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ARTICLE

Correlation between Chemical Analysis and Sensory Committee Tests with Total Fluorescence Characteristics of Jordanian Olive Oil

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Abstract: Olive oil samples extracted from olive trees grown in different un-irrigated areas of Jordan were classified according to their quality using three different techniques. Two of the techniques; namely, chemical analysis and sensory committee tests, are recognized by the International Olive Oil Council (IOC) and considered as classical techniques. The results were compared with those of the total fluorescence spectroscopy that has been recently adopted for olive oil quality analysis. Nine different olive oil samples were subjected to the analysis by the above techniques. The analysis for each sample was repeated twice to ensure consistency of the results. Chemical analysis was used to determine their acidities, peroxide values, ΔK's and polyphenol content parameters. The results of chemical analysis indicated that eight of the samples could be classified as EVOO and one as VOO. Sensory tests indicated that four samples could be classified as EVOO and five as VOO. Total fluorescence spectra results revealed that six samples are EVOO and three samples are VOO. The correlation of fluorescence results to chemical and sensory results indicates the ability of fluorescence technique as a powerful tool for classification of olive oil. Reference samples that were already classified by IOC were tested to ensure the accuracy of the fluorescence technique in recognizing EVOO from other types of olive oil. Moreover, fluorescence analysis proved to be fast and consumes minute amounts of samples and chemicals, in contrary to chemical analysis which is known to be time and chemicals-consuming.

Keywords: Jordan's olive oil, EVOO, Polyphenols, Total fluorescence, Total synchronous fluorescence, Fluorescence fingerprint, PCA.

Introduction

There is much interest in natural diets that contain antioxidants, including polyphenols and tocopherols[1,2]. Olive oil in particular is known to contain a large number of compounds that are useful to our bodies; including antioxidants, such as α-tocopherol and polyphenols. Standard tests were developed and approved by the International Olive Council (IOC) to verify the quality of olive oil products and to ensure that olive oil produced meets the customer's specifications needed[3]. These tests include sensory committees and chemical tests that can be used to classify olive oil into different quality categories (extra virgin, virgin, ordinary, lampante,…etc). On the other hand, there has been increasing interest in the development of new devices accompanied with analytical techniques for oil and food testing, such as high-performance liquid chromatography (HPLC)[4,5], thin layer chromatography (TLC)[6], nuclear magnetic resonance (NMR)[7], gas chromatography (GC)[8] and fluorescence spectroscopy techniques, beside others[9, 10]. Whether a certain technique is successful or not as a quality control technique is determined by its ability to classify

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olive oil into determined categories (EVOO, VOO, ...etc.) that could match the set of requirements (analysis) suggested by IOC using chemical and sensory analysis for classification. In this context, and due to the presence of natural fluorescent components, including phenolic compounds, tocopherols, chlorophylls and vitamins in olive oil, fluorescence techniques were found to be non-invasive, non-destructive, rapid, selective and accurate for characterization and classification of olive oil[11-20]. Our main goal in this study is to correlate fluorescence measurement results with sensory analysis and chemical analysis carried out on various olive oil samples produced from un-irrigated olive trees grown in different parts of Jordan. An excellent match between sensory tests and chemical tests with fluorescence analysis that makes fluorescence techniques is a good choice for classification of olive oil to at least three categories; EVOO, VOO and olive pomace oil (OPO).

Material and Methods

Sampling

Olive oil samples were purchased randomly from nine farmers who pressed their olive fruits in different mills distributed all over Jordan during November of the year 2011. Three samples were collected from each geographic region in Jordan (north, middle and south) for which we fixed parameters that may have roles in the quality of olive oil, such as method of harvesting, time of storage and using cold or heated water in extraction. It must be mentioned that all of the nine samples were obtained from un-irrigated olive trees and each sample was measured and analyzed twice. Olive fruits were pressed using several types of olive mills, most of which are of Italian origin; for example, "Polat Makina" brand is a famous olive mill project in Irbid.

Chemicals and Glassware

Spectroscopic grade hexane was purchased from Tedia Chemicals Company (TCC). Other chemicals and high-purity solvents used in the chemical analysis were purchased from Sigma Aldrich. Fractional distillation was necessary to remove trace impurities in the solvent. Excitation and emission spectra were used to double check the solvent purity. Clean acetone from TCC (99.5%) was used for glassware washing. Samples of the south region were a donation from Al-Toor Company, Jordan. The other samples were obtained either from people (north region) or local oil millers distributed in the middle province. Fused silica cuvettes were purchased from Helma Company. The cell transmittance curve was tested and found to be flat in the spectral range from 200 to 1100 nm. Clear Borosilicate vials for sample storage and preparation were purchased from Wheaton. Chemical tests were performed accordingto the procedure described by the American Oil Chemists' Society (AOCS) or IOC[27].

Absorption Measurement

UV/Vis spectra were recorded using Shimadzu Model 1800 spectrophotometer. The following parameters were set to record all spectra: scanning speed 500 nm/min, spectral band width 1 nm, wavelength accuracy 0.1 nm, photometric range about a maximum of 0.2 absorbance. The instrument includes a silicon photodiode as a photo detector. UV probe PC software (Version 2.33) was used for data acquisition, analysis and graphics. For our samples, UV/Vis absorption measurements in the range 200-900 nm were carried out on olive oil samples using 1.0 cm path length quartz cuvettes[22].

FluorescenceMeasurements

Steady state, excitation-emission, synchro-nous spectra and total fluorescence spectral measurements were carried out on olive oil samples using front face sample cell holder system to overcome the extremely short penetration depth of light beam into the oil samples and to eliminate the self-absorption of emission. All spectral measurements were preformed using an upgraded version of FS-900 fluorometer from Edinburgh Instruments. Full details about the system are described in a previous work[22,24]. The cell holder inside the sample chamber is fitted with external thermostated liquid circulator (cooler/heater) and used to control the sample temperature (-20°C – 70°C) using ethylene glycol-water mixture.

Chemical and Sensory Analysis Tests

Chemical tests were carried out in the laboratory on olive oil samples to determine its quality[25],while acidity tests were conducted to determine the free fatty acid and peroxide values as described by AOCS[26, 27]. The phenol contents of olive oil were determined following the method of Gutfinger with some modifications[28].

Specific absorption coefficients (K-values) for olive oil samples as described by (IOOC, 1968; EU 1995)[29]. The sensory analysis of the samples was carried out by the Jordanian team for olive oil sensory evaluation, using the profile sheet for organoleptic assessment of olive oil according to the International Olive Oil Council (IOC, 1996) protocol[30-31]. The number of panels was eight. They evaluated the sensory attributes that exist in profile sheet for organoleptic assessment of olive oil, with each sample being tested in duplicates. Each sample was assigned a number from 1-9, so that the name of region or mill is absent for the panel.

Results and Discussion

Sensory Tests

The sensory evaluation according to positive attributes percentage was carried out by the Jordanian team (sensory panel) for nine different olive oil samples. According to the team, four samples were classified as EVOO, while the rest were identified asVOO. The results are tabulated in Table 1.

Table 1. Arranging sample results according to positive attributes percentage. S= South, N= North, M= Middle.

Sample code/region sensory test	% favorably (+ve) attributes from medians	Classification
S4	100	EVOO
S5	100	EVOO
S6	100	EVOO
N7	100	EVOO
N8	81.0	VOO
N9	79.0	VOO
M1	60	VOO
M2	86	VOO
M3	92	VOO

Chemical test Results

The acidity, peroxide value (PV), polyphenols and specific absorption coefficients test results are summarized in Table 2. Comparing these results with the main legal limits for classification of olive oil presented inTable 3, the oil samples 1-9 could be classified as tabulated in Table 4. As a conclusion, classification built based on chemical tests only could put almost all the samples as EVOO, except sample M3 that is classified as VOO Please note that sample M3 is recorded as EVOO based on chemical test column in Table4. According to IOC guidelines, chemical tests are not enough to judge on the quality of olive oil when taking into consideration that several chemical tests such as total polyphenols test, for example, have no range limits specified by IOC[3].Therefore, samples should be tested by a sensory team as a complementary technique. According to the sensory tests, samples from the mid region (M1, M2 and M3) were classified as VOO, while samples from the south region (S4, S5and S6) were classified as EVOO and sample from the north region N7 was classified as EVOO, while N8 and N9 were classified as VOO as shown in Table 4.

Table 2. Summary of chemical test results for all samples.

Sample name	Free acidity	Peroxide value	Total polyphenols	Difference specific absorption (Δk)	Classification
S4	0.26	11.72	350	0.008	EVOO
S5	0.38	7.12	383	0.003	EVOO
S6	0.20	4.11	404	0.006	EVOO
N7	0.62	4.35	370	0.002	EVOO
N8	0.40	6.63	405	0.006	EVOO
N9	0.66	6.50	390	0.008	EVOO
M1	0.69	10.46	252	0.005	EVOO
M2	0.60	8.02	239	0.005	EVOO
M3	1.04	10.47	224	0.004	EVOO

Table 3. The main legal limits of authentication parameters of olive oil types[26, 27].

Category	Free acidity	Peroxide value	Total polyphenols	Difference specific absorption (Δk)
EVOO	≤0.8	≤20	-	≤0.01
VOO	≤2.0	≤20	-	≤0.01
LOO	>2.0	-	-	-

Absorption and FluorescenceSpectra of EVOO Olive Oil Samples Used as a Reference in Our Laboratory

It is well known that the electronic absorption spectrum of olive oil extends from 250-750 nm in the UV-Visible region, reflecting the presence of many compounds, like polyphenols, α-tocopherol (vitamin-E), chlorophyll, among many others. The synchronous and total fluorescence combined with chemometric analysis of olive oil were able to show definite regions to represent polyphenols, Vitamin-Eand chlorophyll in the so-called excitation/emission landscape or fingerprint for EVOO. Inspection of more than 200 fluorescence landscapes of different olive oil samples from Jordan and from other countries lead us to believe that a standard excitation/emission landscape graph of EVOO could be generated and represented as can be seen in Figure 1[32]. Region **A**is denoted for Vitamin-E and polyphenols, region **C** for Vitamin-A and Vitamin-B2. Region **B** shows the degradation products and other unknown chemicals. Region **D** shows the chlorophyll content. We believe that deviation from this fluorescence landscape could lead to other brands of olive oil.

Fluorescence of Olive Oil Samples Presented in This Study 1-9

Figure 2 shows the fluorescence landscapes of samples of olive oil 1-9. Inspection of these fluorescence landscapes and by comparison with standard fluorescence landscape of Figure 1 lead us to qualitatively classify them to their brands as shown in Table 4.

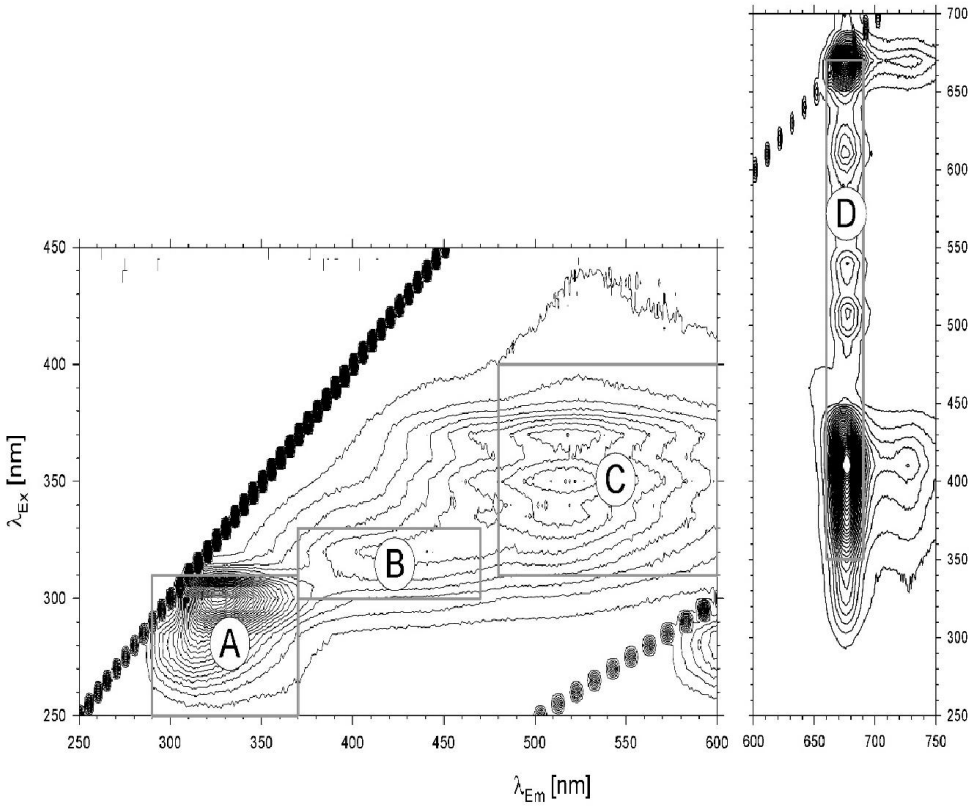

Figure 1. Total fluorescence (contourplot) of an extra virgin olive oil divided into the main four regions: A, B, C and D.

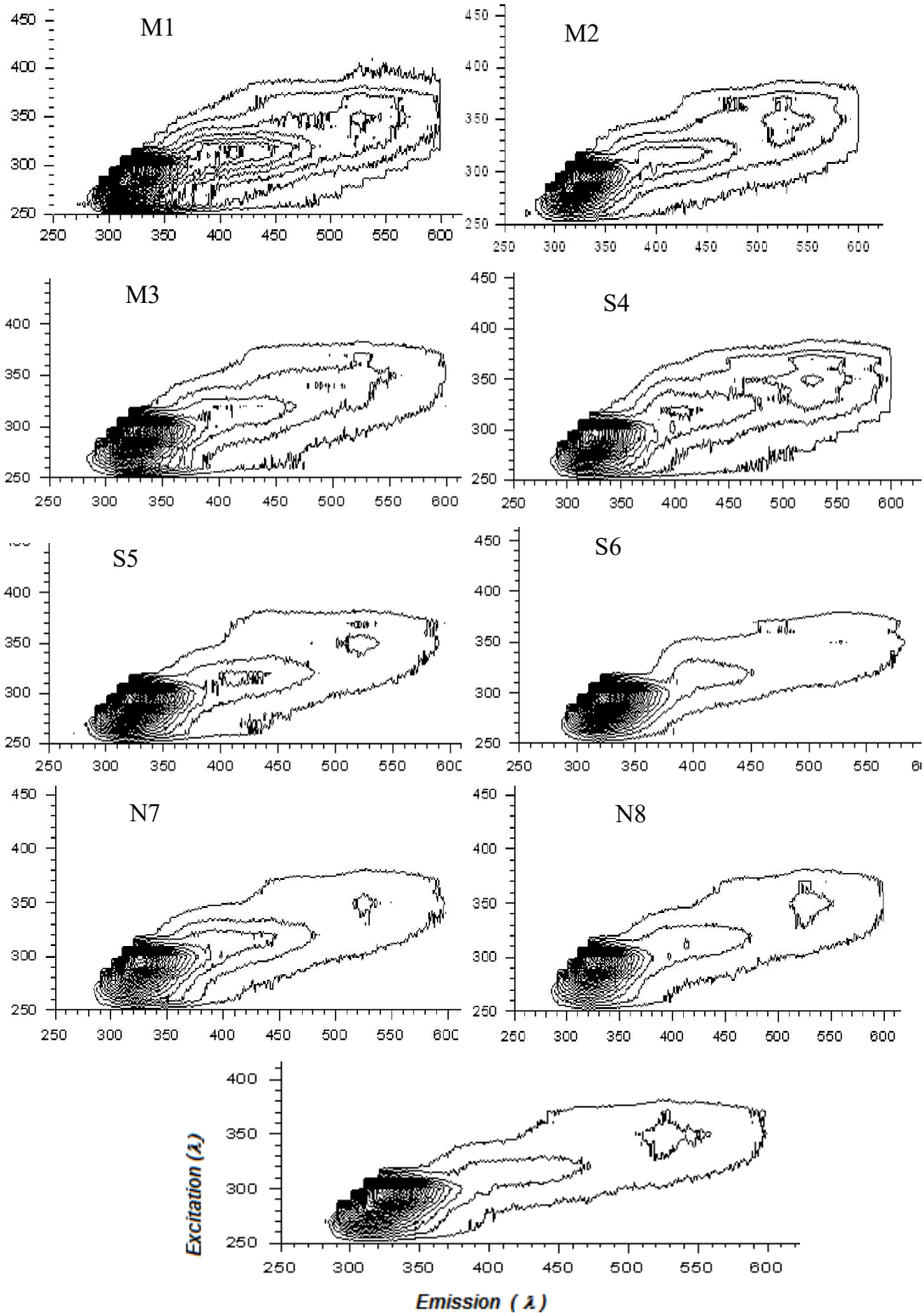

Figure 2. Total fluorescence (contourplot) for the tested samples 1-9. Region D has been omitted, because all samples show the same features of region D.

Table 4. Classification of olive oil samples 1-9 by various techniques.

Sample code/region	*Chemical tests*	*Sensory test*	*Fluorescence*
M1	EVOO	VOO	VOO
M2	EVOO	VOO	VOO
M3	EVOO	VOO	EVOO
S4	EVOO	EVOO	VOO
S5	EVOO	EVOO	EVOO
S6	EVOO	EVOO	EVOO
N7	EVOO	EVOO	EVOO
N8	EVOO	VOO	EVOO
N9	EVOO	VOO	EVOO

Correlation of Fluorescence Spectra with Chemical and Sensory Analysis

The results obtained confirmed our expectation that fluorescence spectroscopy techniques are excellent tools to give a very fast decision on whether a sample of olive oil is VOO, EVOO or not, as shown in Table 4.

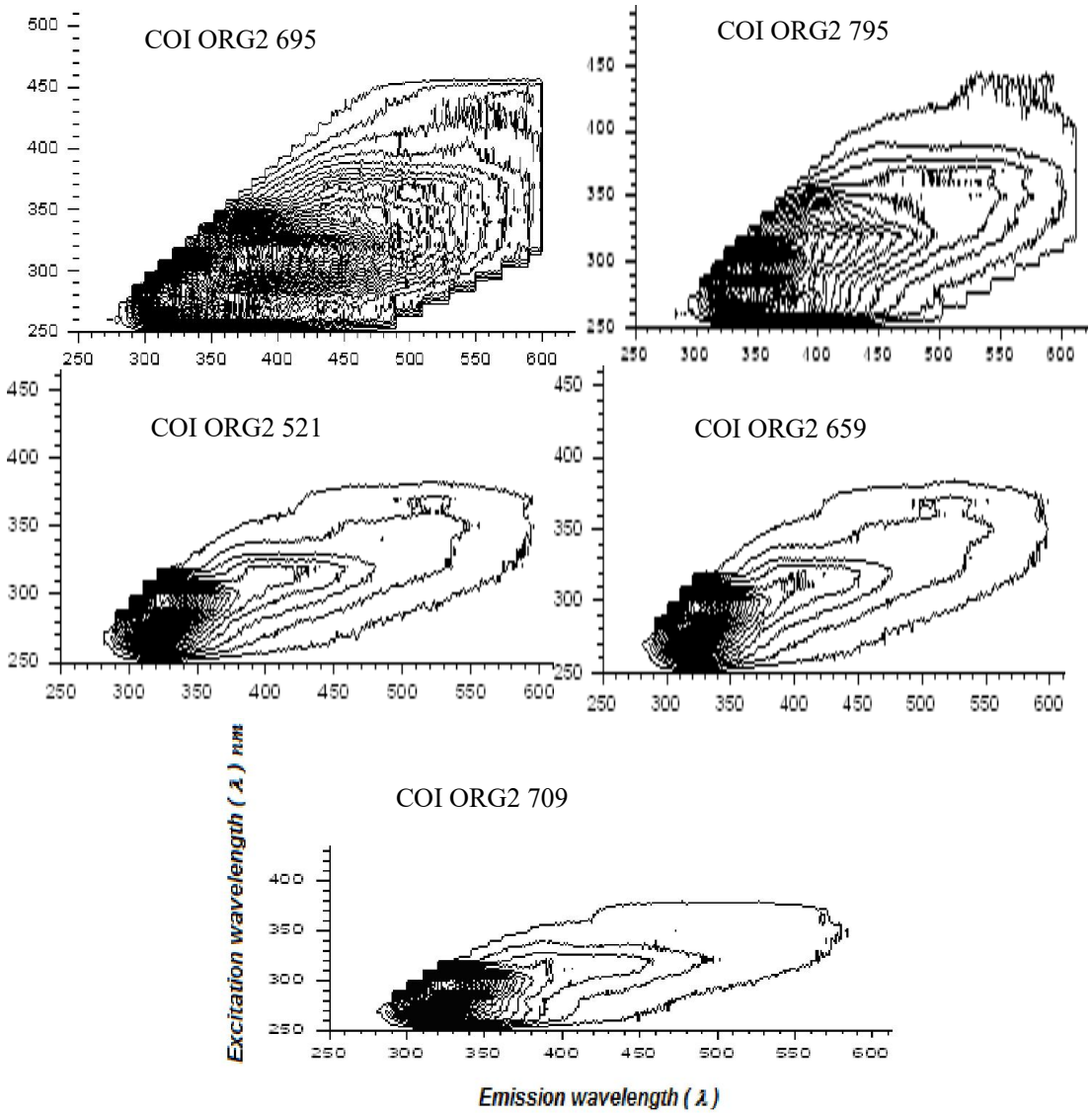

Figure3. Total fluorescence (contourplot) for the reference samples obtained from IOC/Spain.

Confirmation by Using Olive Oil Samples from IOC / Spain and by Principal Component Analysis (PCA)

We performed fluorescence measurements on samples that have been given codes and obtained from IOC/Spain and already classified using chemical and sensory tests, as listed in Table 5. The fluorescence landscapes of these samples are presented in Figure 3 and their classification almost matches that categorized by IOC. This confirms the use of fluorescence spectroscopy as an excellent technique to classify olive oil into at least 3 categoriess; EVOO, VOO or none of them.

Table 5. IOC samples with their names, codes and categories.

Sample name	Code	Category as IOC	Fluorescence classification in our laboratory
M1	COI ORG2 695	LVOO	Neither VOO nor EVOO
M2	COI ORG2 795	OVOO*	Neither VOO nor EVOO
M3	COI ORG2 521	EVOO	EVOO
M4	COI ORG2 659	EVOO	EVOO
M5	COI ORG2 709	VOO	EVOO

*Ordinary virgin olive oil.

To prove the consistency between fluorescence technique with conventional techniques (chemical and sensory tests) as a tool to classify olive oil samples to their categories, we applied principal component analysis at λ_{ex} = 330 nm on reference samples that belong to IOC (Figure 4). The black circles in the left-hand side of Figure 4-a indicates that the sample belongs to the EVOO category (COI ORG2 521 and COI ORG2 659), followed directly by the sample COI ORG2 709 classified as VOO) (black rectangle). The samples COI ORG2 795(VOO) and COI ORG2 695 (LVOO) were separated to the upper right of Figure 4-a. At the same λ_{ex} = 330 nm applied to all samples 1-9, as shown in the score plot presented in Figure 4-b, the samples classified as EVOO or VOO were located at the same region for reference samples already classified as EVOO or VOO. This conclusion gives a strong evidence to classify olive oil to its different categories by fluorescence techniques in less time than that neededby chemical and sensory test.

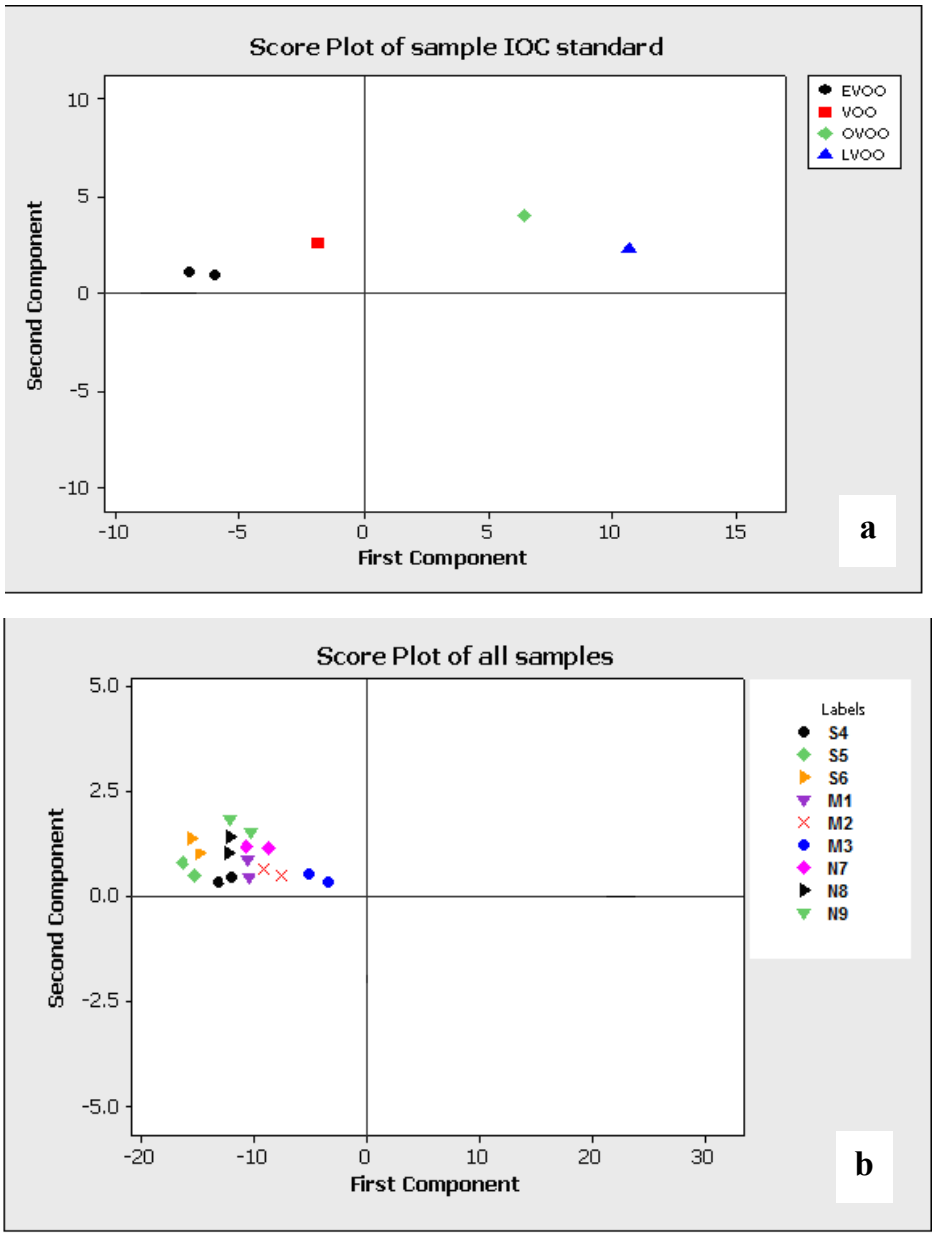

Figure 4.a) PCA for IOC sample separation of EVOO, VOO from other brands; b) PCA model of all samples, 1-9.

Conclusion

Total fluorescence spectroscopy was successfully applied to classify EVOO samples from other types of olive oil; namely, VOO and OP. The results were compared with those of sensory committee and chemical analysis techniques. Direct comparison of the fluorescence results with other techniques for standard olive oil samples indicated that fluorescence is more accurate in differentiating EVOO samples from other olive oil samples. The technique could be improved by using total fluorescence results in PCA to produce graphical plots for grouping each type of oil in a certain graphical region separate from the other types.

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