DOI:10.1021/jf902758d

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Synchronous Fluorescence Spectroscopy: Tool for Monitoring Thermally Stressed Edible Oils

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Total synchronous fluorescence spectra are proposed for monitoring edible oils during thermal stress. Synchronous fluorescence spectra obtained at an 80 nm wavelength interval combined with principal component analysis are suitable for classification of vegetable oil deterioration. Spectroscopic features in the range of 300−500 nm have been used for extra virgin olive, olive pomace, and sesame oil and the range of 320−520 nm has been used for corn, soybean, sunflower, and a commercial blend of oils. The score in the first two principal components explains 91.1% of the data matrix variance for extra virgin olive, sesame, and olive pomace oil and 89.3% for corn, soybean, sunflower, and the commercial blend of oils. The objective of this study is to develop a rapid method for the prediction of edible oil quality during thermal stress. Spectroscopic changes are indicative of oxidative deterioration as measured through wet chemistry methods: peroxide value, *p*-anisidine value, totox value, and radical-scavenging capacity.

KEYWORDS: Synchronous fluorescence spectroscopy; extra virgin olive oil; olive pomace oil; sesame oil; corn oil; soybean oil; sunflower oil; thermal stress; radical-scavenging activity; totox

INTRODUCTION

Edible oils during frying undergo a series of chain degradation reactions producing potentially toxic compounds that also reduce the organoleptic and nutritional values. The primary oxidation products are hydroperoxides that decompose to secondary oxidation products: aldehydes, ketones, hydrocarbons, and alcohols (*1*). The rate of oil degradation increases during exposure to oxygen, light, and heating (*2*). The oxidative stability of oils depends on their composition. Phenolic compounds and tocopherols inhibit or delay the initiation of oxidative chain reactions as they react with lipid radicals, forming inactive products. The chain-breaking capacity of phenolic compounds is higher than that of tocopherols (*3*, *4*). Extra virgin olive oil stability is higher due to its high level of monounsaturated fatty acids and natural phenolic compounds. Other vegetable oils contain high levels of polyunsaturated fatty acids and tocopherols.

Classical methods such as peroxide value (PV), anisidine value (AV), acid value, carbonyl value, 2-thiobarbituric acid value, and total polar materials (*5*) are used for monitoring lipid oxidation and evaluating the quality of frying oils in food industries. Spectrophotometric absorption at 232 and 270 nm is also indicative of the oxidative stage.

Development of rapid and easy to use tests that correlate with standard methods for the evaluation of oil quality during frying was recommended in the third and fourth international symposiums on deep fat frying held at Hagen, Germany (*6*). Tests should be rugged, simple, and safe for use in food processing/preparation areas, providing an objective index of oil degradation.

Spectroscopic techniques combined with multivariate analysis are useful for rapid screening of oil quality (*7*). Infrared and Raman spectroscopy have been recently used for the determination of oil degradation (*8*−*13*). Vibrational techniques lack sensitivity in comparison with fluorescence spectroscopy. Recently, conventional fluorescence spectroscopy has been used for monitoring extra virgin olive oil deterioration. Fluorescence excitation spectra with emission at 330 and 450 nm have been used for monitoring polyphenol and vitamin E evolution and ROOH degradation, respectively, during heating (*14*). In addition, total luminescence spectra and synchronous fluorescence spectra have been used for monitoring changes occurring in olive oil during storage under different conditions (*15*).

Synchronous fluorescence (SyF) in comparison with conventional fluorescence spectroscopy is preferable for the analysis of complex multicomponent samples because it reduces spectral overlaps by narrowing spectral bands and simplifies spectra using a suitable wavelength interval (*16*). In SyF both the excitation and emission monochromators are scanned simultaneously in such a manner that a constant wavelength interval ($\Delta\lambda$) is kept between emission and excitation wavelengths. Total synchronous fluorescence (TSyF) spectra are obtained by plotting fluorescence intensity as a combined function of the excitation wavelength and the wavelength interval.

The aim of this study is to develop a method for the classification of extra virgin olive, olive pomace, sesame, corn, sunflower,

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 Published on Web 09/01/2009

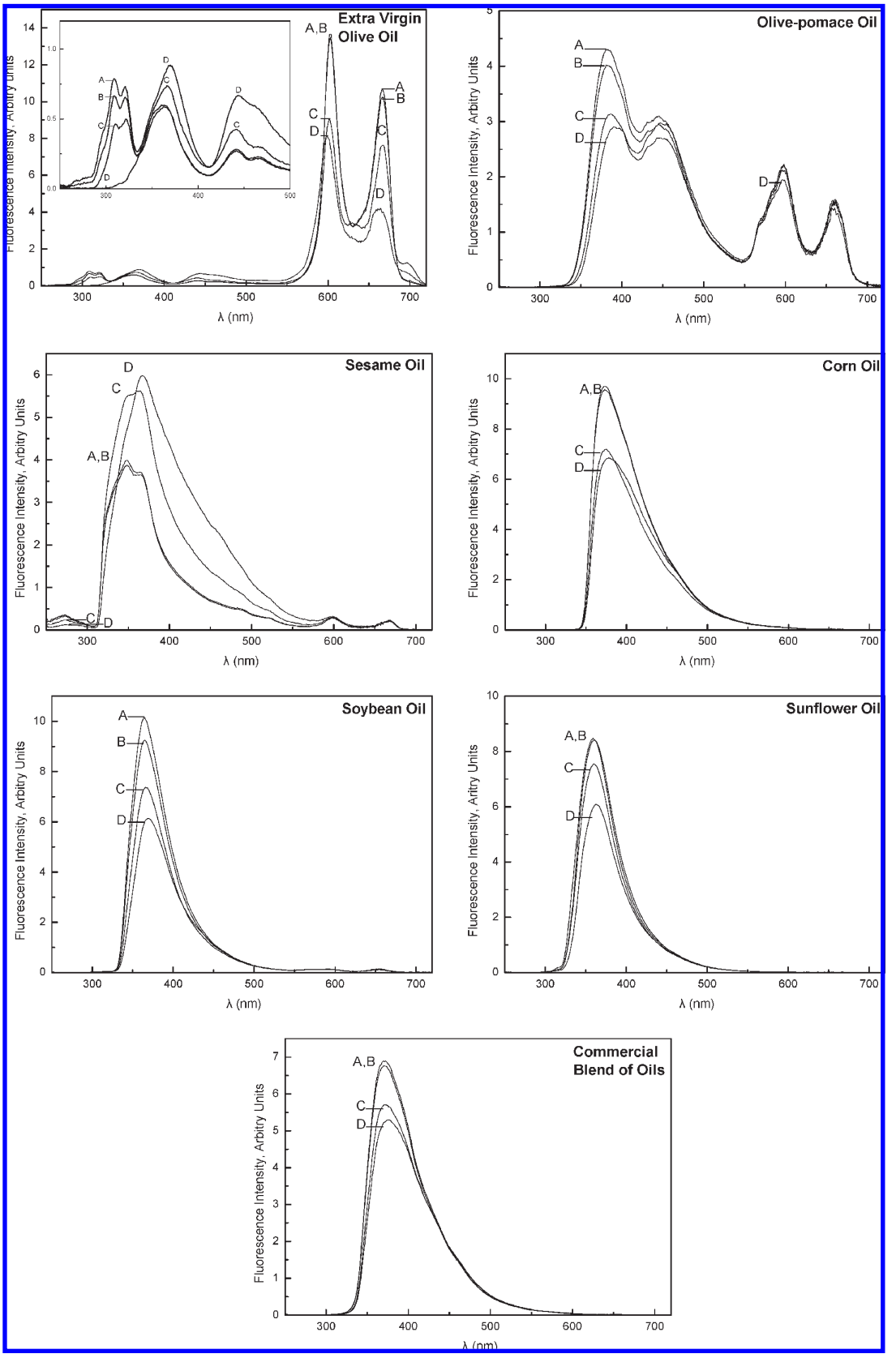

Figure 1. Synchronous fluorescence spectra of extra virgin olive, olive pomace, sesame, corn, sunflower, soybean, and a commercial blend of oils (A) and after 30 min heating at 100 (B), 150 (C), and 190 °C (D).

soybean, and a commercial blend of oils during heating at 100, 150, and 190 °C using synchronous fluorescence spectra. In addition, the study assigns spectroscopic changes to the changes of totox value and total antioxidant capacity.

MATERIALS AND METHODS

Samples and Reagents. One sample each of extra virgin olive, olive pomace, sesame, corn, sunflower, and soybean oils and a commercial blend of oils by Minerva S.A., Athens, Greece, was purchased from a local

shopping center. Hydroxytyrosol and tyrosol were purchased from Extrasynthese, Genay, France, and Acros Organics, Geel, Belgium, respectively. Isooctane, isopropanol, and glacial acetic acid were purchased from Merck, Darmstadt, Germany. Chloroform and ethyl acetate were purchased from SDS and Riedel-de Haën, Seelze, Germany, respectively. All solvents were of analytical grade. Potassium iodide 99.5%, sodium thiosulfate standard solution 0.1 M, starch, and *p*-anisidine 99% were purchased from Ferak, Berlin, Germany; Merck; Riedel-de Haën; and Aldrich, Steinheim, Germany, respectively. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) 90% and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) 97% were purchased from Sigma, St. Louis, MO, and Aldrich, respectively.

Procedure. Edible oil aliquots of approximately 7.5 g were placed in open glass vials of 4 cm height and 2.5 cm internal diameter and heated at 100, 150, and 190 °C using oil baths. Temperature was controlled within ±1 °C. A vial was withdrawn after 30 min and 2 and 8 h. After sampling, the vials were rapidly cooled on ice and stored at −18 °C until analyses and spectra acquisition.

Phenolic compound stock solutions 1.0×10^{-4} M in isopropanol were prepared; 1.0×10^{-6} M working solutions were prepared by diluting stock solutions in 1% w/v olive oil in hexane.

Methods. PV was determined according to the official method reported in the EC regulation (*17*). AV was determined according to International Union of Pure and Applied Chemistry (IUPAC) standard method 2.504 (*18*). Total oxidation value (totox) is calculated as totox = 2 × PV + AV (*19*).

Radical scavenging capacity (RSC) of vegetable oils was measured through the DPPH assay (*20*) as follows: DPPH solution of 5×10^{-3}% w/v in ethyl acetate (absorption approximately 1.4−1.5 at 515 nm) was prepared and kept in the dark. Oil aliquots of around 80−130 mg were weighed in screw-capped glass vials. Four milliliters of DPPH solution was added to the vial, the mixture was shaken, and the reaction was allowed to proceed for 1 h in the dark. The absorbance of samples was measured at 515 nm. To express absorbance changes as Trolox equivalent antioxidant capacity (TEAC) values, a Trolox calibration curve was used.

Instruments. Synchronous fluorescence spectra were acquired by the Jobin Yvon fluorolog-3 spectrofluorometer, which is a fully computer-controlled instrument using a double-grating excitation and a single-grating emission monochromator. The spectrofluorometer uses a 450 W xenon lamp for excitation. Excitation and emission slit widths were set at 2 nm. A quartz cell 10 × 4 × 45 mm from Hellma 104F-QS was used. The emission optical path was 2 mm. Right-angle geometry was used for spectrum acquisition.

TSyF spectra were obtained by measurement in the excitation wavelength region of 250−720 nm; the wavelength interval was varied from 20 to 120 nm in 20 nm intervals. To avoid spectroscopic distortions, spectra were corrected for the excitation lamp, the photomultiplier detector spectroscopic response, and emission and excitation gratings (*21*).

For AV and RSC measurements a Jasco V-550 UV−vis spectrophotometer was used. Quartz cells with a 10 mm optical path were used.

Data Analysis. Statistica software version 6 (StatSoft, 2001) was used for data analysis. Spectra were used after standardization, that is, transformation of the intensity at a specific wavelength by subtracting the mean and dividing by the standard deviation.

Principal component analysis (PCA) is an unsupervised method for exploratory data analysis. It reduces a large number of variables to a small number of principal components that explain the vast majority of variance in the data, reducing the dimensionality of the original data (*22*).

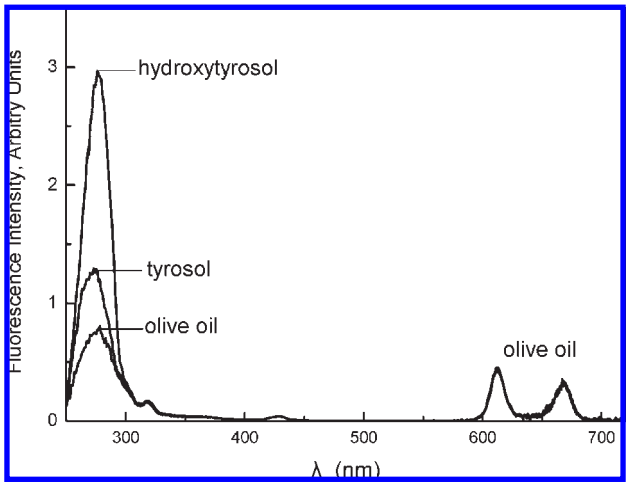

Figure 2. Synchronous fluorescence spectra of hydroxytyrosol, tyrosol, and olive oil using a wavelength interval of 60 nm.

RESULTS AND DISCUSSION

Synchronous Fluorescence Spectroscopy. Initial synchronous fluorescence spectra acquired using an 80 nm wavelength interval and after 30 min of heating at 100, 150, and 190 °C are shown in **Figure 1**. Extra virgin olive and olive pomace oils give fluorescence bands in the region around 570−700 nm attributed to chlorophyll pigments (*23*). These bands decrease after 30 min of heating at 150 and 190 °C. Sesame oil also shows small signals in this region. Corn, soybean, sunflower, and the commercial blend of oils do not show any signal in this region.

Extra virgin olive oil shows three peaks in the region of 350−500 nm that increase after 30 min at 150 and 190 °C. It also shows a double peak in the region around 315−350 nm that decreases after 30 min at 100 and 150 °C and disappears at 190 °C. It is interesting to note the small fluorescence signals in the region lower than 315 nm that disappear after 30 min of heating at 150 and 190 °C. Olive pomace oil shows two peaks in the region of 350−500 nm that decrease during heating at 100, 150, and 190 °C. Sesame oil shows fluorescence signals in the region of 315−500 nm

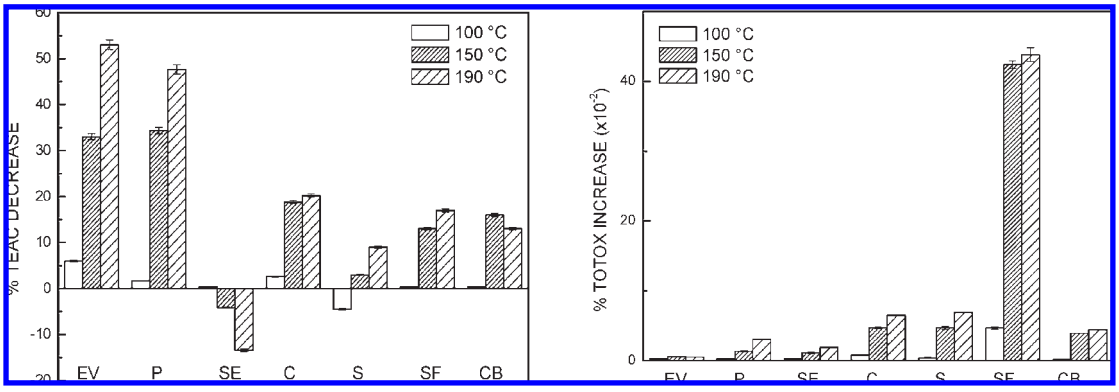

Figure 3. Changes of TEAC and totox values after 30 min of heating at 100, 150, and 190 °C: extra virgin olive (EV), olive pomace (P), sesame (SE), corn (C), soybean (S), sunflower (SF), and commercial blend of oils (CB). Values are means of three replicate measurements accompanied by standard deviations.

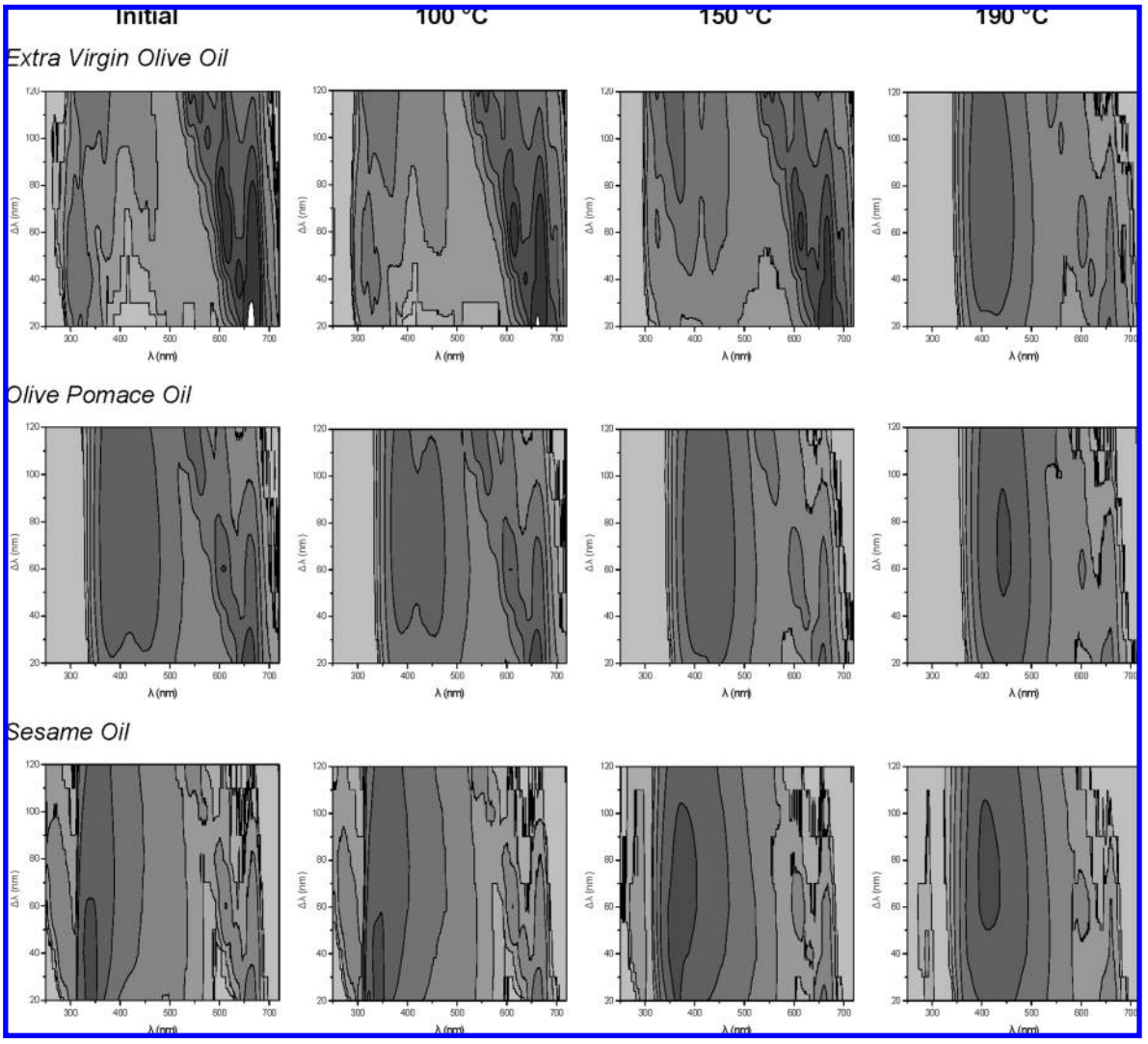

Figure 4. Total synchronous fluorescence spectra contour plots of extra virgin olive, olive pomace, and sesame oils after 8 h of heating at different temperatures. Grayscale indicates fluorescence intensities.

that increase along with temperature. Furthermore, it shows small fluorescence signals in the region lower than 315 nm that decrease during heating at 150 and 190 °C.

Corn, soybean, sunflower, and the commercial blend of oils show a fluorescence band in the region around 340−500 nm that decreases during heating at 150 and 190 °C. For soybean oil, the fluorescence band decreases earlier, after 30 min of frying at 100 °C.

As shown in **Figure 1**, oils that are obtained without any refinement (virgin olive and sesame oil) show fluorescence signals in the region lower than 315 nm. These signals could be attributed to phenolic compounds (*15*). Synchronous fluorescence spectra of two phenolic compounds, tyrosol and hydroxytyrosol, that are present in olive oils are shown in **Figure 2**. Fluorescence signals in this region decrease during heating, presumably due to the consumption of phenolic antioxidants by lipid radicals generated. Olive pomace, corn, soybean, sunflower, and the commercial blend of oils show a decrease of fluorescence bands in the region of 340−500 nm during heating at 150 and 190 °C. Olive pomace and soybean oil fluorescence bands decrease even after 30 min at 100 °C.

The decrease of fluorescence bands in the ranges of 250−350 and 350−400 nm for extra virgin olive and olive pomace oil, respectively, is in accordance with percent TEAC reduction, which is shown in **Figure 3**. Moreover, the percent TEAC increase of sesame oil after 30 min at 150 and 190 °C probably explains the increase of fluorescence signals in the region of 350−500 nm. The decrease of fluorescence signals in the region of 340−500 nm for corn, soybean, sunflower, and commercial blend of oils is in accordance with the TEAC reduction that could be attributed to the decrease of tocopherol. The small increase of fluorescence signals of extra virgin olive oil in the region of 350−500 nm at 150 and 190 °C is in accordance with the small increase of totox value shown in **Figure 3**.

By comparison of oil deterioration, extra virgin olive oil has the highest percent TEAC decrease during heating and the smallest change of totox value, which can be attributed to the effectiveness of phenolic compounds to break free radical chain reactions. Corn, sunflower, and the commercial blend of oils show similar perent TEAC decreases, although sunflower shows the highest totox value increase. It is interesting to note the TEAC increase of sesame oil upon heating at 150 and 190 °C and that of soybean oil at 100 °C. This could be explained by the conversion of natural to more potent antioxidants (*24*, *25*).

Figure 4 shows the initial TSyF contour plots of extra virgin olive, olive pomace, and sesame oils and those acquired after 8 h heating at 100, 150, and 190 °C. The TSyF contour plot of extra virgin olive oil spreads in the wavelength region of 260−720 nm:

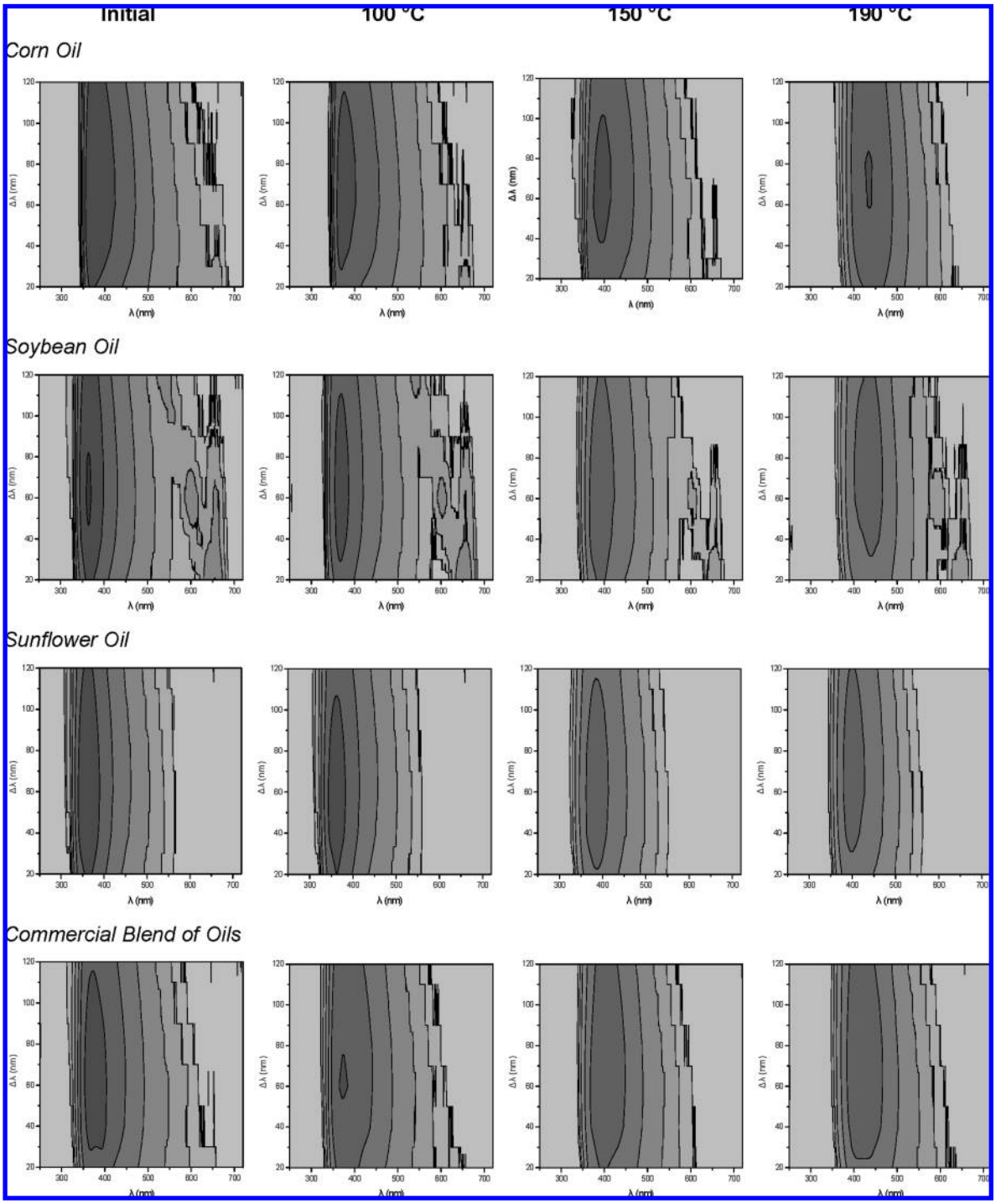

Figure 5. Total synchronous fluorescence spectra contour plots of corn, sunflower, soybean, and a commercial blend of oils after 8 h of heating at different temperatures. Grayscale indicates fluorescence intensities.

the region of 260−350 nm is attributed to phenolic antioxidants and tocopherol, and the region higher than 590 nm is attributed to chlorophyll. The signals below 290 nm disappear after 8 h at 100 °C. At 150 °C, fluorescence signals below 300 nm disappear, whereas the signals in the region of 325−465 nm increase. TSyF contour plots change significantly after 8 h at 190 °C: signals in the region of 265−350 nm disappear, those in the region of 350−500 nm increase, and those in the region of 500−720 nm diminish.

The TSyF contour plot of olive pomace oil spreads in the excitation wavelength region of 325−720 nm. The TSyF contour plot changes slightly after 8 h at 100 °C. At 150 and 190 °C the fluorescence signals in the wavelength region lower than 350 nm disappear, and those in the region of 400−500 nm increase, whereas signals in the region higher than 600 nm diminish. Maximum fluorescence intensities are observed at 190 °C in the region of 430−460 nm using a wavelength interval of 48−92 nm.

The sesame oil TSyF contour plot spreads in the excitation wavelength region of 250−720 nm. The contour plot remains almost the same after 8 h at 100 °C. At 150 and 190 °C fluorescence signals in the region below 325 nm and higher than 580 nm diminish, whereas signals in the region of 350−530 nm increase. Higher intensities are shown in the wavelength region of

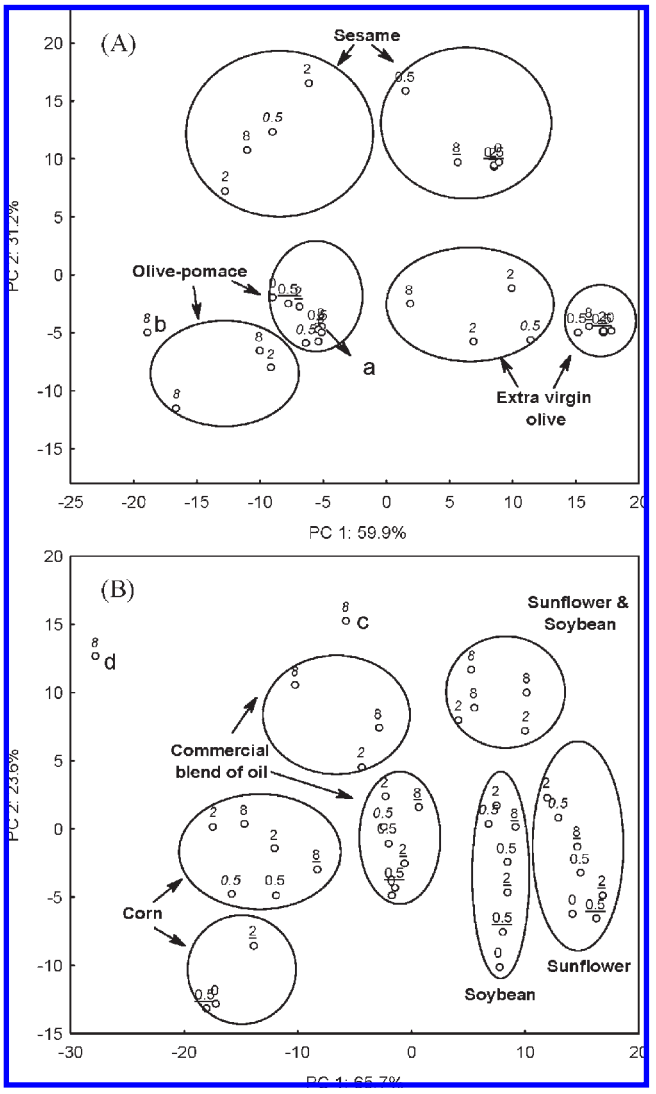

Figure 6. PCA based on fluorescence spectra of extra virgin olive, olive pomace, and sesame oils acquired in the range of 300−500 nm (**A**) and of corn, sunflower, soybean, and a commercial blend of oils acquired in the range of 320−520 nm (**B**) using an 80 nm wavelength interval during the time course of heating at different temperatures. Numbers are the exposure time (h) at 100 (underlined), 150 (regular), and 190 °C (italic); (a) extra virgin olive oil after 8 h at 190 °C; (b) sesame oil after 8 h at 190 °C; (c) soybean oil after 8 h at 190 °C; (d) corn oil after 8 h at 190 °C.

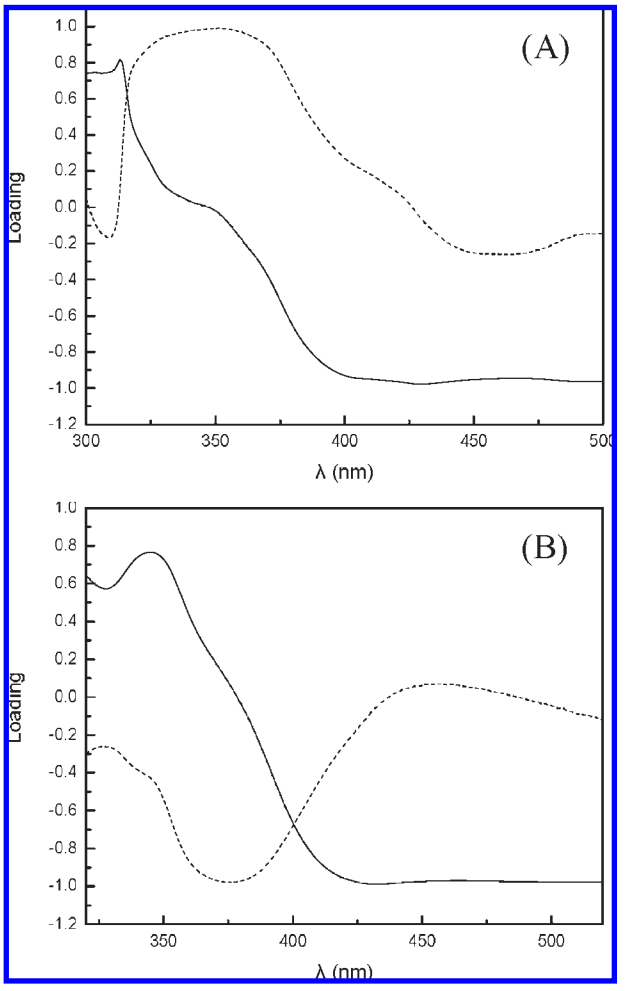

Figure 7. PCA loading plots for extra virgin olive, olive pomace, and sesame oils (**A**) and corn, sunflower, soybean, and a commercial blend of oils (**B**): (continuous line) PC1; (dashed line) PC2.

395−440 nm using a wavelength interval 50−105 nm at 190 °C. It is interesting to note that for extra virgin olive, olive pomace, and sesame oils, fluorescence signals in the region of 400−450 nm increase after 8 h of heating at 190 °C.

Figure 5 shows the initial TSyF contour plots of corn, soybean, sunflower, and the commercial blend of oils and those acquired after 8 h of heating. The corn oil TSyF contour plot spreads in the excitation wavelength region of 335−685 nm. The TSyF contour plot changes slightly at 100 °C. Fluorescence signals in the region lower than 370 nm diminish at 150 °C. At 190 °C the signals lower than 370 nm and higher than 630 nm disappear. During heating at 100, 150, and 190 °C the highest intensities of the contour plots shift from the region of 355−425 nm to 365−405, 375−420, and 425−440 nm, respectively. TSyF contour plots of soybean oil spread in the excitation wavelength region of 315−685 nm. Fluorescence signals in the region of 365−375 nm diminish after 8 h at 100 °C. At 150 °C fluorescence signals in the regions of 345−400 and 630−685 nm diminish. Signals in the region below 365 nm disappear, whereas those in the region of 430−500 nm increase at 190 °C. The TSyF contour plot of sunflower oil spreads in the excitation wavelength region of 305−565 nm. The contour plot does not change significantly after 8 h at 100 °C. Fluorescence signals lower than 350 nm disappear and those in the region of 350−385 nm diminish along with temperature. At 190 °C, signals in the region of 380−435 nm increase. The TSyF contour plot of the commercial blend of oils spreads in the excitation wavelength region of 320−660 nm. Fluorescence signals in the region of 355−410 nm diminish after 8 h at 100 °C. In higher temperatures, fluorescence signals lower than 350 nm disappear and those in the region of 350−385 nm diminish.

In summary, spectra shown in **Figures 4** and **5** change during heating: signals in the region lower than 350 nm disappear and those in the region around 600−700 nm diminish, probably due to the decrease of antioxidant compounds and chlorophyll, respectively. Signals in the region around 400−450 nm increase after 8 h at 190 °C, probably due to the formation of secondary oxidation products.

Principal Component Analysis. Extra virgin olive, olive pomace, and sesame oil differentiation according to stress time is shown in **Figure 6A**. Spectral features in the range of 300−500 nm have been used for PCA. $\Delta\lambda$ 80 nm is chosen for the analysis as the spectroscopic characteristics of edible oils change significantly during the procedure, according to TSyF spectra. The range

selected is in accordance with fluorescence spectra change after exclusion of chlorophyll signals. The score of the first two principal components (PC) explains 91.1% of the data matrix variance. Extra virgin olive oil forms two classes according to stress time. The first class includes the initial spectrum and spectra acquired during heating at 100 °C and after 30 min at 150 °C. The second class includes spectra acquired after 2 and 8 h of heating at 150 °C and 30 min and 2 h at 190 °C. The extra virgin olive oil spectrum acquired at 8 h of heating at 190 °C is mixed with olive pomace oil. Olive pomace oil spectra are categorized according to thermal stress in two classes. The first class includes the initial spectrum and spectra acquired after 30 min at 100, 150, and 190 °C and after 2 h at 100 and 150 °C. The second class includes the remaining spectra. Sesame oil forms two classes. One class includes the initial spectrum and spectra acquired from 30 min to 8 h at 100 °C and from 30 min at 150 °C, whereas the other includes spectra acquired after 2−8 h at 150 °C and after 30 min and 2 h at 190 °C. The sesame oil spectrum acquired at 8 h of heating at 190 °C is not assigned to any class. In conclusion, extra virgin olive and sesame oil spectra shift to negative scores of PC 1 during heating, whereas olive pomace oil spectra shift to more negative scores of PC 1 and PC 2.

PCA classes are in accordance with AV. The first class of extra virgin olive, olive pomace, and sesame oils includes samples of 3.1−5, 4.6−19, and 0.3−4 AV, respectively. The second class includes samples of 15−40, 45−100, and 12−40 AV for extra virgin olive, olive pomace, and sesame oils, respectively. Sesame oil acquired after 8 h of heating at 190 °C reaches 99 AV and is not included in any class. Extra virgin olive oil acquired after 8 h of heating at 190 °C reaches 43.5 AV and is included in the first class of olive pomace oil (4.6−19 AV).

Corn, soybean, sunflower, and a commercial blend of oils are categorized according to thermal stress as shown in **Figure 6B**. The score in the first two PCs explains 89.3% of the data matrix variance. The spectral features in the range of 320−520 nm have been used for PCA. This region is in accordance with TSyF contour plot changes. Corn oil forms two classes that follow thermal stress. The first class includes the initial spectrum and that acquired after 30 min and 2 h at 100 °C. Spectra acquired after 8 h at 100 °C and all spectra acquired at 150 and 190 °C form the second class. The spectrum acquired after 8 h at 190 °C is not assigned to the class. The commercial blend of oils forms two classes. The initial spectrum and those acquired after 30 min and 2 and 8 h at 100 °C, after 30 min and 2 h at 150 °C, and after 30 min at 190 °C are included in the first class. Spectra acquired after 8 h at 150 °C and after 2 and 8 h at 190 °C form the second class. Soybean oil spectra are categorized in two classes. The first class includes the initial spectrum and those acquired from 30 min to 8 h at 100 °C, from 30 min to 2 h at 150 °C, and after 30 min at 190 °C. The second class includes spectra acquired after 8 h at 150 °C and after 2 h at 190 °C. The spectrum acquired after 8 h at 190 °C is not assigned to any class. Sunflower oil also forms two classes that follow thermal stress. The first class includes the initial spectrum and those acquired from 30 min to 8 h at 100 °C, from 30 min to 2 h at 150 °C, and after 30 min at 190 °C. The second class includes spectra acquired after 8 h at 150 °C and after 2 and 8 h at 190 °C. By comparison of the classes in **Figure 6B**, it is clear that spectra shift to positive scores of the second principal component (PC 2) during heating. Moreover, soybean and sunflower oils have positive scores in the first principal component (PC 1) in contrast to corn and the commercial blend of oils.

PCA classes are in accordance with AV. Corn oils included in the first class have around 3.5 AV. Samples with 7−90 AV are in the second class, beyond one sample that has AV 4. Corn oil acquired after 8 h of frying at 190 °C reaches 217 AV and is not included in any class. The commercial blend of oils shows two classes with 5−43 and 100−235 AV. Soybean oil shows two classes that are also in accordance with AV. The first class includes oils with 33−49 AV, whereas the second class includes oils with 135−155 AV. Oil with 294 AV is not included in any class. Sunflower forms two classes with low (0.9−34) and high (100−140) AV.

PCA loading plots in **Figure 7A** show that PC1 gives information in the region lower than 315 nm. As shown in **Figure 4**, extra virgin olive and sesame oils show signals in this region in contrast to olive pomace oil. This could be attributed to phenolic compounds present in oils that are obtained without any refinement (i.e., extra virgin olive and sesame oil) in contrast to olive pomace oil. PC 2 gives information in the region of 315−500 nm where sesame oil gives higher signals.

PCA loading plots in **Figure 7B** show that PC 1 gives information in the region around 350 nm. As shown in **Figure 5**, sunflower gives higher signals in this region in contrast to soybean, corn, and the commercial blend of oils. This explains the higher PC 1 scores in **Figure 6B** for sunflower oil. PC 2 gives information in the region higher than 400 nm. This region is characteristic for stressed corn, soybean, sunflower, and the commercial blend of oils. That is why spectra shift to positive scores in PC 2 during heating (**Figure 6B**).

The above results show that SyF spectra combined with PCA could be used for monitoring edible oil oxidation during the early stage.

In summary, total synchronous fluorescence spectra of vegetable oils change significantly during heating and can be used for monitoring oil deterioration. Fluorescence signals in the region lower than 350 nm disappear and those in the region higher than 600 nm diminish, probably due to the decrease of phenolic antioxidants and chlorophylls, respectively. During heating, signals in the region around 400−450 nm increase, presumably due to the formation of secondary oxidation products. Synchronous fluorescence spectra combined with multidimensional chemometrics are useful for categorizing edible oils according to thermal stress. Results show the potential of SyF for following thermal stress and warrant further research concerning edible oil monitoring during frying.

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Received April 14, 2009. Revised manuscript received August 12, 2009. Accepted August 13, 2009. This work was financially supported by the Greek General Secretariat for Research and Technology and Minerva S.A. (Athens, Greece) through a PENED 2003 grant.