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Evaluation of Virgin Olive Oil Thermal Deterioration by Fluorescence Spectroscopy

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The evolution of the fluorescent compounds during the thermal deterioration of virgin olive oil is not yet well-known. Samples of heated virgin olive oils collected from a fryer every 2 h up to 94 h were analyzed to study their fluorescence spectra as well as the evolution of the concentrations of α-tocopherol and individual phenols by high-performance liquid chromatography (HPLC). The regions of the fluorescence spectra of the heated oils, diluted in hexane at 1%, were explained by the content of these compounds with regression coefficients higher than 0.90 (R^2 adjusted). The fluorescence intensity recorded at 350 nm and the wavelength of the spectrum maximum in the range of 390−630 nm also allowed for the explanation of the increase of the percentage of polar compounds during the experiment. On the other hand, the spectra of the undiluted heated oils indicated that the maximum of the spectrum of any undiluted oil at 490 nm or beyond is related to a percentage of the polar compounds higher than 25%, which is the maximum percentage accepted for edible oils used in frying processes.

KEYWORDS: Olive oil; fluorescence spectroscopy; thermoxidation; polar compounds; phenols; α-tocopherol

INTRODUCTION

Virgin olive oil (VOO), the most commonly used cooking fat in Mediterranean countries, is subjected to culinary practices described in umpteen new and ancient recipes that entail heating processes. These processes are typically boiling, microwave heating, baking, and deep frying, with the latter being the most popular practice. In fact, nearly one-half of lunch and dinner food orders in restaurants and canteens include one or more items fried with edible oils (*1*). During this operation, because of the high temperature and the absorption of oxygen and water, the oil suffers a series of chemical reactions, namely, hydrolysis, oxidation, isomerization, and polymerization (*2*), and these reactions affect not only its sensory and nutritional properties but also its food safety (*3*).

VOO, however, exhibits high resistance to oxidation processes and advantageous frying properties when compared to other vegetable oils because of its fatty acid composition, high in monounsaturated fatty acids (MUFAs) (55−88%) and low in polyunsaturated fatty acids (PUFAs) (2−21%), and the presence of highly antioxidative phenolic compounds that mainly inhibit the production of hydroperoxides (*4*). Concerning other minor compounds, the total content of tocopherols (mainly α-tocopherol) is not particularly high (between 100 and 380 mg/kg) but the ratio between VOO contents of vitamin E and PUFAs (1.2) is very high in comparison to others edible oils, such as sunflower oil (0.91), rapeseed oil (0.59), and soybean oil (0.25), which means an excellent protection of fatty acid double bonds, which are sensitive to oxidation (*5*).

The determination of these minor compounds is of high interest, not only because of their influence over VOO stability and sensory quality but also because of their nutritional relevance in relation to the percentage of total polar compounds, which is the current official parameter in many countries to determine when a given fried oil has to be discarded (*6*). Thus, monitoring the decrease in the concentration of these minor compounds and the increase of degraded compounds in oils by a selective, rapid, and nondestructive technique would give accurate information about the actual level of thermal degradation of oils under thermal stress. Fluorescence spectroscopy meets these characteristics because it is one of the most sensitive and selective techniques (*7*). The applicability of this technique is based on the fact that VOO is a complex mixture of nonfluorescent and fluorescent compounds. Thus, previous studies have revealed that the VOO fluorescence emission spectra contain information about the content of polyphenols and tocopherols (300−390 nm) (*8*, *9*), oxidation products (410−450, 450−480, and 485−540 nm) (*10*, *11*), compounds derived from vitamin E (∼525 nm), conjugated dienes and trienes, K232 and K270, and hydrolysis products (445−455 nm) (*11*). Finally, the emission bands between 600 and 750 nm have been attributed to chlorophylls and pheophytins (*11*, *12*).

Because many of the fluorophores in edible oils are compounds with a very different chemical nature that are directly related to oxidation mechanisms (e.g., phenols and vitamin E), fluorescence spectroscopy is deemed a suitable technique to assess the oxidative degree of heated oils (*13*). Accordingly, this work explores the

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Table 1. Percentage of Polar Compounds in the Samples Heated from 2 to 38 h Together with Spectroscopic and Chromatographic Information

thermal oxidation (hours)	polar compounds (%)	ratio of the maximum band at 350 nm	α-tocopherol[a]	phenols[a,b]	*o*-diphenols[a,b]	secoiridoid derivatives[a,b]
0	2.64	1.00	91.75	216.17	116.84	163.13
2	4.37	0.76	84.39			
4	5.21	0.75	82.32	175.74	55.78	144.26
6	6.19	0.75	78.53			
8	6.66	0.53	71.37	124.55	35.63	99.49
10	8.60	0.41	62.95			
12	9.72	0.40	52.46			
14	10.81	0.39	48.46			
16	11.50	0.40	37.74	87.82	24.33	75.03
18	13.12	0.28	26.98			
20	14.04	0.28	22.02			
22	15.81	0.33	19.98			
24	17.78	0.24	14.21			
26	18.97	0.27	10.00			
28	21.36	0.20	3.90			
29	21.65	0.25	2.06	47.90	17.77	41.53
30	21.66	0.23	0.00			
31	22.44	0.17				
32	23.16	0.18				
33	23.96	0.16				
34	24.56	0.17				
35	24.83	0.14				
36	26.04	0.12		38.55	16.42	33.78
38	28.57	0.11				

[a] Parts per million (ppm). [b] Total.

possibility of fluorescence spectroscopy in the analysis of thermoxidized VOO samples. Thus, the objective of this study is to ascertain whether the fluorescence spectra contain enough information for a better understanding of the evolution of VOO quality during thermoxidation. This information was also evaluated to determine when the oil has to be discarded by correlating the spectrum band with the content of total polar compounds. The assignment of the most relevant spectral bands was supported by chemical analysis carried out in parallel and the results discussed according to the work reported by other authors.

MATERIALS AND METHODS

Samples and Sample Preparation. Two commercial Spanish VOOs, purchased at a local supermarket, were used in the thermoxidation study. This process was carried in a 4 L domestic fryer with an automatic temperature controller (Heidolph EKT 3001, Schwabach, Germany). Each sample of VOO was heated at 190 °C for 94 h in cycles of 8 h per day. A sample of 40 mL was collected every 2 h until the end of the heating process, resulting in a total of 47 samples to be analyzed. A second commercial Spanish VOO was heated under the same conditions to validate the results of the previous experiment. Samples were kept in brown glass vials at 4 °C until further chemical and spectroscopic analyses.

Chemicals. Phenols (caffeic, cinnamic, gallic, *p*-coumaric, syringic, and vanillic acids and tyrosol) and α-tocopherol were purchased from Sigma-Aldrich (St Louis, MO). Solvents methanol (99% purity) and hexane (95% purity) were purchased from Romil (Cambridge, U.K.). Concerning the quantification of phenols and tocopherols by high-performance liquid chromatography (HPLC), acetonitrile (far-UV), orto-phosphoric acid, methanol, hexane, and 2-propanol were of HPLC grade (Romil Ltd., Cambridge, U.K.), while SPE cartridges (3 mL), packed with the diol phase, were obtained from Supelco (Bellefonte, PA). Standards of phenols and α-tocopherol were diluted in methanol at 1% prior to analysis.

Analysis of Total Polar Compounds. The percentage of total polar compounds was determined gravimetrically according to the International Union of Pure and Applied Chemistry (IUPAC) standard method (*14*). Nonpolar and polar fractions were separated from 1 g of oil by silica gel column chromatography (20 g of silica adjusted to a water content of 5%, w/w) hexane/diethyl ether (90:10) and diethyl ether as elution systems. The nonpolar fraction was eluted with 150 mL of *n*-hexane/diethyl ether (90:10, v/v), while the polar fraction was eluted with 150 mL of diethyl ether. Efficiency of the separation was confirmed by thin-layer chromatography (TLC) using *n*-hexane/diethyl ether/acetic acid (80:20:1, v/v/v) and visualized with iodine vapor. The percentage of the polar fraction was calculated by weighing both fractions after the evaporation of the solvents.

Fluorescence Spectroscopy. Spectrofluorimetric measurements were performed with two instruments. The spectra of the entire VOO samples were performed with a RF-1501 (Shimadzu Corporation, Kyoto, Japan) spectrofluorometer, while the same VOO samples diluted in hexane at 1% were analyzed with Cary Eclipse equipment (Varian Ibérica, Madrid, Spain). Both instruments were equipped with a continuous xenon lamp, excitation and emission monochromators, and a photomultiplier. A 10 × 10 mm nonfluorescent cell (3 mL) was used for "right-angle" RA fluorometry, and the excitation and emission slits of 10 nm width were employed for the emission spectra (300−500 nm for VOO samples diluted in hexane and 300−800 nm for the entire VOO samples) excited at λ_{exc}=270 nm. The integration time was 0.1 s, and the wavelength increment was of 1 nm during the spectrum scanning. The experimental conditions allowed spectra of satisfactory intensity, resolution, and signal-to-noise ratio.

Because aggregates are generally present in olive oils, samples were shaken vigorously during 5 min to reduce turbidity to a minimum. After each series of measurements, the cuvette was cleaned using detergent, followed by a rinse with deionized hot water and acetone to dry and eliminate the rest of the fat. Each sample was analyzed in duplicate.

The spectrofluorometers were interfaced to computers for spectral acquisition and data processing. The software was supplied by Varian and Shimadzu. Raw spectra were used as acquired without any pretreatment.

Determination of Tocopherols. Tocopherols were analyzed by following the IUPAC standard method 2432 (*15*). A solution of oil in hexane (10 mg/mL) was analyzed by HPLC (Agilent Technologies 1200) on a silica gel column (Merck, Superspher Si60, particle size of 4 μm, 250 × 4 mm inner diameter), eluting with hexane/2-propanol (99:1) at flow rate of 1 mL/min, with an injection volume of 20 μL. A fluorescence detector (Agilent Technologies 1100) with the excitation wavelength set at 290 nm and emission wavelength set at 330 nm was used.

Determination of Phenols. A standard solution (0.5 mL), made with *p*-hydroxyphenylacetic (0.12 mg/mL) and *o*-coumaric (0.01 mg/mL) acids in methanol, was added to a sample of filtered VOO (2.5 g). A rotary evaporator at 40 °C under vacuum was used to evaporate the solvent, and the oily residue was dissolved in 6 mL of hexane.

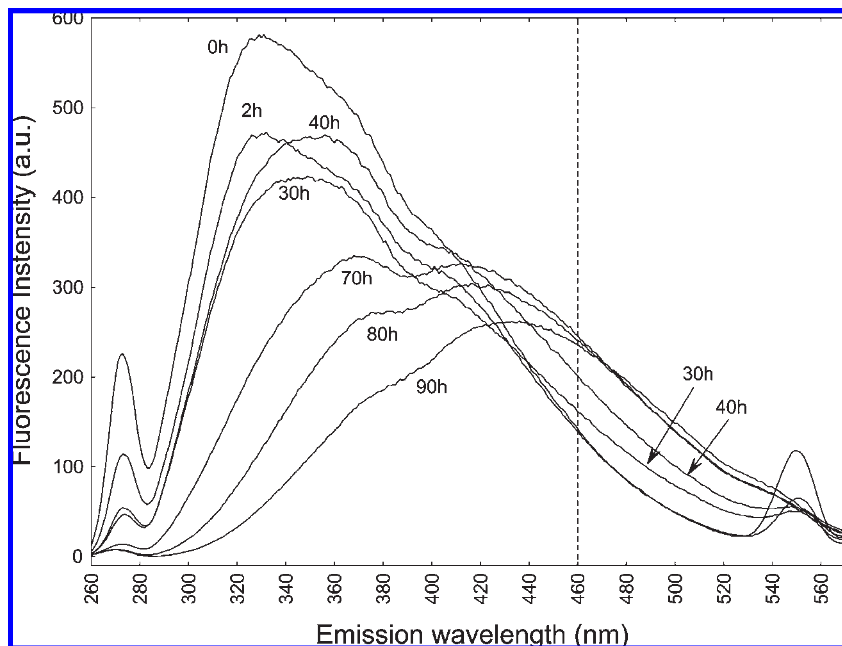

Figure 1. Fluorescence spectra (λ_{exc} = 270 nm) of the VOO (0 h) heated at 190 °C for 2, 30, 40, 70, 80, and 90 h in a fryer.

The diol-bonded phase cartridge was conditioned according to Mateos et al. (*16*). After the sample was loaded, the final residue was extracted with 10 mL of methanol and evaporated at 40 °C under vacuum and the extract was diluted with 500 μL of methanol/water (1:1, v/v). A filtrated aliquot (20 μL) of the final colorless solution was injected onto the HPLC system (an Agilent Technologies 1100 liquid chromatographic system equipped with a diode array UV detector and a Rheodyne injection valve of 20 μL loop). The column was a Lichrospher 100RP-18 column (4.0 mm inner diameter × 250 mm; 5 μm, particle size) maintained at 30 °C. The gradient elution, at a flow rate of 1.0 mL/min, was achieved using the following mobile phases: a mixture of water/ortophosphoric acid (99.5:0.5, v/v) (solvent A) and methanol/acetonitrile (50:50, v/v) (solvent B). The change of the solvent gradient was programmed as follows: from 95% (A) and 5% (B) to 70% (A) and 30% (B) in 25 min, 62% (A) and 38% (B) in 10 min, 62% (A) and 38% (B) in 5 min, 55% (A) and 45% (B) in 5 min, 47.5% (A) and 52.5% (B) in 5 min, and 100% (B) in 5 min, followed by 5 min of maintenance. The chromatographic signals were obtained at 235, 280, and 335 nm.

Quantification of phenols cinnamic acid and lignans was carried out at 280 nm using *p*-hydroxyphenylacetic acid as the internal standard. Quantification of flavones was performed at 335 nm using *o*-coumaric acid as the internal standard. The response factors and recoveries were based on the procedure carried out by Mateos et al. (*16*).

Statistical Analysis. The data were analyzed using principal component analysis (PCA), an unsupervised tool oriented toward modeling the variance/co-variance structure of the data matrix into a model that represents the significant variations and considers the noise as an error. Thus, PCA was used for the classification of samples as well as their description and interpretation according to their spectral data. The next step was to determine if bands of the spectra were fairly correlated with the percentage of polar compounds and the concentrations of chemical compounds, such as α-tocopherol, phenols, *o*-diphenols, and secoiridoid derivatives. The relationships were studied by Ridge Regression ($\lambda = 0.10$). Statistica 7.0 (StatSoft, Tulsa, OK) was used for carrying out all of the statistical analyses.

RESULTS AND DISCUSSION

Thermal deterioration of oils causes changes in the kind and concentration of molecular species present in VOO that alters its quality attributes and nutritional profile to result in potentially toxic compounds. Thus, the current standard applied in most of the countries protects consumers by establishing a maximum limit of 25% of polar compounds (*17*). A value higher than 25% would indicate that the oil can no longer be used for human consumption, and in consequence, it has to be discarded. **Table 1** shows the percentage of polar compounds in the samples of VOOs heated at 190 °C from 0 h (the nonheated genuine oil) up to 38 h, which is the time immediately after the oil has reached 25% of polar compounds. The value of 25% corresponded to 35 h, which points out the maximum time that this oil can be used as a frying oil for that temperature and frying conditions.

Besides the increase of polar materials during heating, the oxidation as a consequence of high temperatures produces an evident change of the VOO fluorescence profile that results from decomposition of the fluorophores. Given the great variety of new compounds forming during oxidation, some of them are expected to be fluorophores. Moreover, lipid oxidation is even a much more complex process, in which polyunsaturated lipids undergo reaction with molecular oxygen to form lipid hydroperoxides (*18*). The lipid hydroperoxides undergo further oxidation, via a complex series of radical reactions, to form eventually aldehydes and ketones among other species. The presence of chlorophylls and their derivative pheophytins, as singlet oxygen sensitizers, enhances VOO oxidation, while tocopherols and carotenoids prevent the oxidative degradation by quenching the reactive oxygen species. VOO also possesses phenols, other natural antioxidants, which affect the thermal oxidation (*18*).

Figure 1 shows the fluorescence spectra of some heated samples diluted in hexane. The time of the thermoxidation oscillated between 0 h (genuine VOO) and 94 h, which corresponded to the end of the experiment. The spectral zone between 290 and 400 nm exhibits high fluorescence signals with a maximum around 330 nm in the genuine VOO. The fluorescence intensity at this wavelength diminishes with the time of heating at the same time that the maximum is red-shifted, e.g., 350−360 nm up to 50 h of heating. Furthermore, the absolute maximum is located around 420−440 nm from 80 h onward.

Because the fluorescence signals observed in the region 300−390 nm are assigned to tocopherols, together with polyphenols (*8*), the information collected from the spectra were compared to the results from HPLC analysis of these compounds. The HPLC analysis of tocopherols with a fluorescence detector showed the maximum intensity at $\lambda_{em} = 330$ nm ($\lambda_{exc} = 290$ nm) (*19*),

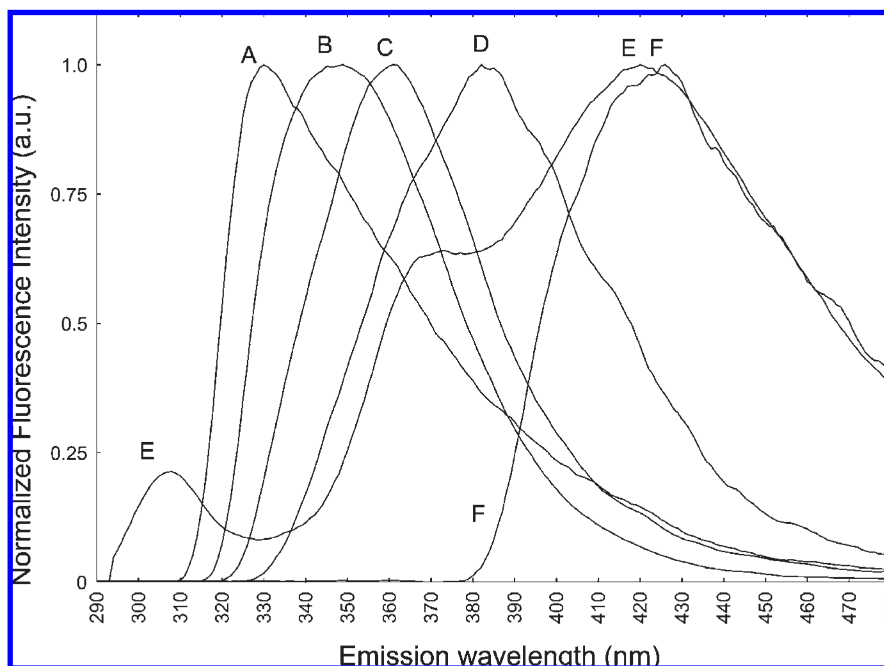

Figure 2. Normalized fluorescence spectra (λ_{exc} = 270 nm) of (A) α-tocopherol, (B) vanillic acid, (C) syringic acid, (D) gallic acid, (E) tyrosol, and (F) *o*-coumaric acid.

Table 2. Emission Wavelengths (nm) of the Maximum Fluorescence Intensity ($I_{fluorescence}$) of Remarkable Phenolic Acids Present in VOO When They Are Excited at 270 nm

phenols	λ_{em}	$I_{fluorescence}$
vanillic acid	349	340.05
syringic acid	361	171.54
gallic acid	382	61.91
p-coumaric acid	416	1.56
cinnamic acid	420	0.96
tyrosol	420	39.18
o-coumaric acid	426	38.32
caffeic acid	457	4.31

which mostly corresponds to α-tocopherol; VOO has tocopherols (α, β, and γ), of which α-tocopherol is the most concentrated one (40−400 mg/kg) (*20*), while tocotrienols are absent (*21*). The concentration of α-tocopherol in the initial VOO sample, determined by HPLC, decreased up to 98% at 29 h of thermal oxidation (**Table 1**). The concentration of α-tocopherol was highly correlated to the percentage of polar compounds (R^2 adjusted = 0.97) because both parameters are affected by the heating length. With regard to the information from the fluorescence spectra, the correlation matrix of the concentration of α-tocopherol and the fluorescent spectrum of the heated samples from 0 to 29 h showed good correlations (>0.80; $p < 0.05$) in the range of 300−380 nm. The highest correlation (0.90; $p < 0.05$), however, was found for the band in the range of 353−355 nm. These wavelengths corresponded to the maximum in most of the spectra of the heated oils. These wavelengths also matched the maximum emission for HPLC analysis (λ_{em} = 330 nm). Thus, the maximum intensity of the fluorescence spectrum of α-tocopherol diluted in methanol at 1% (v/v) (**Figure 2**) was also observed at λ_{em} = 330 nm.

Table 1 also shows that the concentration of α-tocopherol is approximately 50% of the original value after 14 h of thermal stress, a period that is unexpectedly long considering the high temperature and the role of tocopherols in the first moments of oxidation. Similarly, the maximum intensity of the fluorescence spectra (∼350−360 nm) decreases up to 50% after 10 h of thermal oxidation, and the percentage was 15% after 35 h. At this point, the oil contained 25% of polar compounds, and thereby, the oil was not fit for consumption and had to be discarded. The concentrations of α-tocopherol (**Table 1**) as compared to the fluorescence intensity indicate that the fluorescence emission between 290 and 400 nm is not only due to tocopherols (mostly α-tocopherol and its oxidation products) but other compounds that also contribute to the fluorescence intensity. Thus, the concentration of α-tocopherol is almost at trace level after 26 h of thermal oxidation, while at this time, the fluorescence spectra still exhibit significant intensity. Those compounds can be phenols (*8*).

The phenolic compounds of the samples were then quantified by HPLC. The list of phenolic compounds described in VOO varies depending upon the authors (*16*, *22*), although most of the researchers agree that the phenols described in **Table 2** are among the most representative and abundant in VOO. This table shows the wavelengths where the maximum fluorescence intensities of their spectra (λ_{exc} = 270 nm) are registered once they have been diluted in methanol at 1%. This solvent was selected because of its adequate polarity, and it does not affect the fluorescence properties of the selected phenols (*8*). Concerning the percentage of dilution, it was the result of a study with two phenols (vanillic and caffeic acids) that were diluted at 0.5, 1, 5, and 10%; the highest intensity of fluorescence of these phenols was detected at 1%.

The maximum fluorescence intensities (**Table 2**) correspond to five phenols (tyrosol and *o*-coumaric, vanillic, syringic, and gallic acids), whose emission wavelengths oscillate between 349 and 426 nm, which partially explain the maxima of the spectra of **Figure 1**. **Figure 2** shows the fluorescence spectra of those remarkable phenols, and the strange behavior of tyrosol that presents a wide band with two relative maxima, at 305 and 365 nm, versus the narrow band of the *o*-coumaric acid is remarkable.

The analysis of the phenolic compounds by HPLC proved the presence of these compounds, although they were quantified at small concentrations after 36 h of thermoxidation when the percentage of polar compounds was higher than 25% (**Table 1**).

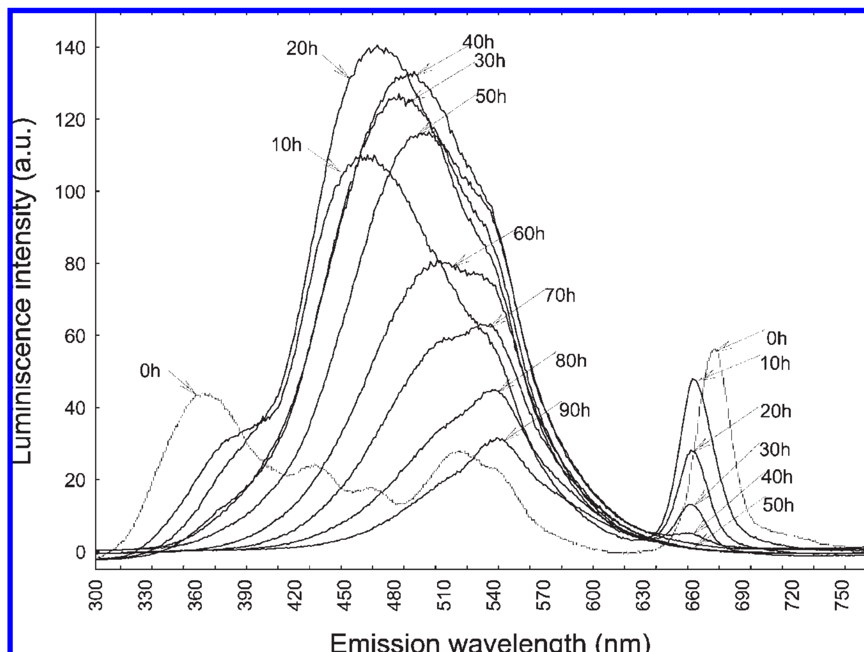

Figure 3. Sequence of fluorescence spectra (λ_{exc} = 270 nm) of a VOO thermoxidized for 90 h.

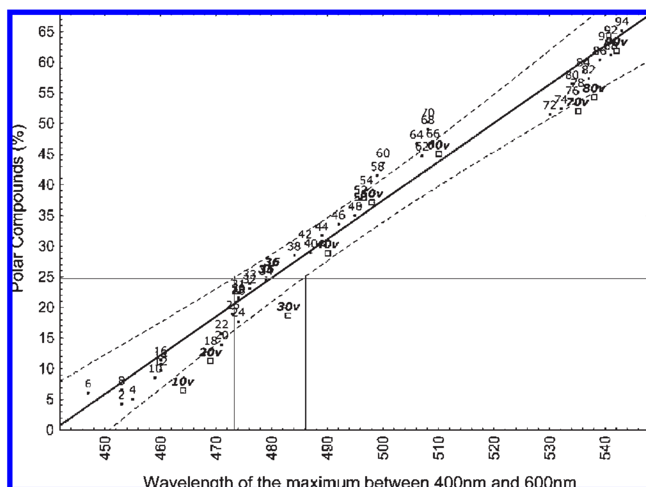

Figure 4. Percentage of polar compounds versus the emission wavelength of the maximum in the band between 400 and 600 nm of the samples thermoxidized from 2 to 94 h. Numbers in bold and italic (10v−90v) correspond to the validation samples.

These results partially agree with Brenes et al. (*23*), who determined that an oil heated at 180 °C for 25 h contained only 15% of polar compounds and some phenols (1-acetoxypinoresinol and pinoresinol) were still present, although some other phenols (e.g., hydroxytyrosol and tyrosol-like substances) have dramatically decreased in the first hours of heating. Gomez-Alonso et al. (*24*) reported similar results for oils used for frying potatoes.

Our experiences, however, show that 1-acetoxypinoresinol is present after 36 h, although its concentration is only 10% of its initial 11.58 mg/kg. The concentration of pinoresinol, however, increases in the first 16 h but decreases abruptly after 29 h of thermoxidation. Almost all of the phenolic compounds (e.g., hydroxytyrosol, vanillic acid, and luteolin) have disappeared before 16 h, while the concentrations of tyrosol and the derivatives of hydroxytyrosol and tyrosol have not even disappeared when oils have reached 25% of polar compounds.

The mathematical studies show that there are good relationships between the behavior of the fluorescence spectra (**Table 1**) expressed as the intensity at 350 nm normalized by the maximum of the genuine VOO (0 h of heating) and the concentrations of tyrosol (R^2 adjusted = 0.96), 1-acetoxypinoresinol (R^2 adjusted = 0.90), the total concentrations of polyphenols (R^2 adjusted = 0.98), *o*-diphenols (R^2 adjusted = 0.93), and secoiridoid derivatives (R^2 adjusted = 0.97). The regression values are slightly worse when the regression concerns the percentage of polar compounds, although R^2 adjusted is still higher than 0.90.

As it was stated above, the maximum intensity of the fluorescence spectra undergoes a shift to higher wavelengths when the time of oil thermoxidation increases (**Figure 1**). An exhaustive analysis of the spectra allows us to observe that the absolute maximum displaces 17 units from 2 h (332 nm) to 30 h (349 nm) of heating and this value reaches 104 units (436 nm) at 90 h. There are in fact two maxima, around 368 and 412 nm, which have approximately the same intensity in the sample heated for 70 h. This displacement and the observed change in the spectrum profile are due to the decrease of the tocopherols and phenols and the increase of the oxidation products of vitamin E, compounds related to K232 and K270 and hydrolysis products (*11*). This mixture hinders to obtain a good relationship between the percentages of polar compounds (% PC) and the fluorescence spectra. The absorbance in an extended band, between 400 and 600 nm, can help to estimate % PC, despite the artifacts associated with the RA technique, so-called primary and secondary inner-filter effects (*25*), if the thermoxidized samples are analyzed undiluted (**Figure 3**).

Inner effects involve the attenuation of the emission intensity because of the absorption of the incident excitation light and the absorption of the emitted light (*7*). This phenomenon occurs in all of the samples, and in consequence, its effect is embedded in the time series study of the thermoxidized samples. Furthermore, the effect of the quenching mechanisms is lower in undiluted samples than diluted samples because there are fewer collisions between the fluorophore and oxygen or other quenchers in viscous samples (*7*) and viscosity increases as the oils are heated. **Figure 3** shows the genuine VOO spectrum (0 h) that presents several bands spread over the central part of their spectra (390−600 nm),

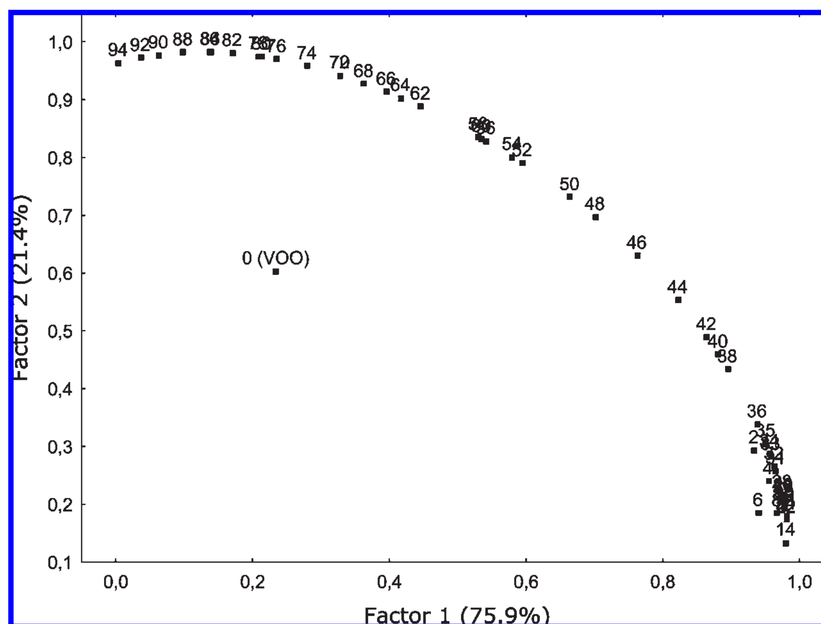

Figure 5. PCA plot of the values of fluorescence intensity (360−690 nm) of the thermoxidized samples. Note that VOO is fresh virgin olive oil.

while there is a broad absorption band in this spectral zone for all of the thermoxidized VOO samples. Observing the zone between 390 and 630 nm, the first maximum intensity of the thermal oxidation samples appears after 2 h of thermal oxidation and a bathochromatic effect takes place as the oil undergoes oxidation, as observed in the spectra of samples diluted at 1% in hexane (**Figure 1**). Thus, the spectrum at 70 h of thermal oxidation shows two maxima with approximately the same intensity, although the second maximum (540 nm) corresponds to the overtone (λ_{exc} = 270 nm) and, therefore, was neglected. The shift of the maxima is well-explained with the increase of polar compounds as the time of thermoxidation increases as well. **Figure 4** shows the result of the correlation between the wavelengths where the spectral maximum was registered and the percentage of total polar compounds. It is important to remark that the spectral changes are studied in the frequency domain, which is much more accurate than the measurements in the amplitude domain. Thus, it can be proposed that a maximum of the spectrum beyond 486 nm means that the content of polar compounds is higher than 25%. This proposal was validated with another VOO heated to 90 h under the same conditions. The latter samples correspond to the numbers labeled with letter “v” in **Figure 4**.

The band between 630 and 750 nm, associated with chlorophylls and pheophytins according to some authors (*11*, *12*), also decreases with the thermoxidation time (**Figure 3**), as pointed out by other authors (*26*). The evolution of the intensity recorded for this band in the thermoxidation experiment was exponential. Thus, the intensity had decreased by 50% after 18 h, and it was only 10% when the percentage of polar compounds reached 25%. Assuming that this spectrum region is associated with pigments, the existence of the quantifiable intensity after more than 38 h of heating might be due to the evolution of the pigment noncolored products (*27*) as well as their protection by phenol compounds that are still present.

To acquire a better knowledge on the evolution of the spectra over time as the oxidation progresses, the statistical procedure of PCA was applied to the values of fluorescence intensity of all of the samples. The results are shown in **Figure 5**. The first two factors explained 97.3% of total variance. The first factor explains 75.9% of the variance and is related to the spectrum zone between 300 and 530 nm, while the second factor (21.4% of variance) is related to the zone between 630 and 690 nm. **Figure 5** shows that the samples with less than 25% of polar compounds are clustered together on the PCA plot and mainly differ in their values by factor 2. On the contrary, the evolution of the most altered samples is mainly expressed by factor 1. Thus, the samples with more than 74 h of heating are independent of factor 2, probably because chlorophylls, pheophytins, and pyropheophytins have disappeared already.

These results are promising because they show the potential utility of fluorescence instruments for monitoring the degree of alteration of thermoxidized VOOs. Despite the fact that the “right-angle” (RA) implementation may cause some observations of self-absorption effects, when compared to front-face fluorescence spectroscopy (*8*), these empirical results prove a good relationship of the registered intensity extracted from different bands in the spectra with the content of total polar materials.

The spectra obtained from either diluted or undiluted samples provide useful information that can be used for a rapid and easy monitoring of thermoxidation processes, such as frying. Although the spectra of undiluted samples are quite different in comparison to those of undiluted samples and may be affected by light absorption phenomena, they are still quite informative of the heating process. Thus, there is a clear relationship between the wavelengths of the spectral maximum and the percentage of total polar compounds. Considering these results and the fact that the fluorophores in VOO are compounds with a clear implication in oxidation, fluorescence spectroscopy can be proposed as an excellent technique for a better understanding of oil oxidation with a straightforward basis, in particular, in frying processes.

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