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Characterization of extra virgin olive oils adulterated with sunflower oil using different physical methods

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This paper describes a study of the usefulness of some physical methods in the detection of adulteration of extra virgin olive oil with controlled concentration of relatively cheap sunflower oil. We have tested three physical methods measuring refractive indices (RI) and their dispersion curves, fluorescence spectra and color parameters that are related to the chemical structure and content of the olive oils. The RI values of the samples were measured with a total experimental uncertainty of less than 3×10^{-4} by the method of the disappearing diffraction pattern for two wavelengths – 405 nm and 532 nm at a temperature 23ºC. Fluorescence spectra were measured using a fiber optic spectrometer (AvaSpec-2038, Avantes) and the samples were excited by light emitting diodes at 370 nm, 395 nm, 425 nm and 450 nm using the set up. The spectrometer's sensitivity is in the (200 – 1100) nm range with a resolution of about 8 nm. The color parameters (index of lightness L^*, a^*, b^*, chroma C^* and hue angle h_ab) corresponding to the uniform color space CIELab, were determined on a Lovibond PFX 880. The color parameters were used for determining the β – carotene and chlorophyll content in the investigated samples. All of the obtained experimental results suggest that the three optical methods presented are correlated and could be useful for a fast detection of sunflower adulteration of extra virgin olive oils. These techniques are sensitive and rapid and also do not require any additional chemical agents.

Keywords: *Refractive index, fluorescence, extra virgin olive oil, sunflower oil, chlorophyll, β – carotene.*

INTRODUCTION

Olive oil is a valuable food product as compared with other vegetable oil products. As a result, the adulteration of olive oil with cheaper vegetable oil becomes a real concern. For this reason, the analysis of edible oils for possible adulterants is very important for food safety and protection of consumers. Various physical and chemical tests have been used to establish the authenticity of olive oil and to detect the level of adulterants in it [1-2]. The most useful are chromatographic methods [4-6]. They offer high sensibility and accuracy, but are also time consuming and expensive. For this reason in our study we suggest three fast and cheap optical methods – refractometry, fluorescence and color measuring - that can be used for determining the adulterants in olive oils. The aim of this paper is the investigation of the extra virgin olive oil with controlled sunflower oil content by proposed three optical methods.

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EXPERIMENTAL

Samples

In this work we have investigated three samples with different characteristics. Samples of extra virgin olive oil (EVOO) were provided by Greece Company and its botanical origin and quality were guaranteed by the supplier. Samples of sunflower oil (SFO) were obtained from local Bulgarian superstore. Binary samples of 50 % EVOO and 50 % SFO were prepared using a KERN ABJ 80 - 4M analytical balance (precision 0.1 mg). All the samples were stored in fully filled and closed glass vessels and kept in the dark in an incubator at a fixed temperature of 25 °C.

Refractive index measurements

The refractive indices (RI) for all the samples were measured by the method of the disappearing diffraction pattern using a laser refractometer at wavelengths of 405 nm and 532 nm. The method and the laboratory device were reported earlier [7]. The several microliters of the samples were put between a reflecting grating and a glass prism with $RI = N$. When the light beam from one of the lasers falls at an angle smaller than the critical angle α_C the beam penetrates through the sample, reaches the reflecting grating and creates a diffraction pattern. In the case when the incidence angle is equal to or higher than the critical angle α_C only one total reflected beam is observed. Therefore, measuring the critical angle α_C as the angle of disappearance of the diffraction pattern we could calculate the RI by the following formula:

$$n = N\sin\left[A \pm \arcsin\left(\frac{\sin\alpha_C}{N}\right)\right] \qquad (1)$$

where *A* and *N* were the prism refractive angle and the refractive index, respectively. In our experiment A = 65°C, N (405 *nm*) = 1.7880, N (532 *nm*) = 1.7480. The experimental uncertainty was Δn = ±0.0003.

Fluorescence spectra measurements

The sources used to measure the fluorescence spectra are 370 nm, 395 nm, 425 nm light emitting diodes (LEDs). A fiber optic spectrometer (AvaSpec-2038, Avantes) with sensitivity in the (200-1100) nm range and a resolution of about 8 nm was used to measure the fluorescence spectra. The oil samples were placed in a cuvette 10 mm x 10 mm and irradiated by LEDs.

Color measuring

Using a Lovibond PFX 880 (UK) colorimeter and a cuvette of a 10 mm length (Recommendations on uniform color spaces, 1971), the color parameters in CIELab colorimetric system have been obtained. All measurements have been carried out at room temperature immediately after opening the oil bottle. , Color coordinates, color parameters *a, b* and brightness *L* of tested samples have been measured. The chlorophyll and β – carotene are calculated by using the transmission spectra in the visible region and values for color parameters by software program developed specially for Lovibond PFX 880 from the producer. Parameters such as chroma $\left(C^*_{ab}\right)$ and hue h_{ab} were defined by Eqns (2) and (3):

$$C^*_{ab} = \sqrt{\left(a^*\right)^2 + \left(b^*\right)^2} \qquad (2)$$

$$h_{ab} = \arctan\left(\frac{b^*}{a^*}\right) \qquad (3)$$

RESULTS AND DISCUSSION

The fluorescence spectra in the visible region of the investigated samples are obtained for excitation wavelength respectively λ_e = 370 nm, 395 nm, 425 nm and 450 nm. The best ration for fluorescence emission vs. excitation intensity is found at λ_e = 395 nm (Figure 1).

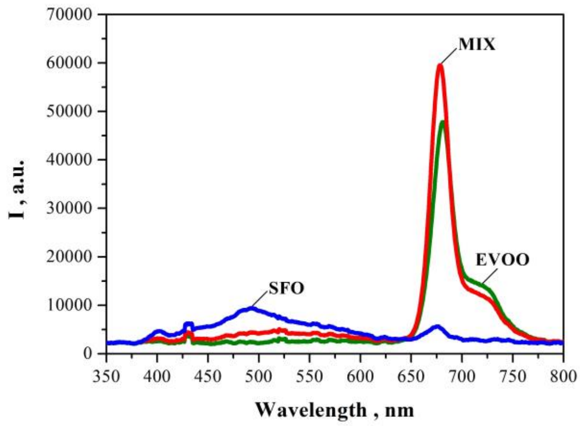

Fig. 1. Fluorescence spectra of the olive oil samples.

There are four fluorescence peaks for investigated samples related to β – carotene at λ = 430 nm; oxidation products at about λ= (500-520) nm; chlorophyll at λ = (675-678) nm; non-determined pigments at λ = 700 nm.

Color parameters for investigated samples are measured in CIELab colorimetric system. The results are present in Table 1.

Table 1. Color parameters for olive oil and its double mixture with sunflower oil.

Samples	SFO	MIX	EVOO
Olive oil concentration, %	**0**	**50**	**100**
L	92.51	90	81.1
a	-1.73	-12.1	-11.7
b	6.93	55.6	81.1
C_{ab}	7.14	56.9	81.9
ΔE	75.68	27	0
h_{ab}	-76	-77.8	-81.8
β–carotene, ppm	2	18.4	34.7
Chlorophyll, ppm	0.011	20.7	5.18

The brightness *L* decreases with increasing of concentration of extra virgin olive oil and content of β – carotene and chlorophyll increases. The influence of green component increases when the content of olive oil is raised. The linear dependence exists between values of *b** and concentration of olive oil. The greatest color difference is between pure olive oil and sunflower oil.

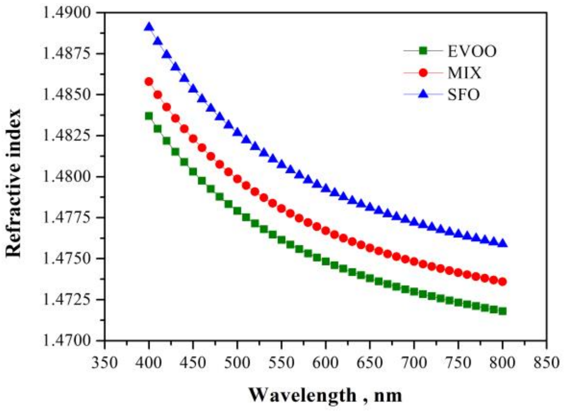

Fig. 2. RI dispersion curves of the olive oil samples.

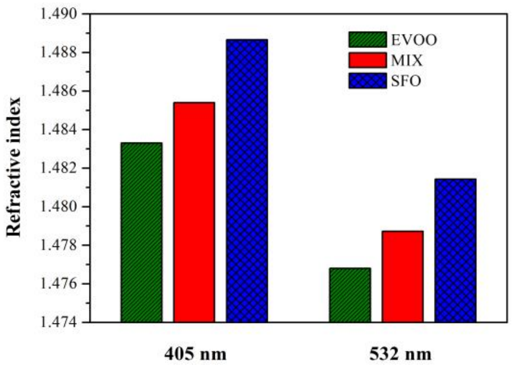

Fig. 3. RI of the olive oil samples for two wavelengths.

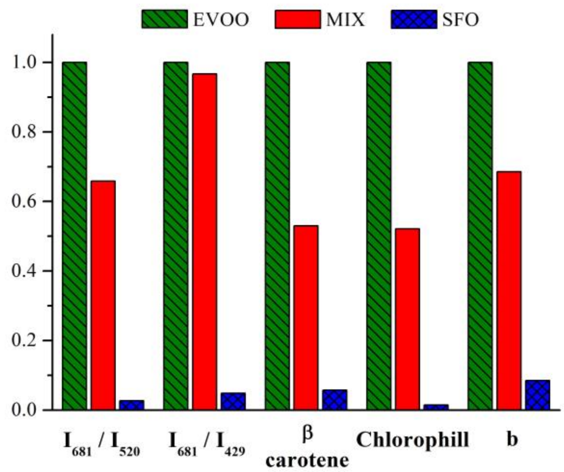

Fig. 4. Normalized experimental data for the olive oil samples.

The data obtained from the two wavelengths RI measurements were used for the construction of dispersion curves using the one-term Sellmeier equation far from the fundamental absorption band[8]. By the one-oscillator Sellmeier's model we can determine the Sellmeier's coefficients s and λ_s from the systems:

$$n_{s_i}^2 - 1 = \frac{s\lambda_i^2}{\lambda_i^2 - \lambda_s^2}, \qquad i = 1,2 \qquad (4)$$

Then we can calculate the RI dispersion curves in the visible spectral range for all samples using the already determined coefficients. Figure 2 presents the obtained dependences for RI.

The refractive indices of the EVOO measured at room temperature are in very good agreement with the results announced in work[9]. The comparison of dispersion curves shows that the RI is the lowest for the EVOO and the RI value increases in direct proportional to adding of SFO as it is shown in Figure 3 for two wavelengths – 405 nm and 532 nm. It is shown that the reduction of the RI is associated with a greater amount of EVOO.

In Figure 4 the normalized changes of the fluorescence peak, the normalized content of chlorophyll and β – carotene and the normalized color characteristics with the increase of the SFO content are presented. In Figure 4 each parameter value is normalized by using the EVOO value as reference.

The increase of the two fluorescence peaks (I_{681}/I_{520} and I_{681}/I_{429}) and the color parameter b associated with a greater amount of EVOO could be related to the increase of chlorophyll and β – carotene too.

In view of the results of the RI and the chemical analysis presented in Figure 3 and Figure 4 the RI reduction could be related to the increase of the chlorophyll content and of β – carotene content.

In future investigations the authors plan to study in more detail the relation between fatty acid content of the samples and the intensity of the fluorescence peaks as well as the relation between chlorophyll and β – carotene contents and RI.

Hence, three independent optical methods – measuring of refractive indices, fluorescence spectra, and color parameters, can be used for identify a pure EVOO and determining the presence of SFO adulterant. The results obtained demonstrate that the measured physical parameters are related to chemical structure and content of the olive oils. So, these methods are useful of the detection of adulteration of extra virgin olive oil with cheap sunflower oil.

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ХАРАКТЕРИЗИРАНЕ НА СТУДЕНО ПРЕСОВАН ЗЕХТИН, ФАЛШИФИЦИРАН С ОЛИО, ЧРЕЗ РАЗЛИЧНИ ФИЗИЧНИ МЕТОДИ

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(Резюме)

В тази статия се изследва полезността на някои физични методи за откриването на фалшификация на студено пресован зехтин с контролирана концентрация на сравнително евтино слънчогледово масло. Тествани са три физични метода за измерване съответно на показателите на пречупване (ПП) и получаване на дисперсионните криви; на флуоресцентните спектри и на цветовите характеристики, които са свързани с химичната структура и съдържанието на зехтините. Стойностите на ПП на образците са измерени с експериминтална неопределеност по-малка от 3×10^{-4} по метода на изчезващата дифракционна картина за две дължини на вълната – 405 nm и 532 nm при температура 23°C.. Флуорисцентните спектри са измерени с влакнестооптичен спектрометър AvaSpec-2038, Avantes, като образците бяха възбуждани със светодиоди, излъчващи съответно на 370 nm, 395 nm, 425 nm и 450 nm. Чуствителността на спектрометъра в областта (200 – 1100) nm е около 8 nm. Изучени бяха цветовите характеристики (L^*, a^*, b^*,C^* и h_{ab}) на олиото в SIELab колориметрична система чрез Lovibond PFX 880. Цветови характеристики бяха използвани за определяне на β - каротин и на съдържанието на хлорофил в изследваните проби. Всички получени експериментални резултати показваха, че трите представени оптически метода са взаимосвързани и биха могли да бъдат полезни за бързо откриване на фалшификация на студено пресован зехтин със слънчогледово олио. Тези техники са бързи и чувствителни, а освен това не изискват използването на никакви допълнителни химически агенти.