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COMPARATIVE STUDY OF SEED COMPOSITION OF WHITE AND RED GRAPE VARIETIES

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ABSTRACT: *The composition of grape seeds was investigated. The content of proteins was found to be 6.3-8.9%, of carbohydrates – 65.5-70.9%, of glyceride oil - 11.6 16.5%. The content of phospholipids, mainly phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidic acids in the oils, was 0.6-0.9%. The sterol content was 0.3-0.4% while β-sitosterol (70.0-72.1%) and campesterol (18.5-19.9%) were the major components. In the phospholipids and sterol esters higher amount of saturated and monounsaturated fatty acids was established than in triacylglycerols. The quantity of palmitic acid was 43.4-59.4%, of oleic – 9.0-14.0% and of linoleic – 10.0-24.5% while in sterol esters, the predominant ones were mainly linoleic (40.7-53.8%), oleic (24.9-35.5%) and palmitic acid (13.0-16.7%). Linoleic (68.5-72.3%) and oleic acids (16.3-18.7%) presented as LLL, LLO and LLP were found to be the main components in the triacylglycerols. The tocopherol content varied from 67.7mg/kg to 290.5mg/kg where α-tocopherol (74.8-84.4%) was the predominant component.*

KEYWORDS: Grape Seed Oil, Lipids, Carbohydrates, Proteins.

INTRODUCTION

Grape seeds comprise about 5% of the fruit weight (Choi and Lee, 2009) and more than 3 million tons of them are discarded annually worldwide (Fernandes *et al*., 2012) (in Bulgaria 10 000 - 20 000 tons annually). They are an important part of the pomace, corresponding to 38-52% of dry matter (Maier *et al.*, 2009). In fact, grape seeds are often referred as a significant agricultural and industrial waste (Luque-Rodriguez *et al.*, 2005; Freitas *et al.*, 2008; Kim *et al.*, 2008; Lutterodt *et al.*, 2011), a by-product of winemaking containing significant amounts of glyceride oil, proteins, carbohydrates, etc.

Grape seed oil is used for salad dressings, marinades, deep frying, flavored oils, baking, massage oil, sunburn repair lotion, hair products, body creams, lip balm and hand creams (Akin and Altindisli, 2011). It is a good source of polyunsaturated fatty acids (PUFAs), vitamins and antioxidants. For this reason it is used for the prevention of a variety of diseases, such as thrombosis, cardiovascular diseases, reduction of cholesterol in serum, dilation of blood vessels, cancer reduction and regulation of autonomic nerves (Luque-Rodrguez *et al.*, 2005; Yi *et al*., 2009).

Razuvaev (1980) established about 8-10% oil, 44-57% cellulose, 3.7% tannins and 1-2% mineral substances. Campos *et al.*, (2008) reported that the grape seeds contain about 40% fibres, 11% proteins, 7% phenolic compounds, as well as sugar and minerals. According to Kamel *et al.*, (1985), the seeds contain 8.2% crude proteins. Mironeasa *et al.*, (2010) announced

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28% cellulose, 4-6% tannins, 10-25% glyceride oil, 2-4% minerals. Licev *et al*., (1974) indicated about 10-11% cellulose, 4-6% tannins, 20% glyceride oil.

The oil content of grape seeds varies between 10-20% (Canbay and Bardakc, 2011; Campos *et al.*, 2008; Schuster, 1992; Ahmandi and Siahsar, 2011; Ohnishi *et al.*, 1990; Baydar and Akkurt, 2001; Schieber *et al.*, 2002; Luque-Rodriquez *et al*., 2005; Martinello *et al*., 2007; Baydar *et al*., 2007; Sabir *et al*., 2012) depending on grape variety and agrocultural conditions.

Phospholipid composition and fatty acid profile of phospholipids were not investigated.

The total sterols in grape seed oil were submitted in considerably small amounts (0.27%) and sterol fraction included 0.5% brasicasterol, 10.3% campesterol, 9.2% stigmasterol, 74.9% β-sitosterol, 5.1% Δ^5-avenasterol (Madawala *et al*., 2012). Cholesterol was not detected. β-Sitosterol was the main component (73.9%) followed by stigmasterol (13.7%) and campesterol (10.6%) according El-Shami *et al*., (1999) and Fedeli *et al*., (1966). There is no information about the ratio between free and esterified sterols, nor about fatty acid composition of the sterol esters.

Triacylglycerols (TAGs) are complex molecules present in all oils and fats. They serve mainly as energy stores, but are also employed as carriers of fatty acids within aqueous solutions such as blood. TAGs are made up of three fatty acids, attached to a glycerol backbone by an ester linkage (Nollet L., 2004). Linoleic and oleic acids predominate in the glyceride oil (Akhter *et. al.,* 2006; Ramos *et. al.,* 2009). Data about triacylglycerol structure were reported by Tuberso *et al.,* (2007). LLP, LLO, and LLL (L - linoleic acid, O – oleic acid and P – palmitic acid residues) were found to be the major components. According to Barron *et al.*, 1988 and Bail *et al.,* 2008, the content of LLL (35.8 % and 34-9-41.8 % respectively) is higher than the one in the data reported by Tuberso *et al.,* (2007) (20.7 %). They also reported higher amounts of other TAG species such as LPL, LOP, LOL and LSL.

The amount of tocopherols in grape seed oil ranged between 153.1-260.5 mg/kg according to Sabir *et al*., (2012) and between 121-829 mg/kg according to Cliszczynska-Swiglo *et al.*, (2007) in the results of different kinds of oils or different oil producers. Kraujalyte *et al*., (2011) and Sabir *et al*., (2012) reported a predomination of α-tocopherol but α-tocotrienol, γ-tocopherol and γ-tocotrienol were also detected.

The aim of the present study is to characterize the general composition of grape seeds including proteins, carbohydrates, ash, the content and composition of biologically active substances such as phospholipids, sterols and tocopherols; as well as fatty acid profile of triacylglycerols, individual phospholipids and sterol esters of glyceride oils isolated from seeds of Bulgarian grape varieties with a view to estimate the values of the oil as functional food with therapeutical effect, salad oil and for preparing cosmetic products and the seeds for source of food supplements.

MATERIALS AND METHODS

Samples. Grape seeds were collected from four grape varieties: *Bolgar, Super ran Bolgar* are white; *Mavroud* and *Shiroka melnishka loza* are red cultivars (vintage 2013). They were provided by the Agricultural University, Faculty of Viticulture and Horticulture, Plovdiv,

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Plovdiv region in South Bulgaria. All harvested grape seeds were subjected to air-drying before the analyses and the moisture content was found to be in the range from 6.2% to 8.2% wt.

Reagents: All reagents are with analytical grade of purity (p. a).

Isolation of glyceride oil. The dried grape seeds were milled by a grinder just before extraction using Soxhlet apparatus with hexane for 8 h (ISO 659, 2009). Solvent recovery was made in rotary distillation unit and the remaining amount of the solvent was evaporated under a stream of nitrogen. The oil obtained was weighted and then the yield was calculated as a percentage and kept in stoppered bottles in the refrigerator.

Determination of protein content. Total proteins were established by Kjeldhal method according to ISO 20483, 2007, using VELP scientifica apparature including DK 6 Heating digester, JP water aspirator and UDK 127 Distillation unit. The percentage of proteins was calculated using Factor 6.25.

Determination of carbohydrate composition. Carbohydrate content was calculated as the difference of mean values: 100 - (percentage in percentage of oil, moisture, protein and ash (Besbes *et al.*, 2004). The amount and composition of the soluble carbohydrates were determinated by high performance liquid chromatography (HPLC) on a Agilent® LC 1220 (USA) instrument equipped with Zorbax Carbohydrate column (150mm x 4.6 mm, 5μm, Agilent) and ZorbaX Reliance Cartridge-guard-column (Agilent) and refractive index detector (RID 1260). The mobile phase was AcN/H_2O 80:20, at a flow rate 1.0 mL/min. The soluble carbohydrates were identified by comparing their retention times with those of standards (individual pure mono- and disaccharides with purity ≥98%).

Determination of ash. From defatted meal, 2 g of each sample were weighed and put in a crucible and then incinerated in an oven at 600^0 C for 6h. The obtained ash was weighed (ISO 2171:2009).

Determination of phospholipids. The air-dried seeds were subjected to Folch extraction according to Christie (2003). After that phospholipids were recovered from glyceride oil by hydration with 1.5-3.0% water by weight to oil at 76-82^0C (Pierce, 1996). The phospholipid classes were isolated by two-dimensional thin-layer chromatography as follows: the sample (0.1 cm^3) was poured out drop by drop on 20 x 20 cm glass plates with 0.2 mm Silica gel 60 G layer impregnated with 1% aqueous $(NH_4)_2SO_4$. In the first direction the plate was developed with chloroform:methanol:ammonia, 65:25:5 (by volume) and in the second – with chloroform:methanol:ammonia:acetic acid:water, 50:20:10:10:5 (by volume) (Zlatanov *et al.*, 2009). The individual phospholipids were detected and identified by spraying with specific reagents according to Christie (2003): Dragendorff test (detection of choline-containing phospholipids); Ninhydrin spray (for phospholipids with free amino groups) and Shiff's reagent (for inositol containing phospholipids). Additional identification was performed by comparing the respective R_f values with those of authentic commercial standards subjected to Silica gel 60 G TLC under identical experimental conditions. The quantification was carried out spectrophotometrically against a standard curve by measuring the phosphorous content at 700 nm after scraping the respective phospholipid spot and mineralization of the substance with a mixture of perchloric acid: sulphuric acid, 1:1 by volume. The calibration curve was constructed by using a standard solution of KH_2PO_4 (1-130 μg/ml as phosphorus).

Determination of sterols. The glyceride oil (sample size of 5g, precisely measured) was hydrolyzed with 50 cm^3 ethanolic KOH (Christie, 2003) for one hour. Then 50 ml of water

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was added and the unsaponifiable lipids were extracted five times with 30 ml n-hexane. The solvent was removed by rotary evaporator. Unsaponifiables were dried in an oven at 105°C for 30 min. Thereafter the sample was dissolved in chloroform and purified by TLC on 0.2 mm Silica gel 60 G plates impregnated with 0.2 N NaOH, mobile phase n-hexane: diethyl ether (1:1). Then sterol composition was determinated by GC with HP 5890 (Hewlett Packard GmbH, Austria) Data Apex Clarity™ chromatography station for Windows ™ (Data Apex Ltd 2006) equipped with 30 m x 0.25 mm DB-5 column (Agilent Technologies, Santa Clara CA, USA) and flame ionization detector. The temperature gradient ranged from 90°C (2 min) to 290°C (15 min) and then increased to 310°C at 4°C/min (hold 10 min). The injector and detector temperatures were 300°C and 320°C. Hydrogen was used as a carrier gas at 0.8 mL/min velocity, split 50:1. Identification was confirmed by comparison of retention times with those of a standard mixture of sterols (Zlatanov *et al*., 2009).

Determination of fatty acid composition. Separation of fatty acid fraction of triacylglycerols, individual phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidic acids) as well as sterol esters was performed using thin-layer chromatography (TLC). Briefly, the sample (1ml solution of phospholipid concentrate) was applied on 20 cm x 20 cm glass plates with 0.2 mm Silica gel 60 G layer impregnated with 1% aqueous solution of $(NH_4)_2SO_4$. The plate was developed with chloroform : acetone : methanol : acetic acid : water 50 : 20 : 10 :10 : 5 (by volume). The bands were visualised by iodine treatment, detected and scraped. After that the individual phospholipids were eluated by Folch reagent (chloroform : methanol 2:1). Sterol esters were isolated from the glyceride oil also by TLC. Sample size of 2-2.5g (precisely measured) was applied on several 20 cm x 20 cm glass plates with 0.5 mm thick Silica gel 60 G layer (*Merck*, Darmstadt, Germany) and developed with n-hexane:diethyl ether:acetic acid (80:20:1) (by volume). The fatty acid composition of phospholipids, sterol esters and triacylglycerols was determined by gas chromatography (GC) of fatty acid methyl esters (FAME) (ISO 5508, 1990) after transmethylation at 50^0C with sulfuric acid in methanol as catalyst (Christie, 2003). Fatty acid methyl esters (FAME) were purified by TLC on 20x20 cm plates covered with 0.2 mm Silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase n-hexane:diethyl ether 97:3 (by volume). Gas chromatographic analysis of them were performed on a HP 5890 apparatus (Hewlett Packard GmbH, Austria) equipped with flame ionization detector (FID), 60 m x 0.25 mm capillary DB-23 column (Agilent Technologies, Santa Clara CA, USA). 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 of 0.8 mL/min; split was 50:1. The identification of FAME was based on external standard mixtures (37 components standard mixture provided by Agilent Technology). Each of the experiments was repeated three times.

Determination of triacylglycerol structure. For the identification and quantification of triacylglycerol (TAG) fraction, preliminary isolated by thin-layer chromatography (TLC) (Christie, 2003) a 3 mg/mL solution in dichloroethane by reserved phase high-performance liquid chromatography (RP-HPLC) was used. The analysis was performed on Agilent 1100 liquid chromatograph (Agilent Technologies, USA) equipped with a Rheodyne injector (7725i Rheodyne, USA) and evaporative light-scattering detector (ELSD, PL-ELS 1000 Polymer Laboratories Inc., Amherst MA, USA) programmed at evaporator temperature of 82^0C and air pressure of 1.2 atm. A ChemStation Rev.B.04.03 (Agilent Technologies, USA) software was used. Separation was performed with 250 x 4.6 mm Inertsil 5 ODS-2 column (Varian Inc., Palo Alto CA, USA). A 150 min step-wise gradient was applied at a flow rate of 1 mL/min with

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mobile phase of: A – dichloromethane, B – acetonitrile, C – iso-propanol and a gradient program as follows: from 10 % A: 80 % B: 10 % C to 17 % A: 83 % B: 10 % C over 40 min, kept at this for 20 min and changed to 35 % A: 55 % B: 10 % C over 90 min. The column temperature was thermostated at 30°C (Agilent 1100 Series G1316A compartment). TAG species in the sample were determined by spikes of authentic standard TAG separated previously in laboratory conditions from natural plant vegetable oils by silver ion TLC (Ag-TLC). The standards were identified by their fatty acid composition. The component molecular TAG varieties (if any) were fixed by RP-HPLC under the same experimental conditions. The size of each peak was estimated according to the relative content of the target component in the sample. The quantification of TAG was done by precise calibration of the ELSD with monoacid standard TAG: LnLnLn, LLL, OOO, PPP, SSS (Ln – linolenic acid, L – linoleic acid, O – oleic acid, P – palmitic acid and S – stearic acid residues). The linear interval of detector response was determined (1.5 to 22 μg TAG in 20 μL) and the respective linear equations were obtained. The TAG quantities were fixed respectively and the evaluated TAG fatty acid composition was compared with that determined directly by GC-FID.

Determination of tocopherols. The total tocopherol content and individual composition were analyzed directly in the oil with a high performance liquid chromatography (HPLC) (ISO 9936, 2006) on a Merck-Hitachi unit (Merck, Darmstadt, Germany) equipped with a 250 x 4 mm Nucleosil Si 50-5 column (Macherey-Nagel, Germany) and fluorescent detector (Merck-Hitachi F 1000). The operating conditions were as follows: mobile phase n-hexane: dioxane =96:4, excitation 295 nm, emission 330 nm, flow rate of 1 ml/min. The peaks were identified by comparing the retention times for the oil samples and pure standard solutions of tocopherols and tocotrienols (Merck Darmstadt, Germany) of known concentracion of them. The amount of tocopherols in the oil was calculated as the sum of tocopherol components and the total content was expressed in mg/kg.

Statistical analysis. All the analyses were made in triplicate. Statistical differences between samples were tested by one-way analysis of variance (ANOVA) using SAS version 8.1 (SAS Institute, Cary, NC, USA). Data were expressed as mean ± SD. The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The general chemical composition of the tested seeds is shown in Table 1

Compounds	**Varieties**			
	Bolgar	***Super ran Bolgar***	***Mavroud***	***Shiroka melnishka loza***
Oil content, %	11.6 ± 0.2	16.5 ± 0.7	15.7 ± 0.5	13.9 ± 0.3
Moisture, %	8.2 ± 0.2	6.5 ± 0.3	7.8 ± 0.3	6.2 ± 0.2
Proteins, %	6.9 ± 0.3	6.3 ± 0.1	8.9 ± 0.4	8.9 ± 0.4
Ash, %	2.4 ± 0.1	2.5 ± 0.1	2.1 ± 0.1	2.8 ± 0.1
Carbohydrates,% -unsoluble -soluble	70.9 ± 2.1 67.3 ± 2.7 3.6 ± 0.1	68.2 ± 1.4 63.1 ± 1.9 5.1 ± 0.2	65.5 ± 2.0 59.2 ± 1.2 6.3 ±0.3	68.2 ± 2.7 64.0 ± 2.6 4.2 ± 0.2

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Table 1 General composition of grape seeds*

*Means ± SD of three determination

The investigated grape seeds contained relatively low amounts of glyceride oil in comparison with other vegetable oils. The highest oil content (16.5%) was found to be in *Super ran Bolgar* seeds. These amounts were similar to data reported early by Karimian *et al* (2012) but lower than the percentages established by Razuvaev (1980). The moisture content ranged between 6.2% and 8.2% and these values were lower than the data reported by Ahmandi and Siahsar (2011). The seeds of red wine varieties - *Mavroud* and *Shiroka melnishka loza* contain higher levels of proteins (8.9%) than the seeds of white cultivars. For all tested varieties the amounts of carbohydrates varies in relatively narrow limits (65.5-70.9%). The main part of them was unsoluble carbohydrates (59.2-67.3%) while a higher amount was detected in *Bolgar*. These data were similar to the results reported earlier by Kamel *et al.,* (1985) and Mironesa *et al.,* (2010) but higher than the percentages for other Bulgarian cultivars announced by Licev *et al.,* (1974) as well as by Razuvaev (1980) which established significantly lower quantities of carbohydrates (44.0-57.0%).

Individual composition of soluble carbohydrates was presented in Table 2

Saccharides	***Bolgar***	***Super ran bolgar***	***Mavroud***	***Shiroka melnishka loza***
Monosaccharides,% wt	24.3 ± 1.0	13.5 ± 0.5	42.2 ±1.7	21.1 ± 0.4
Fructose	16.2 ± 0.5	33.9 ± 1.0	18.7 ± 0.6	20.1 ± 0.6
Glucose	40.6 ± 1.6	43.5 ± 0.9	44.4 ± 1.8	38.2 ± 1.5
Galactose	39.2 ± 1.6	17.6 ± 0.7	30.9 ± 1.2	27.4 ± 1.4
Xylose	2.4 ± 0.1	1.8 ± 0.1	2.7 ± 0.1	3.5 ± 0.1
Rhamnose	1.2 ± 0.1	3.1 ± 0.1	2.8 ± 0.1	10.8 ± 0.3
Arabinose	0.4 ± 0.02	-	0.5 ± 0.02	-
Disaccharides,% wt	56.5 ± 2.3	70.2 ±2.8	47.7 ± 1.4	72.6 ± 2.9
Saccharose	8.4 ± 0.3	4.9 ± 0.2	5.4 ± 1.6	7.8 ± 0.4
Maltose	3.7 ± 0.1	3.0 ± 0.1	2.3 ± 0.1	3.6 ± 0.1
Iso-maltose	87.9 ± 3.5	82.1 ± 3.3	91.5 ± 2.7	85.6 ± 2.6
Trisaccharides,% wt	19.2 ± 0.8	16.3 ± 0.7	10.1 ± 0.4	6.3 ± 0.3

Table 2 Composition of soluble saccharides*****

*Means ± SD of three determination

In the fraction of soluble carbohydrates the major part is disaccharides (47.7-72.6%). Higher quantities of them were detected in the seeds of *Super ran Bolgar* and *Shiroka melnishka loza*. Highest percentages of monosaccharides were established in the seeds of *Mavroud* (42.2%) at the expense of the lower content of trisaccharides (10.1%). Glucose, galactose and fructose were the predominant components in all monosaccharide fractions, while iso-maltose is the main constituent in trisaccharide fractions (more than 82.1%) in all varieties. The content of trisaccharides varies - in white wine varieties their level was found to be considerably higher.

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The observed individual profile of soluble carbohydrates is different to the data reported by Rondeou *et al.*, (2013) for grape pomace. Significantly higher quantities of fructose and galactose (18.7% and 30.0% respectively v/s 0.6% and 3.9% respectively according Rondeou *et. al.*, 2013) were observed in pomaces, at the expense of lower values of glucose and xylose (44.5% and 2.7% v/s 65.5% and 13.9% respectively).

General characteristic of glyceride oils are given in Table 3

Variety	Phospholipids, % wt.	Sterol, %	Sterol fractions, ratio		Tocopherols, mg/kg
			Free sterols	Esterified sterols	
1. Bolgar	0.8 ±0.03	0.4 ± 0.02	94.3 ± 1.9	5.7 ± 0.2	269.5 ± 5.4
2. Super ran bolgar	0.6 ± 0.02	0.3 ± 0.01	93.4 ± 2.8	6.6 ± 0.2	67.7 ± 2.7
3.Mavroud	0.9 ± 0.04	0.3 ± 0.01	93.5 ± 1.9	6.5 ± 0.1	156.3 ± 4.7
4.Shiroka melnishka loza	0.9 ± 0.04	0.3 ± 0.01	97.0 ± 3.9	3.0 ± 0.1	290.5 ± 5.8

Table 3 Content of biologically active substances in the oils*

*Means ± SD of three determination

The sterol content in the oils was found to be similar to the data reported earlier by Madawala *et al*., (2012) and Piironen *et al.*, (2003). The main part of sterol fraction was presented in free form (more than 97.0%) like other vegetable oils as sunflower (Zlatanov et *al.*, 2009) and lalemantia (Zlatanov *et al.*, 2012). Tocopherol content ranges in relatively large limits from 67.7 mg/kg in the oil of *Super ran Bolgar* to 290.5 mg/kg in the oils of other grape varieties. The tocopherol level in other varieties: *Bolgar, Shiroka melnishka loza* and *Mavroud* were close to the quantities established by Sabir *et al.*, (2012).

The samples were also analysed to determine the individual composition of phospholipid fraction (Table 4).

Phospholipids, %	***Bolgar***	***Super ran Bolgar***	***Mavroud***	***Shiroka melnishka loza***
Phosphatidylcholine	16.0 ± 0.5	16.4 ± 0.5	23.8 ± 1.0	16.2 ± 0.6
Phosphatidylinositol	28.0 ± 0.6	49.1 ± 2.0	33.9 ± 1.4	32.2 ± 1.0
Phosphatidylethanolamine	17.3 ± 0.7	13.1 ± 0.5	12.2 ± 0.4	10.3 ± 0.4
Phosphatidic acids	25.3 ± 0.5	8.2 ± 0.4	14.1 ± 0.6	30.2 ± 1.2
Phosphatidylserine	2.7 ± 0.1	2.6 ± 0.1	1.8 ± 0.1	2.2 ±0.1
Lysophosphatidylcholine	1.6 ± 0.1	3.2 ± 0.1	4.2 ± 0.2	1.1 ± 0.04
Lysophosphatidylethanolamine	1.4 ± 0.1	3.4 ± 0.1	2.6 ± 0.1	1.0 ± 0.03

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Diphosphatidylglycerol	2.7 ± 0.1	2.0 ± 0.3	3.7 ± 0.1	2.3 ± 0.1
Monophosphatidylglycerol	2.8 ± 0.1	1.1 ±0.1	1.2 ±0.1	2.4 ± 0.1
Sphingomyelin	2.2 ± 0.1	0.9 ± 0.02	2.5 ± 0.1	2.3 ± 0.1

Table 4 Individual phospholipid composition in grape seed oil*

*Means ± SD of three determination

There is no information about the composition of phospholipids in grape seed oils for comparison but its qualitative profile was found to be close to that of other vegetable oils, such as sunflower, rape etc (Gunstone, 2007). Phospholipids include all classes typical for these oils. The major component was phosphatidylinositol (PI) (28.0 - 49.1%), followed by phosphatidic acids (PA), phosphatidylcholine (PC) and phosphatidylethanolamine (PEA). The qualitive composition of the studied oils was found to be close but sizable differences were established in the quantitative profile. The highest amount of phosphatidylinositol was found in *Super ran Bolgar* variety (49.1%) at the expense of a lower quantity of phosphatidic acids, while in other oils their content varied from 28.0% to 32.2%. The variance of the phosphatidylcholine and phosphatidylethanolamine was in more close limits. In comparison with other plant oils an unusual high content of phosphatidic acids was determined. This fact probably is a result of hydrolysis processes or unfinished stage of their biosynthesis in the oil. A relatively high level of sphingomyelin (0.9-2.5%) was also established while in other oils it was detected in insignificant amounts or in traces. This individual quantitative composition is different from the phospholipid profile of other vegetable oils such as sunflower, rape, corn oil where the phosphatidylcholine and phosphatidylinositol are the predominant components (Gunstone 2003).

General sterol composition of the cultivars used for the investigations is given in Table 5.

Sterols,%	**Varieties**			
	Bolgar	***Super ran bolgar***	***Mavroud***	***Shiroka melnishka loza***
Cholesterol	0.8 ± 0.02	0.7 ± 0.02	0.6 ± 0.02	0.7 ± 0.02
Brasicasterol	2.6 ± 0.1	2.5 ± 0.1	2.3 ± 0.1	2.5 ± 0.1
Campesterol	19.9 ± 1.0	18.5 ± 0.7	19.6 ± 0.6	18.5 ± 0.7
Stigmasterol	3.4 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.2
Δ^7-Campesterol	1.5 ± 0.1	1.1 ± 0.1	1.8 ± 0.1	1.1 ± 0.1
β-Sitosterol	70.0 ± 1.4	72.1 ± 2.2	70.4 ± 2.1	72.1 ± 2.2
Δ^5-Avenasterol	0.5 ± 0.02	0.5 ± 0.01	0.6 ± 0.02	0.5 ± 0.02
Δ^7-Avenasterol	0.8 ± 0.03	0.6 ± 0.02	0.6 ± 0.02	0.6 ± 0.02
Δ^7-Stigmasterol	0.5 ± 0.02	0.5 ± 0.03	0.6 ± 0.02	0.5 ± 0.02

Table 5 Sterol composition of grape seed oil*

*Means ± SD of three determination

The obtained picture clearly shows that the qualitative and quantitative profile of all studied seeds is similar. β-Sitosterol (70.0-72.1%) and campesterol (18.5-19.9%) were found to be the main components, followed by stigmasterol and brasicasterol. In contrast to earlier reports, the

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presence of cholesterol was established and its amount was found to be higher than in other vegetable oils where it was about 0.1-0.2%. The other sterol components - Δ^7-campesterol, Δ^5-avenasterol, Δ^7-avenasterol, Δ^7-stigmasterol were detected in negligible amounts in all investigated varieties of grape seed oils. These results are different to those reported by El-Shami *et al*., (1999) and Fedeli *et al*., (1966) which are also noted predomination of β-sitosterol (73.8%) in sterol fraction but different values of campesterol. A higher content of campesterol (18.5-19.9%) was established at the expense of lower levels of stigmasterol (3.4-3.5%) and Δ^5-avenasterol (0.5-0.6%) in comparison to the results announced by Madawala *et al*., (2012) and El Shami *et al*., (1999) who established 10.3% and 10.6% of campesterol, 9.2-13.7% stigmasterol and 5.1% Δ^5-avenasterol respectively.

The fatty acid composition of the four grape varieties is listed in Table 6.

Table 6 Fatty acid composition of triacylglycerols*

Fatty acids, %	**Varieties**			
	Bolgar	***Super ran Bolgar***	***Mavroud***	***Shiroka melnishka loza***
Lauric ($C_{12:0}$)	0.4 ± 0.02	n.d.	0.4 ± 0.01	n.d.
Myristic ($C_{14:0}$)	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
Myristoleic ($C_{14:1}$)	tr.	n.d.	tr.	0.1 ± 0
Pentadecanoic($C_{15:0}$)	n.d.	0.1 ± 0	0.1 ± 0	tr.
Palmitic ($C_{16:0}$)	11.5 ± 0.2	8.8 ± 0.3	8.8 ± 0.4	10.0 ± 0.3
Palmitoleic ($C_{16:1}$)	0.2± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.3 ± 0.01
Margarinic ($C_{17:0}$)	0.1 ± 0	0.1 ± 0	0.1 ± 0.01	0.1 ± 0.01
Stearic ($C_{18:0}$)	1.0 ± 0.03	0.8 ± 0.03	1.0 ± 0.04	0.7 ± 0.03
Oleic ($C_{18:1}$)	17.6 ± 0.7	17.3 ± 0.4	18.7 ± 0.7	16.3 ± 0.7
Linoleic ($C_{18:2}$)	68.5 ± 1.4	72.2 ± 2.9	70.1 ± 2.1	71.3 ± 2.9
Linolenic ($C_{18:3}$)	0.3 ± 0.01	n.d.	0.2 ± 0.01	0.5 ± 0.02
Arahinic ($C_{20:0}$)	0.2 ± 0.01	0.1 ± 0	0.1 ± 0	0.2 ± 0.01
Gadoleic ($C_{20:1}$)	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.3 ± 0.01
Behenic ($C_{22:0}$)	n.d.	0.3 ± 0.01	0.1± 0.01	0.1 ± 0

*Means ± SD of three determination; n.d.-not detected; tr.-traces

14 fatty acids were identified in the glyceride oils. The qualitative and quantitative profiles of all samples are comparable. Linoleic acid (68.5-72.3%) predominates in the triacylglycerols followed by oleic (16.3-18.7%) and palmitic acid (8.8-11.5%). The content of stearic acid was found to be a very small percentage in comparison with other glyceride oils where it is about 3-5% (Gunstone 2003). The other fatty acids were detected in negligible quantities. These results are close to data reported earlier by other authors (Bail *et al.*, 2008; Lutterodt *et al.*, 2011). Fatty acid composition of grape seed oil is also similar to this of classic sunflower oil, where linoleic and oleic acids are the main components (Gunstone 2003).

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№	Samples / TAG	*Bolgar*	*Super ran Bolgar*	*Mavroud*	*Shiroka melnishka loza*
		%	%	%	%
1	LLL	48.7 ± 1.5	40.4 ± 1.6	43.6 ± 1.7	57.0 ± 2.3
2	LLO	18.5 ± 0.7	23.3 ± 0.9	23.2 ± 0.5	16.4 ± 0.7
3	LLP	11.4 ± 0.5	15.4 ± 0.6	8.4 ± 0.3	10.1 ± 0.4
4	LOO	3.4 ± 0.1	3.8 ± 0.2	5.2 ± 0.2	2.6 ± 0.1
5	LOP	6.3 ± 0.2	6.7 ± 0.3	5.8 ± 0.1	4.5 ± 0.1
6	LLS	4.3 ± 0.2	4.6 ± 0.1	4.7 ± 0.1	3.2 ± 0.1
7	LPP	tr.	tr.	tr.	tr.
8	OOO	2.2 ± 0.1	1.7 ± 0.1	3.0 ± 0.1	2.0 ± 0.1
9	OOP	2.7 ± 0.1	2.3 ± 0.1	3.4 ± 0.2	2.1 ± 0.04
10	LOS	2.5 ± 0.1	1.8 ± 0.1	2.7 ± 0.1	2.1 ± 0.04

Table 7 Triacylglycerol structure of grape seed oils*

*Means ± SD of three determination

The identification and quantification of TAG species indicated the presence of ten triacylglycerol structures. In the four tested varieties was found that the major component is LLL (40.4-57%), followed by LLO (16.4-23.3%) and LLP (8.4-15.4%). These results are different from those found in the literature (Tuberso *et al*, 2007) where these authors reported that the content of LLP was the highest (35.1%), followed by LLO (21.6%) and LLL (20.7%). The other six TAG species - LOO, LOP, LLS, OOO, OOP, LOS were presented in amounts of 1.7% to 6.7%, while the remaining type LPP was detected only in trace.

The fatty acid profile of individual phospholipid classes is presented in Figure 1

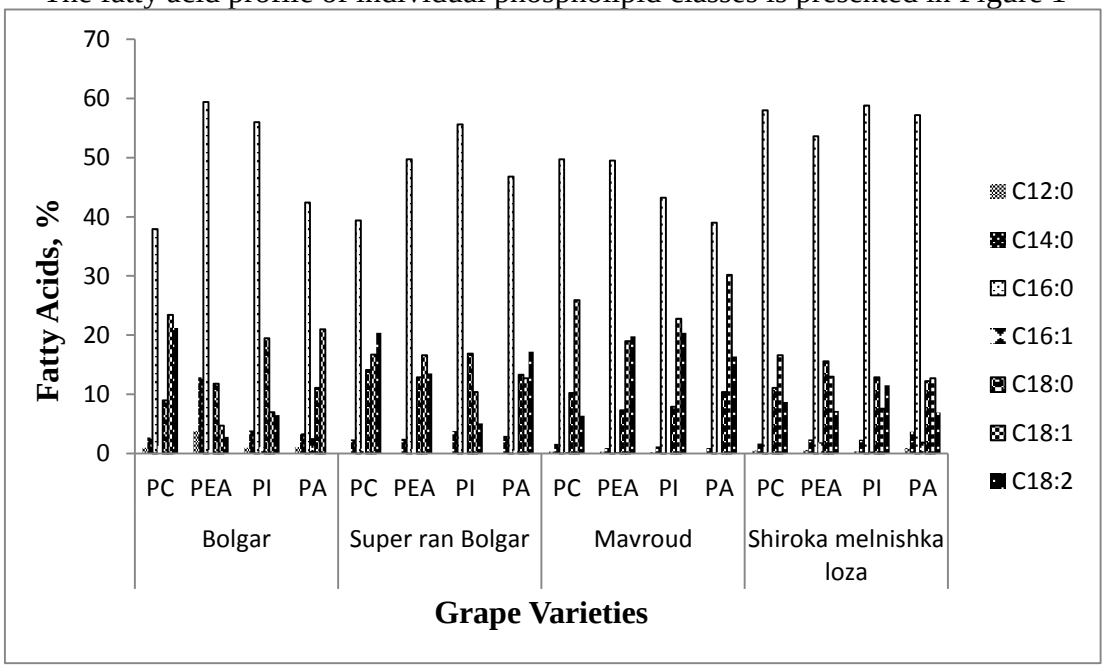

Figure 1 Fatty acid composition of individual phospholipids

PC- Phosphatidylcholine, PEA- Phosphatidylethanolamine, PI- Phosphatidylinositol, PA- Phosphatidic acids

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The results show that the qualitative fatty acid composition of separate individual phospholipids is similar, but the quantitative profile is different. The content of palmitic acid was found to be the major component in all phospholipid classes (37.9-68.0%), followed by oleic (4.7- 30.2%), stearic (7.3-19.5%) and linoleic acid (2.8-20.4%). The highest content of unsaturated fatty acids, mainly oleic and linoleic, was detected in phosphatidic acids (6.9-30.2%) and phosphatidylcholine (6.4-25.9%) while in phosphatidylinositol and phosphatidylethanolamine their amounts were relatively lower (2.8-19.8% and 4.7-22.8% respectively). Identical fatty acids were detected in the fraction of sterol esters. The quantitative profile of the separate varieties is similar. Unsaturated acids, mainly linoleic, (40.7-53.8%) predominated, followed by oleic acid (24.9-35.5%). The content of saturated acids (mainly palmitic and stearic) is 13.0-16.7% and 4.2-6.7% respectively. Insignificant amounts of $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, $C_{20:0}$ and $C_{22:0}$ were detected too.

Figure 2 presents the ratio saturated vs. mono- and polyunsaturated fatty acids in each lipid class. Phospholipids are presented with average value.

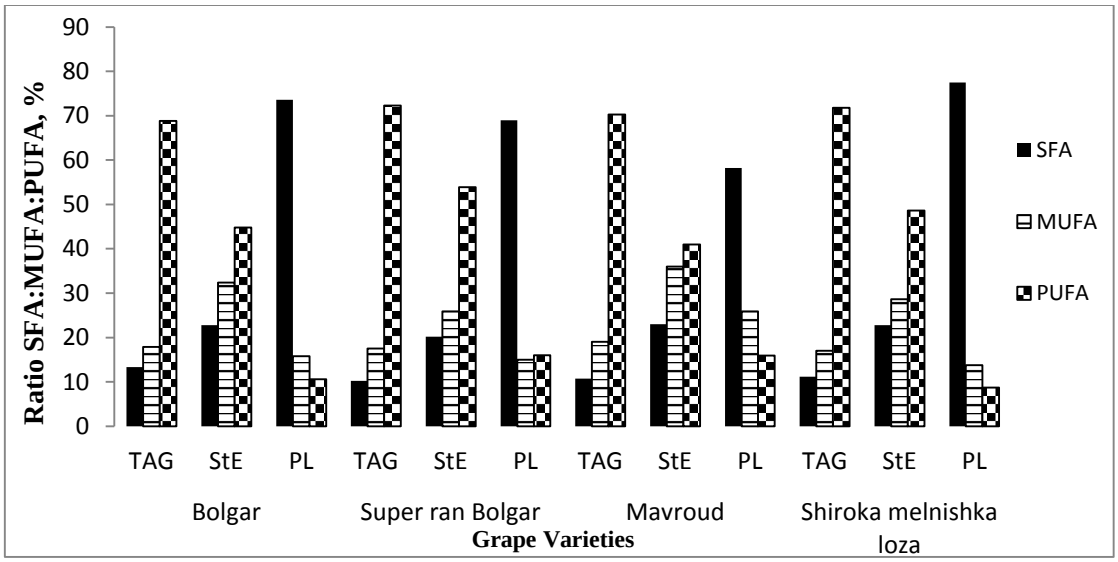

Figure 2 Distribution of fatty acid classes in triacylglycerols, sterol esters and phospholipids

The total content of saturated and monounsaturated fatty acids in sterol esters is higher than in triacylglycerols at the expense of a lower level of polyunsaturated fatty acids. On the other hand, the quantity of saturated fatty acids in sterol esters was found to be significantly lower than in phospholipids where their content was more than 77.5%.

These differences can be due to the different stages of biosynthesis of the triacylglycerols, sterol esters and phospholipids. Phospholipids are synthesized at the first stage and are connected to saturated fatty acids, synthesized at the same stage. Sterol esters are synthesized after that, and they connect with saturated and monounsaturated fatty acids. Finally polyunsaturated fatty acids were synthesized and they were combined in triacylglycerols (Munshi *et al.*, 1982).

The tocopherol composition of the oils is given in Table 8

Table 8 Individual tocopherols composition of seed oil*

*Means ± SD of three determination; tr.- traces

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The qualitative and quantitative compostion in all varieties was found to be similar. α-

Tocopherol	Varieties			
	Bolgar	***Super ran bolgar***	***Mavroud***	***Shiroka melnishka loza***
α-tocopherol	74.8 ± 1.5	76.7 ± 3.1	84.4± 3.4	79.5 ± 2.4
γ-tocopherol	14.0 ± 0.4	23.3 ± 0.9	15.6 ± 0.3	20.5 ± 0.8
γ-tocotrienol	11.2 ± 0.4	tr.	tr.	tr.

Tocopherol (74.8-84.4%) is the main component in tocopherol fraction, followed by γ-tocopherol (14.0-23.3%). These values are close to data reported earlier by Sabir *et al*., (2012) and Kraujalyte *et al*., (2011) who announced that α-tocopherol was also predominated.

CONCLUSION

The results recieved from detailed investigations on the grape seed composition show that the concentration of the seed components (glyceride oil, proteins, carbohydrates and ash) varies depending on the variety in relatively limited ranges. In the soluble carbohydrate fraction predominate disaccharides, mainly iso-maltose, are predominant while in monosaccarides the major constituents were found to be galactose and glucose. Significant differences in oil content between white and red cultivar were not observed. The quantity and individual composition of biologically active substances in the oil of the separate varieties grape seeds (phospholipids, sterols and tocopherols) are identical but it differs distinguishes from data of other regions probably as a result of different climatic conditions for cultivation of the plants. The glyceride oil contains mainly polyunsaturated fatty acids, while in phospholipids and sterol esters saturated and monosaturated fatty acids predominate. The obtained information allows complete estimation of the seeds as a rich source of valuable food substances, mainly proteins and carbohydrates, of the quality of the grape seed oil as a food product with a therapeutical effect as well as a source for preparing cosmetic products.

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