A novel approach to monitor the oxidation process of different types of heated oils by using chemometric tools

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ABSTRACT

The oxidative stability of seven oils with different fatty acid profiles was assessed. Oxidation at 0, 2 and 4h at 180ºC was monitored by measuring the absorbance of thiobarbituric acid reactive substances (TBARS) along the absorption spectrum (300-600 nm), the volatile aldehydes (HS-SPME-GC-MS) and the fatty acid profile (FID-GC).

TBARS absorption spectrum behavior depended on the lipid composition of heated oils. Higher absorbance increments during heating were noticed at 390 nm compared to 532 nm (from 2 to 21 fold higher depending on the oil), pointing to its better sensitivity to detect oxidation. Furthermore, a close relationship between ABS_{390}, the loss of polyunsaturated fatty acids and their corresponding oxidation compounds (volatile aldehydes) was revealed by Principal Component Analysis

Multiparametric equations allowed predicting the formation of volatile aldehydes of heated oils by measuring only two parameters: $TBARS_{390}$ during their heating, and the lipid profile in unheated oils (MUFA, ω-3 and ω-6). Results pointed out the interest of choosing the ABS_{390} when the oxidative evolution of vegetable oils under heating is assessed by the TBARS test.

Key words: lipid oxidation; SPME; TBARS; volatile aldehydes; fatty acids; oil composition.

1. INTRODUCTION

The most important cause of deterioration of oils and fats is oxidation, which does not only reduce shelf life, sensory acceptance and the nutritional value of food, but also produces toxic compounds. Unsaturated and especially polyunsaturated fatty acids are firstly oxidized to form odorless and tasteless hydroperoxides, which are further decomposed to form secondary oxidation products (Papastergiadis, Mubiru, Van Langenhove & De Meulenaer, 2012). Most of them are mainly volatile aldehydes with different unsaturation degree and characterized by low threshold values (Petersen, Kleeberg, Jahreis & Fritsche, 2012). It is well-known that, by oxidation, oleic acid can give rise to heptanal, octanal, nonanal, decanal, (*E*)-2-decenal and (*E*)-2-undecenal; linoleic acid leads to hexanal, 2-heptenal, 2-octenal, (*E,Z*)-2,4-decadienal and (*E,E*)-2,4-decadienal and, lastly linolenic acid produces a greater proportion of (*E,Z*)-2,4-heptadienal and (*E,E*)-2,4-heptadienal (Belitz, Grosch & Schieberle, 2009).

Malondialdehyde (MDA) has been used as representative of non-volatile secondary oxidation products and its determination by the TBARS test measuring the absorbance at 532 nm has been widely used due to its simplicity (Barriuso, Astiasarán & Ansorena, 2013). However, this method exhibits some drawbacks. It has been stated that the TBARS assay is nearly worthless with heat-treated oils and fats (Guillén-Sans & Guzmán-Chozas, 1998) and that this test is reliable only when applied for the determination of MDA in unprocessed foods (Papastergiadis et al., 2012). According to these last authors (Guillén-Sans & Guzmán-Chozas, 1998; Papastergiadis et al., 2012) in thermally treated food, the TBARS$_{532}$ test overestimates the content of MDA because of the interference of other compounds, probably due to the presence of other secondary oxidation products, such as alkadienals. Besides, the overestimation of MDA can also be due to the fact that other types of compounds (carbohydrates, amino acids and

nucleic acids) can react with TBA and absorb at 532 nm (Salih, Smith, Price & Dawson, 1987).

On the other hand, the measurement of MDA is inherently insensitive to monounsaturated fatty acids (MUFA), as oleic acid hydroperoxides contain less than two double bonds. This fact leads to underestimation of oxidation in highly monounsaturated lipids when TBARS test is used (Waraho, Cardenia, Rodríguez-Estrada, McClements & Decker, 2009; Poyato, Navarro-Blasco, Calvo, Cavero, Astiasarán & Ansorena, 2013). Consequently, MDA measurements at 532 nm may not be true measure of oxidative deterioration in certain foods. A better option must be found to easily monitor the evolution of secondary oxidation products, especially during thermal treatments.

It has been reported that, at least, some aldehydes from lipid oxidation may also react with TBA to produce not only red (λ_{max}= 532 nm), but also yellow (λ_{max}= 455 nm) and orange pigments (λ_{max}= 495 nm) (Kosugi, Kato & Kikugawa, 1987). Many alkanals, alkenals and alkadienals produce a yellow pigment with TBA (Guillén-Sans & Guzmán-Chozas, 1998), depending on the time-temperature conditions in which the reaction is forced (Marcuse & Johansson, 1973). In addition, from the safety standpoint, these aldehydes other than MDA are also considered to be important lipid oxidation products due to their toxic effects, and should be considered more than just lipid oxidation markers (Stevens & Meier, 2008; Guillén & Uriarte, 2012).

In this context, the aim of this work was to optimize conditions to follow the oxidation rate of different edible oils subjected to an intense heating by (i) measuring volatile aldehydes by gas chromatography and, (ii) correlating them with the measurement of TBARS at a range of wavelengths (300-600 nm) in order to choose the highest sensitivity for detecting and predicting oxidation intensity. Seven oils with very

different lipid profiles were chosen for the study, to evaluate the influence of the different composition on both parameters.

2. MATERIALS AND METHODS

2.1. Materials

The oils used in this study were Virgin Linseed oil, VL, (Biolasi Productos Naturales S.L.,Guipúzcoa, Spain); Algae oil, AO, (Martek Biosciences Corporation, Columbia, USA); Sunflower oil, SF, (Urzante S.L, Navarra, Spain); High-oleic Sunflower oil, HOSF, (Titan, Sos Corporación Alimentaria, S.A, Madrid, Spain); Extra Virgin Olive oil, EVO1, and Refined Olive oil, RO, (Koipe, Sos Corporación Alimentaria, S.A, Madrid, Spain); and Extra Virgin Olive oil, EVO2, (Carbonell, Sos Corporación Alimentaria S.A, Madrid, Spain).

2-thiobarbituric acid, tetraethoxypropane and fatty acid methyl esters were purchased from Sigma–Aldrich Chemical (Steinheim, Germany). Boron trifluoride/methanol and Butylated hydroxytoluene (BHT) were obtained from Merck (Whitehouse Station, NJ, USA). Potassium hydroxide, hexane, cyclohexanone, hydrochoric acid, trichloroacetic acid and ammonium sulphate were from Panreac (Barcelona, Spain).

2.2. Heating study

1 g of oil was weighed into a 25 mL glass vial. Before sealing, the air was replaced with nitrogen in order to control the lipid oxidation process. The test vials were placed in an orbital shaker (JP Selecta S.A., Rotaterm, Barcelona, Spain) previously stabilized at 180 ºC. The septum was pierced by a needle allowing air exchange during heating. The vials were removed from the hotplate at different heating times (2 and 4 h). Then, the samples were introduced in an ice bath for 15 minutes for cooling and stored in the freezer (-20 ºC) until analysis. Seven types of oils were tested. From each type, three

independent batches were heated at 0, 2 and 4 hours. The 63 different samples were analyzed in triplicate.

2.3. Oil analysis

2.3.1. Fatty acid profile

Fatty acids (FA) were determined in the assayed oils by gas chromatography FID detection, previous preparation of the fatty acid methyl esters derivatives. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (AOAC, 2002). A Perkin-Elmer Clarus 500 gas chromatograph, equipped with a split-splitless injector, automatic autosampler, and coupled to a computerized system for data acquisition (TotalChrom, version 6.2.1) was used. It was fitted with a capillary column SPTM-2560 (100 m×0.25 mm×0.2 μm). The temperature of the injection port was 250 °C and 260 °C for the detector. The oven temperature was programmed to increase from 170 to 200 °C at a rate of 10.0 °C/min and then at rate of 4.0 °C/min to 220 °C. The carrier gas was hydrogen, 2.15 mL/min. The sample size was 0.5 μL and the split ratio was 120:1. The identification 30 fatty acids analyzed was done by comparison of their retention times with those of pure fatty acid methyl esters and the quantification used heptadecanoic acid methyl ester as internal standard. After the quantification of the individual FA, the sums of saturated, SFA, (capric, lauric, miristic, palmitic, stearic, arachidic, and behenic acid), monounsaturated, MUFA, (palmitoleic, oleic, vaccenic, erucic, nervonic and eicosenoic acid), polyunsaturated, PUFA, (linoleic, γ-linoleic, α-linoleic, eicosadienoic, eicosatrienoic, arachidonic, eicosapentaenoic, docosatrienoic, docosapentaenoic (ω-3), docosapentaenoic (ω-6) and docosahexahenoic acid), trans (t-palmitoleic, elaidic, t-linoleic, c,t- linoleic, t,c-linoleic and brassidic acid) and sums of ω-3 (α-linolenic, eicosadienoic, eicosatrienoic, docosapentaenoic, docosahexahenoic acid) and ω-6 (linoleic, γ-linoleic, arachidonic, docosapentaenoic) were calculated.

2.3.2. Determination of volatile aldehydes

The determination of the volatile aldehydes in the headspace of the oils throughout the heating time was carried out by a HS-SPME-GC-MS method. Vials containing 1 g of oil were placed into a water bath maintained at 50 °C. After a period of sample equilibration (15 min), a fiber coated with DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane, 50/30 μm film thickness, Supelco) was inserted into the headspace of the sample and maintained for 60 min (Guillén & Uriarte, 2012). The fiber was desorbed for 15 min in the injection port of a gas chromatograph model HP 6890 Series (Hewlett Packard), equipped with a HP Mass Selective Detector 5973. A fused-silica capillary column (30 m long × 0.25 mm inner diameter × 0.25 μm film thickness, from Agilent Technologies), coated with a non-polar stationary phase (HP-5MS, 5% phenyl methyl siloxane) was used. The operating conditions were as follows: the oven temperature was set initially at 42 °C (5 min hold), increased to 120 °C at 3 °C/min and to 250 °C at 10 °C/min (5 min hold); the temperatures of the ion source and the quadrupole mass analyzer were kept at 230 °C and 150 °C, respectively. Helium was used as carrier gas at 1 mL/min; injector and detector temperatures were held at 250 °C and 280 °C, respectively. Mass spectra were recorded at 70 eV; using scan mode (amu range 33-350). Before performing every extraction, cleanness of the fiber was checked by running a blank and confirming the absence of peaks in the chromatogram. Compounds were identified by previous injection of standards, by their Kovats index and mass spectra or by matching with mass spectra of a commercial library (Wiley 275.L, Mass Spectral Database). Total area for each compound was obtained on basis on the amount of a specific ion for each peak, and taking into account the relative ratio in which this ion is present in each compound

(Petersen et al., 2012; Thomas, Mercier, Tournayre, Martin & Berdagué, 2013). Results are expressed in area $\times 10^5$/ g.

2.3.3. TBARS value

TBARS values were determined according to the method described by Maqsood and Benjakul (2010) with slight modifications. Briefly, the TBARS reagent was prepared by mixing 15 % w/v trichloroacetic acid, 0.0375 % w/v 2-thiobarbituric acid in 0.25 N hydrochloric acid. The oil (0.3 g), distillate water (250 µL), solution 1% BHT (20 µL) and the TBARS reagent (1 mL) were vortexed in a centrifuge tube (20 s), placed in a boiling water bath for exactly 15 min and then cooled in an ice bath to room temperature. Cyclohexanone (2 mL) and 4 M ammonium sulphate (500 µL) were added to the mixture and were vortexed for 30 s. The mixture was centrifuged at room temperature at 4000 rpm for 10 min to allow separation of phases. In the case that solid particles were formed in the interface, the TBARS reaction was repeated using lower amount of sample. After centrifugation, the supernatant was collected and the absorbance was measured between 300 and 600 nm (FLUOStar Omega spectrofluorometric analyzer, BMG Labtechnologies, Offenburg, Germany). The spectra were collected with a resolution of 2 nm. Results were expressed in absorbance units corrected by sample weight.

2.4. Statistical data processing

ANOVA and Tukey b post hoc test were applied to compare the evolution of the lipid profile along the heating treatment with statistical significance set at $p < 0.05$. Pearson correlation test was used to determine correlations among variables (fatty acids, aldehydes, and absorbances at every wavelength between 300-600 nm) and contributed to decide the wavelength selected.

A data matrix (63 x 27) whose rows were the different oil samples analysed (cases: 3 different batches from 7 types of oils at 3 different heating times) and whose columns were the selected analytical parameters assayed was built. A Principal Component Analysis (PCA), was used to achieve a reduction of dimension, retaining the maximum amount of variability present in the experimental data in order to verify the existence of relationships between the analysed oil samples. The data were autoscaled before PCA in order to achieve independence on the different scale factors of the analytical parameters. After studying the primary observation of principal components obtained, a linear regression model was applied in order to relate the production of volatile aldehydes with the TBARS test absorbances (390 and 532 nm). The variables that may affect the regression were introduced into the study following a forward stepwise model (inclusion criteria: $p < 0.05$; exclusion criteria $p > 0.10$). The selected variables were MUFA, ω-6, and ω-3 (in g/100 g oil) present in the unheated oils. The contribution of the variables was tested in the model for each absorbance (390 or 532 nm) and family of volatile compounds (alkanals, alkenals, alkadienals and total aldehydes). The significant interactions between variables were also taken into account to obtain the equations. A cross validation of the regression model was done by means of data splitting (Montgomery, Peck & Vining, 2006). The data set (n = 63) was split at random into an estimation set (n = 42) used to build the regression model, and a prediction set (n =21) that studied the predictive ability. Cross validation estimates the prediction error of the obtained regression models. Several statistics were included to evaluate the quality of the diagnostic approach of the regression models: the coefficient of determination for the regression model (R^2), cross validation (R_{CV}^2) and prediction (R_{pred}^2), and root mean squared error of cross validation ($RMSE_{CV}$) or root mean squared error of prediction

(RMSE$_P$), calculated as follows, based on the observed and predicted values of the analyzed parameters:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y})^2}{n}} \qquad R^2\,pred = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y})^2}{\sum_{i=1}^{n}(y_i - \overline{y})^2}$$

where y$_i$ and ŷ$_i$ are the analytical parameter loads for observation i determined by experimental test and by estimated regression model, respectively; and ȳ represents the mean of the observed data. Besides, the usefulness of regression models was evaluated using the ratio of prediction to deviation (RPD). This parameter expresses the ratio of the standard deviation data set in relation to the root mean squared error of prediction. The regression model was considered an adequate good predictor of analyzed parameters when the RPD value was higher than 2, while a value below 1,5 indicates the useless of the model (Villar, Fernández, Gorritxategi, Ciria. & Fernández, 2014; Kamruzzaman, ElMasry, Sun & Allen, 2012).

Finally, a double-cross validation by reversion of the roles of estimation and prediction data sets was carried out for confirmation purposes of the robustness of prediction regression models.

All statistical analyses were run using SPSS v. 15.0 for Windows program.

3. RESULTS

3.1. Fatty acid profile

The fatty acid profile of the seven studied oils was analyzed throughout the heat treatment and sums of the different fractions were calculated (Table 1). Before heating, as expected, the EVO, RO and HOSF oils were rich in oleic acid (67-75%), whereas SF, VL and AO oil were abundant in linoleic (58%), α-linolenic acid (53%) and DHA (35%), respectively. Regardless the antioxidant amounts present in the oils (data not shown), the heat treatment caused a reduction in the fatty acids content (g FA/100 g oil)

of all studied samples, which mainly affected the PUFA fraction. Thus, a large PUFA reduction (28.66 %) was noticed for algae oil, followed by olive oils (EVO1, 11.17%; EVO2, 13.76%; RO, 7.34%), high-oleic sunflower oil (11.74%) linseed oil (7.93%), and sunflower oil (5.90%). MUFA decreased only in the highly monounsaturated oils, being always this reduction lower than a 2.6% of their initial content.

The evaluation of the absolute weight loss of fatty acids pointed out the DHA suffered the greatest decrease after the 4 h of treatment in AO (10.55 g/100 g oil), whereas in VL, it was α-linolenic who was mainly lost (4.66 g/100 g oil). Linoleic acid was the main PUFA affected by the heat treatment in SF oil (3.41 g/100 g oil). Only a slight reduction in the oleic acid content (1.7, 1.32, 0.76, 1.83 g/100 g oil) was noticed in HOSF, EVO1, EVO2 and RO oils respectively, despite being the most abundant FA in these four oils.

3.2. Volatile aldehydes analysis

HS-SPMS-GC-MS data of major volatile aldehydes isolated from the headspace of the seven oils were followed throughout the heating treatment (Table 2). 5 alkanals, 9 alkenals and 7 alkadienals were identified during the whole process. These compounds represent groups of secondary oxidation products resulting mainly from the autooxidation of oleic, linoleic, α-linolenic acid and DHA (Belitz, Grosch & Schieberle, 2009).

Before heating, only very low amounts of alkanals (hexanal and nonanal) in olive oils were found, while in VL only very low amounts of alkadienals ((Z,E)-2,4-heptadienal and (E,E)-2,4-heptadienal) were detected. No volatile aldehydes in AO, SF and HOSF oil before the heat treatment were obtained.

The thermal degradation of the fatty acids led to many other compounds that increased their concentrations in the headspace along the heating time, showing different aldehyde

profiles depending on the type of oil. The highest increment measured as area counts ($\times 10^5$) was detected in SF followed by EVO oils, RO and VL. HOSF and particularly AO oil gave rise to the lowest amount of total aldehydes.

Alkenals were the main contributors to the total volatile aldehydes in EVO oils at the end of heating (up to 55%) mainly due to the increase of those compounds with 10 or 11 carbon atoms. Alkenals and alkadienals were the predominant volatile compounds in AO (50% each approximately), characterized by short chain compounds (alkenals of 3-5 carbon atoms and alkadienals of 6-7 carbon atoms). In both VL and SF, alkadienals represented more than 65% of total aldehydes content, with (*E,E*)-2,4-decadienal and (*E,E*)-2,4-heptadienal being undoubtedly the principal responsible compounds, respectively. In the case of RO and HOSF oils, the distribution of both aldehydes was very similar, the alkenals around a 45% and the alkanals to a 40% of the total aldehydes, approximately.

3.3. TBARS

The full absorption spectra (300-600 nm) resulting from the TBA reaction of the different oils at the different sampling points are shown in Figure 1. Oils characterized by a high PUFA content (VL, AO and SF) showed a substantial absorbance increase in the 350-450 nm region during heating. 390 nm was the wavelength in which the highest correlation coefficients between the measured yellow absorbances (350-420 nm) and the increments of the different families of volatile aldehydes and compositional variables was noticed. Moreover, the association between the reduction of fatty acids and the TBARS absorbance modification during treatment was higher at 390 nm than that observed at other wavelengths.

However, the values measured at the wavelength traditionally used for TBARS analysis (532 nm) were not so clearly modified. In fact, whereas the maximum values at 532 nm

were lower than 2 absorbance units, results at the proposed target wavelength (390 nm) reached significantly higher values (up to 17 units in the case of SF and around 15 and 7 units for VL and AO, respectively). In SF and VL oils the absorbance increments during the 4 h treatment at 390 nm were around 21 fold the increments observed at 532 nm, whereas in the olive oils they were only 2-3 folds. Thus, the $TBARS_{390}$ produced during the heating process increased the absorbance in a greater extent than $TBARS_{532}$ along the 4 h treatment regardless of the type of oil tested. It should be also noted that, whereas the absorbance at 390 nm always increased along the process, a constant value or even a reduction in the absorbance at 532 nm was detected in the latest 2 hours (Table 3).

3.4. PCA and regressions

Prior to performing PCA, the suitability of autoscaled data for factor analysis was checked. Table S1 (supplementary material) reports the correlation matrix (R) among the twenty seven variables selected. Inspection of this matrix revealed a great number of coefficients higher than 0.400. The determinant value (1.62×10^{-39}) of correlation matrix was low. All variable items showed a significant correlation with, at least, six other variables or more. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.729, exceeding the recommended value of 0.6, and the Barlett's test of Sphericity (value 4659) reached statistical significance ($p < 0.001$), both data supporting the factorability of the correlation matrix. Moreover, the Anti-image Correlation matrix showed low values and the Measures of Sampling Adequacy (MSA) for each individual variable were from 0.541 to 0.902. The matrix was therefore, appropriate for PCA.

Four principal components with eigenvalues exceeding one were extracted according Kaiser Criterion which explained up to 90% of the total variance (40.0%, 23.5%, 19.0% and 7.5%, respectively). The communalities (variance proportion of a variable involved

in the PC space) of every descriptor were found higher than 0.75, except for oleic acid and absorbance at 532 nm (0.465 and 0.624, respectively).

The PC extracted correlation matrix was subjected to the Varimax rotation in order to clarify the assignment of experimental variables. The rotated factor matrix is shown in Table 4. After orthogonal rotation, easier interpretation of the factors was possible.

This four-factor model interpreted reasonably well the associations between the variation of certain fatty acids after 4 h (Δ FA) treatment, the ABS_{390} and the volatile aldehydes formed from every oil studied. Thereby, Figure 2 shows the associations obtained among the analyzed parameters in order to visualize the discriminating efficiency of principal factors. The first factor was characterized by high loadings for ABS_{390} (0.690) and also for those parameters (pentanal, hexanal, 2-heptenal, 2-octenal, 2,4-nonadienal and 2,4-decadienal) (loadings between 0.854-0.975) associated with linoleic acid loss (0.965). Heptanal, octanal, nonenal, 2-nonenal, 2-decenal and 2-undecenal, were the dominating variables (loadings higher than 0.926) in the second factor, followed by oleic acid loss (loading of 0.646), and ABS_{532} also correlating to a lower extent (0.428). The prevailing variables in the third factor, 2-butenal (0.965) and 2,4-heptadienal (0.966), mainly reflected the α-linolenic acid decline (0.948). Additionally, ABS_{390} and ABS_{532} had also significant positive loadings (0.647 and 0.505, respectively) related to this third factor. Finally, the fourth factor was related to DHA (0.961) and its oxidation products (2, 4-hexadienal and 2-propenal with loadings 0.932 and 0.748, respectively). Also, PCA allows visualizing the discriminating efficiency of the rotated principal components in three-dimension scores plot, making easy the observation and interpretation of the findings for the simple understanding. Scatterplot of loadings for assayed oil samples (Figure S1, supplementary material) in the space defined by the principal factors show that different clusters are clearly

distinguished according to the type of oil, setting a differentiation criterion. From visual inspection, a plain separation between linseed, sunflower and olive-based oils in Figure S1a; and algae, sunflower and olive-based oils in Figure S1b, was found.

After studying the primary observation of principal components obtained, the linear regression model applied showed no close relationship between the production of volatile aldehydes and the TBARS test absorbances (390 and 532 nm) using monoparametric equations, therefore a multiple regression model was tested. The Figure S2 (supplementary material) showed the multiparametric character of the relationship between the aldehydes and the absorbance at 390 nm. Numerous multiparametric equations establishing a relationship between the initial lipid profile of oils and the volatile aldehydes formed were calculated. Those in which the highest coefficients of correlation were achieved included the MUFA, ω-3 and ω-6 percentages (Table 5). In all cases, predictive models for volatile aldehydes were related with ABS_{390}. In the case of alkanals (equations 1) and alkenals (equations 2) the lineal model was related to % MUFA, aside from ABS_{390}. The adjustment of the regression was better for alkanals (0.907) in comparison with alkenals (0.789). In the case of alkadienals, the highest regression coefficient was obtained when the equation included the % ω-3 or % ω-6 (R > 0.9). In these biparametric equations, the interaction between the lipidic fraction selected and the ABS_{390} was also included. The best prediction of total aldehydes was achieved by using a multiparametric equation that included the %MUFA as well as % ω-3 (equation 5; R = 0.896) or % ω-6 (equation 6; R = 0.877). As in the previous cases, the interaction between % MUFA and ABS_{390} was significant and included in the equation. On the contrary, predictive models for volatile aldehydes and ABS_{532} did not fit with the real behavior of volatiles concentration, basically due to the slight decrease of ABS_{532} observed during the last 2 h heating in some oils. Consequently, they were

discarded and confirmed the suitability of ABS_{390} to evaluate lipid oxidation in heated oils.

The performance of regression models was evaluated by means of a double cross validation. Table 5 provides also the performance statistics of multiple regression models obtained in comparison with those evaluated in cross validation. The calculated regression coefficients and determination coefficients exhibit a reasonable similarity for all estimated models. In addition, the RMSE of cross validation is close to the root mean squared error of regression model, deducing be a successful as a predictor. Besides the prediction determination coefficients showed that the quantitative prediction is possible for alkanals ($R_{pred}^2 = 0.821$), alkadienals ($R_{pred}^2 = 0.877$ and 0.874) and total aldehydes ($R_{pred}^2 = 0.800$ and 0.786); supported at the same time by the values of RPD greater than 2. In a lesser extent, the alkenals regression model allows to establish a limited used for prediction ($R_{pred}^2 = 0.600$, RPD > 1.5), but explanatory purposes may be claimed. Finally, the little differences in predictive performance and estimated regression coefficients obtained in double-cross validation (data partially showed in Table 5) prove the useful of the developed model to evaluate the analytical parameters in the different types of assayed oils.

4. DISCUSSION

A significant oxidation of oils was evidenced during heating, giving rise to a decrease in FA, especially the highly unsaturated ones. Furthermore, the higher the unsaturation degree of the FA, the higher was its quantitative loss. These data are consistent with the lower oxidative stability of PUFA, and more specifically with the longer chain FA compared to that of MUFA (Belitz et al., 2009). In fact, polymerization can easily take place in high polyunsaturated oils, which leads to a decrease the fatty acids´ content, giving rise to polar compounds and polymers (Choe & Min, 2007).

The formation of volatile aldehydes during heating constitutes a good index of lipid oxidation intensity (Petersen et al., 2012; Ritter & Budge, 2012). A high association between the total volatile compounds formed and the loss of MUFA and PUFA was observed. In particular, high inverse correlations were especially found between the loss of ω-6 fatty acids and alkadienals (R= -0.968) and total volatile aldehydes (R= -0.853). Aldehydes produced were substantially different in quantity and type (alkanal, alkenal or alkadienal) depending on the composition of the lipid matrix. These findings were in agreement with those obtained by Guillén & Uriarte (2012) after the analysis of different edible oils heated at high temperatures for long periods of treatment. The individual volatile aldehydes formed were in accordance with the main FA loss detected in each oil. Oils rich in ω-3 FA (VL and AO) showed (*E,E*)-2,4-heptadienal as the main aldehyde formed. SF, rich in ω-6 showed the highest value for (*E,E*)-2,4-decadienal, and oils rich in MUFA (HOSF, EVO1, EVO2 and RO) produced high amounts of 2-decenal and nonanal.

The secondary oxidation compounds formed colored compounds with TBA, whose absorbance was measured at different wavelengths (300-600 nm) and correlated with the increments of the different families of volatile aldehydes. In the yellow area (350-450nm), the highest correlation coefficients between the measured absorbances and the increments of the different families of volatile aldehydes and compositional variable were found at 390 nm. This wavelength was chosen as the best representative parameter for the assessment of the yellow pigment formed from the reaction between the volatile aldehydes and TBA. Moreover, the sensitivity of the measure was higher at ABS_{390} than at ABS_{532} due to the larger increments of absorbance values at 390 nm throughout the same period of heating. In addition, within each type of oil, results showed that a higher

correlation was found between total aldehydes and ABS_{390} compared to ABS_{532}, showing the better suitability of the absorbance measured at 390 nm.

Although increases in total aldehydes were found for all analyzed oils during the last 2 hours of heating, it is worthy to be mentioned that no absorbance increases at 532 nm were noticed in this period for AO, HOSF, EVO1 and RO using $TBARS_{532}$. This fact means that this parameter was not able to reveal that an oxidation process was taking place during that period, whereas according to the aldehydes analysis and ABS_{390}, the oxidation increased significantly.

Also, the association between the reduction of ω-6 fatty acids and the TBARS absorbance modification during treatment was higher at 390 nm (R= -0.748) than that observed at 532 nm (R = -0.223).

Chemometric techniques have been used to discriminate among experimental data on edible oils (Giacomelli, Mattea & Ceballos, 2006; Cordella, Tekye, Rutledge & Leardi, 2012; Vaclavik, Belkova, Reblova, Riddellova & Hajslova, 2013; Tsiaka, Christodouleas, & Calokerinos, 2013). In our case, PCA evidenced the associations found between the decrease of individual fatty acids, the absorbances (390 and 532 nm) and the volatile aldehydes produced during the heat treatment. The high association of ABS_{390} to the first and the third components, indicated that the major volatile TBA-reactive compounds that gives rise to yellow chromophores, proceed basically from the oxidation of linoleic (highly associated to the first component) and α- linolenic acid (highly associated to the third component). On the contrary, no associations were found between the first component and ABS_{532}. These data are consistent with previous studies in which it was proved that MDA is not always formed in some oxidized systems. Esterbauer and Cheeseman (1990) proposed that linoleic acid is a poor

precursor of MDA, explaining the fact that the decline of this fatty acid is not properly detectable at 532 nm.

PCA also showed a low association between ABS_{390} and the amount of oleic acid (second component). However, in olive oils, the increases of ABS_{390} were also significant during heating because the greater FA decrease was linked to PUFA, not to MUFA, as it has been previously stated. The association of ABS_{532} to the second component was higher than expected. It has been described that TBARS test is insensitive to MUFA oxidation. However, alkenals and alkanals (highly associated to the second component), can also form red pigments in the TBA reaction, (Kosugi et al., 1987) and contributed to this association. For these reasons, it seems that the ABS_{390} measurement is more appropriate than the traditional wavelength (ABS_{532}) to follow the oxidation rate of oxidized lipids, at least under the conditions assayed in the present study.

An interesting possibility regarding the estimation of the intensity of lipid oxidation is to find a mathematical model that could be able to monitor it by finding solid relationships among different parameters related to this process. Richards, Wijesundera and Salisbury (2005) found that PV were well associated with the formation of two volatile compounds (hexanal and (*E,E*)-2,4 heptadienal) during accelerated oxidation of canola oil, developing a suitable model to predict the PV from the amounts of those compounds. Petersen, Kleeberg, Jahreis, Busch-Stockfisch and Fritsche (2012) identified key volatile aldehydes (propanal, (*E,E*)-2,4-hexadienal and *E*-2-heptenal) to differentiate rapeseed oil samples with different oxidative properties. In our study relationships between ABS_{390} and the MUFA amount, ω-3 and ω-6 in the unheated oils were able to predict the amount of different families of aldehydes (alkanals, alkenals

and alkadienals) and also total volatile aldehydes and, in consequence, to estimate the intensity of the oxidation process.

The developed equations showed that the amount of alkanals and alkenals were highly dependent on the initial MUFA content of the oils, whereas in the case of alkadienals, regression was mainly related to the initial PUFA content. This finding is in agreement with previous studies in which the PUFA content had also been described as a good predictor of lipid oxidation (Maestre, Pazos & Medina, 2011; Guillén & Uriarte, 2012).

Our regression models are an easy, simple and good tool to predict the formation and semi-quantification of volatile aldehydes, from the fatty acids profile of oils and the evaluation of TBARS absorbance at 390 nm, particularly when it is carried out under accelerated oxidation conditions (180ºC-4h).

We proposed that measuring TBARS absorbance at 390 nm resulted more sensitive than the measurement making use of 532 nm to monitor lipid oxidation in heated oils, regardless of the lipid profile of the oil evaluated. The formation of volatile aldehydes from lipid oxidation during heating can be predicted from two simple and routine parameters, by measuring the absorbance at proposed wavelength (390 nm) through the TBARS test and assessing the lipid profile of the oil samples.

Our results confirm the validity of using TBARS for assessing oxidation in heated oils if wavelength used is 390 nm.

ABBREVIATIONS

VL, virgin linseed oil; AO, algae oil; SF, sunflower oil; HOSF, high-oleic sunflower oil; EVO1, extra-virgin olive oil (Koipe); EVO2, extra virgin olive oil (Carbonell); RO, refined olive oil; FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances; IS, internal standard.

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