CHEMICAL COMPOSITION AND OPTICAL PROPERTIES OF REFINED SUNFLOWER OIL WITH ADDED VARIOUS OILS

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Received 20 September 2023
Accepted 08 April 2024 DOI: 10.59957/jctm.v59.i4.2024.1

ABSTRACT

Some physicochemical characteristics and elemental composition of refined sunflower oil, as well as linseed oil added to it, were investigated; linseed oil and olive oil; truffle oil and rosemary oil. Fatty acid analysis shows substantial increases in monounsaturated fatty acids with the addition of truffle and rosemary oils (up to about 78 %). With the same supplements, a significant oxidative stability over 20 hours was also observed. High concentrations of chlorophyll were found with the addition of rosemary oils and oils of linseed oil and olive oil. β-carotene was affected three to six times in all supplements compared to the commonly refined oil. Eight elements (Mg, Cr, Mn, Zn, Ni, As, Pd and Cd) were analyzed in the studied oils, no presence of toxic elements As and Cd (< 0.02 mg kg^{-1}), lead was up to 0.04 mg kg^{-1}. The remaining elements vary in different concentrations depending on the additive oils used. The fluorescence spectra of the tested samples were obtained for excitation wavelengths of 380 nm, and the fluorescence maxima allowed to determine the relationship between the optical and chemical properties of the samples. In addition, infrared spectroscopic experiments (ATR and transmittance) were used to investigate the fatty acid profile of the analyzed oil samples.

Keywords: *sunflower oil with additives, fluorescence, IR spectra, elements.*

INTRODUCTION

The sunflower (*Helianthus annuus* L.) is an unpretentious plant suitable for different climatic conditions. Its seeds are the subject of much research due to the composition of fatty acids and secondary components it contains [1]. According to the production method, refined and unrefined oils can be obtained from its seeds (virgin sunflower oil). Unrefined sunflower oil has a pleasant taste and aroma. The peculiarity of this oil is that it has almost no importance in the market and is most often used in the food industry [2]. Refined oil has no pronounced taste and aroma, its main use is in frying and baking. In Bulgaria, it is part of dressings, as well as used independently in different types of salads. Today, sunflower oil faces serious competition as its demand declines due to competition with other oil crops and changing consumer demand.

Olive oil is a food product perfectly combining nutritional and sensory values, and its production and consumption have long exceeded the regions of the Mediterranean Sea [3]. Its fatty acid composition is characterized by a good balance between saturated, monounsaturated, and polyunsaturated acids. It can be consumed raw, thus preserving the content of important nutritional compounds such as vitamins and phenols [4]. Its health benefits are related to: improving glycemic control and weight management [5], prevention of colon cancer, breast cancer, etc. species [6, 7] reduces blood pressure [8] has antimicrobial activity [9].

Flaxseed has established itself as an extremely important, functional and nutritional ingredient. In addition, the linseed oil extracted from the seeds has many and varied scope of application. Lowers blood cholesterol, normalizes blood pressure [10] has antimicrobial, anticancer, immunostimulant, etc. actions [11 - 13]. *In vivo* evaluated the local anti-inflammatory and antioxidant effects of linseed oil on acute inflammation, which used a model of carrageenan-induced edema [14].

Rosemary (*Rosmarinus officinalis*) is known more as a cooking spice. Rosemary oil has a number of beneficial properties for the body, for example, it shows antibacterial and anticancer activity [15 - 17]. Alvarado-García et al. evaluated the effectiveness of using essential oils extracted from *Rosmarinus officinalis* in calming anxiety, depression and improving sleep quality [18]. In food technology, the potential of oil on the microbiological and oxidative stability of Sarshir - a perishable dairy product [19] has been investigated.

Truffle oil is a preferred ingredient for an easy way to add flavor. Truffles are among the most expensive foods and their quality depends on their unique aroma and their oils are very common. For these reasons, most scientific articles relate to the established misuse of truffle-flavored artificial flavorings [20, 21]. Other studies have shown that the use of this oil can be a valuable source of protection against coronary heart disease [22].

The aim of this study is to investigate commercial mixtures based on refined sunflower oil with the addition of different vegetable oils with different fatty acid compositions: linseed oil, olive oil, truffle, and rosemary oils. Some of their characteristics (composition, optical and chemical) important for both nutritionists and consumers were determined and compared.

EXPERIMENTAL

Samples

The following products were purchased from a commercial network (from the same manufacturer).

a) Refined sunflower oil (RSO).
b) Refined sunflower oil + linseed oil (RSO + LO).
c) Refined sunflower oil + truffle oil (RSO + TO).
d) Refined sunflower oil + rosemary oil (RSO + RO).
e) Refined sunflower oil + olive oil + linseed oil (RSO+ OO + LO).

Methods

Determination of the chemical parameters

- Animal and vegetable fats and oils - Determination of peroxide value - Iodometric (visual) endpoint determination (ISO 3960:2017) - BDS EN ISO 3960:2017.
- Animal and vegetable fats and oils - Determination of saponification value (ISO 3657:2020) - BDS EN ISO 3657:2020.
- Determination of oxidative stability - according to [23].

Fatty acid composition

Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 2: Preparation of methyl esters of fatty acids (ISO 12966-2:2017).

Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters (ISO 12966-1:2014).

Determination of fluorescence spectra

A BroLight BIM-6002 fiber optic spectrophotometer with a spectral sensitivity of 200 nm - 1100 nm was used to measure the spectral characteristics of sunflower oil with herbal additives. Due to the relatively weak fluorescence signal of the samples, they were examined without dilution. They were placed between two quartz plates and their fluorescence spectra were taken using 90^o geometry. The entrance slit used is 200 μm, the resolution of the spectrophotometer is approximately 8 nm. The best signal-to-noise ratio and intensity of the fluorescence maximum was found using an LED emitting at 390 nm.

Determination of β-carotene and chlorophyll content

Animal and vegetable fats and oils - Determination of Lovibond colour - BDS ISO 15305:2003.

FT-IR spectroscopy

Infrared spectra were recorded on a Thermo Fischer Nicolet iS50 FT-IR instrument. The analysed sample of 200 μL was placed between two KBr disks and the transmittance spectrum was recorded [23].

Determination of elements in oils

Approximately 0.3 g of each oil was weighed in PTFE vessels. 6 mL of 67 % HNO_3 (supra pure) and 2 mL of 30 % H_2O_2 (supra pure) were added. Microwave digestion was performed as follows: 15 min to reach 200°C and 15 min to hold the temperature. After cooling samples were transferred into a 50 mL volumetric flask and diluted to the mark with deionized water. Blank samples were prepared that way as well. Inductively coupled plasma mass spectrometer "X SERIES 2" - ThermoScientific was used to determine elements concentrations. Multielement standard solution 5 for ICP (TraceCERT®, Merck) and 1000 mg L^{-1} As (TraceCERT®, Merck) were used for the preparation of diluted working standard [24].

RESULTS AND DISCUSSION

Table 1 presents the oil acid composition of the samples, as well as some of their other characteristics.

Lipid oxidation degrades the quality of vegetable oils, leading to the use of synthetic antioxidants to maintain the shelf life and quality of the oils. Oils with a high content of polyunsaturated fatty acids are considered highly susceptible to oxidation [25]. From this point of view, the oxidative stability of oils is an important indicator of their acceptable shelf life. Different sunflower oil values are given in the literature, probably influenced by their fatty acid composition [26, 27]. As can be seen, different additions to RSO affect its value, with additions of rosemary and truffle oil significantly increasing it, while other additions decrease it, most significantly with the addition of linseed oil. Peroxide number (PV) is used to determine the degree of oxidation of vegetable oils, but sometimes its measurement does not give real information about the quality of the oil [28].

Table 1. Physical-chemical properties of the various oil products.

№	Physical-chemical properties	Unit	RSO	RSO + LO	RSO + TO	RSO + RO	RSO + OO + LO
1.	Fatty acid composition: - saturated - monounsaturated - polyunsaturated	%	 10.7 30.9 58.4	 8.8 28.2 63.0	 6.2 77.6 16.2	 6.1 77.2 16.7	 10.2 44.1 45.7
2.	Peroxide value	meq O_2 kg^{-1}	1.05 ± 0.01	2.03 ± 0.01	5.52 ± 0.02	7.52 ± 0.03	8.02 ± 0.05
3.	Saponification value	mg KOH g^{-1}	166 ± 2	161 ± 2	162 ± 2	159 ± 2	168 ± 2
4.	Oxidative stability	h	9.1 ± 0.3	6.5 ± 0.3	Over 20	Over 20	8.3 ± 0.3
5.	Chlorophyll	ppm	0.02 ± 0.005	1.14 ± 0.02	0.22 ± 0.01	1.98 ± 0.03	3.98 ± 0.06
6.	β-carotene	ppm	27.4 ± 0.2	108 ± 2	92.2 ± 1.7	159 ± 5	102 ± 3

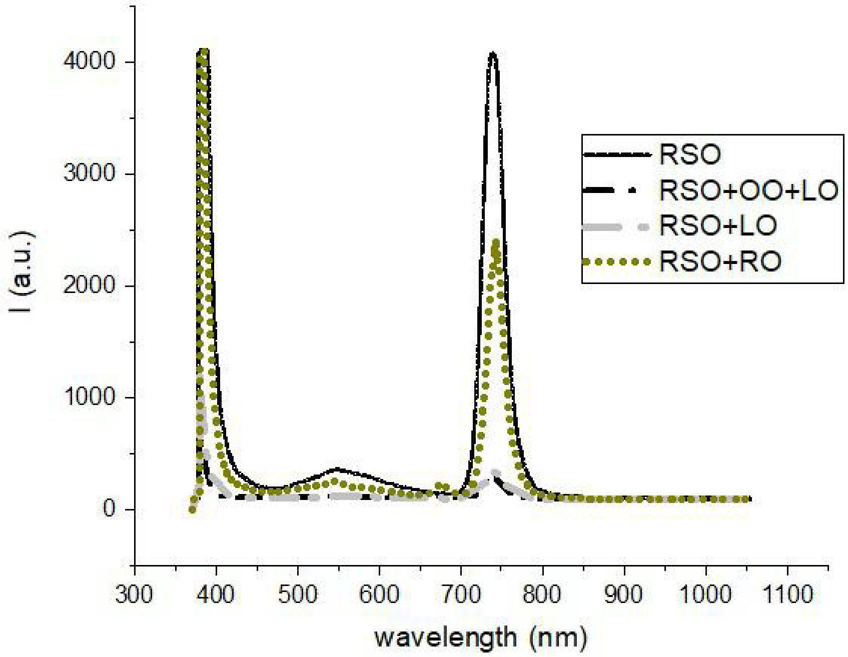


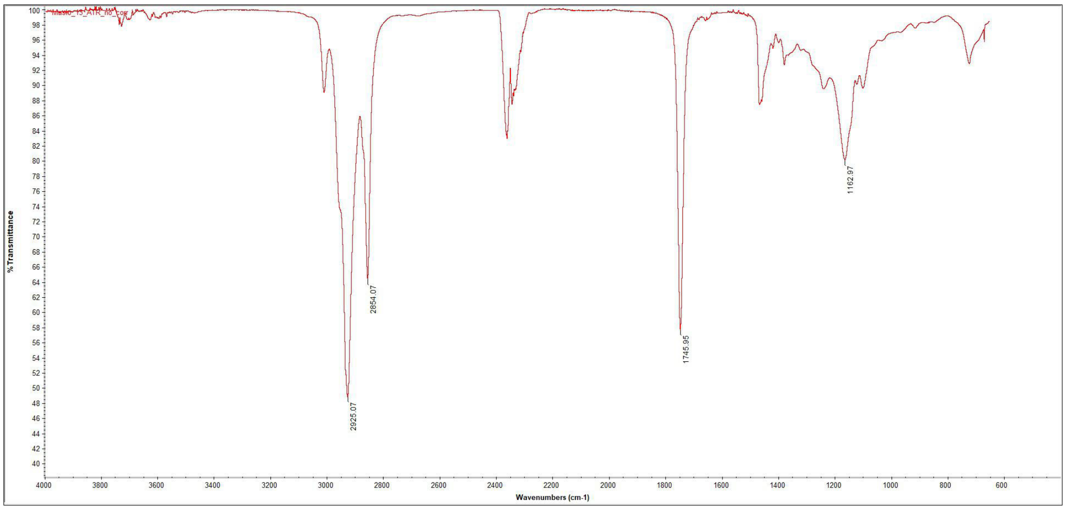
Fig. 1. Fluorescence spectra of the oils.

As can be seen from the results presented in the table for values below approximately 10 mEq O_2 kg^{-1} the oils can be considered fresh [29]. The different additives did not lead to significant changes in the saponification number of the analyzed samples. However, the fatty acid composition is strongly influenced by the addition of truffle and rosemary oils, with an increase in monounsaturated acid concentrations at the expense of polyunsaturated ones.

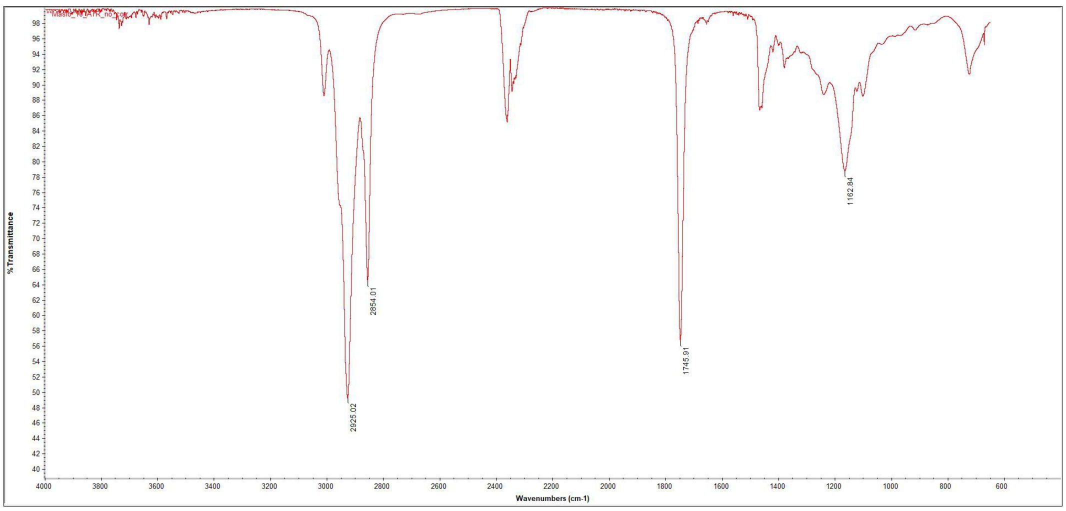
Fig. 1 presents the results of the fluorescence analysis of the studied oils.

The fluorescence spectra of vegetable oils and extra virgin olive oil (EVOO) are characterized by peaks of chlorophyll, carotenoids, and oxidation products of fatty acids [30]. Missing from Fig. 1 is the doublet line of the fluorescence spectrum of relatively low intensity in the 440 - 470 nm region associated with the conjugated hydroperoxide. It is known that in the presence of oxidation in the oil the unconjugated double and triple bonds change to conjugated ones and this further enhances the intensity of the fluorescence spectra [31]. The absence of the indicated maximum means that there is no high amount of oxidation products in the investigated samples. i.e. there is no rancidity and spoilage of the samples. The fluorescence maximum around 550 nm is associated with the β-carotene content [32] or vitamin E [31]. In our case, this maximum exists in samples with lower b-carotene content (RSO and RSO + LO + OO), and therefore can be related to vitamin E content. RSO + LO + OO and RSO + RO samples show relatively weak peak around 650 - 670 nm. This peak in the literature is associated with the content of chlorophyll *a* or *b* [33, 34]. This is confirmed by the quantification of chlorophyll in the tested oils given in Table 1. The indicated samples have respectively 10- and 200-times higher chlorophyll content compared to sunflower oil supplemented with linseed oil. All samples have an emission maximum around 750 nm, which is associated with pigments other than chlorophyll.

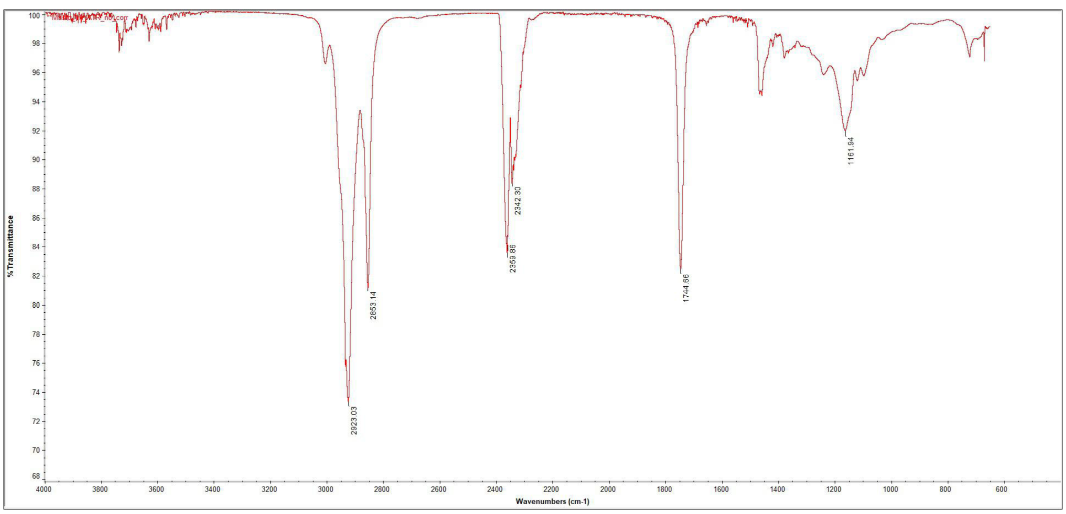
All oils possess absorption bands around 2925 cm^{-1} and 2864 cm^{-1} in IR spectra (Fig. 2). They are below 3000 cm^{-1} and are associated in the literature with alkane chains of fatty acids [35]. Vibrations around 2925 cm^{-1} correspond to asymmetric bond stretching of the methylene group, and around 2864 cm^{-1} are associated with vibrations of symmetric members of the methylene group [36]. In some of the samples there


% Transmittance
Wavenumbers (cm-1)
2925.07
2854.07
1745.95
1162.97

a)


% Transmittance
Wavenumbers (cm-1)
2925.02
2854.01
1745.91
1162.84

b)


% Transmittance
Wavenumbers (cm-1)
2923.03
2853.14
2359.86
2342.30
1744.66
1161.94

c)

d)

e)

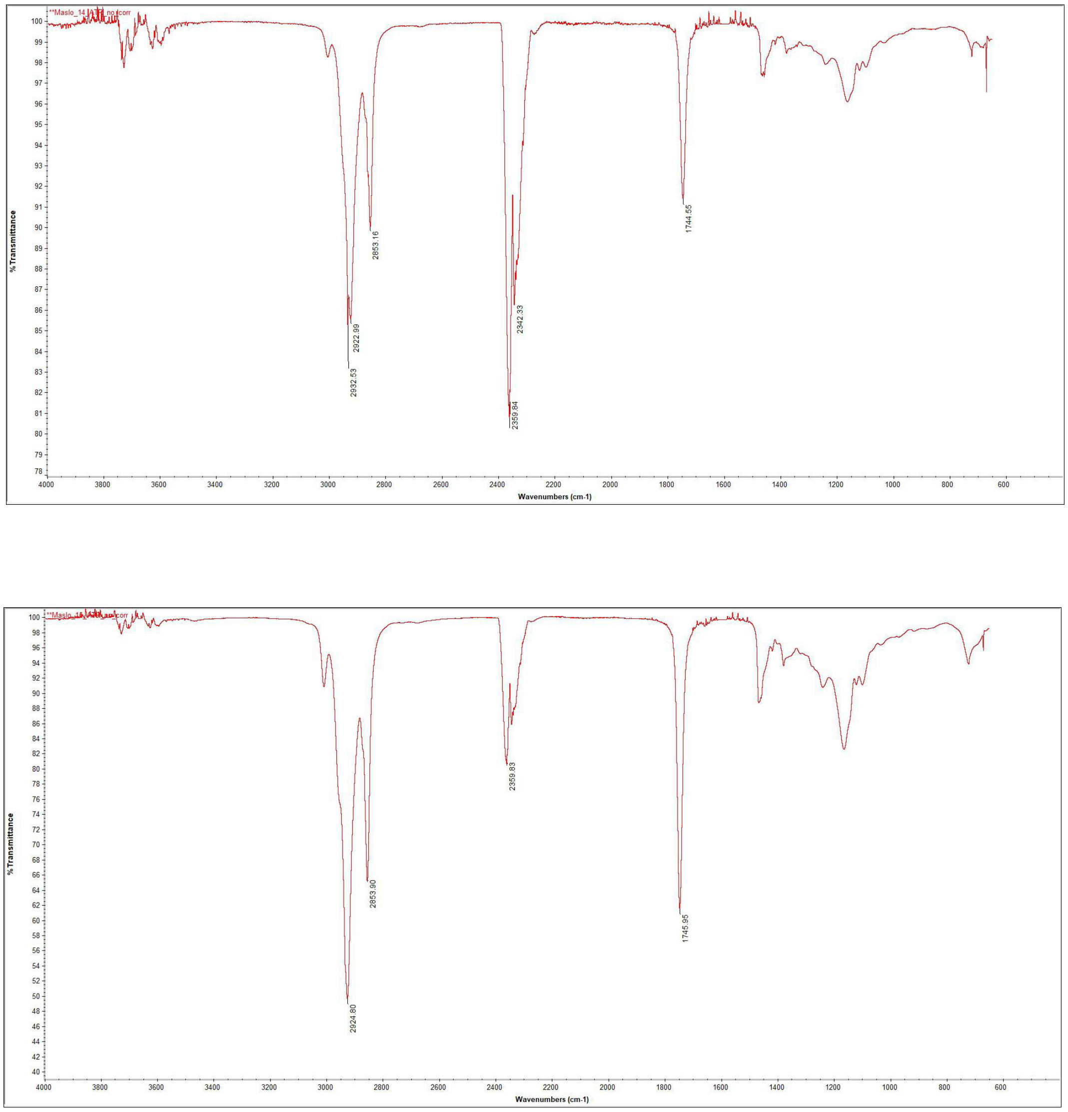


Fig. 2. FT-IR spectra of oils: (a) RSO, (b) RSO + LO, (c) RSO + TO (d) RSO + RO, (e) RSO + OO + LO.

is a slight shift in the indicated peaks related to the degree of unsaturation and the relative state of the fatty acids in the samples [37]. Stretching vibration of C=O groups around 1746 cm^{-1} is explained by the presence of fatty acids in ester forms. Stretching bonds of C-O groups around 1163 cm^{-1} is characteristic of all the studied samples. Only in samples RSO + RO and RSO + TO is observed adsorption around 2343 cm^{-1}, which is probably related to the CO_2 [38].

The only element that is regulated in oils in the EU is Pb = 0.10 mg kg^{-1} [39]. As can be seen from Table 2, in all oils, the concentration of this element is below the permissible norm. Of the elements to be determined, magnesium is the main one with concentrations of 0.95 - 2.95 mg kg^{-1} for all the studied samples. The concentration of this element (1.18 mg kg^{-1}) in RSO is twice lower than that found in Turkish sunflower oil - 2.24 mg kg^{-1} [40]. Magnesium and zinc are the elements whose concentration changes most significantly in the analyzed samples depending on the additive. Similar values for Zn and Mn in RSO have been reported in Turkish sunflower oil [40, 41]. As can be seen from the table, the toxic elements As and Cd are not detected in the samples (< 0.02 mg kg^{-1}) we studied with or without additives.

Table 2. Concentration of Mg, Cr, Mn, Zn, Ni, As, Pd and Cd in the different oils (RSD 3 - 8 %, n = 3).

№	Element	RSO	RSO + LO	RSO + TO	RSO + RO	RSO + OO + LO
1.	Mg, mg kg^{-1}	1.18	4.26	0.95	1.32	2.95
2.	Cr, mg kg^{-1}	0.22	0.15	0.34	0.21	0.32
3.	Mn, mg kg^{-1}	0.05	0.02	0.03	0.04	< 0.02
4.	Zn, mg kg^{-1}	1.12	0.08	0.45	1.67	< 0.02
5.	Ni, mg kg^{-1}	0.04	0.07	0.12	0.11	0.03
6.	Pb, mg kg^{-1}	0.03	< 0.02	0.04	< 0.02	0.04
7.	Cd, mg kg^{-1}	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
8.	As, mg kg^{-1}	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02

CONCLUSIONS

The results show that the addition of different oils to refined sunflower oil can improve its oil acid composition and oxidative stability: rosemary and truffle oil. The saponification value is not significantly affected by the different additives. The presence of the fluorescence maximum of around 550 mn for the RSO and RSO + LO + OO oils can be related to the presence of vitamin E. In all the oils examined, toxic elements such as cadmium and thallium are below 0.02 mg kg^{-1}, and lead does not exceed the norm determined by the European regulation.

Acknowledgements

This study is financed by European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgarian, project № BG-RRP-2.004-0009-C02.

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