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Sterol and fatty acid composition of grape seed oils

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Dedicated to Acad. Dimiter Ivanov on the occasion of his 120th birth anniversary

The content and composition of sterols, sterol esters and fatty acid composition of the triacylglycerol and sterol esters fractions isolated from the seed oil of four Bulgarian grape varieties - *Super ran bolgar, Bolgar, Mavroud* and *Shiroka melnishka loza* - were investigated. The total sterol content was estimated to be 0.3-0.4% with an amount of free sterols - 93.4-97.0% and that of sterol esters - 3.0-6.6%. Sterol composition of both sterol fractions was determined by gas chromatography. β-Sitosterol was the main component in both sterol fractions (66.8-73.2%) followed by campesterol (7.1-24.5%) and stigmasterol (2.7-6.9%). The content of cholesterol and stigmasterol in sterol esters was found to be several times higher than in the fraction of free sterols (1.3-2.0% vs 0.5-0.7 % and 3.2-6.9% vs 2.7-3.3% respectively). On the contrary, the percentage of campesterol in the sterol esters (7.1-14.0%) was about two times lower than in the free sterols fraction (19.1-24.5%). Linoleic acid predominated in all samples of sterol esters followed by oleic and palmitic acids (40.7-53.8 %, 24.9-35.5% and 13.0-16.7% respectively); the content of these acids in the triacylglycerols fractions were in the ranges 68.5-72.3%, 16.3-18.7% and 8.8-11.5% respectively.

Key words: grape seed oil, sterols, sterol esters, fatty acids

INTRODUCTION

The fruits of grape (*Vitis vinifera* L.) are used as food for raw or dried consumption, as well as a source for the production of wine. Grape seeds represent a by-product obtained after winery exploitation and are a cheap renewable source for the production of glyceride oil. The content of glyceride oil in the seeds was found to be about 11-16% [1,2]. Besides, the seeds contain dietary fiber, galic acid, catechin, tannins [3,4]. The oil is rich in essential fatty acids, such as linoleic and linolenic, and contains some bioactive substances: sterols, tocopherols, carotenoids, phospholipids, polyphenols which have a significant role for the determination of its food value [4,5]. The seeds contain relatively small amounts of oil which is usually extracted by solvent but can also be obtained by pressing [6].

Sterols have a beneficial effect for the prevention of atherosclerosis and coronary heart diseases by lowering the levels of cholesterol. El-Shami *et al.* [7] and Fedeli *et al*. [8] reported predomination of β-sitosterol (73.8%) in sterol fraction followed by stigmasterol (13.7%) and campesterol (10.6%). According to Pironen *et al.* [9] the total quantity of sterols in the dry pomace was found to be 1390 mg/kg^{-1}, and the main constituents were β-sitosterol, campesterol and stigmasterol. The recent investigations of Madawala *et al*. [10] announced about 0.27% total sterol content in the oil and the sterol fraction included 0.5% brasicasterol, 10.3% campesterol, 9.2% stigmasterol, 74.9% β-sitosterol, 5.1% Δ^5-avenasterol. Cholesterol was not detected in the oil.

All these investigations have a bearing on composition of total sterol fraction. On the other hand, in glyceride oil, sterols are present in two forms - free sterols and sterol esters.

Sterol composition is an important indicator of the quality of vegetable oils due to the requirement for providing information about the content of cholesterol in foods. However, the information on the type and amount of sterols in grape varieties is rather fragmentary as yet. In addition, separate data on free sterols and sterol esters have not been published so far.

Linoleic acid was found to be the main (63.0-73.1%) constituent in triacylglycerols, followed by oleic (13.7-20.8%) and palmitic acids (6.2-8.5%) according to Fernandes *et al.* [11].

In this connection, the aim of this study was to investigate the seeds recovered from four Bulgarian grape varieties for oil content and to present the

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results about the content and composition of free and esterified sterols separated from the oils as well as the fatty acid composition of sterol esters and triacylglycerol fractions.

MATERIALS AND METHODS

Samples

The seeds of the investigated grape varieties *Super ran bolgar, Bolgar, Mavroud* and *Shiroka melnishka loza* were obtained from the Agricultural University, Faculty of Viticulture and Horticulture, Plovdiv, Plovdiv region in South Bulgaria, crop 2013. The study was carried out on air dried seeds in technical ripeness.

Glyceride oil

The oil was extracted in Soxhlet apparatus with hexane for 8 h [12]. Then the main part of the solvent was removed in a rotary evaporator, the residue of the solvent was evaporated under a stream of nitrogen and the samples were weighed to determine the oil content.

Sterols

The total oil sample (sample size about 200 mg, precisely measured) was applied on a 20 cm x 20 cm glass plate with 0.5 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, 1% solutions in chloroform were prepared. Free sterols were subjected to gas chromatography (GC) without derivatization.

Sterol esters

Sterol esters were hydrolyzed with ethanolic KOH [13]; free sterols were extracted with light petroleum ether and purified by thin layer chromatography under the above conditions. Sterol composition of both sterol fractions was determined without derivatization 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. Hydrogen was the carrier gas at a flow rate of 0.8 mL/min; split 100:1. Identification was performed by comparison of the retention times with those of a standard mixture of sterols [14]. The content of total sterols was determined by gas chromatography under the same conditions with betulin as an internal standard [14].

Fatty acids

The fatty acid composition of the triacylglycerols and sterol esters fractions were determined by GC after transmethylation of the respective sample with 2N methanolic KOH at 50°C according to Christie [13]. 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 capillary DB-23 column (Hewlett Packard GmbH, Austria) and a FID. The column temperature was programmed from 130°C (1 min) to 170°C at 6.5°C/min; at 3°C/min to 215°C (9 min), at 40°C/min to 230°C (1 min); injector and detector temperatures were 270°C and 280°C respectively. Hydrogen was the carrier gas at a flow rate 0.8 mL/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 [15].

All the analyses were made in triplicate. Data were expressed as mean ± SD.

RESULTS AND DISCUSSION

The data about oil content in the seeds and sterol content in the oils are presented in Table 1.

The investigated seeds contain low amount of glyceride oil. These values are close to data reported about some varieties of foreign origin [1,16,20]. The sterol content in the oils was found to be similar to data reported earlier by Piironen *et al*. [9] and Madawala *et al*. [10]. Expectedly, the major part of sterols (more than 90%) was in a free form. Since there is no information about free to esterified sterols ratio in grape seed oil, this profile was compared with sterol composition of other vege-

table oils. The quantity of free sterols was found to be rather higher than that in sunflower [17] and tomato [18] seed oils where this content was about 70-75%.

The qualitative and quantitative composition of the free and esterified sterols is given in Table 2.

The qualitative and quantitative sterol composition of the studied oils isolated from all grape varieties was found to be identical. β-Sitosterol predominated in both sterol fractions and was present in relatively close quantities. The content of campesterol in both fractions was relatively high but in the free sterol fraction its value was about 2 times higher than in sterol esters. On the other hand, the quantities of stigmasterol were about 2 times lower in the fraction of free sterols. A substantial difference in cholesterol content of free and esterified sterols fractions was also found. In sterol esters the cholesterol amount was significantly higher than in the free form. A similar ratio between the content of cholesterol in the free and sterol fractions was reported earlier for other glyceride oils of *Asteraceae* family [17] and for tomato seed oil [18]. This picture about ratio between free sterols and sterol esters is a result of different stages for biosynthesis of free sterols and sterol esters and, on the other hand, of different stages for biosynthesis of cholesterol. The presence of a higher content of cholesterol in sterols esters is ascribed to: (i) the fact that sterol esters are the first to biosynthesize and, (ii) cholesterol is biosynthesized firstly in this sterol fraction and is then used as an intermediate for the synthesis of the other sterols [19].

The other sterol components - brasicasterol, Δ^7-campesterol, Δ^5-avenasterol, Δ^7-avenasterol and Δ^7-stigmasterol - were present in insignificant quantities or in traces in all investigated varieties of grape seed oils.

These amounts of sterols are different from the data reported by El Shami *et al.* [7] and Madawala *et al.* [10], who found the quantities of campesterol to be considerably lower at the expense of the higher values of stigmasterol.

The fatty acid composition of the sterol esters and triacylglycerol fractions are given in Table 3.

Linoleic acid followed by oleic acid predominated in all sterol ester fractions as unsaturated fatty acids. Saturated fatty acids are presented mainly by palmitic and stearic acids. The other constituents are detected in negligible amounts (lower than 1.0%).

Significant differences were established between the separate species. The sterol fraction isolated from *Bolgar* and *Mavroud* seed oils contains higher percentages of oleic acid (32.1% and 35.5% respectively) at the expense of lower levels of linoleic acid (44.6% and 40.7% respectively). On the other hand, the content of oleic acid in the sterol esters of *Shiroka melnishka loza* and *Super ran bolgar* was found to be lower (28.3% and 24.9% respectively) while the quantity of linoleic acid was rather higher (48.6% and 53.8% respectively).

The qualitative and quantitative fatty acid profile of transmethylated triacylglycerols isolated from the oil of all four investigated grape species was similar. Linoleic acid (68.5-72.3%) predominated, followed by oleic and palmitic acids. The ob-

Table 1. Content of oil in the seeds and sterols in the oils*.

Variety	Oil content of seeds, wt %	Sterol content in oil, wt %	Sterol fractions, rel. % from total sterols	
			Free sterols	Esterified sterols
1. Bolgar	11.6 ± 0.2	0.4 ± 0.02	94.3 ± 1.9	5.7 ± 0.2
2. Super ran bolgar	16.5 ± 0.5	0.3 ± 0.01	93.4 ± 2.8	6.6 ± 0.2
3. Mavroud	15.7 ± 0.3	0.3 ± 0.01	93.5 ± 1.9	6.5 ± 0.1
4. Shiroka melnishka loza	13.9 ± 0.6	0.3 ± 0.01	97.0 ± 3.9	3.0 ± 0.1

*Mean ± SD of three determinations.

Table 2. Composition of free and esterified sterols* (rel. %).

Sterols	Varieties							
	Bolgar		*Super ran bolgar*		*Mavroud*		*Shiroka melnishka loza*	
	Free	Esterified	Free	Esterified	Free	Esterified	Free	Esterified
Cholesterol	0.5 ± 0.01	1.6 ± 0.05	0.6 ± 0.02	2.0 ± 0.1	0.5 ± 0.02	1.3 ± 0.04	0.7 ± 0.01	1.9 ± 0.04
Brasicasterol	2.6 ± 0.1	1.8 ± 0.1	2.6 ± 0.1	1.5 ± 0.1	2.4 ± 0.1	1.4 ± 0.04	1.8 ± 0.1	1.9 ± 0.1
Campesterol	20.5 ± 0.4	11.7 ± 0.2	19.1 ± 0.8	7.1 ± 0.1	20.0 ± 0.4	14.0 ± 0.6	24.5 ± 0.7	12.2 ± 0.5
Stigmasterol	3.2 ± 0.1	6.7 ± 0.3	3.3 ± 0.1	6.0 ± 0.1	3.3 ± 0.2	6.9 ± 0.1	2.7 ± 0.1	3.2 ± 0.1
Δ^7-Campesterol	1.3 ± 0.1	2.4 ± 0.05	1.0 ± 0.02	2.9 ± 0.1	1.7 ± 0.1	2.4 ± 0.1	1.8 ± 0.04	2.7 ± 0.1
β-Sitosterol	70.2 ± 1.4	72.5 ± 2.9	72.1 ± 1.4	72.8 ± 2.2	70.4 ± 1.4	70.9 ± 2.1	66.8 ± 1.3	73.2 ± 2.2
Δ^5-Avenasterol	0.4 ± 0.01	1.5 ± 0.1	0.4 ± 0.02	1.5 ± 0.03	0.5 ± 0.01	1.4 ± 0.1	0.4 ± 0.02	2.0 ± 0.1
Δ^7-Avenasterol	0.8 ± 0.02	1.4 ± 0.04	0.5 ± 0.02	2.3 ± 0.1	0.6 ± 0.02	0.8 ± 0.02	0.5 ± 0.02	1.2 ± 0.04
Δ^7-Stigmasterol	0.5 ± 0.02	2.4 ± 0.05	0.3 ± 0.01	3.9 ± 0.1	0.6 ± 0.01	0.9 ± 0.02	0.9 ± 0.02	1.7 ± 0.1

*Mean ± SD of three determinations.

Table 3. The fatty acid composition of sterol esters and triacylglycerols* (rel. %).

Fatty acids	Grape varieties							
	Bolgar		***Shiroka melnishka loza***		***Mavroud***		***Super ran Bolgar***	
	Sterol esters	**Triacyl-glycerols**	**Sterol esters**	**Triacyl-glycerols**	**Sterol esters**	**Triacyl-glycerols**	**Sterol esters**	**Triacyl-glycerols**
Lauric ($C_{12:0}$)	0.1 ± 0	0.4 ± 0.02	0.3 ± 0.01	0.1 ± 0	0.3 ± 0.02	0.4 ± 0.02	0.1±0	-
Myristic ($C_{14:0}$)	0.5 ± 0.02	0.1 ± 0	0.5 ± 0.01	0.1 ± 0	1.3 ± 0.04	0.1 ± 0	0.2 ± 0.01	0.1 ± 0
Pentadecanoic ($C_{15:0}$)	0.2 ± 0.01	-	0.1 ± 0	-	0.3 ± 0.01	0.1 ± 0	0.1 ± 0	-
Palmitic ($C_{16:0}$)	16.7 ± 0.3	11.5 ± 0.5	14.4 ± 0.4	10.0 ± 0.4	16.4 ± 0.7	8.8 ± 0.4	13.0 ± 0.3	8.8 ± 0.4
Palmitoleic ($C_{16:1}$)	0.1 ± 0	0.2 ± 0.01	0.1 ± 0	0.3 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.1 ± 0	0.1 ± 0
Margarinic ($C_{17:0}$)	0.3 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0	0.2 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
Stearic ($C_{18:0}$)	4.2 ± 0.1	1.0 ± 0.03	6.7 ± 0.3	0.7 ± 0.03	3.5 ± 0.1	1.0 ± 0.1	6.1 ± 0.3	0.8 ± 0.03
Oleic ($C_{18:1}$)	32.1 ± 1.3	17.6 ± 0.4	28.3 ± 1.1	16.3 ± 0.7	35.5 ± 1.1	18.7 ± 0.7	24.9 ± 1.2	17.3 ± 0.7
Linoleic ($C_{18:2}$)	44.6 ± 0.9	68.5 ± 2.7	48.6 ± 1.9	71.3 ± 2.9	40.7 ± 1.2	70.1 ± 2.1	53.8 ± 2.2	72.3 ± 2.9
Linolenic ($C_{18:3}$)	0.2 ± 0.01	0.3 ± 0.01	0.1 ± 0	0.5 ± 0.02	0.3 ± 0.01	0.2 ± 0.01	0.1 ± 0	-
Arahinic ($C_{20:0}$)	0.6 ± 0.02	0.2 ± 0.01	0.3 ± 0.01	0.2 ± 0.01	0.8 ± 0.03	0.1 ± 0	0.2 ± 0.01	0.1 ± 0
Gadoleic ($C_{20:1}$)	0.2 ± 0	0.1 ± 0	0.2 ± 0	0.3 ± 0.01	0.3 ± 0.01	0.1 ± 0.01	0.9 ± 0.04	0.1 ± 0
Behenic ($C_{22:0}$)	0.2 ± 0	-	0.3 ± 0.02	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0	0.3 ± 0.01	0.3 ± 0.01

*Mean ± SD of three determinations.

tained data are close to results reported earlier by [20,21].

This fatty acid profile of the transmethylated triacylglycerol fraction is to a considerable extent different from this of sterol esters. The amount of linoleic acid in triacylglycerols of all investigated varieties was higher at the expense of the lower level of oleic acid. On the other hand, the content of palmitic (8.8-11.5%) and stearic acids (0.7-1.0%) showed decreasing in comparison with fraction of sterol esters.

CONCLUSION

Sterol composition of the glyceride oils isolated from seeds of four Bulgarian grape varieties was found to be similar. The main part of sterol fraction was in free form. β-Sitosterol predominated in both sterol fractions but the content of campesterol in free form was higher than in sterol esters at the expense of the lower quantities of cholesterol and stigmasterol. The qualitative fatty acid profile of sterol esters and triacylglycerols was found to be similar, but higher amounts of saturated and mono-unsaturated fatty acids were present in sterol esters than in triacylglycerols.

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СТЕРОЛОВ И МАСТНО-КИСЕЛИНЕН СЪСТАВ НА МАСЛО ОТ ГРОЗДОВИ СЕМКИ

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(Резюме)

Изследвано е съдържанието и състава на стеролите, стероловите естери, техния мастно-киселинен състав, както и този на триацилглицероловите фракции, изолирани от семената на четири български сорта грозде: *Супер ран Болгар, Болгар, Мавруд и Широка мелнишка лоза*. Общото съдържание на стероли в маслото е 0.3-0.4%, като свободните стероли са 93.4-97.0%, а стероловите естери-3.0-6.6%. Индивидуалният състав е определен чрез газова хроматография. Основният компонент в двете стеролови фракции е β-ситостерол (66.8-73.2%), следван от кампестерол (7.1-24.5%) и стигмастерол (2.7-6.9%). Съдържанието на холестерол и стигмастерол в стероловите естери е няколко пъти по-високо, отколкото във фракциите на свободните стероли (1.3-2.0% срещу 0.5-0.7% и 3.2-6.9% срещу 2.7-3.3%). Обратно, съдържанието на кампестерол в стероловите естери (7.1-14.0%) е около два пъти по-ниско отколкото в свободната форма (19.1-24.5%). Основните мастни киселини, които преобладават в стероловите естери са линолова (40.7-53.8%), олеинова (24.9-35.5%) и палмитинова (13.0-16.7%); съдържанието на тези киселини в триацилглицеролите е съответно 68.5-72.3%, 16.3-18.7% и 8.8-11.5%).