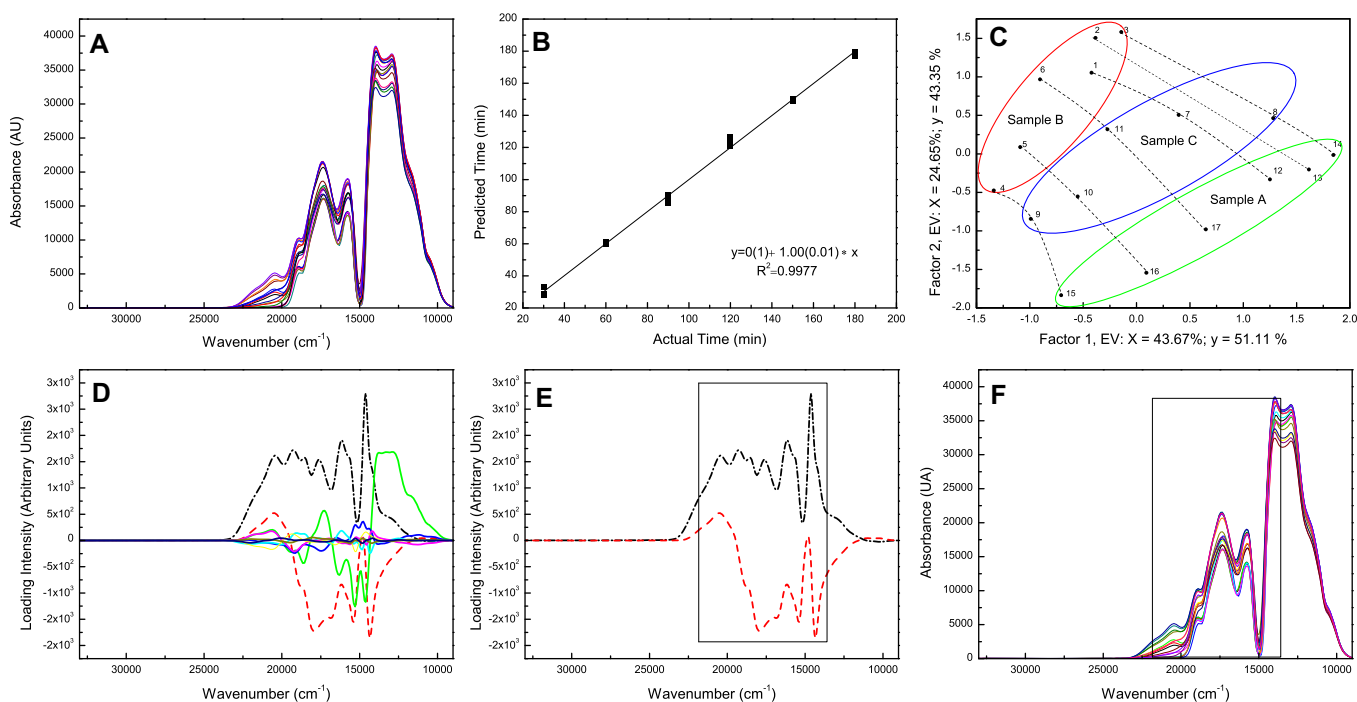

Fig. 3. (A) NIR spectra (33,000–9000 cm^{-1}). (B) Actual vs. predicted heating time values in the calibration set. (C) First and 2nd PLS factor score plot, see reference number in Table 2. (D) First 9 PLS loadings vectors. (E) First and 2nd PLS loadings vectors. (F) NIR spectra in the selected wavenumber range.

stability, while sample A and sample C presented a OSI time decrease of 28.19% and 24.75%, respectively (Fig. 1, part B).

3.2. Spectral analysis

The analysis of the variations in the FT-MIR spectra is not very easy, because these changes are very weak and there are so many peaks and shoulders without resolution (Fig. 2, part A). For a better visualisation of trends, the compression of the spectral information is needed. Principal component analysis (PCA) is one of the most used tools for this kind of works. Although, in this particular case it has not been possible to find any correlation between the PCA scores and the heating time.

Therefore, PLS was used, because it is a tool capable of addressing decomposition of the spectra to the dependent variable (heating time) (Carlson & Gautun, 2005). Since PLS was unable to find any acceptable correlation between oil spectra evolution and heating time using full spectrum FT-MIR, we used a wavelength selection algorithm (Xu & Schechter, 1996). The region 1296.1–912.3 cm^{-1} was selected as the optimum for regression (Fig. 2, parts A and B). Then we proceeded to analyse the score plots of significant PLS factors to see which of them was correlated with both the original composition of the oil and the evolution in time (Fig. 2, part C and Table 2). A segmentation was pointed out to 3rd and 4th PLS factor (Fig. 2, part C), which can distinguish among the different oils A, B and C. Moreover, the first (0–90 min) and the last (120–180 min) heating times into each kind of oil could be distinguish, as is showed with a dashed line. Once these factors were individualised as the most relevant ones, their loadings were evaluated (Fig. 2, parts D and E). Two regions were identified as principal influential ones: 1245–1180 cm^{-1} and 1150–1030 cm^{-1}, as shown in Fig. 2, part F. These regions comprise the bands belonging to –CH_2– (1238), C–O (1238, 1138, 1118, 1097 and 1033 cm^{-1}), being C–O reported by Lerma-García, Simó-Alfonso, Bendini, and Cerretani (2011), as one of the regions more affected by oxidation.

In the case of the NIR spectra, shown in Fig. 3, part A, there were not many peaks but the analysis of the spectral variations was also complicated due to the low resolution among them.

Therefore, for better visualisation of trends, PLS and PCA were applied. UV–NIR spectra did not show any correlation between PCA scores and the heating times, but PLS was able to find an acceptable correlation (see Fig. 3, part B) with full spectrum NIR. Because of that, we proceeded to analyse score plots of significant PLS factors (Fig 3, part C and Table 2) to see which of them were correlated with both the original composition of the oils A, B and C and the evolution in time. A segmentation was evidenced to 1st and 2nd PLS factor, which distinguished among the different oils. In addition, the different heating times appear sorted in ascending way with the 2nd PLS-factor for A, B and C oils (Fig. 3, part C and reference number in Table 2). This fact shows that the 2nd PLS-factor is very important for the correlation. Once these PLS factors were detected as the most relevant, their loadings were evaluated (Fig 3, parts D and E). The major influence region was individualised in: 2200–1325 cm^{-1} (Fig. 3, part F).

4. Conclusions

This work describes a study of thermal stress on three virgin olive oils. Evaluation of oxidative stability under forced conditions, determination of k_{232} and k_{270} and spectral properties were explored along the thermal treatment. Fatty acid composition was explored at the beginning of the work. The three samples underwent a significant increase of k_{270} with heating, while a common trend for k_{232} among samples was not evidenced. Before the thermal treatment, the three samples showed high differences among

OSI values. At the end of the thermal treatment sample B, with highest initial OSI time, highest oleic acid and lowest amounts of polyunsaturated FA content, registered the lowest decrease (9.64%) in terms of OSI time. The opportunity to adopt rapid spectroscopic methods (Vis–NIR and FT-MIR) in the control of cooked olive oils has been explored by using the chemometric analysis PLS. The most significant regions included the bands related to –CH_2– (1238) and C–O (1238, 1138, 1118, 1097 and 1033 cm^{-1}) groups, concluding that C–O was one of bands more affected by oxidation. The spectroscopic data elaboration by PLS was able to distinguish among the different oils and the different heating times. This approach could be useful for monitoring the oxidative status of cooked oils, both in industrial scale or in food-catering field.

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