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STEROL COMPOSITION OF OILS FROM BULGARIAN VARIETIES OF SUNFLOWER

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Abstract

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The composition of sterols and sterol esters and the fatty acid composition of sterol esters in the glyceride oil isolated from the seeds of nine Bulgarian sunflower varieties were examined. The total sterol content was estimated to be 3-4 g.kg^{-1} with the amount of free sterols - 658–790 g.kg^{-1} and that of sterol esters - 471–700 g.kg^{-1}. b-Sitosterol was the main component in both sterol fractions followed by stigmasterol and campesterol. Palmitic acid was the main fatty acid component (225–485 g.kg^{-1}) in sterol ester fraction, followed by oleic (203–420 g.kg^{-1}) and linoleic (81–176 g.kg^{-1}) acid.

Key words: sunflower oil, biologically active substances, fatty acids, sterols

Introduction

Sunflower (*Helianthus annus L.*) is among the most important oilseed crops in the world. The nutritional quality of the glyceride oil is very high, ranking the oil among the best commodity oils. Sunflower oil is widely used as salad oil, cooking and frying oil and in margarine production. The high nutritional value of the traditional sunflower oil is due to the high level (600-750 g.kg^{-1}) of the biologically active linoleic acid (*cis* 9, *cis* 12-18:2) and the low level of saturated fatty acids (Cole et al., 1998; Skoric et al., 2008). The oil is rich in sterols and tocopherols which are natural antioxidants and synergists preventing to some extent the autoxidation of lipids at ambient temperature.

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Sterol composition is an important indicator of the quality of vegetable oils due to the requirement for provide information about the content of cholesterol in foods. However, the information on the type and amount of sterols in new sunflower varieties is rather fragmentary as yet. In addition, separate data on free sterols and sterol esters; have not been published so far. In the other hand the knowledge of two sterol fractions wield data which, from a food technological, nutritional and a food legislative point of view, may be more useful than the total sterol pattern.

In this study the results on the content and composition of free and esterified sterols, and the fatty acid composition of sterol esters in oil samples isolated from the seeds of nine Bulgarian sunflower varieties are presented.

Materials and Methods

Samples

Seeds of the investigated sunflower varieties origin from the Plovdiv region in South Bulgaria, crop 2006. The study was carried out on air dried seeds in technical ripeness.

The oil was extracted in Soxhlet apparatus with hexane for 8 h. Then the solvent was removed in a rotary evaporator and the residue was weighed to determine the oil content.

Sterols

The total oil sample (sample size of 200 mg, precisely measured) was applied on a 20 cm x 20 cm glass plate with ca. 1 mm thick silica gel 60 G layer (*Merck*, Darmstadt, Germany) and developed with hexane-acetone, 100:8 (by volume) to a front of 19 cm. Free (R_f=0.4) and esterified (R_f=0.8) sterols were detected under UV light by spraying the edges of the plate with 2',7'-dichlorofluorescein and then they were scraped, transferred to small glass columns and eluted with diethyl ether. The solvent was evaporated under a stream of nitrogen, the residue was weighed in small glass containers to a constant weight. Finally, 2 % solutions in hexane were prepared. Free sterols were subjected to gas chromatography (GC) without derivatization. Sterol esters were hydrolyzed with ethanolic KOH (Ivanov et al., 1972), free sterols were extracted with light petroleum ether and purified by TLC under the above conditions. Sterol composition was determined on HP 5890 gas chromatograph (*Hewlett Packard GmbH*, Austria) equipped with 25 m x 0.25 mm HP5 capillary column (*Agilent Technologies*, Santa Clara CA, USA) and flame ionization detector (FID). The following temperature gradient was applied: from 90°C (held for 2 min) to 290°C at 15°C/min then to 310°C at 4°C/min and held at this temperature for 10 min; the injector temperature was 300°C and the detector temperature was 320°C. Nitrogen was the carrier gas at a flow rate of 0.8 cm^3/min; split 100:1. Identification was performed by comparison of the retention times with those of a standard mixture of sterols (*ISO* 12228,1999).

Fatty acids

The fatty acid composition of sterol esters was determined by GC after transmethylation of the respective sample with 2N methanolic KOH at 50°C according to Metcalfe and Wang (1981). Fatty acid methyl esters (FAME) were purified by silica gel TLC on 20 cm x 20 cm plates covered with ca. 1 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

Table 1

Content of oil in seeds and sterols in sunflower oils*

Sunflower variety	Content of oil in seed, $g.kg^{-1}$	Content of sterols in oil, $g.kg^{-1}$	Sterol fractions, $g.kg^{-1}$	
			Free sterols	Esterified sterols
Diamant	493	4	3.45	0.55
Maritza	480	3	2.64	0.36
Mura	438	3	2.54	0.46
Musala	468	3	2.14	0.86
Merqury	430	4	3.36	0.64
Perfect	506	4	3.42	0.58
Montana	448	3	1.88	1.12
San Luca	456	4	3.27	0.73
Albena	466	4	2.6	1.4

*Mean of three determinations

30 m x 0.25 mm capillary InnoWax column (cross-linked PEG, *Hewlett Packard GmbH*, Austria) and a FID. The column temperature was programmed from 165°C to 240°C at 4°C/min and held at this temperature for 10 min; injector and detector temperatures were 260°C. Nitrogen was the carrier gas at a flow rate of 0.8 cm^3/min; split was 100:1. Identification was performed by comparison of the retention times with those of a standard mixture of fatty acid methyl esters subjected to GC under identical experimental conditions (*ISO 5008*, 2000).

Results and Discussion

The data about oil and sterol contents of the samples are presented in Table 1 and reveal that seeds contain significant amount of glyceride oil (430-506 $g.kg^{-1}$). The sterol content in the oil was 3-4 $g.kg^{-1}$. This value is close to the sterol content in other sunflower oils (*CODEX-STAN* 210, 2003, 2005).

Expectedly, the major part of sterols was in a free form (625-879 $g.kg^{-1}$). The qualitative and quantitative composition of the free and esterified sterols is given in Table 2. β-Sitosterol predominates in both sterol fractions – 658-790 $g.kg^{-1}$ in free sterol fraction and 471-700 $g.kg^{-1}$ in sterol esters. The amount of stigmasterol components (total content of 90-183 $g.kg^{-1}$ in free sterol fraction and 90-204 $g.kg^{-1}$ in sterol esters) was relatively high. Significant quantities of campesterol and D^5-avenasterol were also found.

These amounts of sterols are different from the data reported by *Homberg* and *Bielefeld* (1989). Similarly, -sitosterol and stigmasterol were found in higher amounts and D^5-avenasterol - in lower amounts compared to the results of that authors (Homberg and Bielefeld, 1989).

A substantial difference in cholesterol content was found in free and esterified sterols. In sterol esters the cholesterol amount was significantly higher (6-45 g. kg^{-1} respectively) than in the free form (1-2 $g.kg^{-1}$). Similar results were reported earlier for other glyceride oils of *Apiaceae* (Zlatanov et al., 1998) and for tomato seed oils (Tiscornia et al., 1976; Kiosseoglou and Boskou, 1989). Total content of cholesterol in these oils is about 5-6 $mg.kg^{-1}$.

The other sterol components were present in insignificant quantities or in traces in all investigated varieties of sunflower.

Table 2

Sterol composition of free and esterified sterols, $g.kg^{-1}$*

Sterols, $g.kg^{-1}$	Variety																	
	Diamant		*Mariza*		*Mura*		*Musala*		*Merqury*		*Perfect*		*Montana*		*San Luca*		*Albena*	
	free	esterified	free	esterified	free	esterified	free	esterified	free	esterified	free	esterified	free	esterified	free	esterified	free	esterified
Cholesterol	1	45	1	7	1	6	1	7	1	6	1	7	2	7	1	7	1	6
Campesterol	66	46	69	62	85	85	83	93	91	101	92	96	80	65	111	120	102	155
Stigmasterol	130	123	96	96	68	68	77	77	99	89	115	100	71	79	109	93	89	109
D^7 -Campesterol	-	-	31	31	41	41	69	69	42	52	8	27	20	76	16	62	44	60
β -Sitosterol	790	471	739	700	689	664	706	650	689	644	700	689	710	677	663	598	658	559
D^5 -Avenasterol	5	234	22	62	68	88	47	77	37	67	6	37	80	56	30	42	26	50
D^7 -Stigmasterol	5	81	16	16	33	33	7	7	17	17	56	20	9	23	26	35	32	46
$D^{7,25}$ -Stigmasterol	1	-	18	18	10	10	6	6	14	14	12	13	20	13	25	28	45	10
D^7 -Avenasterol	2	-	8	8	5	5	4	14	10	10	10	11	8	4	19	15	3	5

*Mean of three determinations

Table 3
Fatty acid composition of sterol esters*

Variety	Fatty acid, g.kg^{-1}									
	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Diamant	27	8	225	5	5	125	420	160	20	5
Maritza	55	46	467	4	22	30	203	156	9	8
Mura	53	48	441	3	51	72	224	81	8	19
Musala	31	35	449	3	47	56	235	107	9	28
Merqury	48	59	391	3	14	61	259	153	3	9
Perfect	19	26	312	6	26	58	324	176	19	34
Montana	14	21	257	7	21	118	375	147	14	26
San Luka	43	32	485	5	11	38	205	143	13	25
Albena	31	38	304	2	18	80	380	115	6	26

* Mean of three determinations

Table 3 presents the fatty acid composition of sterol esters.

Palmititic (225-485 g.kg^{-1}) and oleic acid (203-420 g.kg^{-1}g) predominate in sterol esters, followed by linoleic acid (81-176 g.kg^{-1}). This fatty acid composition is different from that of triacylglycerols. The percentage of saturated fatty acids (mainly palmitic and stearic) were 395-684 g.kg^{-1} while the content of the saturated fatty acids in triacylglycerols was substantially lower (84-110 g.kg^{-1}) (Zlatanov et al., 2008). These data are in agreement with those reported earlier (Kiosseouglou and Boskou, 1989; Zlatanov et al., 1998). According to Munshi et al. (1982) the difference between the fatty acid compositions of triacylglycerols and sterol esters is due to different phases of the biosynthesis of these compounds and is closely related to the stages of biosynthesis and accumulation of fatty acids. Sterol esters are synthesized on an early stage of seed development, followed by accumulation of triacylglycerols. The early biosynthetic stage is characterized also by the high content of palmitic and stearic fatty acids, which, accordingly, are accumulated in sterol esters.

Conclusion

The total sterol content in the investigated sunflower oil is close to the values of other sunflower oils. The main part of sterols (more then 60%) is presented in free form and b-sitosterol predominates in both sterol fractions. The percentage of saturated fatty acid (mainly palmitic and stearic) in sterol esters is several times higher then in the corresponding triacylglycerols. The information about sterol composition may be useful for estimation of nutritional value of sunflower oil.

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