Quick Fluorescence Method for the Distinguishing of Vegetable Oils

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Abstract: This paper presents the possibilities offered by fluorescence spectroscopy for the identification of vegetable oils such as soybean, sunflower, flax, walnut, corn, almond, sesame, olive and pumpkin oils. The probes under study have been excited with two types of sources: a laser diode (LD) and light-emitting diodes (LEDs) emitting in the UV and in the visible range. Total luminescence spectra were recorded by measuring the emission spectra in the range 350-720 nm at excitation wavelengths from 375 to 450 nm. The excitation-emission matrices have been obtained and two basic fluorescence regions in the visible have been outlined. On this basis the fluorescence spectra of the oils have been subdivided into three categories depending on the prevalence of the fluorescence maxima. The samples show differences in their fluorescence spectra. The latter fact shows that fluorescence spectroscopy can be used for the quick identification of edible oils. The fatty acid, the tocopherol, the beta-carotene and chlorophyll contents in the analyzed oils have been studied. It is shown that some of the types of oils differ significantly from each other by the first derivatives of their fluorescence spectra. There also exist color differences between the groups of vegetable oils under study.

Keywords: Vegetable oils, fluorescence spectroscopy, excitation-emission matrices, fatty acid composition, colorimetric parameter, optical properties.

1. Introduction

There is a growing interest in the study of the chemical composition of vegetable oils since knowledge of oil allows the assessment of the quality of the products on the market. It is known that tocopherols and carotenoids act on the oxidation stability of oils, while chlorophyll is responsible for the photooxidation [1]. The quantitative real time evaluation of vegetable oils is most often done by an *in situ* analysis using a variety of chromatographic techniques requiring time, expensive consumables and chemicals. The alternative of these methods are the spectroscopic methods which are low-cost and time saving. Recently, fluorescence has been used for the analysis of fish oil [2] and to

evaluate fried vegetable oils [3]. There are a number of works that investigate the contents of vegetable oils using fluorescence spectroscopy [4-6]. In the cited papers the samples were excited using a 950 W xenon lamp in combination with a monochromator to select a particular wavelength. Unlike most of the works related to fluorescence spectroscopy of vegetable oils, excitation sources used in this work are low-cost laser diode (LD) and light-emitting diodes (LEDs) emitting from 370 nm to 450 nm.

The objective of the present paper is to establish a connection between the optical properties of vegetable oils and their physic-chemical characteristics, as well as to test to what a degree fluorescence spectroscopy can be used as a fast method for the identification of vegetable oils without the use of additional chemical reagents.

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2. Materials and Methods

2.1. Materials

Samples of commercially available vegetable oils such as sunflower, flax, sesame, pumpkin, soybean (Bulgaria), corn (Turkey), walnut, olive oil (Greece), hazelnut oil (Italy), were purchasable from supermarket.

All oils have been purchased from local market and kept for several days at 16 °C in the dark. The measurement is performed immediately after opening the bottles to avoid oxidation of the sample.

2.2 Used Equipment

The basic scheme for the observation and measurement of fluorescence signals is shown in the Fig. 1. Since fluorescence is often very weak and is in all directions, then fluorescence signals are measured in a direction orthogonal to the excitation shown in the figure with a blue arrow.

The particular experimental set-up used in our experiments is shown in Fig. 2. Fluorescence and scattering spectra are measured using fiber-optic spectrometer AvaSpec 2048(Avantes), the Netherlands with a spectral sensitivity within the 250-1,100 nm range. The resolution of the spectrometer $\mu\lambda$ is about 8 nm for a 200 μm input slit. An optical fiber with a diameter of 200 μm is used to bring light to the probe and to measure the scattered and fluorescent light. A collimator with a lens of an aperture $D = 5$ mm is used to gather more light and send it to the receiver. To block stray light, the cuvette holder is supplied with a cap.

In the present study, the excitation-emission matrices are obtained not by the use of a Xenon lamp and a monochromator, but by the use of the above LEDs and laser diode. The advantage of the use of LEDs is the simplicity and cost effectiveness of the measurement set-up. The use of a fiber-optic spectrometer allows us to obtain the spectrum in immediate proximity to the sample which eliminates the need to prepare solutions with n-hexane. The data

Fig. 1 Basic set-up for the observation of fluorescence spectra.

from the fiber-optic spectrometer was exported to ASCII code and excitation-emission matrixes were obtained with using Matlab software.

2.3. Methods

Fatty acid composition was determined by gas chromatography (GC) after transmethylation Christie [7]. Fatty acid methyl esters (FAME) were purified by silica gel TLC on 20 × 20 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 m × 0.25 mm (I.D.) capillary InnoWax column (cross-linked PEG, Hewlett Packard GmbH, Austria) and a Flame ionization detector (FID). The column temperature was programmed from 165 °C to 240 °C at 4 °C min⁻¹ and held at 240 °C for 10 min; injector and detector temperatures were 260 °C. Nitrogen was the carrier gas at a flow rate of 0.8 cm³ min⁻¹, 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 [8].

For all samples the transmission spectra within the interval from 400 nm to 750 nm have been measured by means of Lovibond PFX 880 Solar way, Amesbury, UK. The specified colorimetric system has been selected and used because it is more suitable for working with pigments due to the fact that it is simpler to use in practice and gives a very good assessment of the resulting colors obtained by mixing pigments. The samples were poured in 10 mm wide cuvettes. The color

Fig. 2 Experimental set-up for the observation of fluorescence spectra.

parameters (index of lightness L^* , chroma C^* and hue h_{ab}) corresponding to the uniform color space CIELab [9].

Tocopherols in the oils were determined directly by high performance liquid chromatography (HPLC) on a “Merck-Hitachi” (Merck, Darmstadt, Germany) instrument equipped with 250 mm $\times$ 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector “Merck-Hitachi” F 1,000. The operating conditions were as follows: mobile phase of n-hexane:dioxan 96:4 (by volume), flow rate 1 cm³ min⁻¹, excitation 295 nm, emission 330 nm. A 20 μ L 1% solution of crude sunflower oil were injected. Tocopherols were identified by comparing the retention times with those of authentic individual tocopherols. The tocopherol content was calculated on the basis of tocopherol peak areas in the sample vs. tocopherol peak area of standard α -tocopherol solution as shown in reference [10].

Lovibond PFX 880 has been used for determining the β carotene and chlorophyll content in the investigated oils. The device features a special program through which from the readings obtained from the RYBN color scale, designed for determining the color characteristics of transparent products, the β carotene and chlorophyll contents in the product are determined.

3. Results and Discussion

The fluorescence spectra of vegetable oils were obtained by excitation with two types of sources: LD and LEDs. There are two spectral regions in which

fluorescence spectra of vegetable oils are observed:

The first is the interval 400-600 nm with a maximum at 494 nm;

The second is in the interval 650-750 nm with a maximum at 678 nm.

We can subdivide the oils under study into three groups depending on which of the two basic and one intermediate regions the fluorescence spectra are found in. We denote by $I_0(\lambda_i)$ the maximum value of the intensity of scattered light of the excitation radiation, while with I' (maximum at 494 nm) and I'' (maximum at 678 nm), we denote the maxima of the fluorescence spectra of the first and the second regions.

Group I: A strong transformation of the excitation light into fluorescence of the first spectral region, so that $I' \geq I_0(\lambda_i)$ for the lower wavelengths. A typical representative of this group is sunflower oil as well as corn and soybean oil. Similar results for virgin olive oil are obtained in the research [11] and according to authors fluorescence maximums are due to oleic acid. According to Poulli [12], the virgin olive oil is constituted of low levels of saturated fatty acids (16%) and high levels of unsaturated fatty acids, mainly oleic acid (64%) in contrast to seedoil which has high levels of polyunsaturated fatty acids, mainly linoleic acid. For this group, the fluorescence peak of the second spectral region is very weak, i.e., $I''/I_0 \ll 1$.

The results presented for the oil samples under study are obtained as an average value of five samples of data from fluorescence spectra, color characteristics, and fatty-acid contents. For most of the vegetable oils, the

Fig. 3 Fluorescence spectra for corn oil with a different botanic origin.

data obtained is from samples of common origin (country) but from different producers. However, corn and olive oil are from different countries. The individual fluorescence spectra of the oils in the study are not presented because they do not differ significantly. Fig. 3 shows the fluorescence spectra of corn oil samples (originating from Turkey and Italy) obtained for sample excitation at $\lambda = 450$ nm.

Color and fatty-acid content of the samples under study are presented in Table 1.

The difference in colors does not lead to a change in the shape of the excitation-emission matrices, but may only cause a difference in the fluorescence peak intensity. This is explained by the differential absorption of the samples, which differ by color. Fig. 4 presents the transmission spectra of the oils under study.

Group II: This is an intermediate group and is characterized by the observation that the scattered and the fluorescence light of both regions are comparable. Typical representatives are sesame and hazelnut oil samples. For this group, the fluorescence peak of the second region is very weak, i.e., $I''/I_0 \geq 1$, while $I'/I_0 \leq 1$.

Group III: This group is characterized by a strong fluorescence peak of the second region, caused basically by the presence of chlorophyll. For this region

Table 1 Fatty-acid content and color parameters in corn oil with different origin.

Type of oils	Corn oil (Italy)	Corn oil (Turkey)
L^*	93.32	95.24
a^*	-2.34	-4.89
b^*	7.10	22.5
β -carotene ppm	2.1	5
$C_{16:0}$	0.07	0.09
$C_{16:1}$	-	-
$C_{18:0}$	-	0.01
$C_{18:1}$	0.30	0.26
$C_{18:2}$	0.61	0.60
$C_{18:3}$	0.01	0.03

the ratio are $I''/I_0 > I'/I_0 > 1$. The oils falling into this group are flax, walnut pumpkin and olive oil. The long-wavelength fluorescence band from 660 nm to 700 nm with excitation at 350-420 nm, present in olive oil (very low intensity) and flax oil, is characteristic of the pigment fluorescence of the chlorophyll group, which includes chlorophylls *a* and *b* and pheophytins *a* and *b* [4]. Tocopherols are present in oils in variable amounts depending on the type of oil [1]. Pigments of the chlorophyll group occur mainly in crude oils obtained directly by the extraction of oilseed.

Fig. 4 Transmission spectra of corn oil with different botanic origin.

The fluorescence spectra of the indicated groups of several vegetable oils are shown in Fig. 5 for excitation wavelength $\lambda = 425$ nm, where we obtain the strongest differences.

The fatty acid composition of the samples is presented in Table 2.

Flax oil has the highest content of ω -3 fatty acids, α -linolenic $C_{18:3}$ while the richest in ω -6 fatty acids are the oils from the first group. Olive oil has the highest content of oleic $C_{18:1}$ and the lowest of linoleic $C_{18:2}$ acid. It is difficult to establish the presence of adulterants from the third group in olive oil solely on the basis of fatty acid composition because it, too, has a high content of oleic acid. Therefore in the course of the present study optical methods such as fluorescence spectrometry and colorimetry are used to identify differences in oils apart from the chemical analysis.

Oleic acid shows a fluorescence band at 405 nm where both butyric and linoleic acid show fluorescence bands at 273 and 325 nm, respectively. It is clear that the fluorescence intensity in the region 520-650 nm used for data analysis, originates from oleic acid [11].

A large part of fluorescence in vegetable oils is caused by the presence of chlorophyll groups, β -carotene and tocopherols. The parameters indicated of the samples are shown in Table 3.

The fluorescence peak that reveals the quantity of tocopherols in the samples is not observed because it needs excitation at a lower wavelength in the UV. A relation exists between the oxidizing stability of oils and the content of tocopherols. Walnut and the flax oils are an exception to the rule. They have a high content of tocopherols, but the larger part is α -tocopherols, responsible for the vitamin value, while the content of γ -tocopherols in the tocopherol fraction are related to the oxidizing stability.

The weak fluorescence peak around 672 nm for the sunflower oil is caused by the presence of chlorophyll-*a*. No such peaks were discovered in corn and soybean oil since they do not contain or contain very little chlorophyll-*a*. Walnut and olive oil are the richest in chlorophyll due to what they exhibit the highest intensity of fluorescence in the 675-678 nm. Sesame, hazelnut and flax oils exhibit the same peak, but due to the small quantity of chlorophyll and the peak vary between 1,200 and 1,700 relative units. Fluorescence peaks around 520-560 nm are caused by the presence of β -carotene. Walnut oil is the richest in β -carotene and it exhibits the highest fluorescence peak at $\lambda = 450$ nm. Hazelnut and flax oils exhibit weaker fluorescence peaks because they have three times less β -carotene. The absence of a clear peak in pumpkin oil

Fig. 5 Fluorescence spectroscopy of vegetable oils.

Table 2 Fatty acid composition of the vegetables oil samples.

Type of oils	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Sunflower	0.066	0.004	0.01	0.304	0.61	0.006
Soybean	0.094	-	0.042	0.226	0.574	0.006
Corn	0.08	-	-	0.28	0.64	-
Olive	0.193	0.009	0.04	0.694	0.052	0.002
Walnut	0.142	0.006	0.04	0.334	0.389	0.062
Flax	0.137	0.004	0.007	0.29	0.16	0.34
Pumpkin	0.182	-	0.07	0.245	0.358	0.1
Sesame seed	0.12	0.007	0.03	0.685	0.156	-
Hazelnut	0.094	0.004	0.018	0.57	0.30	0.006

is related to the strong absorption in this region. For a comparison, pumpkin oil transmits between 4% and 7% in the 520-560 nm range versus 59% to 78% for the walnut oil. The first derivatives of fluorescence spectra with excitation at $\lambda = 425$ nm are shown in Figs. 6a-f.

The transmission spectra in the indicated visible range are shown in Fig. 7.

Table 3 Content of chlorophyll *a*, β -carotene and tocopherols in vegetable oils.

Type of oils	Chlorophyll <i>a</i> , (ppm)	β -Carotene (ppm)	Tocopherols (mg/%)
Sunflower	0.045	2.29	79
Soybean	0.019	2.64	218
Corn	0	4.80	106
Olive	33.60	6.092	-
Walnut	1.701	33.69	350
Flax	1.138	12.47	214
Pumpkin	0	58.76	-
Sesame seed	0.022	7.89	222
Hazelnut	0.014	22.32	292

Transmission is the strongest with corn, sesame and sunflower oils. Corn and sunflower oils do not exhibit a clear absorption band, while sesame exhibits one in the 400-500 nm range. For olive and pumpkin oil, a clear absorption band is observed in the 650 nm to 700 nm

Fig. 6 First derivatives of fluorescence spectra with 425 nm excitation.

range, and it is higher for olive oil because of the higher contents of chlorophyll-*a*. A similar absorption band exists for flax oil, but it is weaker because of the lower content of chlorophyll-*a*.

These results and dependencies are simplified estimations of what the analysis excitation-emission spectra can provide. Excitation-emission spectra are

three-dimensional representation of fluorescence emission spectra for different values of the excitation wavelength, which for the three chosen groups of oils are shown in Fig. 8.

The comparison of these three-dimensional excitation-emission spectra, which define the corresponding excitation-emission matrix, shows that

Fig. 7 Transmission spectra in the visible range.

Fig. 8 Excitation-emission matrices for vegetable oils.

they are highly individual and discernable, which means that use of appropriate mathematical methods described in the literature can help to efficiently discriminate the different types of oils.

We have tried to establish color differences with the intention of discerning the oils in cases of cheap adulterants. For the purpose the luminosity L^* and color parameters (a^* , b^*) for each of the samples, the color differences ΔE with olive oils. The data is presented in Table 4.

The smallest color differences are between walnut and hazelnut oils, which mean that they can not be discerned by color if present as adulterations in olive oil. Comparatively greater color differences exist between olive oil on the one hand and soybean, corn and sunflower oils on the other which means that colorimetric analysis can be helpful to discern the presence of these oils as adulterants in olive oil. However, the method is not capable of a quantitative determination of such adulterants.

Fig. 9 shows the fluorescence spectra of four extra virgin olive oils from Greece, Turkey and Spain. Their fatty-acid contents are listed in Table 5.

Olive oils are characterized by different colors, hence it is important to compare their color characteristics which for the four samples we present in Table 6 shown the average (AVG), the standard deviation (S) value, as well as the relative variation

Table 4 Color parameters for vegetable oils.

Type of oils	L^*	a^*	b^*	ΔE
Sunflower	86.28	-1.95	7.15	73.03
Soybean	93.66	-2.83	9.12	70.44
Corn	94.48	-5.19	15.84	63.44
Walnut	86.43	-8.3	78.71	10.12
Flax	71.92	-6.47	105.85	36.33
Pumpkin	27.41	16.49	43.78	54.22
Sesame seed	94.43	-6.35	26.91	53.21
Hazelnut	96.8	-7.9	76.5	6.66
Olive	94.87	-13.89	78.68	-

S/AVG in percent. We see that the largest differences are in the brightness L^* , and in the yellow-blue axis b^* parameters, the rest being less than 10%.

Fig. 10 below shows the excitation emission matrices of olive oil samples 1 and 4 (Fig. 10a and b) on one hand, and two other olive oil samples adulterated with sunflower oil, on the other. The graphs of the former are quite similar, while the latter show definite differences. Mathematical procedures to analyze the matrices will be discussed elsewhere.

4. Conclusions

The results obtained allow us to formulate the following conclusions.

The fluorescence spectra of edible vegetable oils in the visible range provide a basis for grouping of vegetable oils in three basic groups depending on the fact, which of the fluorescence peak dominates.

Fig. 9 Fluorescence spectra of four extra virgin olive oils from as follows: 1-Greece, 2-Turkey and 3, 4-Spain.

Table 5 Fatty-acid contents for olive oils from different origin.

Fatty acid (%)	1	2	3	4
C _{14:0} Myristic acid	-	-	-	0.001
C _{16:0} Palmitic acid	0.193	0.139	0.148	0.158
C _{16:1} Palmitoleic acid	0.007	0.001	0.001	0.01
C _{18:0} Stearic acid	0.036	0.024	0.019	0.015
C _{18:1} Oleic acid	0.684	0.787	0.763	0.764
C _{18:2} Linoleic acid	0.052	0.042	0.047	0.036
C _{18:3} Linolenic acid	0.002	0.002	0.002	0.002

Table 6 Color parameters of the four samples of extra virgin olive oil.

Type	x	y	X	Y	Z	L*	a*	b*
1	0.3627	0.4802	58.66	65.93	12.77	84.96	-13.89	78.68
2	0.4298	0.4839	65.78	74.14	13.23	88.92	-14.5	84.51
3	0.4574	0.5063	58.77	65.22	4.64	84.51	-11.55	105.17
4	0.4388	0.4913	61.45	68.83	9.79	86.39	-13.38	89.34
AVG	0.42	0.49	61.17	68.53	10.11	86.20	-13.33	89.43
StDev	0.04	0.01	3.34	4.05	3.95	1.99	1.27	11.37
%	10%	2%	5%	6%	39%	2%	10%	13%

Fig. 10 Excitation-emission matrices: of olive oil samples 1 (a) and 4 (b), and two olive oil samples adulterated with sunflower oil c) and d).

Excitation-emission fluorescence spectra are very individual and can be used to identify the type of oil.

The above excitation-emission spectra can be obtained using low-cost UV and visible LEDs.

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