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Chemical composition of seeds from organically grown tobacco plants

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Bulgaria is a country with agricultural traditions in terms of growing and producing tobacco. Looking for alternative uses of tobacco and trends towards ecological production of major agrarian crops from 2016, the production of tobacco under organic farming conditions has begun in Bulgaria. Oriental tobacco seeds from subgroup Basmi, variety Krumovgrad 58, grown in a certified bio-field in Bulgaria were collected, ground, and analyzed. The chemical composition and lipid biological active components of the organic tobacco seeds from two consecutive years (2020 – 2021) were studied and compared to conventionally grown tobacco seeds from the same variety. The oil content and biologically active compounds in seeds were examined. The lipid content of bio seeds was 41.7 and 38.4%, respectively. The main components in the triacylglycerol fraction were palmitic (11.20 – 14.20%), oleic (14.40 – 17.70%), and linoleic acids (64.40 – 68.20%). The iodine value of the studied tobacco oils was of the same order 134 – 138 g I_2/100 g. Tobacco seed oils are rich in linoleic acid and have beneficial properties for industrial and plant protection purposes. The ratio between unsaturated and saturated fatty acids in tobacco seed oil was 84.0:16.0 and 83.5: 16.5, respectively. The total tocopherol content was between 317 – 325 and 291 – 307 mg/kg in the first and second year, respectively, γ- and α-tocopherol predominating in the tocopherol fraction. The oxidative stability was about 10 – 13 hours. β-Sitosterol predominated in the sterol fraction and phosphatidylinositol was the main isolated phospholipid.

Keywords: organically grown tobacco, tobacco seed oil, lipid composition

INTRODUCTION

Bulgaria is a country with agricultural traditions in terms of growing and producing tobacco. Mainly, oriental varieties of tobacco are grown, with the aim of putting them into tobacco products for smoking. Looking for alternative uses of tobacco and trends towards ecological production of major agrarian crops from 2016, the production of tobacco under organic farming conditions began in Bulgaria. The Institute of Tobacco and Tobacco Products in the village of Markovo developed a „Technology for organic tobacco production" and certified a biofield at its Experimental Tobacco Station in the town of Gotse Delchev [1]. Organic tobacco is a new industrial plant product that is processed and grown in special certified biofields, without the use of conventional plant protection products [2]. Organic farming does not allow or completely exclude the use of synthetic fertilizers, pesticides, and growth regulators. The soil is maintained by using plant residues, manure, green manure, and biological plant protection is used to control pests [3].

In the tobacco production process, seeds are known as agricultural waste. The seeds of the tobacco plant are very small, but they come in an extremely large quantity per plant. They can be preserved for a long time if they are stored in dry conditions [4]. By processing tobacco seeds, two possibly valuable products can be produced: the oil and the cake. Tobacco cultivars give a good yield of oil, ranging up to 40% of the total seed mass, and the remaining part consists of crude fiber, protein, starch, and inorganic material [5]. Oil is free of nicotine, has a low proportion of saturated fatty acids, and contains health-beneficial compounds such as tocopherols and sterols. The main fatty acids found in the tobacco oil are oleic and linoleic acids. The dominant compounds in the sterol fraction in the oriental tobacco seeds that have been reported before were β-sitosterol, stigmasterol, and campesterol [6]. Tobacco oil from seeds of oriental varieties of tobacco from the region of Bulgaria has been studied, but there are no studies on the lipid composition of seeds grown under organic farming conditions from this region.

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The aim of the study is to investigate if there are differences in the lipid composition between oil obtained from organically grown tobacco seeds the variety Krumovgrad 58, Bulgaria and oil obtained from conventional growing tobacco seeds of the same variety.

MATERIALS AND METHODS

Tobacco seeds from oriental tobacco, variety Krumovgrad 58 were used – ones grown in a special certified biofield in Goce Delchev (Institute of Tobacco and Tobacco Products) (bio) and conventionally grown tobacco (con) on a field in Kozarsko (Institute of Tobacco and Tobacco Products). All analyses were performed with seeds from two subsequent years of harvest – 2020 and 2021. The results were expressed as mean values of the two years of harvest with standard deviation.

The tobacco oil was obtained from 25 grams of ground seeds extracted with n-hexane (300 mL) using a Soxhlet extractor for 8 h at 70°C. The solvent was removed, using a rotary evaporator, at 60°C. The oil sample was weighed, closed under a nitrogen stream, and stored in a refrigerator until further analysis [7].

To determine the fatty acids of triacylglycerol`s, preesterification with methanol in the presence of sulfuric acid was done [8, 9]. The obtained fatty acid methyl esters (FAMEs) were identified on an Agilent 8860 gas chromatograph equipped with a capillary DB Fast FAME column, (30 m × 0.25 mm × 0.25 μm (film thickness)) and a flame ionization detector (FID). The injector and detector temperatures were set at 270°C and 300°C; nitrogen was the carrier gas. The column temperature was from 70°C (1 min), at 6°C/min to 180°C, and at 5°C/min to 250°C, and the split ratio was 50:1. A standard Supelco, USA, mixture (FAME mix 37 components, Supelco, USA) was used for the identification of FAMEs.

The unsaponifiable fractions were determined according to the ISO 18609 standard [10]. Sterols were isolated from the unsaponifiable matter by thin-layer chromatography (TLC) [11] and their total content was determined spectrophoto-metrically at a wavelength of 597 nm. The individual sterol composition was determined on HP 5890 gas chromatograph equipped with DB 5 (25 m × 0.25 mm × 0.25 μm (film thickness)) capillary column and FID. Temperature gradient was from 90°C (3 min) up to 290°C at a rate of 15°C/min and then up to 310°C at a rate of 4°C/min (10 min); detector temperature: 320°C; injector temperature: 300°C and carrier gas was hydrogen. Identification was performed by comparing retention times with those of a standard mixture of sterols (Acros Organics, New Jersey, USA) [12].

Individual tocopherols were determined on a Merck-Hitachi (Merck, Darmstadt, Germany) high-performance liquid chromatograph (HPLC) with fluorescence detection (excitation at 295 nm and emission at 330 nm) and Nucleosil Si 50-5 (250 mm × 4 mm) column. The mobile phase used was n-hexane:dioxane, 96:4 (*v/v*) and the flow rate was set at 1 mL/min [13].

Phospholipids were isolated from the seeds according to Folch *et al*. [14] using extraction with a mixture of chloroform and methanol (2:1, *v/v*). Two-dimensional TLC was used to determine the individual phospholipids [15]. Total phospholipid content was determined spectrophotometrically at 700 nm after mineralization of the lipid fraction with a mixture of perchloric and sulfuric acid (1:1, *v/v*) [16].

Oxidative stability was determined by measuring the induction period using conductometric detection of volatile compounds. Rancimat apparatus Methrom 679 (Methrom, Switzerland) was used at 100°C and air flow rate 20 L/h [17].

All measurements were performed in triplicate (n = 3) and the results were presented as mean value ± standard deviation (SD).

RESULTS AND DISCUSSION

Almost 50 % of the chemical content of the tobacco seed is reported as lipids. That is the reason tobacco seeds are rich in oil fraction. The main components of the oil fraction are fatty acids, sterols, phospholipids, and tocopherols, shown in Table 1. The data showed that there was a difference between the oils obtained from the two types of growing – 40.1 % (bio) and 39.4 % (con). It makes an impression that the seeds from harvest 2021 had lower oil yield in both variants of agrarian conditions (Table 1). The yield of oil was close to the reported values for tobacco seeds from Pakistan (40.6 %), Iraq (22 – 45%), Italy (30 – 40%) and higher from those reported from Iran (13.7%) and Serbia (27.8 – 31.3%) [18]. Zlatanov *et al*. [6] have examined Bulgarian oriental tobacco seeds and reported between 37.9 – 41.3 % yields of oil. The obtained result is typical for oriental tobacco. The yield of tobacco oil was good in comparison to other oils such as canola (37 – 41%), sunflower (25 – 47%) and safflower (38 – 48 %) [19].

Table 1. Content of bioactive compounds in tobacco seed oil

Compounds	Tobacco seed							
	Krumovgrad 58 (bio)				*Krumovgrad 58 (con)*			
Year of harvest	*2020*	*2021*	*AVE*	*SD*	*2020*	*2021*	*AVE*	*SD*
Oil in the seeds, %	41.7	38.4	40.1	2.3	42.3	36.5	39.4	4.2
Unsaponifiable matter, %	2.9	1.9	2.4	0.8	2.7	2.8	2.8	0.1
Sterols, %	0.8	1.2	1.0	0.3	0.8	1.2	1.0	0.2
Tocopherols, mg/kg	325	291	308	24	317	307	312	37
Phospholipids, %	1.5	1.7	1.6	0.3	1.6	1.8	1.7	0.4

Table 2. Fatty acids composition of tobacco seed oil

Fatty acids, %		Tobacco seed					
		Krumovgrad 58 (bio)			Krumovgrad 58 (con)		
Year of harvest		*2020*	*2021*	*AVE±SD*	*2020*	*2021*	*AVE±SD*
$C_{14:0}$	*Myristic*	0.10	0.10	0.10±0.00	0.10	0.10	0.10±0.00
$C_{15:1}$	*Pentadecanoic*	0.10	N/D*	0.10±0.00	N/D	0.30	0.30±0.00
$C_{16:0}$	*Palmitic*	14.20	12.00	13.10±1.20	13.80	11.20	12.50±1.30
$C_{16:1}$	*Palmitoleic*	0.10	0.20	0.15±0.05	0.10	0.20	0.15±0.06
$C_{17:0}$	*Margaric*	0.10	0.20	0.15±0.03	0.20	0.30	0.25±0.04
$C_{17:1}$	*Heptadecenoic*	0.30	0.40	0.35±0.05	0.20	0.30	0.25±0.08
$C_{18:0}$	*Stearic*	2.30	2.10	2.20±0.10	2.90	3.70	3.30±0.40
$C_{18:1}$	*Oleic*	17.30	17.70	17.50±0.20	17.20	14.40	15.80±1.40
$C_{18:2}$	*Linoleic*	64.40	65.90	65.20±0.80	64.70	68.20	66.50±1.70
$C_{18:3}$	*Linolenic*	0.50	0.60	0.65±0.20	0.50	0.60	0.55±0.04
$C_{20:0}$	*Arachidic*	0.10	0.20	0.15±0.05	N/D	0.20	0.20±0.00
$C_{20:1}$	*Gadoleic*	0.20	0.10	0.15±0.04	0.10	0.10	0.10±0.00
$C_{22:0}$	*Behenic*	0.20	0.40	0.30±0.05	0.20	0.30	0.25±0.03
$C_{22:1}$	*Erucic*	0.10	0.10	0.10±0.00	N/D*	0.10	0.10±0.00
SFA, %		17.00	15.00	16.00	17.20	15.80	16.50
MUFA, %		18.10	18.50	18.30	17.60	15.40	16.50
PUFA, %		64.90	66.50	65.70	65.20	68.80	67.00

*SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; N/D –not detected

The amount of total sterols and tocopherols was higher than that reported by others in the ranges of 1.0% sterols and 308 – 312 mg/kg tocopherols [20]. The content of phospholipids was found to be 1.6% and 1.7% in the first and second year of analysis, respectively. These values are similar to previous studies of tobacco seeds [20].

One of the main components in oils are fatty acids (FA). Fatty acids were identified and expressed as percentagea of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The amount of PUFA was higher than the other FA – 65.70 % for Krumovgrad 58 (bio) seeds and 67.00 % for Krumovgrad 58 (con). The average values for SFA an MUFA in the oil of bio seeds was equal to 16.00 – 18.30%, while in the conventional one SFA were 16.50 % and MUFA – 16.50%. Individual FA content was determined, shown in Table 2.

The main saturated fatty acid was palmitic 13.10 % average (bio) and 12.50 % (con). In 2020 seeds had higher content of palmitic acid for the varieties. Stearic acid was the other saturated fatty acid that had higher content – 2.20 and 3.30% in Krumovgard 58 (bio) and Krumovgard 58 (con) variety, respectively, during the two years of analysis. The palmitic acid in tobacco seed oil was half of that reported by Zlatanov *et al.* for Bulgarian tobacco varieties (16.40 – 27.70 %) [6]. The main fatty acid is linoleic acid which is polyunsaturated. Linoleic acid is essential in the diet, as it is incorporated into cell membranes and it is involved in the synthesis of compounds that are responsible for regulating blood pressure, as well as for inflammatory response. In addition, PUFAs are considered beneficial for health

due to their ability to reduce total cholesterol and body fat [19]. The content of linoleic acid was 64.40 – 65.90% for Krumovgarad 58 (bio) and 64.70 – 68.20% for conventional one. Oleic acid was reported as an acid with high content in tobacco seed oil. In the analyzed samples, that fatty acid was with medium value between 17.30 – 17.70% for the bio variety and 14.40 – 17.20% for the conventional tobacco seed oil.

Lipids are usually complex mixtures containing minor components that may catalyze or inhibit oxidation and because primary oxidation products are labile, they are easily converted to secondary products. Oxidation of lipids is promoted by factors such as elevated temperature, presence of light or extraneous materials such as metals or other oxidation initiators [21]. The oxidative stability is an important parameter to evaluate the storage period of vegetable oils during which they keep their nutritive quality. It depends mainly on the fatty acid composition (content of unsaturated fatty acids) and the presence of tocopherols and phenolic compounds as substantial native antioxidants. The oxidative stability of tobacco seed oils, determined by the Rancimat test, was found to be very similar to that of sunflower oil – linoleic type, with induction period at 100°C of 12.6 h and 10.1 h, for Krumovgrad 58 (bio) and Krumovgrad 58 (con), respectively. The higher oxidative stability of Krumovgrad 58 bio is a result of the lower content of linoleic acid (Table 2).

Antioxidants prevent lipid oxidation and they occur either naturally in the lipid mixture, such as vitamin E (tocopherols and tocotrienols, four species of each exist, *α, β, γ, δ*) or they are deliberately added synthetic compounds. There is growing interest in the natural forms of vitamin E because they are promising compounds for maintaining a healthy cardiovascular system and blood cholesterol level [22]. The natural sources of tocopherols are cereal and seeds rich in lipids [23, 24]. Tocopherol content in samples from tobacco seed oil grown under organic production conditions was 308±24 mg/kg, while in conventionally grown tobacco seeds 312±37 mg/kg as mean value of two years of harvest (Table 1). The oil from tobacco seeds Krumovgrad 58 (bio) had higher content of total tocopherols – 325 mg/kg for harvest 2020, according to results obtained from conventionally grown tobacco seeds – 317 mg/kg for harvest 2020. Krumovgrad 58 tobacco seeds showed higher total tocopherol content compared to the data reported in the literature, regardless of the cultivation method. The individual tocopherol content was determined for the two ways of cultivation. *α*- Tocopherol and *β*-tocopherol were not determined. *γ*- Tocopherol was found in the oil in the range of 27.3 – 48.5% of the total tocopherol content and *δ*-tocopherol predominated between 51.5 – 72.7%. Krumovgrad 58 (bio) tobacco seeds oil had lower levels of *γ*-tocopherol than conventionally grown variety for the two years of harvest (Figure 1). Amounts of tocopherols in tobacco seed oil have been reported between 2 – 195 mg/kg [6]. Maryland tobacco seeds were reported to contain *α*- and *γ*- tocopherol with a total value between 70.636 – 217.730 μmol/kg. The results of the study are typical for *Nicotiana spp.* from Bulgarian region reported by Popova *et al.* but in the present study *δ*-tocopherol predominated [18, 25]. The levels of tocopherols in the different plant oils varied over a wide range – 11 and 3468 mg/kg. The total tocopherol content from tobacco seed Krumovgrad 58 (bio) oil was close to those reported for celery oil 126.8 mg/kg, poppy oil – 123.5 mg/kg and red palm oil – 121.6 mg/kg.

Sterols are an important group of components contained in the unsaponifiable fraction of a vegetable oil. The most common plant sterols or phytosterols are: *β*-sitosterol, Δ^7-campesterol, Δ^7-stigmasterol and Δ^5-brassicasterol [26]. The total sterol content in samples of tobacco seed oil from variety Krumovgrad 58 (bio) and (con) was near 1 % from the total lipid content. It can be compared to the sterol content in other oriental tobacco seeds varieties grown in Bulgaria, where the results showed the presence of sterols between 0.4 – 0.8% [6]. Sterols identified in high quantity in tobacco seed oils were *β*-sitosterol, campesterol, stigmasterol and cholesterol (Figure 2).

Figure 2 presents the levels of identified sterols in tobacco seed oil from conventionally and organically grown tobacco seeds from variety Krumovgrad 58 in two years of harvest as mean value in percentage. Harvest 2021 showed lower content of identified sterols for the two varieties and even some of them were not detected at all.

β-Sitosrerol (63.1 – 64.2%) was the main sterol in the tobacco seed oil with no significant difference between years of harvest and no matter the way of cultivation. It is near to the reported content of *β*-sitosrerol in tomato seed oil – 53 - 58% [27].

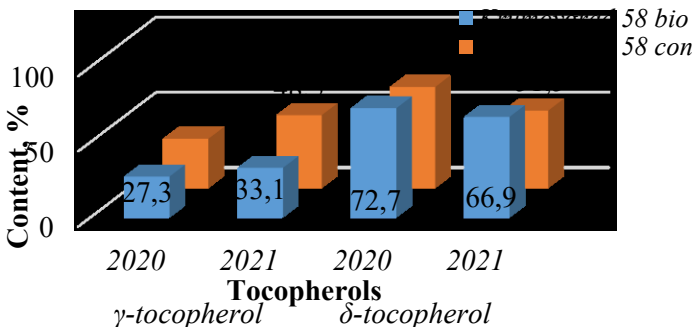

Fig. 1. Individual tocopherol content in tobacco seed oil from conventionally and organic production of tobacco seeds in two years of harvest

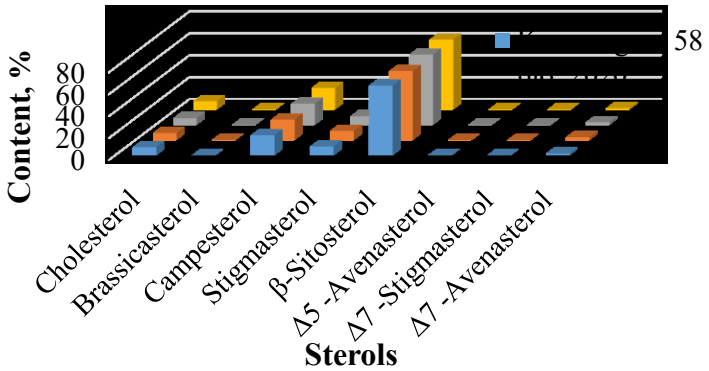

Fig. 2. Sterol content in tobacco seeds oil in two year of harvest and as mean value of the two years

Campesterol was determined as a mean value for the two years of harvest in tobacco seeds oil from Krumovgrad 58 (bio) – 18.6%, while in tobacco seed oil from Krumovgrad 58 (con) it was by 1 % higher. Stigmasterol and cholesterol were with close values – 8.3 % (bio) and 7.5% (con) for stigmasterol and 7.1% (bio) and 6.9% (con) for cholesterol, respectively. Stigmasterol was present with a higher value (7.5 – 8.3%) in a comparison to other edible vegetable oils (1.8% in blackberry seed oil; 0.3% in blueberry seed oil; 1.3% in cranberry seed oil, 1.2% in red raspberry seed oil; 2.3% in strawberry seed oil and 2.4% in kiwi seed oil) [28]. The sterol content in tobacco seeds oil of the examined variety is comparable to grape seed oil reported by Garavaglia *et al.* [29].

The other part of the lipid content in the tobacco seeds are phospholipids. Due to their wide occurrence in foods and their pro- and antioxidant effects, phospholipids have the potential as multifunctional additives in food, pharmaceutical and industrial applications [30]. They are a large group of lipid compounds reported in tobacco seeds oil between 0.2 – 1.7% [24, 31]. The oil from tobacco seeds in the current research had 1.5 – 1.8% of phospholipids. The individual phospholipid content in tobacco seed oil from the conventionally and organically grown tobacco is presented in Fig. 3.

There were no differences in the identified phospholipids. The content of phosphatidylinositol (PI) was the highest – 43.0% (bio) and 41.7 % (con), followed by phosphatidylcholin (PC) – 27.3% (bio) and 29.9% (con) and phosphatidic acids – 10.1% (bio) and 8.6% (con). The levels of phosphatidyletanolamine, phosphatidylserine and other phospholipids were equal (from 1.0% to 2.5%).

CONCLUSION

The present study determines the seeds of the investigated tobacco variety Krumovgrad 58, grown in Bulgaria, as a potential source of glyceride oil rich in biologically active components. The chemical composition of seeds from organically grown tobacco plants from Krumovgrad 58 (bio) was examined and compared with the seeds from the same conventionally grown variety. The oil from organically grown tobacco seeds had a higher yield of oil and better oxidative stability than the conventionally grown tobacco seeds. The tobacco seeds are favorable for the production of vegetable oil whose oxidative stability is similar to that of sunflower oil – linoleic type. There was a higher tocopherol content in the tobacco seed oil from Krumovgrad 58 (bio).

The information about biologically active substances such as polyunsaturated fatty acids, tocopherols and phospholipids may be useful for determination of the nutritional value of this oil. The tobacco seed oil is a valuable source of healthy glyceride oil for human consumption and can be used in food and cosmetic industry in the future.

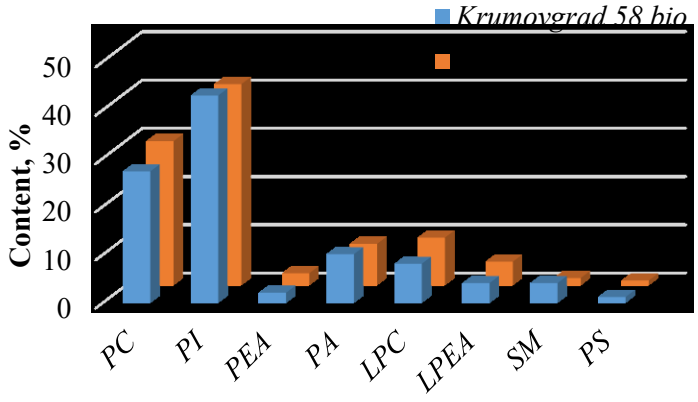

Fig. 3. Individual phospholipid content in tobacco seed oil from conventional and organic production in the two years of analysis.

*PC – Phosphatidylcholine; PI – Phosphatidylinositol; PEA – Phosphatidylethanolamine; PA – Phosphatidic acids; LPC – Lysophosphatidylcholine; LPEA – Lysophosphatidylethanolamine; SM – Sphingomyelin; PS – Phosphatidylserine.

REFERENCES

1. H. Bozukov, M. Kasheva, Y. Kochev, D. Vitanova, *Bulg. J. Agric. Sci*, **25** (4), 633 (2019).
2. H. Bozukov, *Bulg. Tobac.*, **1-2**, 8 (2018).
3. G. Mitev, *Tr. J. Sci.*, **17** (1), 572 (2019).
4. M. Z. Ashirov, U. M. Datkhayev, D. A. Myrzakozha, H. Sato, K. S. Zhakipbekov, N. A. Rakhymbayev, B. N. Sadykov, *Sci. World J.*, **2020** (2020).
5. I. T. Stanisavljević, D. T. Velič Ković, Z. B. Todorović, M. L. Lazić, V. B. Veljković, *Eur. J. Lipid Sci. Technol.*, **111** (5), 513 (2009).
6. M. Zlatanov, M. Angelova, G. Antova, *Bulg. J. Agric. Sci.*, **13** (5), 539 (2007).
7. ISO 659, Oilseeds. Determination of oil content (Reference method), 2014.
8. ISO 12966-1, 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, 2014.
9. ISO 12966-2, Animal and vegetable fat and oils. Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids, 2011.
10. ISO 18609, Animal and vegetable fats and oils. Determination of unsaponifiable matter. Method using hexane extraction, 2000.
11. S. Ivanov, P. Bitcheva, B. Konova, *Rev. Fr. Corps Gras*, **19** (3), 177 (1972).
12. ISO 12228-1, Animal and vegetable fats and oils. Determination of individual and total sterols contents. Gas chromatographic method, 2014.
13. ISO 9936, Animal and vegetable fats and oils. Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography, 2016.
14. J. Folch, M. Lees, G. H. Sloane-Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
15. R. Schneiter, G. Daum, Analysis of yeast lipids, Humana Press Inc., Totowa N. J., 2006.
16. ISO 10540-1, Animal and vegetable fats and oils. Determination of phosphorus content. Part 1: Colorimetric method, 2014.
17. ISO 6886, Animal and vegetable fat and oils. Determination of oxidation stability (Accelerated oxidation test) , 1996.
18. V. Popova, Z. Petkova, T. Ivanova, M. Stoyanova, L. Lazarov, A. Stoyanova, T. Hristeva, M. Docheva, V. Nikolova, N. Nikolov, V. Zheljazkov, *Ind. Crops Prod.*, **117** (3), 375 (2018).
19. M. A. Ali, M. A. Sayeed, R. K. Roy, S. Yeasmin, A. M. Khan, *Asian Jour. of Biochem.*, **3**, 203 (2008).
20. M. Zlatanov, M. Angelova, G. Antova, *Agric. Bohem.*, **38** (2) 69 (2007).
21. F. Gunstone, J. Harwood, Occurrence and characterization of oils and fats, in: The Lipid Handbook, CRC Press, Boca Raton, USA, 2007, p. 37.
22. M. L. Colombo, *Molecules*, **15** (4), 2103 (2010).
23. A. Blanco, G. Blanco, Med. Biochem., 1st edn., 2017, p. 645.
24. E. Aksoz, O. Korkut, D. Aksit, C. Gokbulut, *Flavour Fragr. J.*, **35** (5), 504 (2020).
25. Z. Xie, M. Whent, H. Lutterodt, Y. Niu, M. Slavin, R. Kratochvil, L. Yu, *J. Agric. Food Chem.*, **59** (18), 9877 (2011).
26. A. Blanco, G. Blanco, Med. Biochem., 1st edn., 2017, p. 99.
27. A. M. Giuffrè, M. Capocasale, *Int. Food Res. J.*, **23** (1), 116 (2016).
28. V. Van Hoed, N. De Clercq, C. Echim, M. Andjelkovic, E. Leber, K. Dewettinck, R. Verrhé, *J. Food Lipids*., **16** (1), 33 (2009).
29. J. Garavaglia, M. M. Markoski, A. Oliveira, A. Marcadenti, *Nutr. Metab. Insights*, **16** (9) 59 (2016).
30. B. Y. Guo, B. Wen, X. Q. Shan, S. Z. Zhang, J. M. Lin, *J. Chromatogr. A*, **1074** (1-2), 205 (2005).
31. M. Zlatanov, M. Angelova, E. Ivanova, *SP of PU – Chem.*, **34** (5) 145 (2006