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Physico-chemical properties of sunflower oil enriched with ω-3 fatty acids

K. Nikolova[1*], M. Perifanova-Nemska[1], G. Uzunova[1], T. Eftimov[2], G. Antova[2], A. Aladjadjiyan[3], V. Plachkova[2], W. Bock[4]

[1]*University of Food Technologies, Plovdiv, Bulgaria,*
[2]*Faculty of Physics and Faculty of Chemistry, Plovdiv University, Plovdiv, Bulgaria*
[3]*Agricultural University, Plovdiv*
[4]*Université du Québec en Outaouais, Gatineau, Québec, Canada.*

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The physico-chemical properties (fatty acid composition, content of tocopherols, oxidation stability, color parameters etc) of linolic type sunflower oil enriched with ω-3 fatty acids by the addition of flaxseed oil have been investigated. It has been found that for sunflower oil enriched with flaxseed oil the linolic acid content increases from 0.1 % to 8. 11%. Due to the low oxidation stability of linolic oil, the overall oxidation stability decreases for the sample with addition of 20 % flaxseed oil, but this decrease is not essential and the obtained enriched sunflower oil has a good ratio of the ω-3/ω-6 fatty acids. It has been established that the enrichment of sunflower oil with 20% flaxseed oil leads to rise of linolic acid content to 8.11 %. Obtained enriched sunflower oil has good ratio of ω-3/ω-6 fatty acids. Oxidation stability of sunflower oil with a 20% addition of flaxseed oil declined from 10 h to 6h. The addition of flaxseed to sunflower oil does not enrich it essentially with chlorophyll, but causes a significant increase of β-carotene, which is an important component for the human health. Linear regression models between the intensity of fluorescence spectra in the UV range and the general content of tocopherols and β-carotene has been found.

Keywords: sunflower oil, flax oil, ω-3 fatty acids, fluorescence, fatty acid content

INTRODUCTION

Vegetable oils are an indispensable food component. They are a source of substances needed for humans and indispensable fatty acids, phospholipids, fatty soluble vitamins, sterols etc [1]. From the point of view of biological activity, the value of vegetable oils is determined from the presence of indispensable fatty acids, linolic (ω-6) and linoleic (ω-3) as well as from the quantity and quality of the tocopherol contents and other biologically active components.

The nutrition of a healthy human being as well as in the case of different chronic diseases not only the presence of poly non-saturated fatty acids, but also the ratio ω-6/ω-3 of fatty acids is of essential significance. According to certain authors [2, 3], for healthy people the ratio ω-6/ω-3 is recommended to be 10:1, while in the case of a diet it is recommended 3:1 to 5:1.

Sunflower oil is traditionally used in Bulgarian households. Flaxseed oil is characterized by high contents of α-linolic acid and is the only source of ω-3 fatty acids of vegetable origin. The mixtures of vegetable oils, enriched with ω-3 fatty acids can be used for the production of different type of mayonnaises, sauces and other emulsion products [4].

The objective of the present work is to study the dependence between the biological value of the sunflower oil enriched with ω- 3 fatty acids as well as their physico-chemical indicators.

MATERIALS AND METHODS

Samples for investigation have been prepared by mixing popular Bulgarian sunflower oil „Biser" with cold pressed flaxseed oil of Turkish origin in volumic concentrations 10% and 20%. The products for samples were commercially available and purchased from the food stores and were stored in dark place and at room temperature. The samples were analyzed immediately after being prepared.

Fatty acid content of the vegetable oils has been determined using gas chromatography (GC) method used previously [4-5]. Fatty acid methyl esters (FAME) were purified by silica gel 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 m x 0.25 mm (inner diameter) capillary InnoWax column (cross-linked to polyethylene

* To whom all correspondence should be sent:
E-mail: kr.nikolova@abv.bg

glycol, Hewlett Packard GmbH, Austria) and a fire ionization detector.

The column temperature was programmed to rise from 165ºC to 240ºC by step 4ºC/min and held at this temperature for 10 min; injector and detector temperatures were 260ºC. Carrier gas was nitrogen at a flow rate 0.8 cm^3/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 [6].

Tocopherols in the vegetable oils were determined directly by high performance liquid chromatography (HPLC) [7] on a "Merck-Hitachi" (Merck, Darmstadt, Germany) equipment with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector "Merck-Hitachi" F 1000. The operating conditions were as follows: mobile phase of *n*-hexane: dioxan 96:4 (by volume), flow rate 1 cm^3/min, excitation 295 nm, emission 330 nm. 20 µl 1% solution of oil in hexane were injected. Tocopherols were identified by comparing the retention times with those of authentic individual tocopherols.

The oxidation stability has been determined using the Rancimat 679 equipment at temperature of 100 °C with a volume rate of air flow of 20 dm^3/h [8] .

The degree of oxidation of vegetable oils has been determined by ultraviolet (UV) spectroscopy that provides information about the primary products of the oxidation. The absorption of a 0.2% solution of isooctane at λ = 232 nm and λ = 268 nm has been measured using a Spectrophotometer S – 26, Boeco, Germany.

The optical properties of samples were studied using the following equipment:

- The color parameters were directly taken with Lovibond PFX 880 (Tintometer Limited, UK) in both colorimetric systems XYZ and CIE Lab. Transmission spectra in the visible range have been recorded using a 10 cm long cuvette (recommended for the study of refined vegetable oils). A software program developed specially for the equipment allowed determination of chlorophyll and *β*-carotene.
- The fluorescence spectra in UV and visible regions were measured on Fiber optic spectrometer (AvaSpec-2038, Avantes). Its sensitivity is in the range 200 – 1100 nm and it has resolution of about 5 nm. The oil samples were placed in 10mm cuvette and irradiated by light emitting diodes (LEDs) having emission wavelength 305 nm, 370 nm, 395 nm, 425 nm and 450 nm. Fluorescence spectra in visible region have been taken from a direction orthogonal to the line of transmission, as shown in the Figure 1.

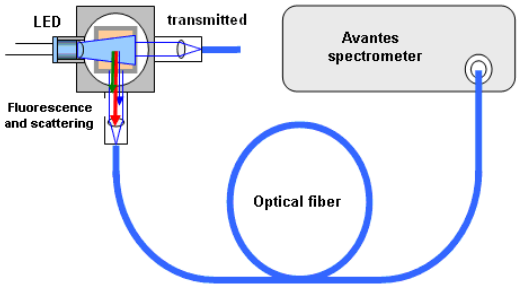

Fig. 1. Experimental set-up for the observation of fluorescence spectra.

UV spectra were obtained on micro layer of the studied samples formed between two quartz plates with detecting optical fiber placed directly in contact with the oil sample as shown in Fig.2.

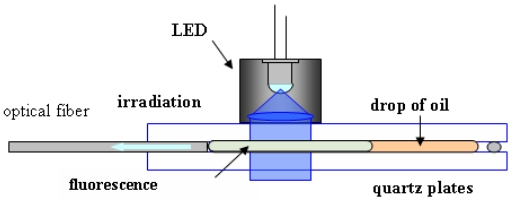

Fig. 2. Representation of the scheme of UV fluorescence using glass plates.

Statistical analysis of results:

Three samples per treatment were measured. Differences were considered significant for $p < 0.05$. One way analysis of variance (ANOVA) was performed. The average results are presented in the corresponding tables.

RESULTS AND DISCUSSIONS

Fatty acid composition is the most important characteristic of vegetable oils as related to its nutrition value, as well as to its oxidation stability. The results for fatty-acid contents of the tested samples are presented in Table 1.

The sunflower oil sample under study is of the linolic type. In this type the linoleic acid dominates over the oleic fatty acid. From nutrition point of view chosen oil is a suitable sample for enrichment because linolic acid belongs to the essential fatty acids that are indispensable for the human organism. Flax oil is rich in linoleic acid (*ω*-3). Its addition to sunflower oil in proportions of 10% and

Table 1. Fatty acid composition of sunflower oil, flaxseed oil and their mixture

Fatty acids, %	Sunflower oil	Flax oil	Sunflower oil + 10 % flax oil	Sunflower oil + 20 % flax oil
$C_{8:0}$	-	-	0.93 ± 0.02	-
$C_{14:0}$	-	-	-	0.1 ± 0.01
$C_{16:0}$	7.35 ± 0.12	6.28 ± 0.14	7.35 ± 0.17	6.5 ± 0.14
$C_{16:1}$	1.04 ± 0.04	-	0.17 ± 0.01	0.10 ± 0.01
$C_{18:0}$	3.39 ± 0.08	4.15 ± 0.05	3.15 ± 0.10	2.91 ± 0.12
$C_{18:1}$	32.16 ± 1.14	20.85 ± 1.05	33.51 ± 1.04	29.94 ± 1.24
$C_{18:2}$	56.06 ± 1.74	28.39 ± 1.28	50.50 ± 1.72	51.73 ± 1.71
$C_{18:3}$	traces	40.33 ± 1.62	3.75 ± 0.20	8.11 ± 0.17
$C_{22:0}$	-	-	0.64 ± 0.02	0.61 ± 0.02

* $C_{8:0}$- caprylic acid; $C_{14:0}$- myristic acid; $C_{16:0}$ – palmitic acid; $C_{16:1}$ - palmitoleic acid; $C_{18:0}$ – stearic acid; $C_{18:1}$ – oleic acid; $C_{18:2}$ – linoleic acid; $C_{18:3}$ – linolenic acid; $C_{22:0}$– behenic acid

Table 2. Content of tocopherols and tocopherol composition of sunflower oil, flax oil and their binary mixtures

Tocopherols (T) and Tocotrienols (T-3), %	Sunflower oil	Flax oil	10 % flax oil in sunflower oil	20 % flax oil in sunflower oil
α - T	100 ± 2.7	19.5 ± 0.23	90.9 ± 1.96	79.7 ± 3.2
γ-T	-	59.1 ± 1.12	4.5 ± 0.11	13.5 ± 0.44
γ- T-3	-	21.4 ± 1.07	4.6 ± 0.16	6.8 ± 0.23
Total, mg/kg	588.6	447.7	555.1	514.1

20% leads to an increase of linoleic acid content in sunflower oil with 3.75% and 8.11 % respectively, while in the non-enriched sunflower oil it reaches 0.1% at most (See Table 1). The content of the oleic acid in the enriched with flax oil is not significantly lower than that of pure sunflower oil sample. The latter allows expecting that the oxidation stability of the enriched oils under study will be essentially altered. Due to the low oxidation stability of flax oil, the oxidation stability of mixed sunflower oils decreases smoothly with raising the addition of flaxseed oil (See Fig. 3)

The sample of sunflower oil with 20% flax oil provides good ω-6/ω-3 ratios of fatty acids. This combination provides also better oxidation stability.

In addition to the fatty acid composition of the studied samples the tocopherol content has been studied as well. The tocopherol content and composition of oil is directly defined by high-performance liquid chromatography with fluorescence detection. The results are presented in Table 2.

As seen from Table 2 the general quantity of tocopherols in the studied oil samples changes linearly with the addition of flaxseed oil.

As seen from both figures 3 and 4 linear regression model shows a dependence of the tocopherol content (y) on the induction period (x) expressed as $y = 20.63\,x + 393.7$ with a correlation coefficient $R^2 = 0.99$.

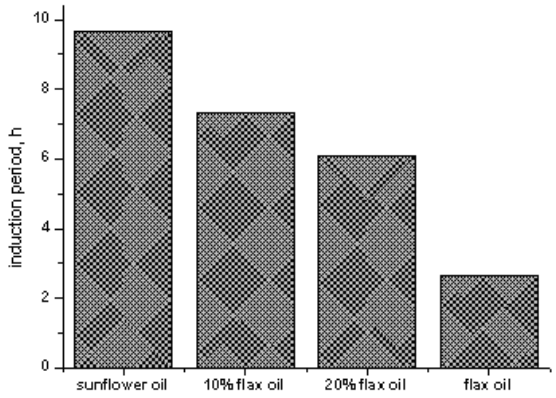

Fig. 3. Oxidation stability of sunflower oil and its binary mixtures.

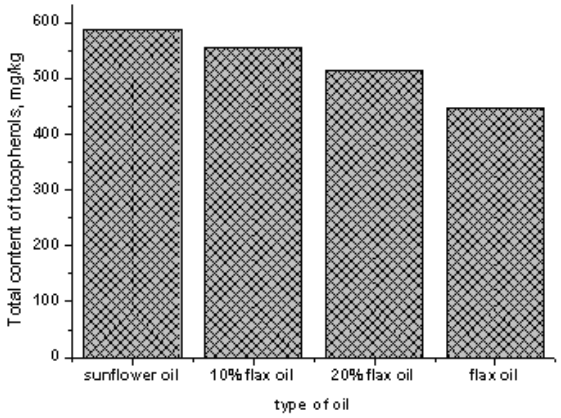

Fig. 4. Total content of tocopherols of sunflower oil and its binary mixtures

Observed comparatively good oxidation stability of the sample with 20% addition of flax oil can be explained with the presence of γ-tocopherol and trienol in the mixture.

In addition to improved nutrition value (high content of fatty solvent vitamins A, D, E and K, indispensable fatty acids) and oxidation stability, the enriched oils must have acceptable color indicators for the customer. The data for the color parameters of the samples in the two colorimetric systems are shown in Fig. 5.

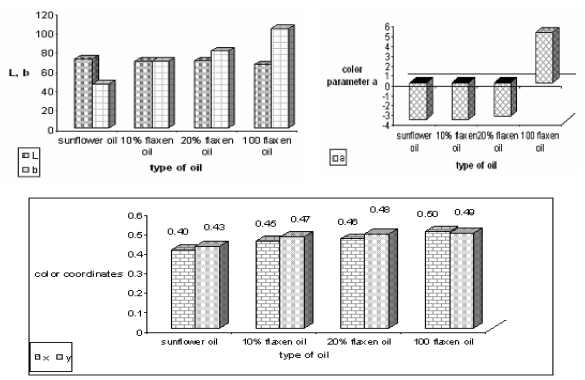

Fig. 5. Color parameters of enriched, non-enriched sunflower and flaxseed oil

The data for the color parameters show that the red component ($a^* > 0$) is predominant only in flax oil, while in all of the rest the green one dominates. The addition of flaxseed oil in greater quantities (20 %) causes the increase of red component. Although flaxseed oil is characterized by a lower brightness compared to sunflower oil this does not lead to an essential change in outer appearance of trade products. The enriched samples have a clearly expressed yellow color. ‘

With the increase of the content of flax oil the b* component rises essentially, which is proved by the red-yellow-blue (RYB) system, used especially to determine the colour of vegetable oils. From the obtained experimental data it is clear that the yellow component is 19 and 27 relative unit (r.u.), respectively for the 10% and 20% flax oil samples, while for sunflower oil it is 7.7 r. u.

The degree of oxidation of studied samples is estimated through indirect determination of primary oxidation products (peroxides) of absorption of conjugated diene structures which are formed from linoleate units using UV spectroscopy at 232 nm and from the absorption of conjugate triene structures at 268 nm. “Fresh” oils of linoleic type are characterized with absorption of conjugate dienes lower than 5.8 [9].

The results of measurements are presented in Table 3, where A_{232nm} and A_{268nm} are absorption maxima in relative units at 232 nm and 268 nm, respectively.

Table 3. Absorption of conjugate dienes of carbonyl compounds.

Type	A_{232nm}	A_{268nm}
Sunflower oil	1.57	0.45
Flax oil	1.94	0.83
10 % flax oil + sunflower oil	1.61	0.51
20 % flax oil + sunflower oil	1.55	0.55

According to the conjugated dienes, the vegetable oils can be defined as “fresh”. The highest absorption value is obtained for pure flaxseed oil, but it lies in the limits for fresh oils. Similar value (around 2.11) for its absorption is reported in [10]. The undesirable oxidation during technological processes is low - absorption for pure sunflower and enriched oils lies in a narrow range between 0.45 and 0.55. The value of the dienes in flaxseed oil is higher.

Fluorescence spectra of investigated oils in the visible diapason have been recorded at four irradiation wavelength - 370 nm, 395 nm, 425 nm and 450 nm. On figure 6 only the spectra at λ=425 nm have been presented, because at this wavelength the differences in the fluorescence maximum intensities and the wavelength at which it is observed are most clearly expressed.

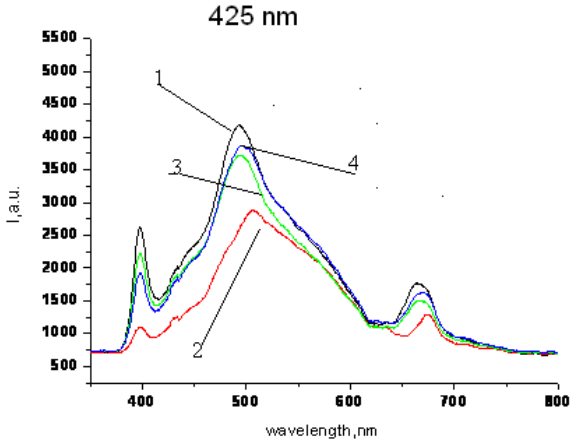

Fig. 6. Fluorescence spectra at λ=425 nm for flaxseed oil, sunflower oil and its mixtures. *1-sunflower oil; 2-flax oil; 3- 10%flax oil in sunflower oil; 4-20% flax oil in sunflower oil*

Two clearly expressed maxima have been observed for all investigated samples. One maximum is broad with high intensity belongs to the range $\lambda \in (490 \div 510)$ nm, the other one with rather lower intensity - to the range $\lambda \in (660 \div 675)$

nm. Latter one is due to the chlorophyll content in studied oils, while the first can be attributed to product of oil oxidation, as well as to the content of oleic acid. The comparison with data in Table 1 shows that the lowest fluorescence intensity is accounted for flaxseed oil – at 509 nm. In flaxseed oil the content of oleic acid is lowest (20.87 %), compared with samples with sunflower oil. A relation between the intensity of the fluorescence peak at 500 nm for excitation with λ=425 nm and absorption in UV diapason at λ=270 nm has been observed. The following correlation was found $I_{500} = -3151.x + 5458.9$ with correlation coefficient $R^2 = 0.949$ (x is absorption of the sample at λ=270 nm).

The addition of flaxseed oil to sunflower oil does not enriches it essentially with chlorophyll, but leads to expressive rise of β – carotene, that is important component for human health. β – Carotene and chlorophyll content in studied samples are presented in figure 7.

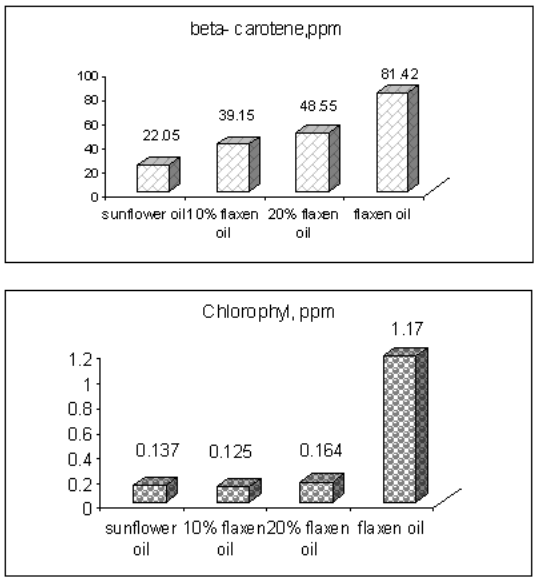

Fig. 7 β – carotene and chlorophyll content in flaxseed oil, sunflower oil and binary mixtures

Fluorescence spectroscopy in UV diapason is related mostly to the existence of pigments and tocopherols in studied samples. The fluorescence spectra for excitation with 305 nm are shown at figure 8.

Three maxima have been observed – one in the region 332-342 nm, the second at 430 nm and the third in the range 485-490 nm. Both first and second are related to the tocopherol content, the last is attributed to the presence of β – carotene in the samples.

The tocopherol content decreases in the samples of pure sunflower oil and those in enriched with flaxseed oil. The ratio of intensity of excitation wavelength λ = 305 nm and that of fluorescence at λ =340 nm (first fluorescence maximum) is related to the tocopherol content of studied samples can be described by following equation $I_{305} / I_{340} = 0.002.x - 0.4772$, where x is the total tocopherol content. The correlation coefficient is $R^2 = 0.916$

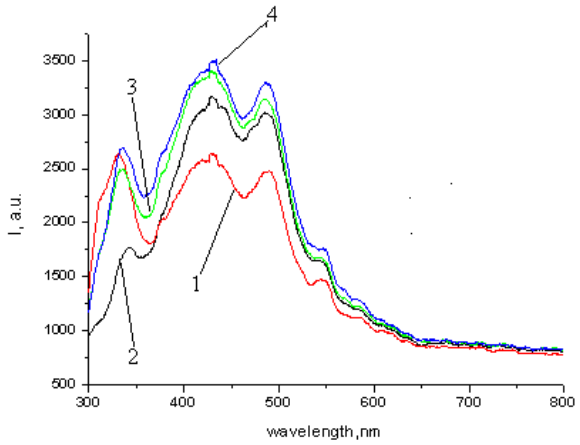

Fig. 8 The fluorescence spectra at 305 nm for sunflower oil, flaxseed oil and binary mixtures. *1-sunflower oil; 2-flax oil; 3- 10%flax oil in sunflower oil; 4-20% flax oil in sunflower oil*

The third fluorescence maximum observed at excitation wavelength λ = 305 nm suggests good correlation with □-carotene content. The linear regression models for the fluorescence maxima at λ = 485 nm can be presented respectively with $I_{485} = 32.001.x + 1805.4$, and $R^2 = 0.97$, where x represents β-carotene content in studied samples

CONCLUSSIONS

The addition of flaxseed oil with volume concentration 10% and 20% to sunflower one leads to enrichment of mixed sunflower oil with linolic acid (*ω*-3) with concentration respectively 3.75% and 8.11%. The pure sunflower oil does not content linolic acid.

The addition of flaxseed oil with volume concentration 20% to sunflower one provides for high ratio *ω*-3 /*ω*-6 of fatty acids, but brings to decrease of oxidation stability.

Enriched sunflower oil mixtures do not change essentially its chlorophyll content, while the content of β-carotene significantly rises. Their brightness slightly decreases compared with pure sunflower oil, but addition of flaxseed oil does not lead to significant change in trade appearance of the products.

Relation between fluorescence intensity in the visible region at 500 nm and oxidation products, defined through UV spectroscopy, is established to be: $I_{500} = -3151.x + 5458.9$ (x is absorption of the sample at λ=270 nm) with correlation coefficient $R^2 = 0.949$.

The following correlation dependences have been established:

-Between total content of tocopherols (y) and the induction period (x) of oxidation y = 20.63 x + 393.7 with correlation coefficient R2 = 0.99.

-Between the fluorescence maxima at λ = 485 nm at excitation wavelength λ = 305 nm and β-carotene content: $I_{485} = 32.001.x + 1805.4$, R2 = 0.97

-Between the ratio of intensity at excitation wavelength λ = 305 nm and that of fluorescence at λ =340 nm (first fluorescence maximum) and the tocopherol content of studied samples described by following equation: $I_{305} / I_{340} = 0.002.x - 0.4772$ with correlation coefficient $R^2 = 0.916$.

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ФИЗИКО-ХИМИЧНИ СВОЙСТВА НА СЛЪНЧОГЛЕДОВО МАСЛО, ОБОГАТЕНО С ω -3 МАСТНИ КИСЕЛИНИ

К. Николова[1], М. Перифанова-Немска[1], Г. Узунова[1], Т. Евтимов[2], Г. Антова[2], А. Аладжаджиян[3], В. Бок[4]

[1]Университет по хранителни технологии, Пловдив,
[2]Пловдивдски Университет „Паисий Хилендарски", Пловдив
[3]Аграрен университет, Пловдив
[4]Университет на Квебек в Отава, Гатино, Квебек, Канада

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(Резюме)

Изследвани са физико-химичните свойства (мастнокиселинен състав, токофероли, оксидантна стабилност, цветови параметри и др.) на слънчогледово масло от линолов тип при обогатяването му с мастни киселини чрез добавяне на ленено масло. Снети са флуоресцентните спектри във видимата и УВ област на спектъра, изследвано е поглъщането на пробите в УВ диапазона. Установено е, че при обогатяване на слънчогледово масло с 20% ленено масло количеството на линоленовата киселина нараства от 0.1% до 8.11%. Полученото обогатено слънчогледово масло има добро съотношение на ω-3/ω-6 мастни киселини. Оксидантната стабилност на слънчогледово масло с 20% добавка на ленено масло намалява от 10h на 6h. Добавянето на ленено масло към слънчогледовото масло не го обогатява съществено с хлорофил, но води до значително увеличаване на съдържанието на β-каротен, който е много важен компонент за човешкия организъм. Установени са линейно-регресионни модели между интензитетите на флуоресцентните спектри в УВ диапазона и общото съдържание на токофероли и β-каротен.