TECHNICAL COMMUNICATIONS

Fluorescence Spectra Measurement of Olive Oil and Other Vegetable Oils

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Fluorescence spectra of some common vegetable oils, including olive oil, olive residue oil, refined olive oil, corn oil, soybean oil, sunflower oil, and cotton oil, were examined in their natural state, with a wavelength of 360 nm used as excitation radiation. All oils studied, except extra virgin olive oil, exhibited a strong fluorescence band at 430–450 nm. Extra virgin olive oil gave a different by interesting fluorescence spectrum, composed of 3 bands: one low intensity doublet at 440 and 455 nm, one strong at 525 nm, and one of medium intensity at 681 nm. The band at 681 nm was identified as the chlorophyll band. The band at 525 nm was at least partly derived from vitamin E. The low intensity doublet at 440 and 455 nm correlated with the absorption intensity at 232 and 270 nm of olive oil. The measurements of these fluorescence spectra were quick (about 5 min) and easy and could possibly be used for authentification of virgin olive oil.

Although the ability of olive oil to emit fluorescence radiation has been known from the beginning of this century, it was only in 1925, when the mercury lamp equipped with the Wood filter was made commercially available, that this property was applied for authenticity determination of olive oil (1). The use of Wood lamp was accepted as a U.S. official method for identification of olive oil adulteration (2). Under the Wood lamp, virgin olive oil gave a yellow to orange fluorescence. A refined olive oil, on the other hand, gave a white to light blue fluorescence. The fluorescence emission of olive oil was studied extensively for at least 30 years. Papers published during this time period are summarized by Nicoletti (3). The use of Wood lamp for detection of vegetable oil adulteration was used intensively until the 1960s. However, the use of gas chromatography for determination of fatty acid methyl esters and sterols vastly surpassed the validity of Wood lamp results, and the use of oil fluorescence as an analytical method was substantially abandoned.

For the last 20 years, the use of fluorescence for detection of vegetable oil adulteration has not received much attention. Only 2 papers came to our attention from this time period. Wolfbeis and Leiner in 1984 (4) characterized 4 types of edible oils by their total fluorescence spectra (fluorescence topograms) using solutions of the oils in *n*-hexane. Nicoletti (3) also studied the fluorescence spectra of virgin olive oil, refined olive oil, and olive residue oil after decolorization and dissolution in isooctane. In both cases, the similarities between fluorescence spectra from different oils were rather significant, and these spectra were not used for diagnostic purposes.

A major drawback in the use of the Wood lamp was that intensity and color of the fluorescence light emitted were subjectively determined by the eye of the analyst. In recent studies on oil fluorescence, the Wood lamp was substituted by scanning fluorimeters, and fluorescence spectra were run from solutions of oils in suitable solvents (3, 4). In these cases, fluorescence spectra of different vegetable oils recorded were more or less similar. We believed that the use of solvents could possibly modify the fluorescence emission of oils. To avoid solvent interference, we studied fluorescence spectra of different vegetable oils from samples of native, undiluted oils.

Experimental

Oil Samples

Authentic samples, including 20 olive oil, 5 sunflower oil, 5 cottonseed oil, 5 soybean oil, 5 corn oil, 5 refined olive oil, and 5 olive residue oil samples were kindly supplied by the Greek General Chemical Laboratories of State. When not in use, oil samples were kept in the refrigerator at 0°C. To avoid solvent interference in oil fluorescence, we used oils in their native form without any treatment, except filtration if necessary.

Analytical Methods

A Perkin-Elmer scanning fluorimeter (Model 235 A) was used in this study. Titratable acidity, peroxide value, and the coefficients of specific extinction at 232 and 270 nm were determined according to the relevant AOAC methods (5).

Acidity value, % of oleic acid, was determined by titration with 0.1N KOH of a solution of oil (10 g) in a previously neutralized solvent mixture of ethanol–ethyl ether (1 + 1) with phenolphthalein indicator (1% in ethanol) added.

Specific extinction coefficients at 232 nm (K_{232}) and 270 nm (K_{270}) were determined from absorption of 1% solution of oil in cyclohexane at 232 and 270 nm, respectively,

Received October 27, 1999. Accepted by JL May 2, 2000.

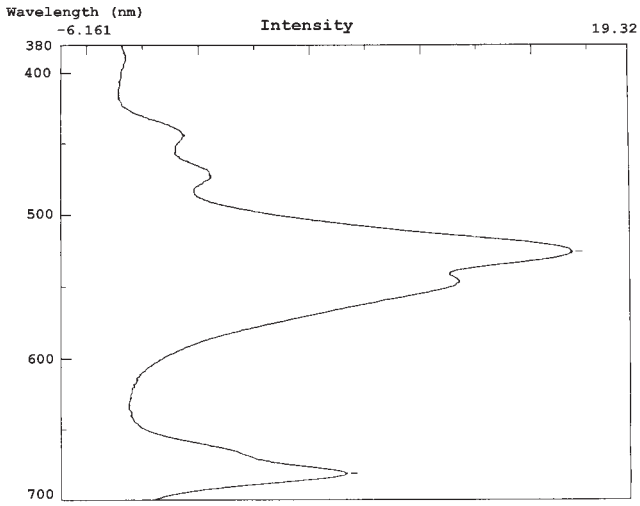

Figure 1. Fluorescence spectrum of extra virgin olive oil (acidity = 0.67, K_{270} = 0.11).

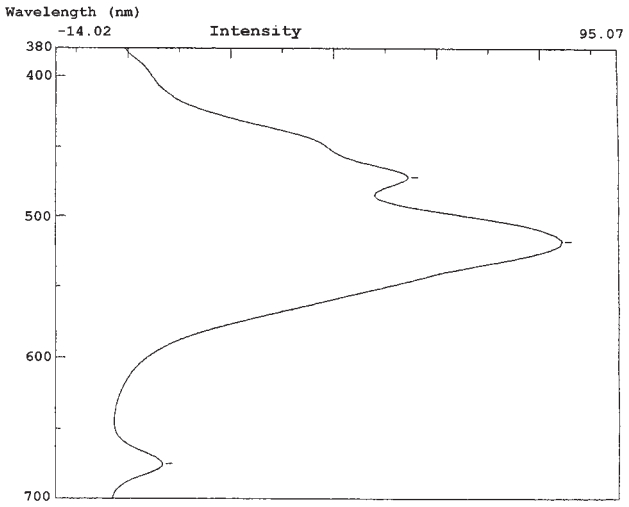

Figure 2. Fluorescence spectrum of extra virgin olive oil (acidity = 1.4, K_{270} = 0.40).

with 1 cm path length cuvette. Measurements were made with a UV spectrophotometer (Hewlett-Packard 8452).

Peroxide value, expressed in milliequivalents of active oxygen per kilogram (meq/kg) oil, was determined by dissolving 2 g oil in 25 mL acetic acid–chloroform solution, followed by addition of potassium iodide. The solution was placed in darkness for 5 min and was titrated with 0.01N sodium thiosulfate solution after addition of soluble starch as indicator.

To select the best excitation wavelength in the fluorimeter, a Wood lamp with a mercury vapor lamp as its fluorescence excitation source was used. From the *Handbook of Chemistry and Physics* (6) the wavelengths of 254 and 365 nm were chosen as maximum intensity mercury spectral lines. Excitation at 254 nm created problems (excessive noise) and was abandoned. Excitation at 365 nm gave excellent results and was used as the excitation wavelength throughout the study. Emission fluorescence spectra were recorded from 400 to 700 nm.

Rapid Deterioration of Olive Oil

For rapid deterioration, olive oil samples were heated under a stream of air; 300 mL oil was placed in a beaker and heated in a water bath at 70°C. A stream of air from a vacuum pump, with a flow rate of 200 mL/min was directed to the oil surface through a Pasteur pipet. Oil samples were taken for analysis at regular intervals. After withdrawal of each oil sample, the tip of the Pasteur pipet was lowered to keep the distance of 5 cm from the oil surface constant.

Results and Discussion

Fluorescence Spectra of Vegetable Oils

Fluorescence spectra of extra virgin olive oil, refined olive oil, olive residue oil, sunflower oil, cotton seed oil, corn oil, and soybean oil were studied. Representative spectra are presented in Figures 1–4.

All vegetable oils examined, except extra virgin olive oil, had more or less the same fluorescence spectrum. A representative spectrum of this group of oils is presented in Figure 3. Their fluorescence spectra had only one broad peak with a base width of 400–550 nm, and a top width of 430–450 nm. The intensity of the peak was between 100 and 400 fluorescence arbitrary units. On the other hand, the fluorescence spectra of extra virgin olive oil samples were quite different (Figures 1, 2, 4, 5, 7), having 4 peaks and low overall intensity compared with other vegetable oils. Their fluorescence spectra consisted of 2 weak to medium peaks (depending on the oxidation state of the oil) at 445 and 475 nm, one strong peak at 525 nm, and one weak to medium peak at 681 nm. Intensity

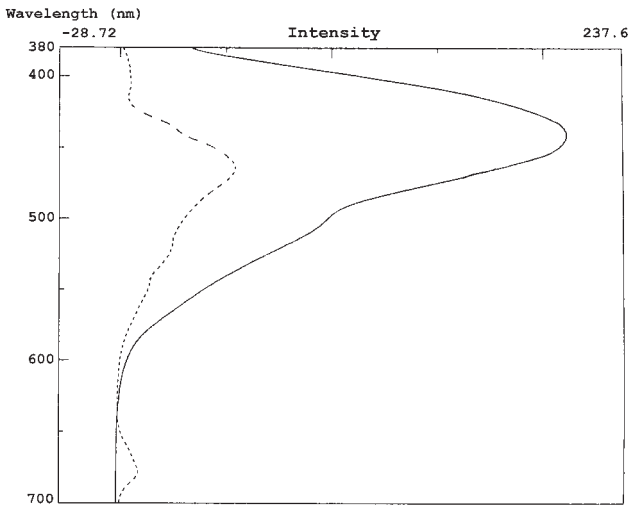

Figure 3. Fluorescence spectrum of refined olive oil with chlorophyll added. (a) Continuous line: neat refined olive oil; (b) broken line: refined olive oil with chlorophyll added.

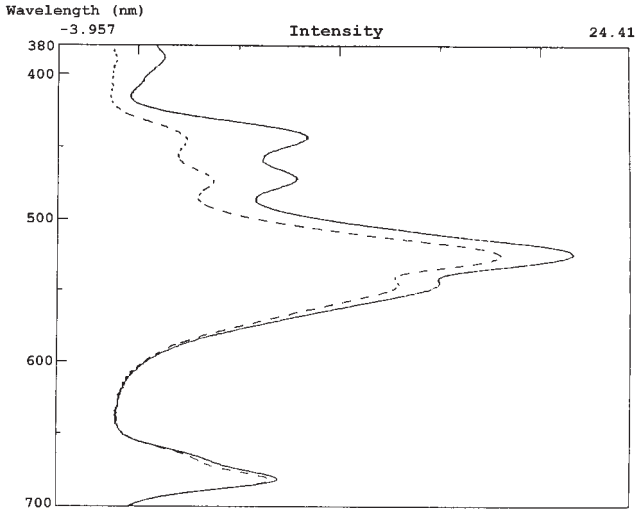

Figure 4. Fluorescence spectrum of extra virgin olive oil with vitamin E added. (a) Broken line: neat extra virgin olive oil; (b) continuous line: extra virgin olive oil with vitamin E acetate added.

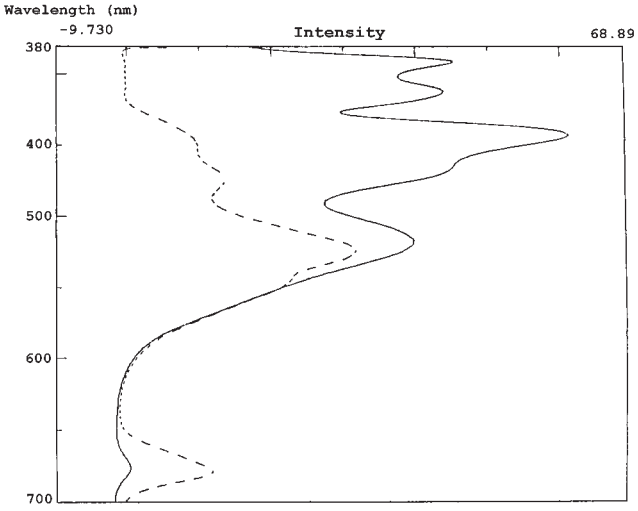

Figure 6. Fluorescence spectrum of virgin olive oil after heating at 70°C for 4 h under stream of air. (a) Broken line: neat extra virgin olive oil; (b) continuous line: extra virgin olive oil after 4 h heating.

of the strongest peak at 525 nm was 30–50 fluorescence arbitrary units.

Virgin Olive Oil Fluorescence Spectra

Peak at 680 nm.—Virgin olive oil emission fluorescence spectra have a weak to medium peak at 680 nm. Fluorescence at this wavelength was typical of native chlorophyll (*7*). This information was further checked as follows: (*1*) Pure chlorophyll was added in a sample of refined olive oil that was completely devoid of any peak at 680 nm (Figure 3). The mixture showed a medium intensity peak at 680 nm. (*2*) Chlorophyll addition to samples of virgin olive oil resulted in increase of the 680 nm peak. (*3*) During the heating experiment of virgin olive oil, the fluorescence peak at 680 nm was drastically decreased (Figures 5, 6), accompanied by a concomitant disappearance of the green color of heated olive oil. We believe that the existing information (*7*) and our experiments prove that the peak at 680 nm originates from the chlorophyll content of the oil.

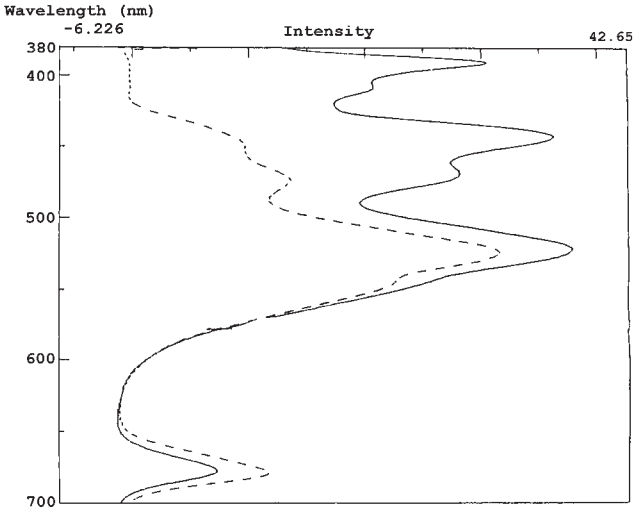

Figure 5. Fluorescence spectrum of virgin olive oil after heating at 70°C for 2 h under a stream of air. (a) Broken line: neat extra virgin olive oil; (b) continuous line: extra virgin olive oil after heating for 2 h.

Another interesting feature of the fluorescence spectra was the decrease of intensity of the strong peak at 440 nm in the spectrum of refined olive oil (Figure 3) and in the spectra of heated virgin olive oil (Figures 5, 6). Figure 3 shows that the intensity of this peak was decreased after addition of pure chlorophyll, from 220 to 50 units. In contrast, during the heating experiments of virgin olive oil, the intensity of the 520 nm peak increased following the concomitant decrease of the chlorophyll peak at 681 nm (Figures 5, 6). Consequently, chlorophyll acts as fluorescence quencher, and this was taken into consideration during the study of the extra virgin olive oil fluorescence spectrum.

Peak at 525 nm.—This was the most intense peak of the fluorescence spectrum of virgin olive oil. It also appeared in all fluorescence spectra of vegetable oils as a shoulder at 525 nm, in the slope of the large peak with a top at 430–450 nm. Its intensity was strongly influenced by the intensity of the chlorophyll peak. Wolbreis and Leiner (4) believe that major peaks in the fluorescence spectrum of olive oil originate from parinaric acid (8), chlorophyll, and vitamin E (9). However, to our knowledge, parinaric acid does not exist in any measurable amount in virgin olive oil, and its existence was not tested further. Initial experiments with 2 lots of pure vitamin E were unsuccessful because they were already oxidized, giving a major peak at 440 nm. We, therefore, used vitamin E acetate instead because it is much more stable to at-

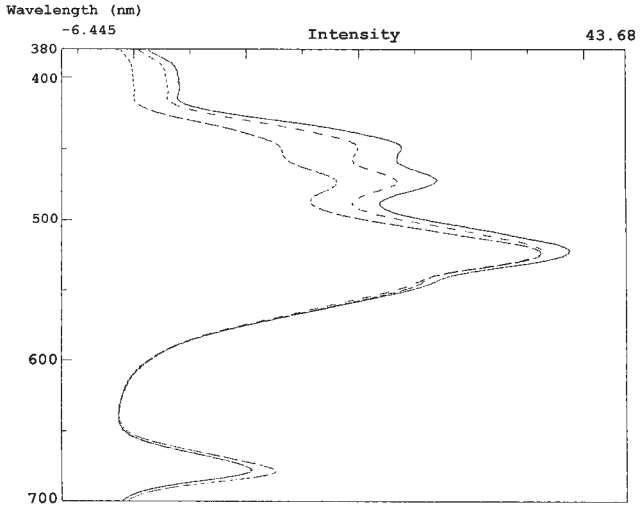

Figure 7. Fluorescence spectrum of extra virgin olive oil neat and after addition of refined olive oil. (a) Lower broken line: neat extra virgin olive oil; (b) upper broken line: extra virgin olive oil with 5% refined olive oil added; (c) continuous line: extra virgin olive with 10% refined olive oil added.

mospheric oxidation. The fluorescence spectrum of vitamin E acetate was very similar to that of vitamin E except that compared with the major peak at 440 nm for vitamin E, that of vitamin E acetate was higher (Figure 4). The addition of vitamin E acetate to a sample of virgin olive oil (Figure 4) gives 2 broad fluorescence peaks under the conditions used (excitation at 365 nm), at 440 and 525 nm, suggesting that the peak at 525 nm was at least partly derived from vitamin E. Because we were unable to extract vitamin E from the examined oils and confirm or reject the partial identification of the 525 nm peak, this peak was identified as originating, partly at least, from the vitamin E content of vegetable oils.

Medium to weak peaks at 445 and 455 nm.—These peaks appear in all virgin olive oil samples but with different intensities (Figures 1, 2, 5, 6). Examination of samples with chlorophyll peaks of approximately equal intensity showed that intensities of the peaks at 445 and 455 nm may correlate with the values of K_{270}, K_{232}, and acidity of relevant oil samples. To further study possible correlation between K_{270} and fluorescence peak intensities, 7 samples of virgin olive oil were examined (Table 1). A good correlation existed between K_{270} and peak intensity at 445 nm. The equation was $y = 811.5x^2 - 100x + 6.77$, with $R^2 = 0.965$. Correlation between K_{270} and fluorescence peak at 520 nm had an equation of $y = 861.5x^2 - 7.3x + 16.3$, with $R^2 = 0.89$.

To identify possible relationships between K_{232}, K_{270}, acidity, and fluorescence intensity, a quick oxidation process was applied to one olive oil sample. The oil was heated at 70°C for 5 h under a stream of air. Samples taken at different times were analyzed for K_{270}, K_{232}, and acidity (Table 2). As the value of parameters related to the oxidation state of the oil (conjugated dienes [K_{232}] and trienes [K_{270}]; 10, 11) and hydrolysis products (acidity) were increased, the intensity of the 445 nm peak increased proportionally. Calculations for determination of the best fitting curve between K_{232} and the ratio of peaks at 445 and 520 nm (considering the 520 nm peak as stable) gave $R^2 = 0.991$ and the regression equation: $y = 1.27x - 2.26$. Calculations for determination of best fitting curve between K_{270} and the ratio of peaks at 445 and 520 nm gave $R^2 = 0.991$ and the regression equation $y = -2.08x^2 + 3.98x - 0.10$. The same calculations between acidity and ratio of peaks at 445 and 520 nm gave $R^2 = 0.954$ and equation $y = -11.24x^2 + 34.34x - 24.01$. Acidity did not change much during this experiment. All vegetable oils (except virgin olive oil) had very low acidity values (between 0.1 and 0.3), but exhibited strong fluorescence peaks at 445 nm, suggesting that the very good R^2 found may be coincidental because of the narrow range of acidity values. Therefore, this last correlation was dropped.

We suggest that fatty acid oxidation products, which are measured by UV absorption at 232 and 270 nm, are responsible for the intensity of the fluorescence peak at 445 nm. The very small fluorescence peak at 445 nm of good quality virgin olive oil that contains mainly monounsaturated fatty acids and a high content of phenolic antioxidants, could be partly due to its tocopherol content and partly to its very low percentage of

Table 1. Relations of K_{270} and fluorescence peak intensities of virgin olive oil samples

		Peak intensities (arbitrary units)		
Sample No.	K_{270}	445 nm	520 nm	681 nm
1	0.35	69.3	114.2	0.5
2	0.11	8.2	29.2	9.7
3	0.19	16.4	40.4	12.2
4	0.16	4.2	21.7	11.6
5	0.11	7.9	35.1	11.3
6	0.25	39.4	87.9	8.1
7	0.16	9.6	31.5	11.6

Table 2. Ratio of fluorescence intensities of 445 and 520 nm peaks, K_{232}, K_{270}, and acidity of virgin olive oil sample heated at 70°C for 5 h under stream of air

Time, h.min	Intensity ratio of 445/520 nm peaks	K_{232}	K_{270}	Acidity (in elaic acid)
0	0.31	2.10	0.13	1.13
0.30	0.43	2.13	0.14	1.13
1.00	0.58	2.23	0.19	1.13
1.30	0.77	2.34	0.23	1.18
2.00	0.95	2.49	0.29	1.18
3.00	1.22	2.75	0.43	1.23
4.00	1.54	2.99	0.64	1.31
5.00	1.83	3.14	0.92	1.31

oxidation products. On the contrary, the very large fluorescence peak at 445 nm exhibited by all other vegetable oils studied, could be due to their large percentages of polyunsaturated fatty acids and to their much higher percentages of oxidation products (12). This increase of fluorescence peak at 445 nm during the quick oxidation of a very good quality virgin olive oil sample is seen in Figures 5 and 6.

A very significant negative correlation existed between absolute intensities of 445 and 681 nm (chlorophyll) peaks with $R^2 = 0.973$ and equation $y = -0.22x + 14.77$. The validity of this correlation was not evaluated further.

The results showed that in the spectral region of 445–455 nm, vitamin E had low fluorescence; oxidation and other decomposition products of vegetable oils generally had strong fluorescence. This higher oxidation state of all vegetable oils compared to virgin olive could be the reason that all these vegetable oils have a sole intense fluorescence peak at 430–450 nm.

Use of Fluorescence Spectra for Identification of Virgin Olive Oil Authenticity

The large differences between fluorescence spectra of virgin olive oil and all other vegetable oils (including refined olive oil) led us to study the effect of adding different vegetable oils on the fluorescence spectrum of virgin olive oil. In a series of preliminary experiments, refined olive oil, sunflower oil, cottonseed oil, soybean oil, corn oil, and olive residue oil were added at 5 and 10% in different samples of virgin olive oil, and changes in the intensities of fluorescence peaks at 445 and 455 nm were measured. In all cases, fluorescence intensities of these peaks increased (Figure 7). Relevant measurements in virgin olive oil showed that fluorescence intensities of these peaks increased by 28–32% with 5% addition and by 18–23% with 10% addition of vegetable oils.

Conclusions

The fluorescence spectra of most common edible vegetable oils were measured under native conditions. Under the experimental conditions used, fluorescence spectra of virgin olive oil were quite different from those of all other vegetable oils. These spectra can be measured quickly and easily, without any pretreatment of the oil sample, and could be possibly used to identify virgin olive oil.

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