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Araştırma Makalesi / Research Article

DETECTION OF CORN AND SUNFLOWER OIL ADULTERATIONS OF OLIVE OILS USING FLUORESCENCE SPECTROSCOPY

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ABSTRACTS

A novel approach for the detection of admixtures of vegetable (corn and sunflower) oils in commercially available olive oils on a qualitative level is presented. Chemical analysis of the contents of fatty acids in each of the model systems under study is performed. The dependence of the ratio of the two main fluorescence intensity peaks F_1 / F_2 and that of the linoleic to oleic acids $C_{18:2} / C_{18:1}$ is studied. It is shown that an increase of the concentration of the adulterant the maximum of the first fluorescence peak exhibits a wavelength shift. Fluorescence analysis can be used for a qualitative real time determination of the presence of adulterants in olive oil by comparison of the observed particular fluorescence spectrum with those from a database of spectra of olive oils and adulterants from a particular region.

Keywords: [1]corn oil, [2]fatty acid composition, [3]fluorescence, [4]sunflower oil, [5]olive oil

INTRODUCTION

Olive oil is an important edible product basically manufactured in Mediterranean countries. Its price is higher compared to the rest of the edible vegetable oils. Because of that and the similarity of the contents, olive oil is often adulterated with the above mentioned vegetable oils. The detection of the type and the quantitative contents of the adulterant is not a simple task. Recent research activities in this area can be subdivided into several main groups. In the first the samples are chemically treated in order to determine their contents: fatty acids, triglycerides (Bucci R et all 2002, Ollivier D et all 2003) and sterols (Leardi R et all 1987) . In the second group of studies the detection of adulterants in olive oil is done by spectroscopic measurements in the infrared without chemical treatment of the sample **(**Yang H et all 2001) , Fourier

transformation spectroscopy (Lopez-Diez E C et all 2003) and Raman spectroscopy (Ozen B et all 2002). To prove the type of the adulterant chemical methods, such as gas chromatography or high performance liquid chromatography (Aparicio R et all 2000 Andrikopoulos N K et all 2001) are used. The latter methods are precise, but expensive, requiring time and highly qualified researches, using expensive reagents. During the recent years fluorescence spectroscopy has been successfully used to analyze certain components in olive oil such as tocopherols, chlorophyll, phenols, riboflavin etc (Zandomenenegli M et all 2005, Kyriakidis MB et all 2000).

The objective of the present paper is to propose offer a fast and low-cost method for the detection of corn and sunflower oil adulterants in olive oil using real time fluorescence spectroscopy.

MATERIALS AND METHODS

SAMPLE

Samples of pure extra virgin olive oil (Turkey), sunflower oil (Bulgaria) and corn oil (Italy) commercially available in Bulgaria were studied after preliminary research of the fatty acid composition of oils of this type available in Bulgaria. Their fatty acid compositions are presented in Table I

TableI. Fatty acid composition of the tested oils

Fatty acids	**Olive oil, %**	**Sunflower oil, %**	**Corn oil, %**
$C_{12:0}$	-	0.1	0.1
$C_{14:0}$	-	0.2	0.1
$C_{16:0}$	17.1	13.2	15.7
$C_{16:1}$	0.2	0.2	0.1
$C_{17:0}$	0.3	0.1	0.1
$C_{18:0}$	3.0	6.4	2.6
$C_{18:1}$	72.0	36.9	33.9
$C_{18:2}$	6.8	41.8	43.1
$C_{18:3}$	0.3	0.2	0.5
$C_{20:0}$	0.3	0.2	3.4
$C_{22:0}$	-	0.7	0.4

Prior to the analysis the samples were stored in dark bottles without headspace at room temperature. A pure sample of each edible oil was analyzed. Different admixtures at various ratios (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 80:20, 90:10-admixture: extra virgin olive oil-EVO) of these oils were prepared and were investigated.

INSTRUMENTS ANS SOFTWARE

Figure 1 below shows the experimental set-up used to measure the fluorescence spectra. The sources used are 370, 395, 425 nm light emitting diodes (LEDs). A fiber optic spectrometer (AvaSpec-2038, Avantes) with a sensitivity in the 200 – 1100 nm range and a resolution of about 5 nm was used to measure the fluorescence spectra. The oil samples were placed in a cuvette (10mm x10mm) and irradiated by laser diodes (LDs) or light emitting diodes (LEDs). Fluorescence spectra were taken from a direction orthogonal to the line of transmission, as shown in the figure 1.

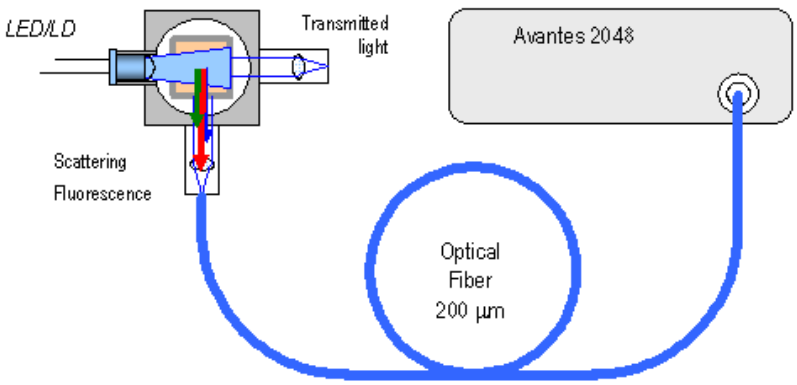


Fig. 1. Experimental set-up for measuring fluorescence.

Fluorescence excitation-emission matrix (EEM) was obtained for each mixture, containing sunflower oil and olive oil. Excitation and emission ranges were correspondingly $\lambda_{ex.}$ = 370-440 nm and $\lambda_{em.}$ = 400 - 800 nm. Data were exported to ASCII code and processed with MatLab 7.12.0.635 (R2011a) software.

FATTY ACID COMPOSITION

The fatty acid composition of oils was determined by GC after transmethylation of the respective sample with 2N methanolic KOH at 50°C according to *Christie* (Christie WW 2003) . Fatty acid methyl esters (FAME) were purified by TLC on 20x20 cm plates covered with 0.2 mm Silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase *n*-hexane:acetone 100:8 (by volume). GC was performed on a HP 5890 (Hewlett Packard GmbH, Austria) gas chromatograph equipped with a 30 mm x 0.25 mm (I.D.) capillary InnoWax column (cross-linked PEG, Hewlett Packard GmbH, Austria) and a FID. The column temperature was programmed from 165°C to 240°C at 4°C/min and held at this temperature for 10 min; injector and detector temperatures were 260°C. Hydrogen was the carrier gas at a flow rate 0.8 ml/min; split was 100:1. Identification was performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions (Animal and vegetable fat and oils 2000).

RESULTS

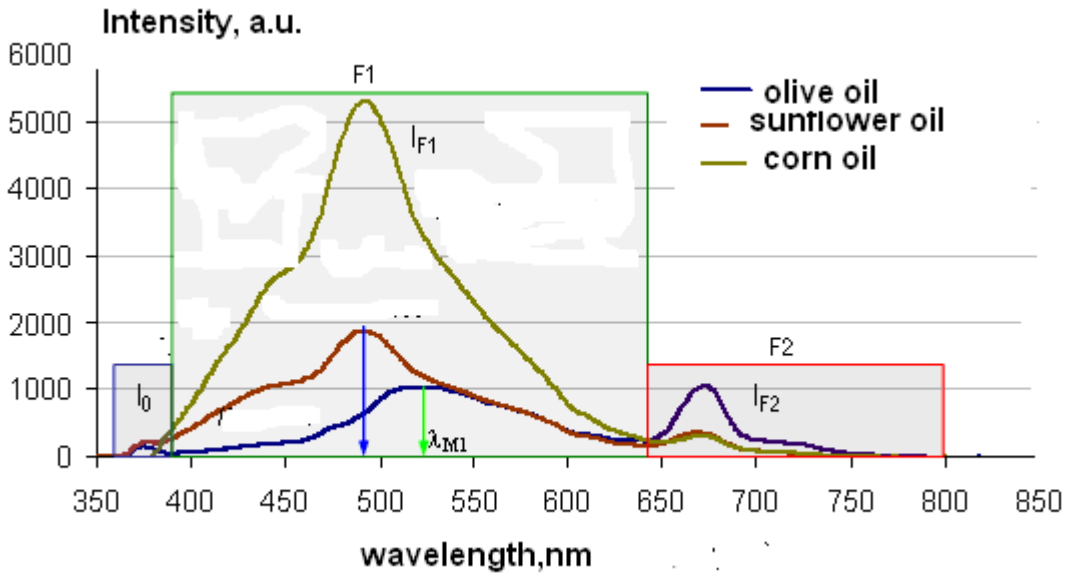
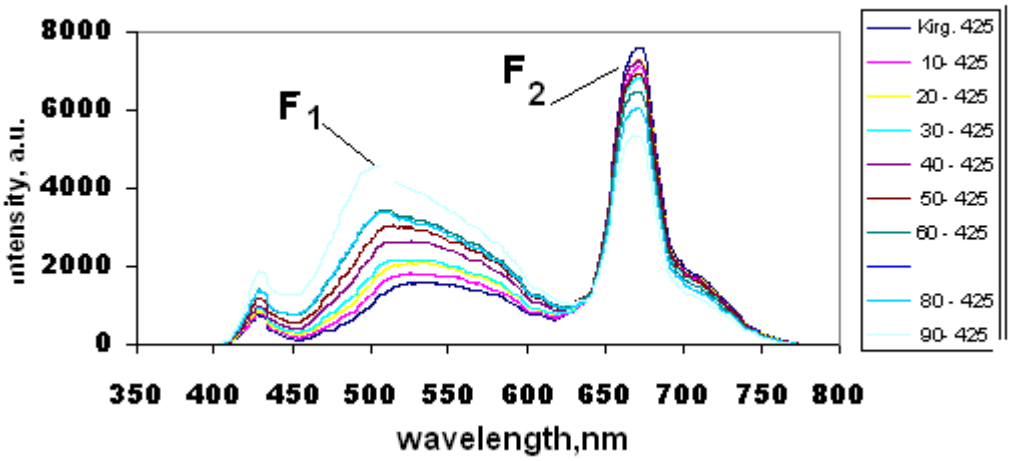
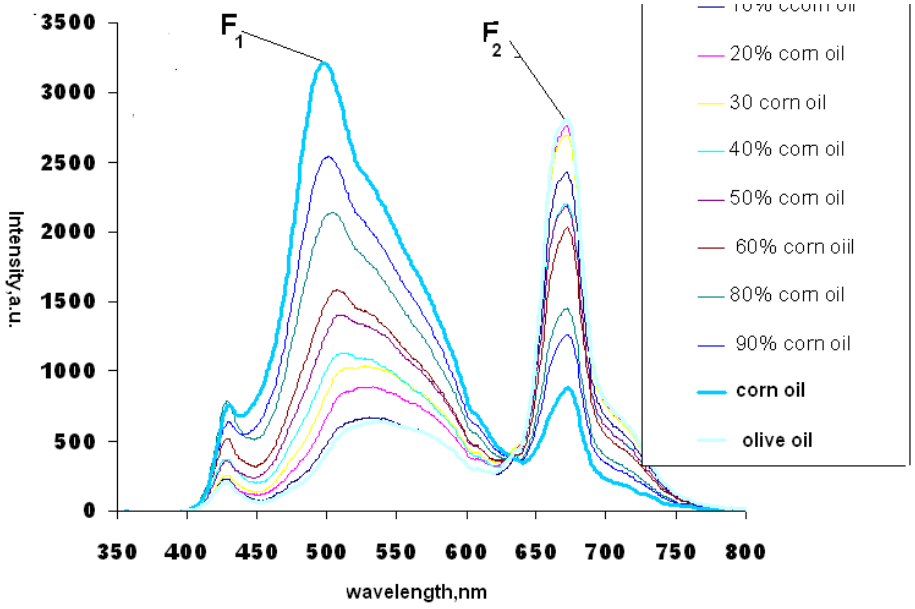


Fig. 2. Fluorescence spectra of olive oil, corn and sunflower oils for 370 nm LED excitation



(a)



(b)

Fig. 3 Fluorescence spectra of mixture of vegetable and olive oils with excitation at $\lambda = 425$ nm**: for sunflower and olive oil or for corn and olive oil**

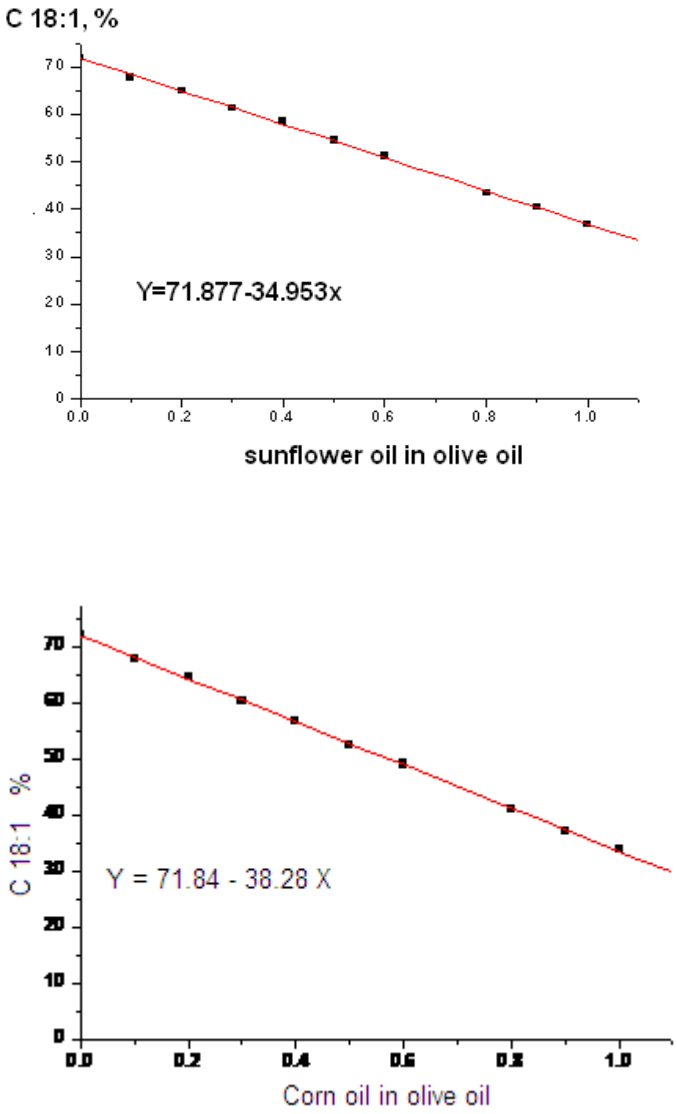


Fig 4 Linear dependence between the concentration of the adulterant and the contents of oleic acids $(C_{18:1})$

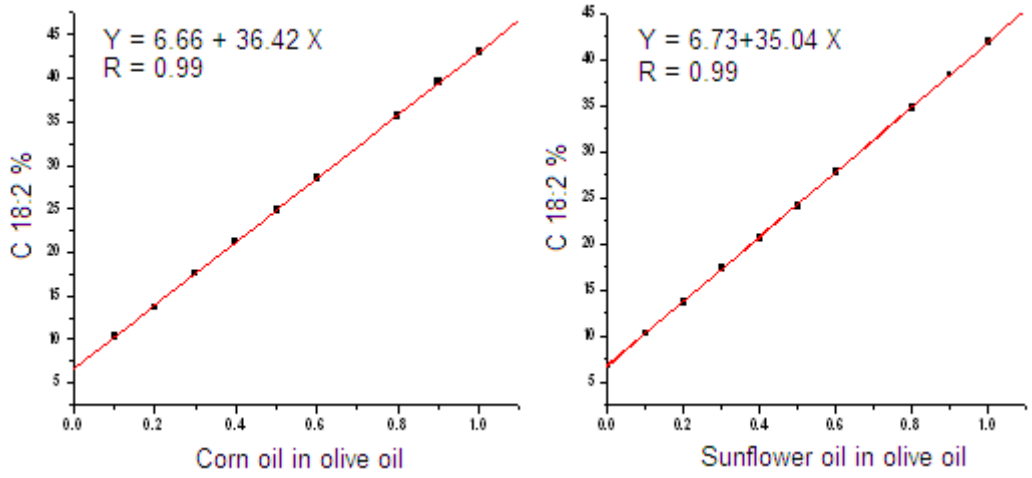
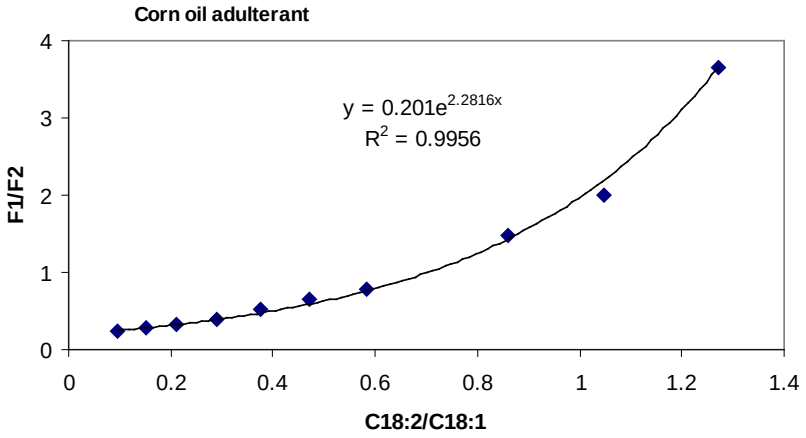
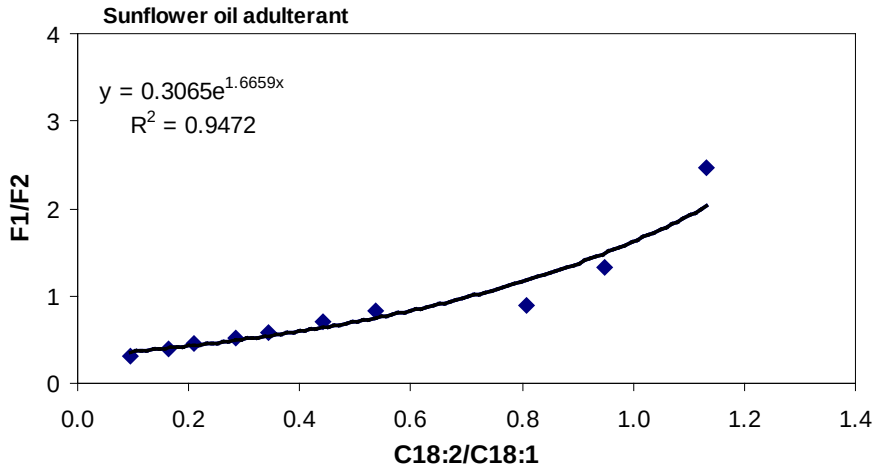


Fig 5 Linear dependence between the concentration of the adulterant and the contents of linoleic acids $(C_{18:2})$



(a)



(b)

Fig. 6.. Exponential dependencies for the olive and corn oil mixtures or for the olive and sunflower oil mixtures.

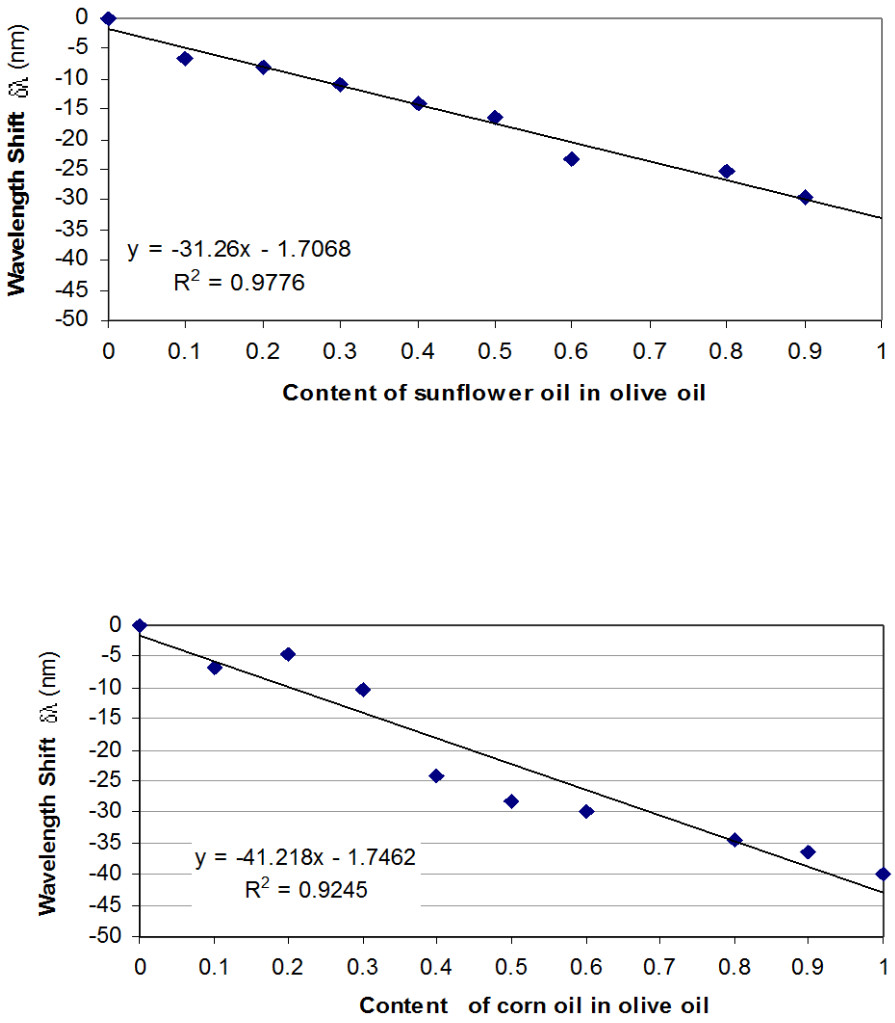


Fig. 7. Dependences oils of wavelength shift $\delta\lambda$ of the maximum of the fluorescence peak and content of adulterant

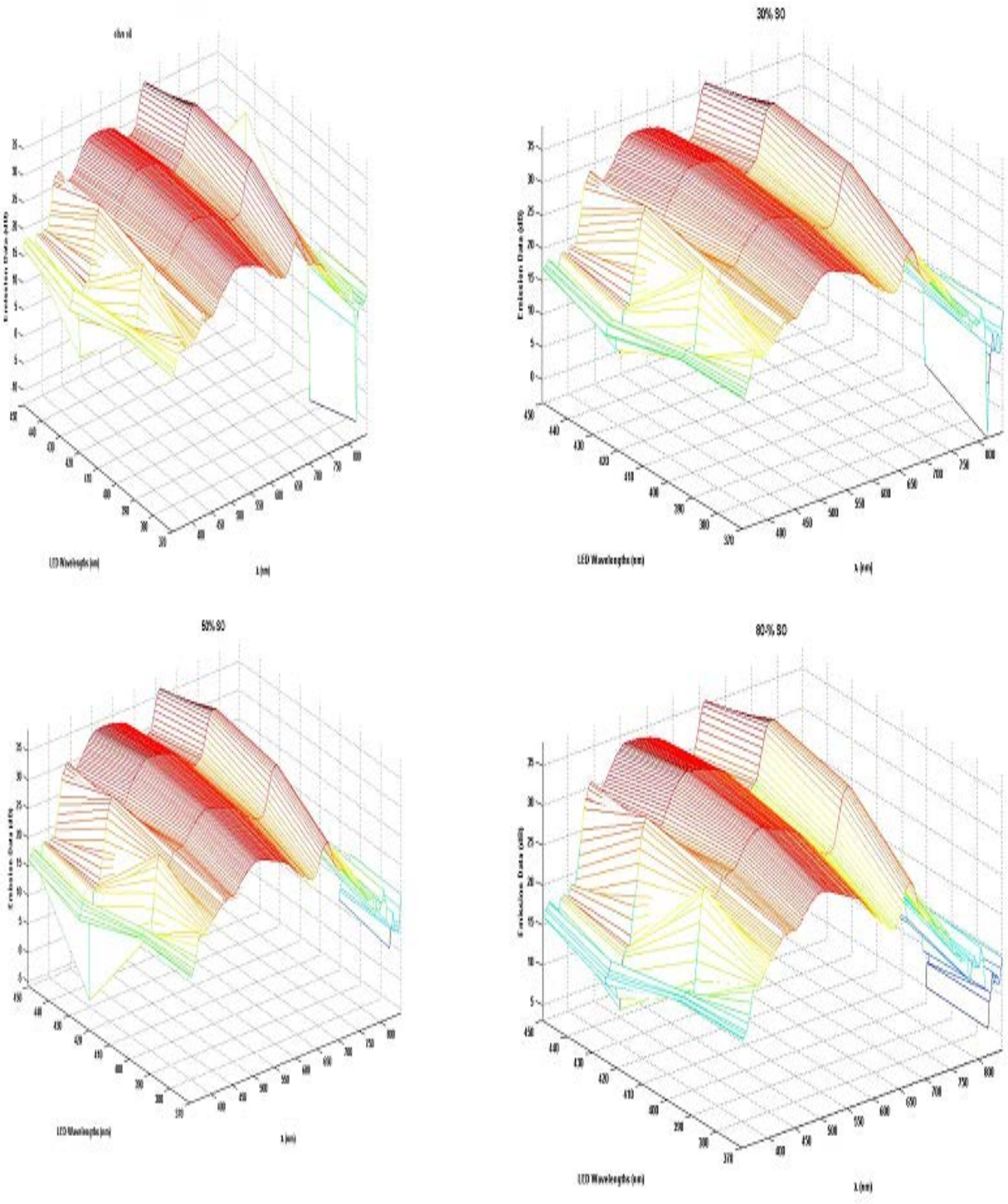


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Fig 8. EEM for olive oil and its mixtures with sunflower oil

Table II

Fatty acids, %	**Olive oil**	**Sun-flow-er oil**	**10%**	**20%**	**30%**	**40%**	**50%**	**60 %**	**80%**	**90%**
$C_{12:0}$	-	0.1	<0.01	<0.01	<0.01	<0.01	0.1	0.1	0.1	0.1
$C_{14:0}$	-	0.2	<0.01	<0.01	0.1	0.1	0.1	0.1	0.2	0.2
$C_{16:0}$	17.1	13.2	16.6	16.3	15.9	15.5	15.1	14.8	14.2	13.6
$C_{16:1}$	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
$C_{17:0}$	0.3	0.1	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.1
$C_{18:0}$	3.0	6.4	3.3	3.7	4.0	4.4	4.6	5.0	5.5	6.0
$C_{18:1}$	72.0	36.9	67.8	65.0	61.2	58.5	54.5	51.2	43.5	40.4
$C_{18:2}$	6.8	41.8	11.0	13.7	17.4	20.1	24.1	27.5	35.2	38.3
$C_{18:3}$	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2
$C_{20:0}$	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2
$C_{22:0}$	-	0.7	0.2	0.3	0.4	0.4	0.5	0.5	0.6	0.7
SFA	**20.7**	**20.9**	**20.7**	**20.8**	**20.9**	**20.9**	**20.9**	**20.9**	**20.9**	**20.9**
USFA	**79.3**	**79.1**	**79.3**	**79.2**	**79.1**	**79.1**	**79.1**	**79.1**	**79.1**	**79.1**
UFA	**72.2**	**37.1**	**68.0**	**65.2**	**61.4**	**58.7**	**54.7**	**51.4**	**43.7**	**40.6**
UFA	**7.1**	**42.0**	**11.3**	**14.0**	**17.7**	**20.4**	**24.4**	**27.7**	**35.4**	**38.5**

SFA - Saturated fatty acids; USFA - Unsaturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids

Table IV

Type	Dependences
Olive oil+ Corn oil	$\frac{F_1}{F_2} = 27.153.e^{-0.0689C_{18:1}}$; $R^2 = 0.971$
	$\frac{F_1}{F_2} = 0.1175.e^{0.0722C_{18:2}}$; $R^2 = 0.973$
Olive oil +Sunflower oil	$\frac{F_1}{F_2} = 13.956.e^{-0.0541C_{18:1}}$; $R^2 = 0.946$
	$\frac{F_1}{F_2} = 0.2217.e^{0.0488C_{18:2}}$; $R^2 = 0.922$

Table III

Fatty acids, %	**Olive oil**	**Corn oil**	**10%**	**20%**	**30%**	**40%**	**50%**	**60%**	**80%**	**90%**
$C_{12:0}$	-	0.1	<0.01	<0.01	<0.01	<0.01	0.1	0.1	0.1	0.1
$C_{14:0}$	-	0.1	<0.01	<0.01	<0.01	<0.01	0.1	0.1	0.1	0.1
$C_{16:0}$	17.1	15.7	17.0	16.9	16.7	16.5	16.4	16.3	16.0	15.8
$C_{16:1}$	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1
$C_{17:0}$	0.3	0.1	0.3	0.3	0.2	0.2	0.2	0.2	0.1	0.1
$C_{18:0}$	3.0	2.6	3.0	2.9	2.9	2.8	2.8	2.8	2.7	2.6
$C_{18:1}$	72.0	33.9	67.6	64.6	60.2	56.8	52.3	49.0	41.1	37.1
$C_{18:2}$	6.8	43.1	10.8	13.7	17.9	21.2	25.4	28.5	36.1	40.1
$C_{18:3}$	0.3	0.5	0.3	0.3	0.4	0.4	0.4	0.4	0.5	0.5
$C_{20:0}$	0.3	3.4	0.6	0.9	1.2	1.6	1.8	2.2	2.8	3.1
$C_{22:0}$	0.2	0.4	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.4
SFA	**20.7**	**22.4**	**21.1**	**21.2**	**21.3**	**21.4**	**21.7**	**22.0**	**22.2**	**22.2**
USFA	**79.3**	**77.6**	**78.9**	**78.8**	**78.7**	**78.6**	**78.3**	**78.0**	**77.8**	**77.8**
MUFA	**72.2**	**34.0**	**67.8**	**64.8**	**60.4**	**57.0**	**52.5**	**49.1**	**41.2**	**37.2**
PUFA	**7.1**	**43.6**	**11.1**	**14.0**	**18.3**	**21.6**	**25.8**	**28.9**	**36.6**	**40.6**

SFA - Saturated fatty acids; USFA - Unsaturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids

DISCUSSION

Fluorescence spectra of corn, sunflower, olive oil as well as their binary admixtures were obtained for three excitation wavelengths (370 nm, 395 nm and 425 nm). For $\lambda = 370$ nm the fluorescence spectra of olive, corn and sunflower oils are shown in Fig. 2. This particular wavelength has been chosen because the shifts of fluorescence peaks of the pure components of the binary systems are most clearly discernable. In these figures, the first peak of intensity *I* is caused by the scattering of the excitation wavelength, followed by two fluorescence peaks of intensities F_1 and F_2 correspondingly in the 400-600 nm and in the 650 nm ranges.

Fig 2 Fluorescence spectra of olive oil, corn and sunflower oils for 370 nm LED excitation

It is clearly seen from the figure that the fluorescence spectra of olive oils differ from those of vegetable (corn and sunflower) oils. Olive oil exhibits a weaker peak around 525 nm and another around 680 nm. The former is associated with the presence of vitamin E while the latter - with that of chlorophyll, while vegetable oils (corn and sunflower) exhibit a single peak in the 400-550 nm range caused by the greater amount of polyunsaturated fatty acids. Compared to olive oil, the intensity of the peak in the 400-550 nm range for vegetable oils shifts to lower wavelengths. For the 680 nm peak caused by the chlorophyll contents, the following formula is valid $I_{corn} < I_{sunflower} < I_{olive,.}$, Refined vegetable oils contain almost no or very small amounts of chlorophyll. The ratio F_1/F_2 is a measure of the prevalence of vegetable oil over olive oil.

Fig. 3 a, b) presents fluorescence spectra of binary mixtures of olive oil and sunflower oil or of olive and corn oil for and excitation at $\lambda = 425$ nm. Fluorescence spectra for the rest of the excitation wavelengths is not shown because they resemble those shown in Fig. 3 and provide no additional information.

Fig. 3 Fluorescence spectra of mixture of vegetable and olive oils with excitation at $\lambda = 425$ nm**: a) sunflower and olive oil; b) corn and olive oil.**

The addition of cheaper vegetable oils to olive oil causes an increase of the fluorescence in the 450nm to 600 nm spectral range an and a shift in the wavelength of maximum intensity by around 30 nm. Fluorescence emission is stronger for the sunflower and olive oil mixture, rather than for the corn and olive oil mixture Tables II and III presents data about the fatty acid composition of the samples.

Table II. Fatty acid composition of double mixtures from olive oil and sunflower oil

Table III. Fatty acid composition of double mixtures from olive oil and corn oil

The increase of the fluorescence peak intensity in the 450-600 nm range can be associated with the increase of the content of linoleic acid. We have tried to relate the

fluorescence peak intensity and the fatty acid content of the samples. The addition of the sunflower oil to olive oil cause a decrease in the relative content of palmitic acid ($C_{16:0}$).The adulteration of olive oil with corn oil however leads to changes in the contents of oleic and linoleic acids. There is a linear dependence between the concentration of the adulterant and the contents of oleic and linoleic acid as seen in Fig. 4 a,b and Fig. 5 a,b.

Fig 4 Linear dependence between the concentration of the adulterant and the contents of oleic acids $(C_{18:1})$

Fig 5 Linear dependence between the concentration of the adulterant and the contents of linoleic acids $(C_{18:2})$

Using Origin 7.0 software we have identified the maximum intensities for each of the fluorescence peaks for an excitation wavelength of 425 nm. Exponential dependencies of the ratio of fluorescence peaks on the relative contents of the oleic and linoleic acids have been found. On adding corn and sunflower oil the F_1 / F_2 ratio as a function of oleic acids ($C_{18:1}$) exponentially decreases exponentially increases as function of linoleic acids ($C_{18:2}$). Fig. 6 represent the dependencies of F_1 / F_2 as a function of the ratio of linoleic/oleic acids. As is clearly seen the increase of the content of sunflower or corn oil leads to an exponential increase of the F_1 / F_2 ratio.

Table IV. Existing dependencies between the ratio of fluorescence peaks and the contents of oleic and linoleic acids.

There also exist relationships between the fluorescence peak ratios $y = F_1 / F_2$ and the relative content ratio $x = C_{18:2} / C_{18:1}$ of linoleic to oleic acids. The latter ratio is a measure of the prevalence of sunflower oil over olive oil. The dependencies were found to be exponential. The analytic form and graphic shape of the mixtures of olive and sunflower oil as well as olive and corn oil are shown in Fig. 6. Because the *y(x)* dependence is exponential, the fluorescence peak ratio *y* is a stronger indication of the prevalence of sunflower over olive oil than the chemical contents ratio *x*. Since the *y* ratio is a normalized quantity the detection of adulteration does not depend on the intensity of the excitation source.

Fig. 6. a). Exponential dependencies for the olive and corn oil mixtures. b). Exponential dependencies for the olive and sunflower oil mixtures.

For all excitation wavelengths we observe a clear wavelength shift $\delta\lambda$ of the maximum of the fluorescence peak in the 450-550 nm intervals. Fig. 7 presents the dependences of $\delta\lambda$ from the concentration of the adulterant for each of the model systems (olive oil + sunflower

oil) and (olive oil + corn oil) for a 425 nm excitation. Addition of vegetable oils to olive oil causes a wavelength shift of the fluorescence peak to lower wavelengths.

Fig. 7. Dependences oils of wavelength shift $\delta\lambda$ of the maximum of the fluorescence peak and content of adulterant sunflower or corn oils

The excitation emission matrices (EEM) for olive oil and mixtures, containing different concentration of sunflower oil, are presented in Fig. 8

Fig. 8 EEM for olive oil and its mixtures with sunflower oil

Virgin olive oil present two low intense peaks at 440nm and 480 nm, one intense peak at 580 nm. The two low intensity peaks are caused by the higher oxidizing stability of olive oils, which can be explained by the greater content of monounsaturated fatty acids. The addition of sunflower oil to olive oil leads to the increase of the area under the peak around 580 nm. In the presence of more than 40 % of sunflower oil in olive oil fluorescence peak intensities at 440 nm and 480 nm are almost imperceptible.

A wide peak between 415 nm and 600nm can be seen in the figure. The latter fact can be explained with the increase of oxidation in olive oil cased by the addition of vegetable oils and the formation of larger quantities of hydroperoxides.

CONCLUSIONS

In the present paper we have studied fluorescence spectra from admixtures of olive oil with sunflower and corn oil and have identified specific dependencies of the fluorescence peaks and the fatty acid composition as well as the adulteration percentage. Two basic fluorescence peaks around 525 nm and 680 nm have been identified.

The fluorescence peak ratio F1/F2 has been found to increase exponentially with the linoileic to oleic acid content ratio.

A negative wavelength spectral shifts of the first fluorescence peak with the increase of the adulterant content has been established.

Three dimensional excitation emission fluorescence spectra have been plotted and they show different reliefs that can be used for identification analysis. The area under the F_1 peak strongly increases due to the increase in the linoleic acid ($C_{18:2}$) content as the percentage of the sunflower adulterant becomes higher. Simultaneously, the area under the F_2 peak diminishes due to the lower content of chlorophyll in refined sunflower oil. The results obtained indicate that fluorescence spectroscopy can be used for a fast, non-destructive and low-cost analysis to identify the presence of adulterants on olive oil. Adulterant concentrations up to 20% can easily be established.

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