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Grape seed flour of different grape pomaces: Fatty acid profile, soluble sugar profile and nutritional value

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Abstract: The aim of this study was to determine fatty acid and soluble sugar profiles of the grape seed flour originated from non-fermented dried pomace of international and autochthonous grape varieties in order to estimate their potential nutritional value. The grape seed flours were obtained from the grapes harvested in technological maturity. It has been shown that grape seed flours contained significant quantities of unsaturated fatty acids (UFAs), especially linoleic fatty acids, whose content ranged from 61.15 - 83.47 %. Oleic acid mostly contributed to the content of monounsaturated fatty acids, while the stearic acid was the most abundant saturated fatty acid (SFA). Among polyunsaturated fatty acids, mainly ω-6 FAs, were the most represented. The tested grape seed flours had the high UFA/SFA ratio (3.63-11.09), low atherogenicity (0.04-0.13) and thrombogenicity (0.16-0.47) indices. Fifteen different sugars were found in analysed samples with the total concentration ranging from 40588 to 91319 mg/kg seed with fructose and glucose as the most abundant. Principal component analysis based on the content of FAs and soluble sugars revealed unique composition of the seed flour of Prokupac variety. These findings indicate that the tested grape seed flours is a good source of nutritionally valuable FAs and sugars that can play an important role in the formulation of a new functional food products.

Keywords: *Vitis vinifera*, marc, soluble carbohydrates, long-chain organic acids, index of atherogenicity, index of thrombogenicity.

INTRODUCTION

Grape is the most extensively cultivated fruit crop in the world, commonly used for wine production. However, the use of grapes in the winemaking industry

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leads to accumulation of large quantities of seed and skin by-product (known as pomace) which is approximately 10–30 % of the total grape mass that has been processed.[1] Depending on the winemaking process, the composition of the pomace is different. In the production of red wines, the entire disintegrated grape mass is included in the alcoholic fermentation while for the production of rosé and white wines only the juice is fermented.[1] It is known that grape berry contains nutritional and health promoting compounds such as carbohydrates, fatty acids, vitamins, minerals and polyphenols.[2] During wine production most of these prominent compounds are extracted into grape juice or wine, but significant amount remains trapped in the grape pomace.[3] Recently, numerous studies have shown that the revalorisation of these by-products is possible and that interesting as well as useful products, for the food industry, can be obtained.[4–6] The value-added products containing specific compounds, antioxidants (such as polyphenols), minerals, dietary fibres or minimally processed products such as grape pomace flours have been proposed for enrichment of food.[1,6]

Grape seeds, constituents of grape pomace, have been used for decades in production of grape seed oil and are widely commercialized in some countries. According to previous research, unsaturated fatty acids such as linoleic and oleic are dominant in the grape seed oil, while among the saturated fatty acids mainly palmitic and stearic fatty acid are present.[7–9] High content of essential fatty acids in the seed oil makes it suitable as functional food ingredients, since they contribute to reduced risk of various diseases.[10] Also, grape seed oil is very deficient in ω-3-fatty acids.[8,9] Besides the oil, Yedro *et al.*[11] observes that grape seed as a lignocellulosic residue consisting mostly of three fractions such as hemicellulose, cellulose and lignin, whereas Beres *et al.*[1] suggest that seed contains about 40 % of the fibre, as well as significant amounts of polyphenols, mainly catechin and proanthocyanidins. So far, for the isolation of the target compounds from the grape seed the complex extraction procedures that require a lot of equipment, time and are less suitable for an industrial application, have been used.[6] More and more studies are emphasizing the importance of the use of whole grape seed or its flour and powder, where different groups of chemical constituents are combined together and enable intense fortification, which may result in better functional properties of value-added products.[6,12] Therefore, the whole grape seed is an interesting by-product that can be used for fortification and incorporation of nutrients into food.

Nowadays, there are several categories of foods such as cereal,[4,12] dairy[13] or meat products[14] that are successfully enriched with grape seed flour and powder. Studies on enrichment of products such as bread,[4] biscuits[12,15] or pancakes,[5] with grape seed flour pay special attention to polyphenols due to their antioxidative properties and role in the sensorial acceptability of the product as well as on dietary fibres. Nevertheless, other compounds present in the seeds like lipids or

components concentrated on the surface of the seed such as sugars also affect the nutritional value of the final product and can have an important function and impact on human health.[1,6] Namely, on the surface of seed separated from the pomace, immediately after grape pressing, some amount of soluble sugars remain, because the seeds were in permanent contact with the pulp. It is known that most of the total soluble grape sugars are concentrated in pulp or grape juice.[16] In grapes, depending on the variety and climatic conditions, the soluble sugar content may vary from 12 to 28 %,[17] with glucose and fructose as predominant. Besides, in most of the berry cultivars sucrose is present in traces.[2,18] Thus, it is very important to define fatty acids (FAs) and soluble sugar profiles of grape seed flours which are intended to be an integral part of food product.

Knowing that the composition of grape seeds depends on several factors, such as variety, location, harvest time, *etc*., the aim of this work was to determine fatty acid and soluble sugar profiles of the grape seed flour originated from non-fermented dried pomace of international and autochthonous grape varieties aiming to estimate their potential nutritional value. In that sense, SFAs, UFAs, monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), UFA/SFA ratio, index of atherogenicity (*IA*) and index of thrombogenicity (*IT*) were calculated. In order to obtain a more detailed insight into the structure of the data and identify similarities and specificities of grouping of objects principal component analysis (PCA) and hierarchical cluster analysis (HCA) were also performed based on the contents of fatty acids, as well as the soluble sugar content detected in various samples of grape seed flour. The obtained results will be valuable for profiling a new functional food product enriched with whole grape seed products.

EXPERIMENTAL

Chemicals and materials

Supelco 37 Component FAME mix standard was purchased from Supelco (Bellefonte, USA). Sugar standards were obtained from Tokyo Chemical Industry, TCI (Europe, Belgium). Other chemicals and solvents were of analytical grade.

Technological parameters of analysed grapes

Technological parameters were determined on sample of 50 berries of each analysed grapes. Total soluble solids (TSS) was analysed by refractometer (ATC 0-32 Brix, Huixia Supply Co.,Ltd, China), titrable acidity (TA) expressed as g/L of tartaric acid was estimated by AOAC method 942.15[19] and pH was determined on pH meter (Consort, Belgium).

Preparation of grape seed flour

In total, the seven samples of seeds from different grape varieties, four red varieties: „Hamburg", „Prokupac", „Merlot", „Cabernet Sauvignon" and three white varieties: „Smederevka", „Riesling Italien" and „Tamjanika" were examined. Samples were obtained from vinery located in Aleksandrovac, center of Župa district, Serbia. Various grape varieties were harvested in the technological stage of maturity suitable for the production of wine. Fresh

grape pomace samples of all grape variety were collected after pressing. Then, pomaces were immediately dried in an drying oven (Thermo Scientific Haraeus, MA, USA) at 60 °C for 72 h (final water content about 15 %). Thereafter, the seeds were manually separated from the skin and were ground in a small laboratory coffee grinder (Bosch MKM 6003 UC, BSH Hausgeräte GmbH, Munich, Germany). The grape seed flours were maintained at –20 °C in vacuum-packed plastic containers until further analysis.

Preparation of grape seed flour samples for GC and HPAEC analysis

Extraction of lipids from seeds flour (approximately 0.5 g), was carried out using 10 ml hexane in ultrasonic bath for 30 min at 40 °C. Thereafter, the extraction was continued with stirring on a magnetic stirrer at 40 °C for 1 h. Then, lipid extracts were filtered through Whatman No.1 filter paper and supernatant was collected. Extractions were carried out in duplicate and both supernatants were combined and evaporated to dryness by rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany) under reduced pressure at 40 °C. After evaporation, the residues were dissolved in 6 ml hexane and used for further GC analysis.

For soluble sugar analysis, defatted seed flour (approximately 0.1 g) was extracted with 10 ml 80 % methanol containing 0.1 % HCl. Samples were stirred for 1h on a mechanical shaker (Thys 2, MLW Labortechnik GmbH, Seelbach, Germany) at room temperature and additionally, for another 1h, treated on a water bath at 38 °C. Then, samples were centrifuged at 4000 rpm for 10 min and supernatant was collected. Thereafter, supernatants were evaporated to dryness and residues dissolved in 10 ml milli-Q water.

GC analysis of FAs

FAs composition of different grape seed lipid extracts were determined using capillary gas chromatography (GC instrument Agilent Technologies 6890 (USA) with flame ionization detector (GC-FID) previously described by Kostić *et al.*[20] The FAME's were determined using capillary gas column SP-2560 (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm, Supelco, Bellefonte, PA, USA). The following conditions were applied: injector temperature, 250 °C; detector temperature, 260 ºC; carrier gas, helium at flow rate of 5 mL/min; injection volume 1 μL; injector split ratio set at 20:1; the column temperature was: 50 °C, 5 min to 240 °C, 20 min with temperature rate of 4 °C/min. The analysis run was 72.5 min. The identification and quantification of the FA was done by the FAME mix standard. Fatty acid content was expressed in relative quantities as mass % of total detected fatty acids.

From these data, the nutritional quality parameters such as SFAs, UFAs, MUFAs, PUFAs and UFA/SFA ratio were determined. Additionally, index of atherogenicity (IA) and index of thrombogenicity (IT) were calculated according to Eqs. (1) and (2):[21]

$$\mathrm{IA} = \frac{A + 4B + C + E}{\sum \mathrm{MUFA} + \sum \omega 6 + \sum \omega 3} \tag{1}$$

$$\mathrm{IT} = \frac{B + C + D + E}{0.5\sum \mathrm{MUFA} + 0.5\sum \omega 6 + 3\sum \omega 3 + \sum \omega 3 / \sum \omega 6} \tag{2}$$

A – the content of C12:0; B – the content of C14:0; C – the content of C16:0; D – the content of C18:0; MUFA – the content of MUFA, $\omega 6$ – the content of $\omega 6$ fatty acid; $\omega 3$ – the content of $\omega 3$ fatty acids; E – the content of *trans*-fatty acids.

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD) for sugars analysis

Composition of the soluble sugar of defatted seed flour of different grape varieties were determined using HPAEC/PAD, according methodology previously described by Gašić *et al*.[22] Briefly, DIONEX ICS 3000 DP liquid chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a quaternary gradient pump (Dionex), ICS AS-DV 50 autosampler (Dionex) and Carbo Pac®PA100 pellicular anion-exchange column (4×250 mm, particle size – 8.5 μm, pore size – microporous,<10 Å (Dionex), was used for sugar analysis at 30 °C. The electrochemical detector consisted of gold as the working and Ag/AgCl as the reference electrodes. The mobile phase consisted of the following reagents: 600 mM sodium hydroxide (A), 500 mM sodium acetate (B) and ultrapure water (C). The linear gradient (flow rate, 0.7 mL/min) was: 0–5 min, 15 % A, 85 % C; 5.0–5.1 min, 15 % A, 2 % B, 83 % C; 5.1–12.0 min, 15 % A, 2 % B, 83 % C; 12.0–12.1 min, 15 % A, 4 % B, 81 % C; 12.1–20.0 min 15 % A, 4 % B, 81 % C; 20.0–20.1 min 20 % A; 20 % B; 60 % C; 20.1–30.0 min 20 % A; 20 % B; 60 % C. Before the analyses, the system was preconditioned with 15 % A, 85 % C, for 15 min. The sample injection volume was 25 μL. The quantification of carbohydrate concentration was obtained from the calibration curves of pure compounds as already reported by Gašić *et al*.[22]

Statistical analysis

Results were reported as means of three measurements ± standard deviation. Differences between mean values were estimated using Tukey's-test, at level of significance $p < 0.05$ in Statistica software v. 6.0 (Statsoft Co., Tulsa, OK, USA). Principal component (PCA) and hierarchical cluster (HCA) analyses were performed in the software package PLS ToolBox, v. 6.2.1, Matlab 7.12.0 (R2011a). All data were auto-scaled before the multivariate analysis.

RESULTS AND DISCUSSION

Technological parameters of analysed grapes

Sugars, expressed as soluble solid contents, acids and pH of grape pulp usually used to estimate technological maturity of grapes. These are very important parameters for quality of wine as well as quality of seed extracts.[9,23] Technological parameters depend on the cultivar, production area and viticultural practices.[24–26] As can be seen in Table I, the total soluble solids, titrable acidity and pH of analysed grape berries varied from 19.3 to 25.5 °Bx, 6.56 to 10.78 g/L of tartaric acid and 3.36 to 3.85, respectively. These data were comparable to other results obtain for grapes harvested in technological maturity.[23–26]

Fatty acid profile of grape seed oils

Results obtained for the relative content of FAs in different grape seed oils are presented in Table II. On the basis of the obtained results, in total, twelve fatty acids were identified in grape seed oil samples. FAs such as linoleic, oleic, stearic and palmitic were found in all examined samples. The linoleic acid, as unsaturated fatty acid, was dominant and ranged from 61.15±0.36 to 83.47±1.15 % depending on the variety. The highest concentration of linoleic acid was detected in seed oil of international grape varieties such as Hamburg and Riesling

Italian. Among the indigenous varieties Tamjanika seed was the best source of this UFA.

TABLE I. Technological parameters of analysed grape berries (TSS; TA; pH); the results in the table were presented as mean±standard deviations (mean±*SD*; $n = 3$); the same letters in the same row are not significantly different according to Tukey's test, $p < 0.05$. Abbreviations: TSS – total soluble solids); TA – titrable acidity

Sample	TSS, g of sucrose / 100 g	TA, g of tartaric acid/L	pH
	°Bx		
Smederevka	19.5±0.3^{a}	10.78±0.94^{a}	3.36±0.02^{a}
Italien Riesling	23.6±0.2^{b}	6.56±0.1^{b}	3.81±0.01^{b}
Tamjanika	22.3±0.5^{c}	7.03±0.94^{b}	3.83±0.01^{b}
Hamburg	20.2±0.2^{ae}	7.50±0.1^{bc}	3.76±0.02^{c}
Prokupac	19.3±0.1^{a}	8.43±0.1^{cd}	3.85±0.01^{b}
Merlot	25.5±0.1^{d}	9.37±0.2^{de}	3.56±0.01^{d}
Cabernet Sauvignon	21.1±0.9^{e}	10.31±1.88^{ae}	3.40±0.005^{a}

TABLE II. Fatty acid composition of analysed grape seed flours; n.d. – stands for not detected; the same letters in the same row are not significantly different according to Tukey's test, $p < 0.05$. Abbreviations: (C16:0) – palmitic; (C16:1) – palmitoleic; (C17:0) – heptadecanoic; (C18:0) – stearic; (C18:1ω9c) – oleic; (C18:2ω6c) – linoleic; (C18:2ω6t) – linoleaidic; (C20:2) – *cis*-11,14-eicosadienoic; (C20:3ω6) – cis-8,11,14-eicosatrienoic; (C20:3ω3) – *cis*-11,14,17-eicosatrienoic; (C22:0) – behenic; (C22:1ω9) – erucic

Fatty acid	Smederevka	Italien Riesling	Tamjanika	Hamburg	Prokupac	Merlot	Cabernet Sauvignon
	Fatty acid content, %						
C16:0	4.38±0.1^{ad}	3.57±0.09^{b}	3.36±0.07^{b}	3.41±0.08^{b}	7.94±0.18^{c}	4.69±0.27^{a}	4.14±0.07^{d}
C16:1	n.d.	n.d.	0.40±0.001	n.d.	n.d.	n.d.	n.d.
C17:0	n.d.	n.d.	0.86±0.04	n.d.	n.d.	n.d.	n.d.
C18:0	6.53±0.11^{a}	4.80±0.09^{b}	4.58±0.25^{bc}	4.10±0.07^{c}	8.27±0.13^{d}	5.83±0.21^{e}	6.59±0.32^{a}
C18:1ω9c	8.57±0.17^{a}	8.24±0.18^{ab}	7.74±0.4b^{e}	6.14±0.17^{cf}	9.72±0.24^{d}	7.10±0.1^{e}	6.40±0.26^{f}
C18:2ω6c	73.61±1.05^{a}	83.39±1.26^{b}	83.07±1.09^{b}	83.47±1.15^{b}	61.15±0.36^{c}	76.98±1.17^{a}	75.61±2.62^{a}
C18:2ω6t	n.d.	n.d.	n.d.	n.d.	2.15±0.06^{a}	1.24±0.05^{b}	1.40±0.07^{c}
C20:2	0.90±0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C20:3ω6	1.00±0.01^{a}	n.d.	n.d.	0.35±0.01^{b}	2.09±0.1^{c}	0.80±0.02^{d}	1.19±0.02^{e}
C20:3ω3	1.47±0.04^{a}	n.d.	n.d.	0.89±0.04^{b}	n.d.	n.d.	1.44±0.03^{a}
C22:0	2.06±0.05^{a}	n.d.	n.d.	0.76±0.02^{b}	5.4±0.25^{c}	1.36±0.03^{d}	1.97±0.01^{a}
C22:1ω9	1.43±0.03^{a}	n.d.	n.d.	0.87±0.05^{b}	3.25±0.12^{c}	2.01±0.09^{d}	1.25±0.01^{a}

Similar results were obtained by other authors who agreed that linoleic acid is the most abundant FA in oil of grape seeds of grape varieties grown in Serbia[27,28] or other countries.[7–9,29–31] Thus, Malićanin *et al.*[27] found that the content of linoleic acid in seeds of the Cabernet Sauvignon variety grown in Serbia was in range from 73.10–75.30 %, whereas in Prokupac variety ranged from 69–

–81 %.[28] Lachman *et al*.[9] obtained results for content of linoleic acid in range from 68.10 to 78.18 % depending on the year of harvest, whereas Beveridge *et al*.[30] recorded the highest content of linoleic acid in the seed oil of Merlot variety. Oleic acid mostly contributed to the content of MUFAs and its content varied from 6.14±0.17 to 9.72±0.24 %, while the stearic acid was the most abundant SFA in all analysed samples with content from 4.1±0.07 to 8.27±0.13 %. Both FAs were registered in the highest amount in indigenous variety Prokupac. However, significant differences among the results for oleic fatty acid in grape seed oil could be found in the literature. For example, these results are in agreement with those obtained by Lachman *et al*.,[9] while Pardo *et al*.[29], registered significantly higher amount of oleic acid in the seed oil of red grape varieties grown in Spain (16.07–24.88 %) and Malićinin *et al.*[27] for seeds of Cabernet Sauvignon grown in Serbia (12.60 to 13.80 %). Results for content of palmitic and stearic acids fall within similar ranges reported by Beveridge *et al*.[30] (6.35 to 8.62 % for palmitic and 3.60 to 5.26 % for stearic acids, depending on extraction solvents and conditions), Fernandes *et al*.[8] (6.17 to 8.50 % and 4.09 to 5.91 %, respectively) or Zdunić *et al*.,[28] obtained for clones of the Prokupac variety (3 to 8 % for stearic acid and 2 to 4 % for palmitic acid). However, according to other reports, palmitic acid is the dominant SFA in most of grape seed oils, which is not correlated with this study.[7–9,29,31] Furthermore, seed oil of variety Prokupac contained significant amount of linoleaidic, erucic, behnic and *cis*-8,11,14-eicosatrienoic fatty acids compared to that of other analyzed varieties. Other identified FAs, known as „rear fatty acids“ were present in traces and their content can often serve as a marker for the characterization of different grape varieties. For example, palmitoleic and heptadecanoic FAs were identified only in the seed oil of variety Tamjanika. Additionally, the presence of *cis*-11,14-eicosadienoic FA was recorded in Smederevka seed oil.

Soluble sugar profile of defatted grape seed flours

The composition of soluble sugars extracted from defatted grape seed flours are presented in Table III. According to results of HPAEC analysis, the concentration of fifteen different sugars was determined in all analyzed samples. Their total concentration varied from 40588 to 91319 mg/kg seed. Monosaccharides were dominant and their concentration was in ranges from 39090 to 89659 mg/kg seed, while disaccharides and trisaccharides are present in traces. In addition to the predominantly presence of monosaccharides, such as glucose and fructose, it is important to note that the sucrose concentration was higher compared to other detected di- and tri-saccharides. The residual carbohydrates, that remain in the pomace after the disintegration and pressing of grapes, are mainly water soluble (monosaccharides and oligosaccharides) and water insoluble structural polysaccharides from the cell wall.[32] In most studies the monosaccharide composition of

TABLE III. The soluble sugar profile of defatted grape seed flours (mg/kg seed); the same letters in the same row are not significantly different according to Tukey's test, $p < 0.05$. Abbreviation: monosaccharides (MS); disaccharides (DS); trisaccharides (TS); glucose (Glc); fructose (Fru); saccharose (Sac); trehalose (Tre); maltose (Mal); arabinose (Ara); turanose (Tur); gentiobiose (Gent); isomaltose (Ism); panose (Pan); isomaltotriose (Ismt); maltotriose (Malt); melibiose (Mel); rafinose (Raf); melesitose (Mele)

Sugar	Smederevka	Italien Riesling	Tamjanika	Hamburg	Prokupac	Merlot	Cabernet Sauvignon
	Sugar concentration, mg/kg (mean ± standard deviation)						
				MS			
Ara	23±1^{a}	18±1.3^{b}	11±0.6^{c}	22±1.4^{a}	35±2^{d}	3±0.3^{e}	17±0.7^{b}
Glc	46777±896^{a}	37827±829^{b}	41415±636^{c}	38351±682^{b}	31456±342^{d}	19350±348^{e}	33316±427^{f}
Fru	42859±1526^{a}	37731±618^{b}	42911±915^{a}	41259±263^{a}	35562±339^{b}	19737±516^{c}	32818±694^{d}
Σ	89659	75576	84337	79632	67053	39090	66151
				DS			
Tre	11±0.7^{ae}	9±0.7^{a}	155±8^{b}	32±2^{cf}	387±10^{d}	45±3^{c}	24±1.1^{ef}
Ism	24±1.2^{a}	10±0.9^{b}	36±0.8^{c}	36±2.2^{c}	26±2.3^{a}	42±0.9^{d}	39±1.9^{cd}
Mel	13±0.8^{a}	4±0.3^{be}	9±0.3^{c}	3±0.2^{b}	4±0.3^{be}	7±0.3^{d}	5±0.4^{e}
Sac	955±11^{a}	3737±140^{b}	2445±34^{c}	4146±59^{d}	4413±26^{e}	1009±18^{a}	1763±64^{f}
Gent	1±0.1^{a}	2±0.2^{b}	1±0.2^{a}	n.d.	2±0.3^{b}	3±0.3^{c}	n.d.
Tur	116±5^{ad}	122±9^{a}	241±12^{b}	178±11^{c}	106±8^{ad}	94±3^{d}	200±13^{c}
Mal	59±4^{a}	74±8^{b}	91±7^{c}	68±6^{ab}	65±2.9^{ab}	41±3^{d}	70±3^{ab}
Σ	1179	3958	2978	4463	5003	1241	2101
				TS			
Raf	339±8^{a}	249±20^{b}	340±9^{a}	249±11^{b}	335±14^{a}	181±6^{c}	277±9^{b}
Mele	15±0.6^{a}	16±0.8^{a}	19±0.8^{b}	14±0.9^{a}	19±1.2^{b}	6±0.3^{c}	22±1.9^{d}
Ismt	20±1.1^{a}	93±6^{b}	10±0.7^{ce}	5±1^{c}	83±7^{d}	11±0.4^{ac}	19±1.2^{ae}
Pan	84±5^{ae}	55±4^{ac}	309±13^{b}	28±2^{c}	448±24^{d}	47±2^{d}	100±7^{e}
Malt	23±1.1^{a}	26±2^{ac}	16±0.9^{b}	30±2.2^{ce}	7±0.4^{d}	12±0.4^{b}	32±2.3^{e}
Σ	481	439	694	326	892	257	450
Total	91319	79973	88009	84421	72948	40588	68702

the grape pomace, after intensive hydrolysis of complex lignocellulosic polysaccharides, was analyzed. However, according to our knowledge, the concentration of soluble sugars in the defatted seed flour obtained from non-fermented dried pomace have been rarely investigated until now, but the different results for total soluble sugar concentration in grape pomace, obtained after grape pressing, could be found in the literature. According to Beres *et al.*,[33] the carbohydrate concentration of grape pomace flour of the Red Pinot variety was 196800 mg/kg, while Sousa *et al.*,[34] reported that the pomace flour of Benitaka variety contained 292000 mg/kg of carbohydrate with a respectable amount of glucose and fructose, 79500 and 89100 mg/kg, respectively. Also, according to Corbin *et al.*,[32] the content of water soluble carbohydrates in pomace of varieties Cabernet Sauvignon and Sauvignon blanc were 4.6 and 37.6 mass %, with the dominant pre-

sence of glucose and fructose. In addition, Wang *et al.*[35] found the total sugar concentration of 368000 mg/kg in grape pomace. González-Centeno *et al.*,[36] showed that the fresh pomace of ten different grape varieties after pressing and maceration have soluble sugar concentration in range of 20000 to 62000 mg/kg, with the sugar concentration of those of varieties Cabernet Sauvignon and Merlot, 23000 and 24000 mg/kg, respectively.

These data suggest that significant amount of soluble sugars remaining in grape pomaces after pressing of grapes which resulted to their significant amount on the surface of pomace seeds and consequently in the grape seed flour. Thus, the apart from fatty acid profiles, it is also desirable to determine the content of soluble sugars in grape seed flour intended for enrichment of the food product. Dominant detected monosaccharides such as glucose or fructose are typical energy components, and their quantity is not negligible.

Nutritional quality parameter of grape seed flours

Nutritional quality parameter such as SFAs, MUFAs, PUFAs and ratio of UFAs/SFAs, were determined and given in Table IV. The content of SFAs was in range from 8.27 (Hamburg) to 21.61 % (Prokupac). The content of MUFAs in seed of all investigated samples were 7.01 (Hamburg)–12.97 % (Prokupac), while the content of PUFAs were in range from 65.39 (Prokupac) to 84.71 % (Hamburg). According to the obtained results, grape seeds of different varieties can be an important source of various FAs, primarily PUFAs with high prevalence of ω-6 FAs. This research also confirmed that grape seeds are deficient in the content of ω-3 FAs.

TABLE IV. Nutritional quality parameters of grape seed flours; SFAs – saturated fatty acids; UFAs – unsaturated fatty acids; MUFAs – monounsaturated fatty acids; PUFAs – polyunsaturated fatty acids; UFA/SFA – the ratio of the content of UFA to the content of SFA; *IA* – index of atherogenicity; *IT* – index of thrombogenicity

Nutritional parameter	Smederevka	Italien Riesling	Tamjanika	Hamburg	Prokupac	Merlot	Cabernet Sauvignon
	Fatty acid content, %						
SFAs	12.97	8.37	8.80	8.27	21.61	11.88	12.7
UFAs	86.98	91.63	91.21	91.72	78.36	88.13	87.29
MUFAs	10	8.27	8.14	7.01	12.97	9.11	7.65
PUFAs	76.98	83.39	83.07	84.71	65.39	79.02	79.64
	UFA/SFA, ratio; *IA* index; *IT* index						
UFA/SFA	6.71	10.95	10.36	11.09	3.63	7.42	6.87
IA	0.05	0.04	0.04	0.04	0.13	0.07	0.06
IT	0.23	0.18	0.17	0.16	0.47	0.27	0.26

It is known that SFAs increase low-density lipoprotein (LDL) cholesterol and thus increases the possibility of the risk of cardiovascular disease[30], while

the inclusion of products that have high content of MUFAs and primarily PUFAs in the diet, have hypocholesterolemic potential, *i.e.*, protective effect against coronary heart and artery diseases.[10,37] The availability of essential ω-3 and ω-6 FAs in nutrition is associated with the normal physiological functions of the membrane and the regulatory cell signals.[10] Therefore, according to Baydar *et al.*,[31] grape seeds that are rich in linoleic acid can be a highly valuable source of dietary fat. By comparing the content of SFAs and UFAs, it was found that the UFA/SFA ratio, has significantly higher values than 1.6 for all tested samples (from 3.63 to 11.09) indicating that grape seeds can be characterized as potentially good dietary supplement (WHO/FAO, 2003).[38]

Additionally, the values for IT and IA are defined, which enable a better classification of different foods.[37] IA showing the inhibition of the aggregation of plaque and the reduction of esterified FAs, cholesterol, and phospholipids, which prevents the appearance of coronary diseases. IT is showing the tendency to form clots in the blood vessels.[39] Analysed grape seeds had low atherogenic (0.04–0.13) and thrombogenic indices (0.16–0.47). The highest indices were obtained for seed oil of Prokupac variety and the lowest for that of Hamburg and Tamjanika varieties. The obtained *IA* values were similar to that of sunflower oil (0.07) but lower than those of olive (0.19) and oat oils (0.17–0.19), whereas *IT* values were in the range of those obtained for the same oils (olive, 0.4, sunflower, 0.20 and oat, 0.30–0.34).[30] These findings indicated that the tested grape seed flours could be a good source of nutritionally valuable FAs that plays an important role in the prevention of cardiovascular diseases.

Principal component analysis (PCA)

PCA based on contents of SFAs and UFAs in various samples of grape seed results in three-component model explaining 90.70 % of the total variance among data. The results obtained by analysing the first two principal components (Table II) are shown in score and loading plots (Fig. 1a and b).

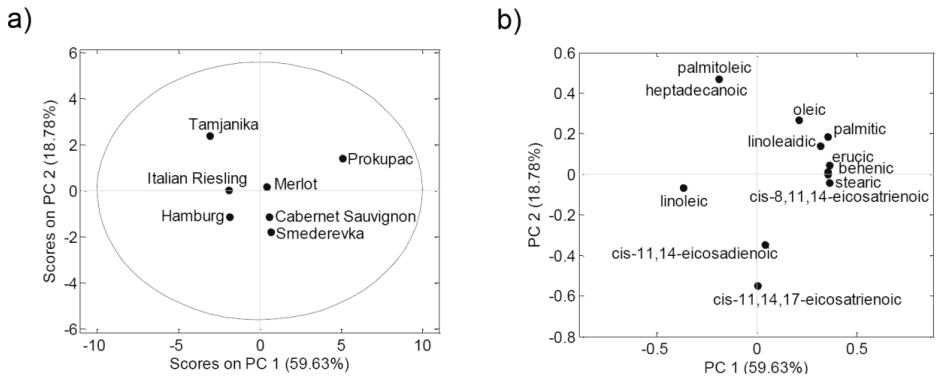

Fig. 1. Score (a) and loading (b) plot.

The score plot (Fig. 1a) shows the separation of three groups of objects. Seeds of samples Tamjanika and Prokupac are separated from other samples and form groups I and II. The third group consists of other analysed samples (Fig. 1a, Table II). Palmitoleic and heptadecanoic acids have the strongest positive influence along the PC2 axis on the separation of seed sample Tamjanika, which is in accordance with the fact that content of these acids is the highest in this sample (Fig. 1, Table I), while erucic, *cis*-11,14-eicosadienoic, *cis*-8,11,14-eicosatrienoic and *cis*-11,14,17-eicosatrienoic FAs, which were not identified in this sample, have a negative influence in the separation of this sample along the PC2 axis. The highest content of oleic, palmitic, linolelaidic, behenic, stearic, erucic and *cis*-8,11,14-eicosatrienoic FAs in the Prokupac variety have the strongest positive effect along the PC1 axis on the separation of this variety. Linoleic acid has a negative influence along the PC1 axis, as its concentration is the lowest in the Prokupac sample, compared to all others, while acids *cis*-11,14-eicosadienoic and *cis*-11,14,17-eicosatrienoic were not identified in this sample. As for the third group of objects (Fig. 1a), by comparing the results of PCA and HCA analyses (Fig. 2a) and based on the contents of linoleic and palmitic acids it can be concluded that there is a similarity between samples Merlot and Cabernet Sauvignon (Table I), as well as between samples Hamburg and Italian Riesling (Table II) which separated them in two different sub-clusters (Fig. 2a). Further, HCA on distance 5 results in the separation of samples of three clusters. The first cluster consists of all analysed samples except Pokupac and Tamjanika.

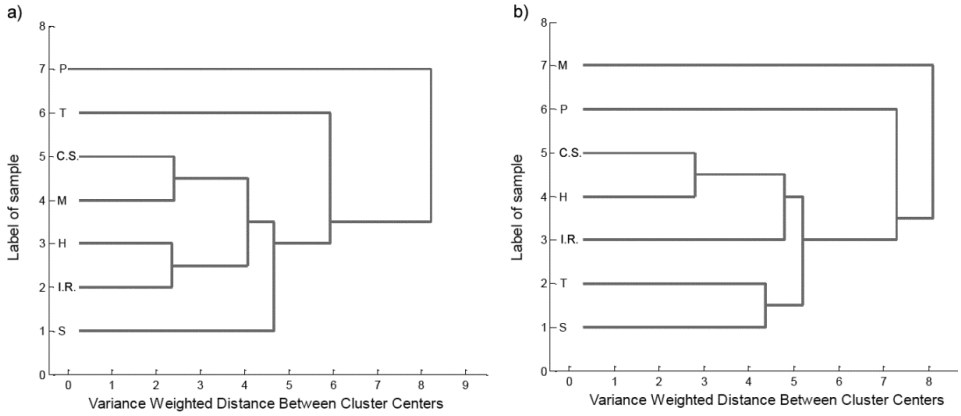

Fig. 2. a) HCA dendrogram of fatty acids; b) HCA dendrogram of soluble sugars; Abbreviations: M – Merlot, P – Prokupac, C.S. – Cabernet Sauvignon, H – Hamburg, I.R. –Italian Riesling, T – Tamjanika, S – Smederevka.

PCA based on sugar concentrations in various samples of grape seed result in three-component model that explains 77.63 % of total variance among data. Results obtained by analysing the first two principal components (Table III) are

shown in score and loading plots (Fig. 3 a and b). Three groups of objects can be seen in the score plot (Fig. 3a). Samples Merlot and Prokupac are separated from other samples (group III) and comprise group I and group II (Fig. 3a, Table II).

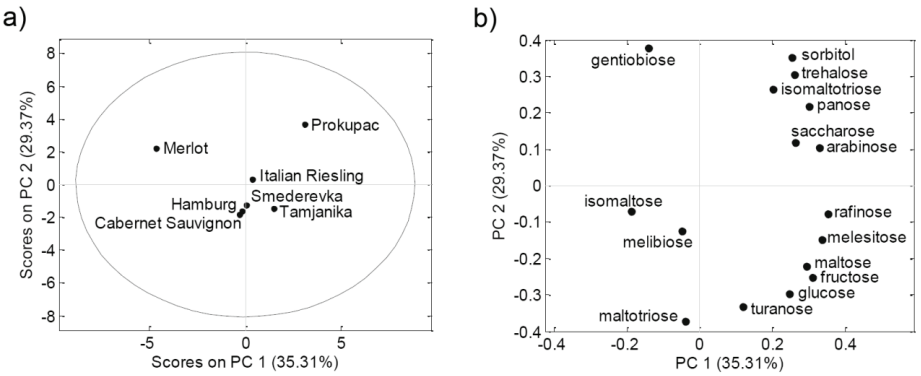

Fig. 3. Score (a) and loading (b) plot.

Rafinose, melesitose, maltose, fructose, glucose, and turanose have the most positive influence along the PC1 axis on the separation of sample Merlot in which the concentration of these sugars is the lowest (Fig. 3, Table III), while melibiose and isomaltose, the concentration of which is the highest in this sample, have a negative influence along the PC1 axis. Gentiobiose, the concentration of which is the highest in Merlot, has the most positive effect on the separation of this sample along PC2 axis. The separation of sample Prokupac along the PC2 axis is most positively influenced by trehalose, isomaltotriose, panose, saccharose and arabinose, which concentrations are the highest in this sample. Maltotriose, the concentration of which is the lowest in sample Prokupac, has the strongest negative effect along the PC2 axis. The separation of the third group of objects (Fig. 3a), which consists of other analysed seed samples, is influenced by rafinose, melesitose, maltose, fructose, glucose, and turanose. The results obtained by the hierarchical cluster analysis are shown in dendrogram (Fig. 2b). HCA at distance 6 results in the separation of samples in three clusters. The first cluster consists of sample Merlot, the second one of Prokupac, and the third of other analysed samples, which is in accordance with the results of PCA. Dendrogram also shows that within the thirds cluster, at the distance 5, two sub--clusters can be separated. The first sub-cluster consists of samples Smederevka and Tamjanika, while the other consists of samples Cabernet Sauvignon, Hamburg, and Italian Riesling.

CONCLUSION

The grape seed flours were obtained from the grapes harvested in technological maturity. All of tested grape seed flours showed favorable FA compo-

sitions, *i.e.*, low levels of SFAs, and high level of UFAs, especially linoleic fatty acids (61.15 – 83.47 %). The highest content of linoleic acid was detected in grape varieties Hamburg, Riesling Italian and Tamjanika. The oleic acid was the most abundant MUFA, whereas the stearic acid was the most represented SFA. Both fatty acids were registered in the highest amounts in variety Prokupac. Furthermore, seed oil of variety Prokupac contained significant amount of linoleaidic, erucic, behenic and *cis*-8,11,14-eicosatrienoic FAs. The significant amount of soluble sugars was registered in all analysed grape seed flours ranged from 40588 to 91319 mg/kg seed. Fifteen different sugars were found in samples among which glucose and fructose were the major ones. The PCA of the content of fatty acids revealed the separation of two autochthonous grape varieties, Prokupac and Tamjanika from each other and from the other analyzed samples, whereas PCA of the soluble sugar concentration differentiated Prokupac and Merlot as separate groups from the other grape varieties. Unique fatty acids and soluble sugar profiles of seed flour of autochthonous grape variety Prokupac together with good nutritional quality parameters indicate to its possible use as a new functional food ingredient.

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ИЗВОД

КОШТИЦЕ ГРОЖЂА РАЗЛИЧИТИХ КОМИНА: МАСНО-КИСЕЛИНСКИ ПРОФИЛ, ПРОФИЛ РАСТВОРЉИВИХ ШЕЋЕРА И НУТРИТИВНА ВРЕДНОСТ

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Циљ овог истраживања је да се одреди масно-киселински профил и профил растворљивих шећера брашна семенки грожђа добијеног од нефементисане осушене комине интернационалних и аутохтоних сорти грожђа у циљу одређивања њихове потенцијалне нутритивне вредности. Брашно је добијено од сменки грожђа убраног у технолошкој зрелости. Показано је да брашно коштица грожђа садржи значајну количину незасићених масних киселина (UFA), посебно линолне, чији се садржај кретао од 61,15–3,47 %. Олеинска киселина је највише допринела садржају мононезасићених масних киселина, док је стеаринска киселина била најзаступљенија засићена масна киселина (SFA). Од полинезасићених масних киселина претежно су ω-6 FA детектоване. Анализирана брашна коштице грожђа имала су висок однос UFA/SFA (3,63–11,09), низак атерогени (0,04–0,13) и тромбогени (0,16–0,47) индекс. У анализираним узорцима је пронађено петнаест различитих шећера, чији се укупни садржај кретао од 40588 до 91319 mg/kg коштице, са моносахаридима, глукозом и фруктозом као најзаступљенијим. Анализа главних компоненти на основу садржаја FA и растворљивих шећера открила је јединствен састав брашна семенки сорте Прокупац. Ова истраживања

указују да анализирана брашна грожђа могу бити добар извор нутритивно вредних масних киселина и шећера који могу имати важну улогу у формулацији нових функционалних прехрамбених производа.

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