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# Detection of adulteration in olive oils using optical and thermal methods

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Quantification of olive oils is an important issue because of frequent adulteration with cheaper oils such as soybean, sunflower or canola. The standard chemical methods used to determine the chemical content of the oils are usually time-consuming, laboratory intensive and expensive. Therefore, we have tested three physical methods measuring refractive indices and their dispersion curves, fluorescence spectra and DSC spectra that are related to the chemical structure and content of the olive oils. These methods are fast, easy to perform and do not require any additional chemical agents. Two groups of olive oils – pure and adulterated, were investigated.

**Keywords:** refractive index, fluorescence, DSC, olive oils, adulteration

## INTRODUCTION

Olive oil is one of the best vegetable oil used in nutrition, medical treatment and cosmetics. The accumulated clinical data prove the beneficial effect of olive oil as a nutrient reducing the risk of heart diseases, skin diseases, breast, prostate and gastrointestinal cancer formation, etc. and is included as an ingredient in a number of pharmaceutical and cosmetic mixtures. Due to its higher price virgin olive oil is often adulterated with cheaper vegetable oils. Olive oil contains a lower level of saturated and higher level of non-saturated fatty acids [1], basically oleic acid (64%), while vegetable oils have a higher level of linoleic acid [2]. Additions of vegetable oils in extra virgin olive oil are usually detected using gas or liquid chromatographic methods [3-4]. The latter methods are expensive, using numerous chemical agents, demanding lengthy analysis and qualified personnel which poses the need for other fast, cheaper and real time methods. An alternative to chromatographic methods are spectroscopic methods which are fast, cheap but with lower sensitivity [5]. Infrared spectroscopy also needs preliminary preparation of sample [6-7]. On the other hand, refractive index and the FTIR spectra of virgin coconut and virgin olive oil were successfully used to determine purity of olive oils [8] and adulteration with sunflower or corn oils [9]. The analysis performed in [10] showed that the refractive index of the olive oils treated by the different enzymes slightly changes.

In recent years fluorescence spectroscopy has been intensively used to study the origin and contents of olive and vegetable oils [11-12] as well as the detection of soy and sunflower oils in olive oil [13].

The objectives of the present work are: to study the capabilities of a group of physical methods such as fluorescence spectroscopy in the visible spectral range, differential scanning calorimetry (DSC) and laser refractometry to discern extra virgin from adulterated olive oil without the use of chemical agents; to propose improved non-traditional fluorescence and refractometric methods for olive oil analysis using low-cost light emitting (LED) and laser diodes (LD).

## MATERIALS AND METHODS

Three types of commercially available extra virgin olive oils (EVOO) from Italy, Spain and Greece were chosen for analysis and their characteristics were compared with three different salad mixtures of olive oil (SMOO) with sunflower and soy bean oils. The samples were measured immediately after opening the bottle to avoid accelerated oxidation processes.

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Three methods were used: DSC, refractometry, and fluorescence.

A differential scanning calorimeter SETARAM 141 (France) was used for thermal analysis of the olive oils. The samples were cooled at a rate of 5°C.min$^{-1}$, from room temperature to -40°C and were kept for 3 minutes to crystallize the sample. After this procedure, melting curves were recorded to 75°C at a heating rate of 5°Cmin$^{-1}$. The resulting DSC thermograms were analyzed by SETARAM software, melting temperature and enthalpy were determined. Melting temperature was considered as the temperature of the extreme.

The refractive index (RI) values of the samples were measured with a total experimental uncertainty of less than $2\times10^{-4}$ by the method of the disappearing diffraction pattern [14] for three laser wavelengths – 405 nm, 532 nm and 635 nm at a temperature of $t$ = 20°C. The data obtained from the three wavelengths RI measurements were used for the construction of dispersion curves using the one-term Sellmeier equation far from the fundamental absorption band [15]. By the one-oscillator Sellmeier's model used for 3 combinations of RI pairs measured at three wavelengths we can determine the Sellmeier's coefficients from the systems:

$$n_{\mathrm{si}}^2 - 1 = \frac{s.\lambda_{\mathrm{i}}^2}{\lambda_{\mathrm{i}}^2 - \lambda_{\mathrm{s}}^2}, \quad \mathrm{i}=1,2; \mathrm{i}=1,3; \mathrm{i}=2,3 \qquad (1)$$

Then we can calculate the average Sellmeier's coefficients $s$ and $\lambda_s$ for all the samples in order to obtain more accurate dispersion dependences for the studied liquids.

Fluorescence spectra were measured using a fiber optic spectrometer (AvaSpec-2038, Avantes) and the samples were excited by LEDs at 370 nm, 395 nm, 425 nm and 450 nm using the set-up, shown in Fig. 1. The spectrometer's sensitivity is in the 200 – 1100 nm range with a resolution of about 5 nm. The oil samples were placed in a 10mm x 10mm cuvette and irradiated by LDs or LEDs. Fluorescence spectra were taken from a direction orthogonal to the line of transmission (Fig. 1).

The pigments have been determined without diluting the sample with a cuvette of 1 cm thickness by using the Lovibond PFX 880 instrument (Tintometer Limited, UK). Special software of the equipment has been used to process the data from the red-yellow-blue (RYB) color scale and to calculate the contents of $\beta$-carotene and clorophyll in the samples.

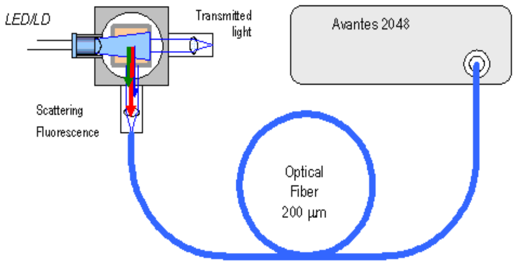


**Fig. 1.** Experimental set-up for the observation of fluorescence spectra.

All analysis were carried out in triplicate and the average data are presented in tables. Differences between means at the 95% ($p < 0.05$) confidence level were considered statistically significant**.**

## RESULTS AND DISCUSSIONS

Fluorescence peaks for olive oils are the highest in the spectral range 671 nm - 675 nm and are associated with the presence of chlorophyll [15]. For all salad mixes this peak is weaker due to the higher contents of vegetable oils which have less chlorophyll. It is known that if chlorophyll is less than 1 ppm, olive oils contain a large quantity of sunflower or soy bean oils. Their presence causes a stronger peak at around 500 - 550 nm, related to the increase of oxidation products caused by the increased content of linoleic acid. The fatty acid content was independently measured by traditional chemical methods and the results were compared. The comparison shows that the adulterated samples contain a lower level of oleic and a higher level of linoleic acid.

The content of β-carotene is rather high, while for salad mixtures it decreases. It is comparable to that of olive oils for only one sample possibly due to artificial vitamin E enrichment (Table 1).

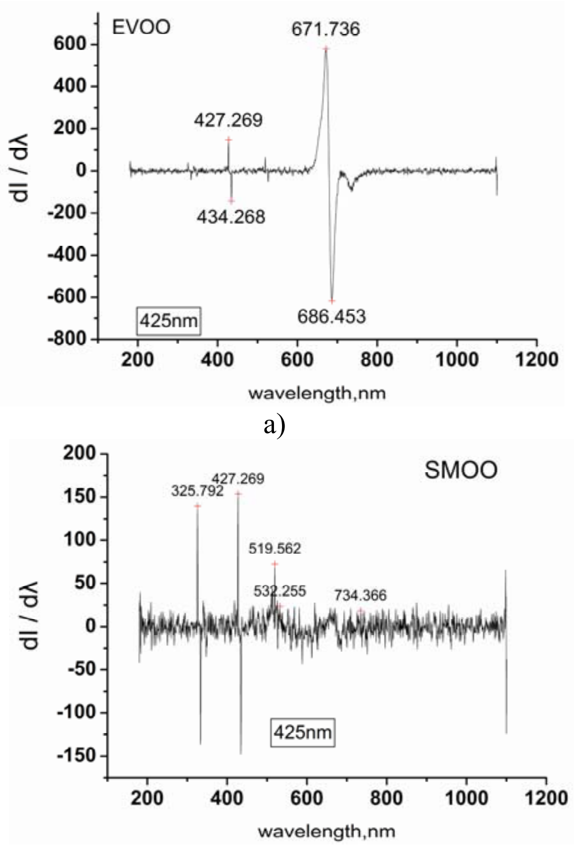
Since fluorescence spectra are similar, to better discern extra virgin olive oils from salad mixtures we calculate the first derivatives of the averaged fluorescence intensities (dI/dλ) results for the three natural olive oils and the three salad mixtures. The dependences of the first derivatives of fluorescence intensity on the wavelength for two types of olive oils are presented in Fig. 2.While the fluorescence peak in the first derivative caused by chlorophyll is clearly observable for the EVOO, for SMOO we observe mostly noise with weak peaks in the range from 430 nm to 550 nm due to oxidation products in the vegetable oils.

**Table 1.** Data on the pigments and Sellmeier's coefficients in olive oils.

| Type | B-carotene, ppm | Chlorophyll,ppm | $s$ | $\lambda_s$ |
|---|---|---|---|---|
| EVOO 1 | 48,74 | 4,656 | 1,111 | 112 |
| EVOO 2 | 52,64 | 3,744 | 1,114 | 113 |
| EVOO 3 | 51,21 | 7,152 | 1,111 | 111 |
| SMOO 1 | 3,18 | 0,07 | 1,134 | 106 |
| SMOO 2 | 47,45 | 0,175 | 1,132 | 107 |
| SMOO 3 | 35,67 | 0,155 | 1,133 | 108 |

**Table 2.** Data from DSC analysis of the olive oils samples.

| Type | $T_1$, °C | $T_2$, °C | $T_3$, °C | $T_4$, °C | $T_5$, °C | ΔH, J/g |
|---|---|---|---|---|---|---|
| EVOO 1 | | | | -4.57 | 5.31 | 66.79 |
| EVOO 2 | | | | -4.99 | 5.80 | 80.92 |
| EVOO 3 | | | | -4.12 | 4.67 | 82.56 |
| SMOO 1 | -29.72 | -22.41 | -9.22 | | | 59.41 |
| SMOO 2 | -28.97 | -22.67 | | | | 59.20 |
| SMOO 3 | | -24.16 | -10.47 | | | 89.30 |



a)

**Fig. 2.** First derivatives of the fluorescence spectra of salad and natural olive oil for an excitation wavelength of 425 nm.

Using the Sellmeier's coefficients - $s$ and $\lambda_s$ (Table 1) obtained from the RI data we calculated the dispersion curves of RI for all samples in the spectral range (400-800) nm and plotted them in Fig.3. The refractive indices of the virgin olive oils measured at room temperature are in very good agreement with the results announced in [8]. It was found for extra virgin olive oils that the refractive index and the Sellmeier's coefficient $s$ are obviously lower and the Sellmeier's coefficient $\lambda_s$ are obviously higher than ones for salad olive oils as shown in Fig.3 and Table 1. These changes could be associated with the presence of adulterants in salad olive oils. Similar increase of the extra virgin olive oil refractive index with addition of an adulterant as refined olive oil, refined olive pomace oil, sunflower oil and corn oil was estimated in [9].

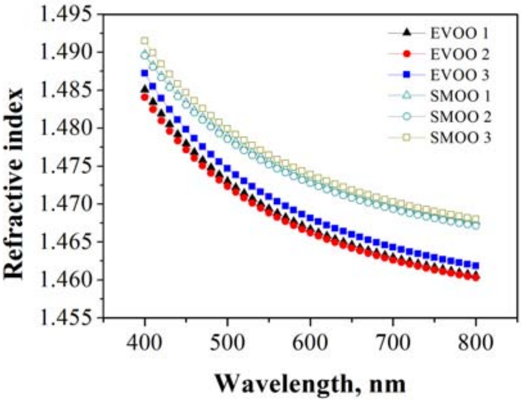


**Fig. 3.** RI dispersion curves of the olive oil samples.

Melting range and DSC curve shape result from combined effects between fatty acid composition, polymorphism and thermal history (Fig. 4). The extra virgin olive oil has characteristics melting range from -20°C to 10°C with 2 melting temperatures of the order of $T_4$=-5°C and $T_5$=5°C. In contrast the salad oil melts in the range of -35°C to 5 °C and there are three endotherm extreme of the order of $T_1$=-29°C, $T_2$=-22 °C and $T_3$=-10°C respectively. All the values for melting temperatures and enthalpies of the samples examined are presented in Table 2.

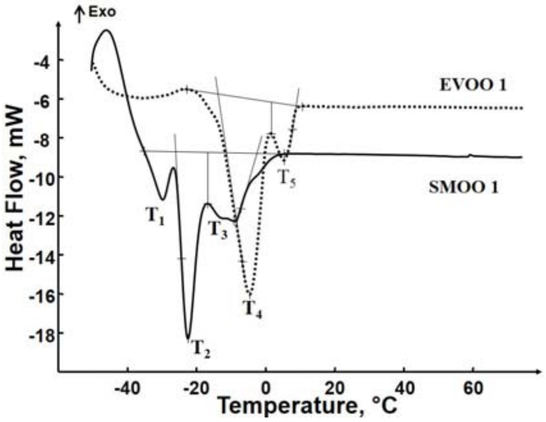


**Fig. 4.** DSC thermograms of the olive oil samples.

The results obtained for the melting temperatures of extra virgin olive oils are in good agreement with previous researches [16-17]. The low temperature melting is due to presence of adulterants – most probably sunflower oil, which has melting temperatures at -29°C and -22°C. The absence of the highest melting temperature could be explained by the decrease of the lipid fraction with higher saturation degree (disaturated triacylglycerides, monosaturated triacylglycerides, palmitic and stearic acid) that is expected to melt in the temperature range of the minor shoulder peak [18]. The shift of the major peak to lower temperature could be associated with the presence of adulterants. It may be hypothesised that the change of the chemical composition of the salad olive oils induced by increasing percentages of sunflower oil may have influenced the formation of different and less stable polymorphic crystals than those of pure oil, which also may melt at lower temperature [18].

## CONCLUSIONS

Comparative analysis shows that all pure olive oils exhibit a higher fluorescence peak at 670 nm while the adulterated have a higher peak in the 490 nm – 540 nm range. Correspondingly the refractive indices of pure olive oils are lower compared to the adulterated. The melting temperatures of the pure samples were of the order of -5°C and +5°C while those of the adulterated ranged in the -10°C to -29°C temperature interval.

These preliminary results suggest that all the presented physical methods are correlated and could be useful for a fast detection of adulteration and discriminating among olive oils of different commercial categories.

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# ОТКРИВАНЕ НА ПРИМЕСИ В ЗЕХТИН ЧРЕЗ ОПТИЧНИ И ТЕРМИЧНИ МЕТОДИ

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## (Резюме)

Качеството на маслиновото масло е важен проблем, защото много често то се примесва с някои евтини растителни мазнини като соево, слънчогледово или рапично олио. Стандартните химически методи, използвани за определяне на химичния състав на маслата са бавни, скъпи и изискват специално оборудване. Затова ние предлагаме три физични метода, измерващи показателя на пречупване и дисперсионните криви, флуоресцентните спектри и DSC спектрите, които са свързани с химичната структура и съдържание на маслиновото масло. Тези методи са бързи, лесни за употреба и не изискват химически реагенти.