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Synchronous fluorescence spectroscopy for analysis of vegetable oils

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Synchronous fluorescence spectroscopy is a well established and proven method for analysis in many disciplines. This research shows the potential of the technique for analysis of vegetable oils. The synchronous fluorescence spectra of two different types of oils - sunflower and extra virgin olive oil, were acquired in the excitation wavelength region 220-800 nm and for wavelength intervals from 0 to 100 nm in 10 nm interval. The study includes the spectra of samples heated for different time intervals - unheated oil, 0 min (oil moved from the oven when just boiled), 5 min boiling and 10 min boiling oil respectively. It's shown that the method gives good results and allows assessment of the quality and constituents of the oils according to the heat treatment applied to samples. Processes such as oxidation may lead to production of potentially toxic compounds for human body. That's way the method is very important and useful.

Key words: synchronous fluorescence spectroscopy, vegetable oils, extra virgin olive oil, sunflower oil, heat treatment, oxidation

INTRODUCTION

Vegetable oils constitute a very large and important group of foods. This is why considerable interest is manifested in the study of their quality and content. The need of rapid method for their quality assessment and detection of deteriorations and adulterations of the products is increasing [1-3]. The reasons are not only of a moral nature. Potentially toxic compounds can be produced during for example a heat treatment of the products or exposure to light - secondary oxidation products [4-7].

Spectroscopic techniques are very widely used and new applications of them are found. They are well established for analysis of multicomponent systems such as oils and allow better differentiation of the compounds of the system than other used techniques. For example NMR spectroscopy [3], FTIR [8,9], nuclear magnetic resonance [10], liquid and gas chromatography techniques [2,6,11-13] are used for oil analysis. Some of these techniques lack sensitivity [9]. Other advantages of the spectroscopic techniques are that there is no need of expensive materials and any pretreatment of the oil samples [6,13]. The standard fluorescence techniques are insufficient for the analysis of vegetable oils because the spectra shows big overlapping of the bands attributed to different constituents [9,13]. That's way synchronous fluorescence spectroscopy is applied for this type of analysis. Furthermore the time for collecting synchronous spectra of one sample is decreased compared with total luminescence spectra [13].

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The technique is based on simultaneously scanning of the excitation and emission wavelength with constant difference between them maintained - wavelength interval $\Delta\lambda$. The idea is more information to be acquired for the complex system by decreasing the overlapping of the spectra and preferentially amplifying strong fluorescence bands by using different wavelength interval [6,9,13,14]. With this method it is possible to distinguish fluorescence bands of tocopherols and phenols for example [15].

Three main groups of fluorophores were observed – vitamin E group, phenols and chlorophylls.

Results are shown by plotting the intensity as combined function of the other two variables – $\Delta\lambda$ and λ_{ex}. We use total synchronous fluorescence spectra (intensity as a function of excitation wavelength) and also contour plots of synchronous spectra ($\Delta\lambda$ as a function of λ_{ex}) where linking points are curves with the same intensity.

Synchronous fluorescence spectroscopy is used for monitoring of other types of foods and nutritional products – brandy [16], milk [17,18], beer [19], energy drinks [20], honey [21] and also petroleum product [22]. The obtained results also give the possibility for monitoring of these products.

METHOD AND MATERIALS

Measurements were made by the Jobin Yvon fluorolog-3 spectrofluorometer. The instrument is fully-computerized and uses a Xenon lamp as a source for excitation. The wavelength range was set at 220-800 nm in excitation and emission. The slits were set at 3 nm and the increment was set at 1 nm for both excitation and emission measurements. For

the needs of the synchronous spectra a constant wavelength difference between the excitation and emission wavelengths was maintained – $\Delta\lambda$. The wavelength offset $\Delta\lambda$ was set at the range 0–100 nm in 10 nm intervals. All of the acquired spectra were corrected for the spectral response of the system - photomultiplier, Xenon lamp and excitation/ emission gratings.

The samples were set in quartz cells - 10 mm optical length was used for the measurements.The spectra were collected for 8 samples separated into two groups according to the oil type – sunflower oil and extra virgin olive oil. Each group includes 4 samples depending on the heat treatment applied – unheated oil, 0 min (oil moved from oven when just boiled), oil boiling for 5 min and oil boiling for 10 min.

Synchronous fluorescence spectra are obtained by plotting the intensity as a function of wavelength interval and excitation wavelength. Contour plots of the total synchronous fluorescence spectra are also shown. The shape and the intensity of the bands of the spectra depend on the wavelength interval $\Delta\lambda$.

The results were constructed using the Origin Software Version 8.0.

RESULTS AND DISCUSSION

In synchronous fluorescence spectra usually one fluorophore is presented in one peak [9,23]. Compared with total luminescence spectra significant amplification of signals of particular bands is gained [9]. This is how simplification is obtained.

Synchronous fluorescence spectra are obtained by plotting the intensity as a function of wavelength interval and excitation wavelength. The shape and the intensity of the bands depend on the used wavelength interval. Contour plots of the total synchronous spectra are also shown.

The results obtained for extra virgin olive oil samples are shown in Fig. 1. The fluorescence bands of unheated sample are concentrated in the wavelength regions 300-400 nm and 550-720 nm. The most intensive peak is observed around 675 nm. Just boiled sample gives signals in the wavelength range 300–720 nm and contours are concentrated in the region 300–400 nm and 600–720 nm. Again the peak with maximum intensity is 675 nm but decreasing in the intensity of the band is spotted. Olive oil boiling for 5 minutes shows results at the wavelength region 320–700 nm with maximum at 320-550 nm range in excitation and another one in the range 600–720 nm. Additional decreasing of the intensity of the peak around 675 nm is observed but also and appearing of new bands in the region 400–450 nm in excitation. The last sample, sample boiling for 10 min, shows fluorescence bands concentrated in the region 300–650 nm with maximum in the range 400–425 nm. The intensity of already observed long wavelength band in the region 600-720 nm is significantly decreased and the sample shows very low fluorescence intensity band around 650 nm. We noticed dramatically changes in the constituent of the extra virgin olive oil during the thermal treatment.

In Fig. 2 are shown the results obtained for sunflower oil. The unheated sample shows fluorescence bands in the region 300–550 nm with maximum around 360 nm and gives another maximum in the wavelength region 650–690 nm – 675 nm in excitation. The signals for just boiled sample are registered in wavelength range 300-550 nm with maximum around 360 nm and in the range 650–690 nm – again at 675 nm in excitation. Compared with the unheated sample we can observe decrease in the fluorescence intensity of the maximum around 350 nm and that one at 675 nm. The other two samples give results in the same wavelength ranges but major reduction of the intensity of the maximums is observed. Furthermore the bands with maximum intensity are shifted to longer wavelengths.

The results can be analyzed and conclusions for the nature and changes in the composition of the oils during the process can be made.

Vegetable oils include three main groups of natural fluorophores – tocopherols, chlorophyls and phenols [13].

The group of the phenolic compounds includes oleic acid, linoleic acid, palmitic acid, vanillic acid, syringic acid, gallic acid, p-coumaric acid, caffeic acid and etc. [9,15]. Olive oils are rich of phenolic compounds but the amount of these fluorophores dramatically decreases during refining [15].

The other big group of fluorescent compounds is vitamin E group which contains α-, β-, γ-, δ-Tocopherol and α-, β-, γ-, δ-Tocotrienol. The most recently present fluorophore from the listed is α-Tocopherol [9]. Tocopherols are the most important antioxidants in the oils [11].

Fluorescence properties of tocopherols and phenols are very similar [15]. The short excitation wavelength range 300–400 nm can be attributed to both tocopherols and phenols. As evidence for that the shape and the form of the spectra are similar to those of pure α-tocopherol as reported by [13,15].

The last mentioned group of natural fluorescence constituents of the oils is those of chlorophylls and pheophytins which includes chlorophyll *a*, chlorophyll *b*, pheophytin *a* and pheophytin *b*. These compounds are responsible for the long wavelength bands with maximum at 675 nm [1,2,4,6,9,13,15]. The fluorescence properties of the pigments are very similar and that's way signals in the wide wavelength range 600–720 nm are detected [13]. There is presence of the chlorophyll group in all studied samples. The observed differences in the intensity of the band are explained with their involving in the oxidation process caused by the heat [5-7].

During the heat treatment is observed significantly decreasing in the chlorophylls amount and appearing of new bands attributed to their oxidative forms and particularly from products formed by the reactions between amino-phospholipids and aldehydes [6,15]. Among the primary oxidation products are hydroperoxides which further degrade to secondary products: aldehydes, alcohols, hydrocarbons and ketons as was mentioned by [6]. It's important to notice that the known products formed during oxidation of vitamin E group are all non-fluorescent [15]. Changing in the content of tocopherols and phenols is also detected.

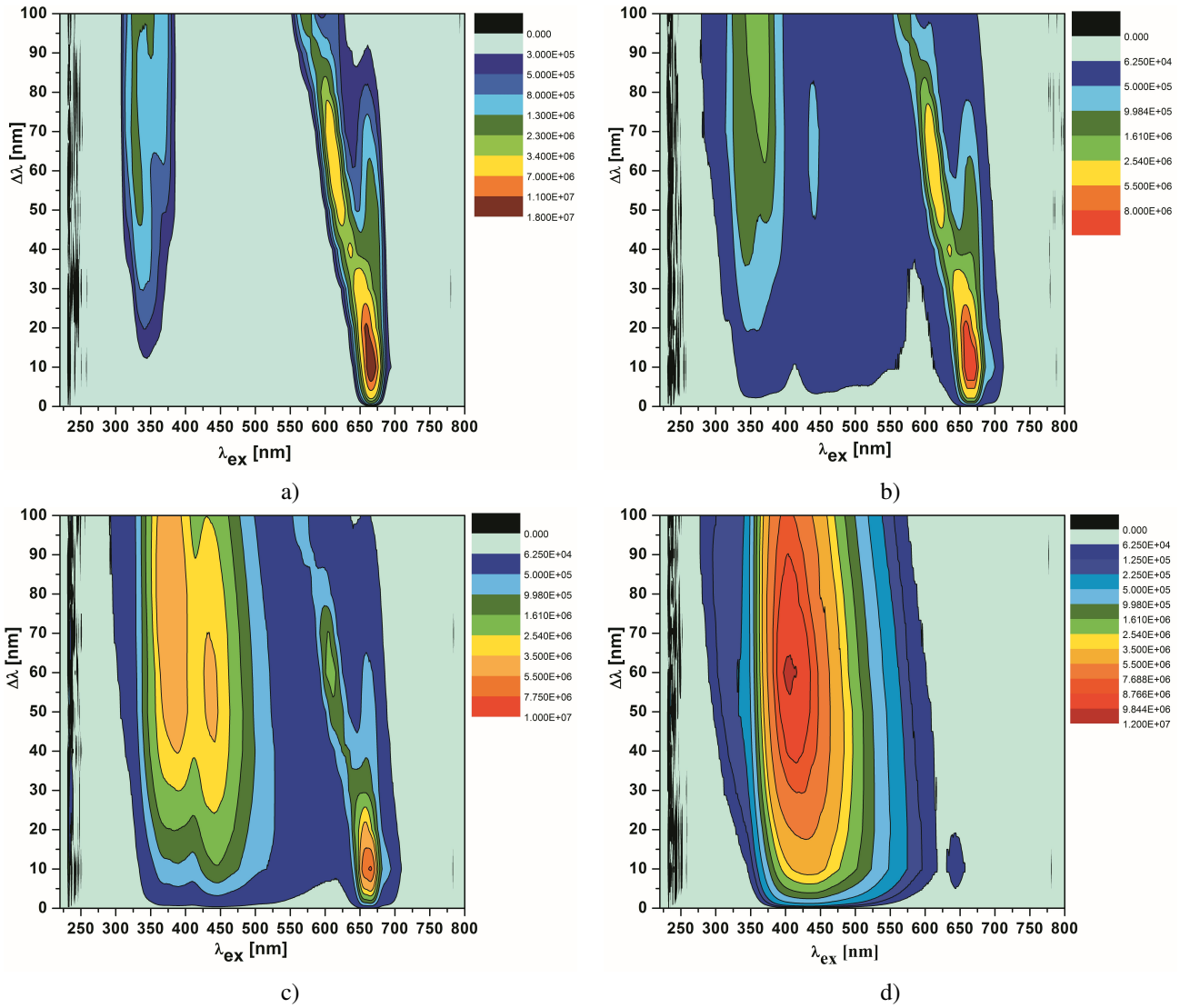

Fig. 1. Contour plots of total synchronous fluorescence spectra of extra virgin olive oil: unheated sample (a), just boiled sample (b), boiling sample for 5 min (c) and boiling sample for 10 min (d).

In Fig. 3 are demonstrate once more the changes in the synchronous spectra of the samples depending on the heat treatment applied. The spectra show that the short wavelength maximum is slowly shifted to longer wavelengths. For extra virgin olive oil the maximum is moved from 350 nm for unheated sample to 450 nm for 10 min boiling sample. The same result is achieved for sunflower oil samples. As it was mentioned the reason is involving of chlorophyls and phenols in the oxidation and formation of oxidative products [7]. The band around 450 nm is ascribed to hydroperoxide producing during the heating process [7].

On the other hand Fig. 3 can be used for comparison of the properties of the two types of oil. The same wavelength interval is applied for the both graphics - 80 nm. Extra virgin olive oils shows splitting of the short wavelength band. This can be used as a fin-

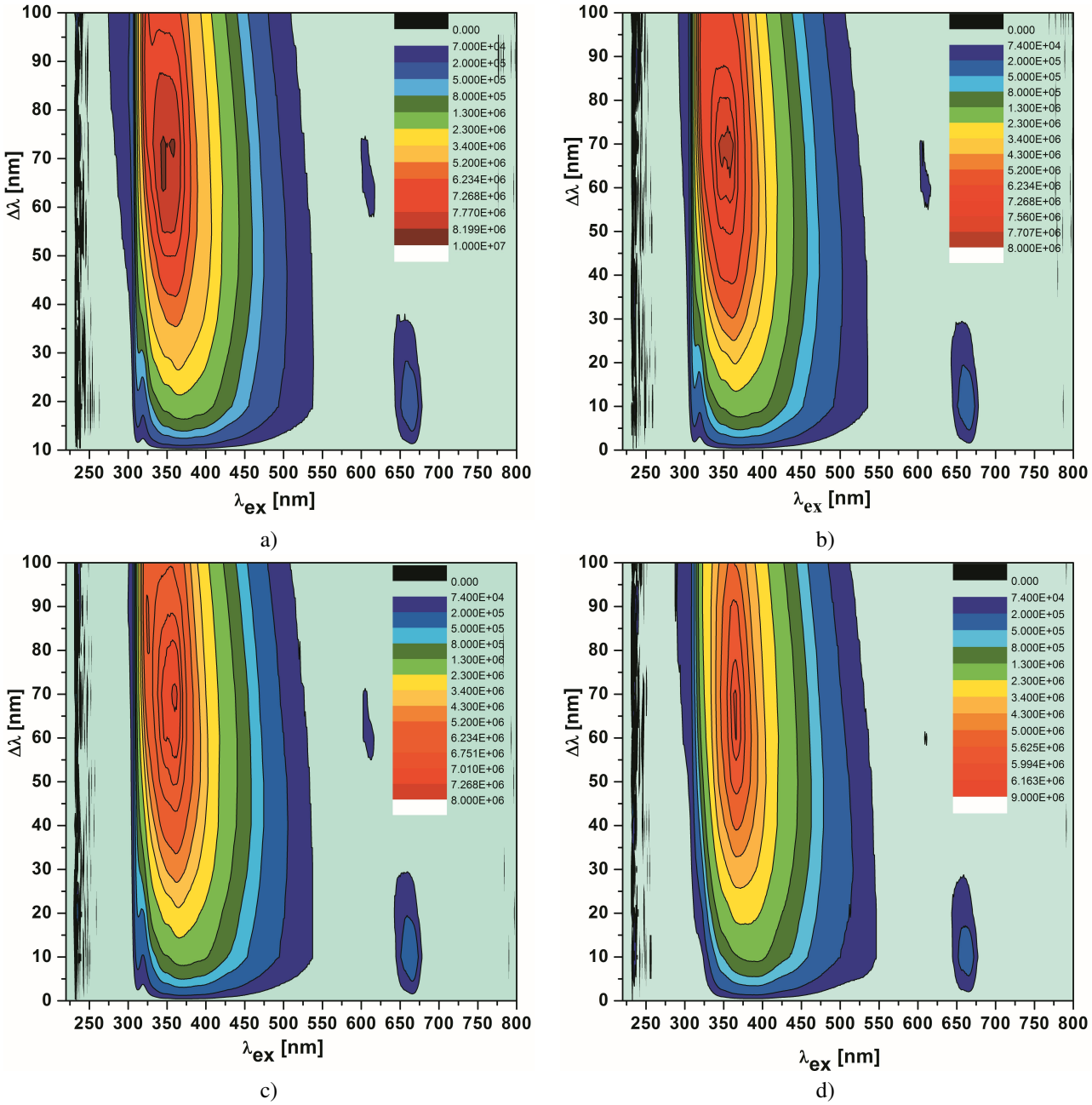

Fig. 2. Contour plots of total synchronous fluorescence spectra of sunflower oil: unheated sample (a), just boiled sample (b), boiling sample for 5 min (c) and boiling sample for 10 min (d).

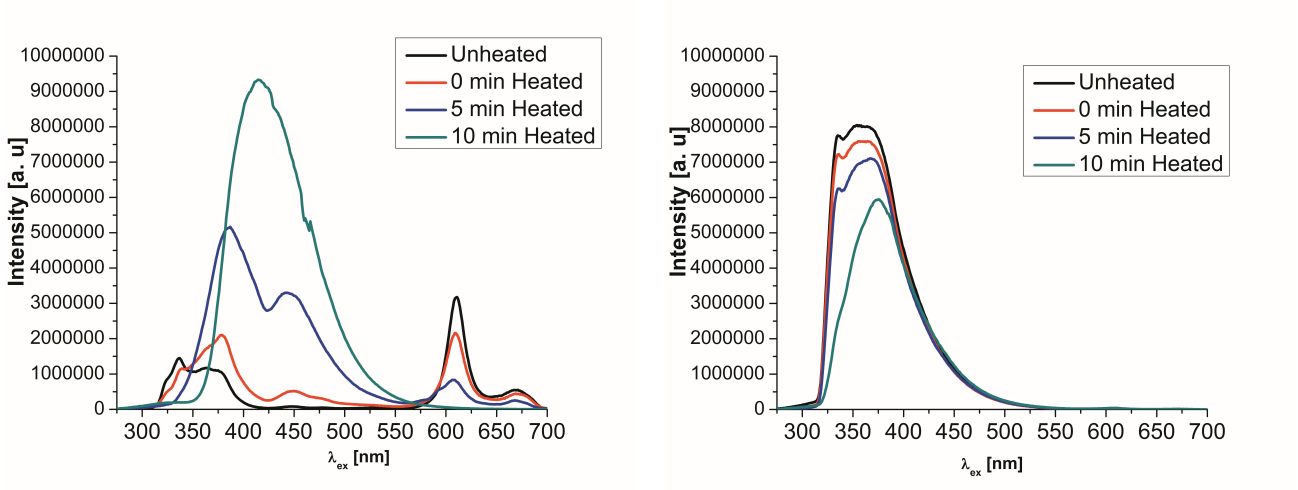

Fig. 3. Synchronous fluorescence spectra of extra virgin olive oil (a) and sunflower oil (b) recorded for wavelength interval $\Delta\lambda$ 80 nm.

gerprint for authentication – refined oils don't give the same splitting [7]. This is possible because oleic acid is presented in extra virgin olive oils in high amounts [1,2]. With increasing of the time of heat treatment the bands are shifted to longer wavelength interval. For sunflower oil only one peak is detected in the short wavelength area attributed to linoleic acid [1,2,9]. Good separation between different types of vegetable oils is achieved with detailed determination of particular bands acquired in the spectra of the products.

The oxidation affects the organoleptic and nutritional properties of the oils. It leads to considerable deterioration of the products such as degradation of their beneficial constituents and consequent producing of toxic compounds [6].

As it was reported by [7] that the oil degradation is controlled not only from the heat but also by the mass transfer which depends on the oil origin.

The same effect, producing of oxidative products, is observed during the storage of oils or exposure to light and the acquired results are similar [4,6,7].

CONCLUSION

Synchronous fluorescence spectroscopy can be successfully applied as a rapid technique in analysis of vegetable oils. Compared with other already used techniques it has advantages in spectra acquisitions, decreasing of the experiment time and lack of prior preparation of the studied samples.

In this research are shown the changes in the constitution of the oil during a heating process. The intensity of the bands attributed to main fluorophores in the vegetable oils - phenols, tocopherols and chlorophylls, is decreasing with increasing of the time of thermal treatment. Producing of potentially toxic compounds because of the degradation of natural antioxidants and formation of secondary oxidation products is detected.

The existence of a threat for the human health makes this issue very important and serious.

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СИНХРОННА ФЛУОРЕСЦЕНТНА СПЕКТРОСКОПИЯ ЗА АНАЛИЗ НА РАСТИТЕЛНИ МАСЛА

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(Резюме)

Синхронизираната флуоресцентна спектроскопия (СФС) е доказан и широко използван метод за изследвания и анализ в много дисциплини. През последните години се наблюдава значително увеличение на приложенията на СФС при анализа на храни и хранителни продукти. Флуоресцентните анализи се използват за изследване на различни течности, като вино, бренди, оцветители, растителни масла, мед и други.

Растителните масла, в това число и зехтините, са една от основните групи хранителни продукти, за които флуоресцентният анализ се прилага успешно. Методът използва наличието на естествени флуорофори като фенолни съединения, токофероли, феофитини и техните оксидирани продукти.

Растителните масла са многокомпонентни системи и затова стандартните флуоресцентни техники, базиращи се на снемането на единичен спектър на възбуждане и на излъчване, са недостатъчни за такива проучвания. В такъв случай се прилагат синхронно-флуоресцентни техники за подобряване на аналитичната способност на този тип изследвания.

Направени са измервания на набор от образци на растителни масла, използвайки спектрофлуориметър FluoroLog3 и възбуждане в областта 200-650 nm. Получени са емисионни спектри в областта 220–850 nm и за $\Delta\lambda$ от 10 до 100 nm със стъпка на синхронно сканиране 10 nm.

Показано е разграничаването на рафинираните от нерафинираните масла, както и възможността за детекция на влошаване на качествата на маслата в процеса на рафиниране. Това проучване представя способността за лесен анализ и оценка на качеството на масла. Получените до момента резултати, показват възможността за анализ на растителни масла чрез бърз и надежден метод, основаващ се на синхронизирана флуоресцентна спектроскопия. Предвидени са допълнителни проучвания, включващи по-голяма база данни и приписване на наблюдаваните флуоресцетни максимуми на химичните компоненти на различните типове растителни масла, както и на странични продукти, получени при оксидация или топлинна обработка на продуктите.

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