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Varietal differences of Prokupac, Evita and Čokot Zemun based on their anthocyanins content in grape skin extract

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Abstract

In this study we have analyzed the anthocyanin composition of skin extracts of three red grape varieties Prokupac, Evita and Čokot Zemun in order to distinguish these cultivars based on their anthocyanin profile. Also, mechanical analysis of grape bunches and berries was performed. According to our results, seventeen anthocyanins were identified using LC-MS technique and quantitative differences were recorded using HPLC-DAD method. The highest content of total anthocyanins was obtained for Evita variety and the lowest one was recorded in Prokupac. Also, clear differences were observed in anthocyanins ratios. In comparison to Prokupac and Evita varieties, Čokot Zemun was characterized with a high content of cumaroyl derivatives of anthocyanin compounds, while high levels of acetylated derivatives were recorded in Prokupac. Data reported in this study represent a certain contribution to a

database of mechanical properties and chemical composition of grape varieties originating from Balkan.

Keywords: anthocyanin, grape, mechanical analysis, Prokupac, Evita

1. INTRODUCTION

The quality of wines and grapes is significantly determined by their polyphenolic profile and composition.[1] As the main compounds of wine's polyphenolic complex anthocyanins are responsible for wine color. Their interconnected proportions significantly impact its stability. During steps of wine maturation and aging their content is constantly decreasing through various chemical reactions. As highly reactive species they are modified to create stable pigments through reactions with flavonols, acetaldehyde molecule, as well as with some low molecular weight compounds such as pyruvic acid. [2]

Anthocyanins are primarily located in the grape skin, with the exception of Teinturier grape varieties in which they could be also found in the grape flesh.[3] The occurrence of heterosides increases the solubility of anthocyanins in water, making their diffusion easier from grape skin into must and wine during maceration. In *Vitis vinifera* L., anthocyanins present 3-*O*-monoglucosides of five main groups of anthocyanidins (malvidin – Mv, delphinidin – D, peonidin – Pn, petunidin – Pt and cyanidin – Cy) which further experience acylation and/or polimerization.[4]

Profile, as well as the concentration of anthocyanin compounds, are under the influence of numerous factors such as variety, cultivar, maturity, climate, terroir, agricultural practice and yield. Previous studies showed that non-genetic factors have a greater effect on the concentration of individual anthocyanins, while their proportions remain practically

constant.[5] The ratio of accumulated anthocyanins is closely connected with genetic characteristics of the grapevine variety. Having this in mind, anthocyanins can be considered as chemical markers. Their content has been applied in previous chemotaxonomic studies on grapevine.[6-9] In these studies different relationships among the anthocyanin groups of compounds were calculated and considered as parameters for cultivars differentiation. Dimitrovska et al.[8], showed that the relationship between coumaroilated and acetylated anthocyanins was significantly higher in Vranec compared to Merlot and Cabernet Sauvignon grapevines. Similarly, Nunez et al.[6], showed that this ratio allows three varieties (Tempranillo, Cabernet-Sauvignon and Graciano) to be distinguished.

This study aimed to investigate anthocyanin profile as well as various ratios of anthocyanin compounds in grape skin extracts of three varieties originated from Serbia: Prokupac, Evita and Čokot Zemun. The analysis of anthocyanins was performed using HPLC-DAD and LC-MS techniques.

2. RESULTS AND DISCUSSION

2.1. Mechanical analysis of grape bunches and berries and quality analysis of grape juice

In this study mechanical analysis of both clusters and berries was performed, as well as the analysis of the chemical composition of the grape must in terms of sugar, acid content, pH and glycoacidometric index (Table 1). The influence of variety and harvest season on investigated parameters was analysed, while the influence of growing location was minimized since investigated varieties were grown in the same vineyard. Knowledge of the mechanical structure of grapes is important for the characterization of the technological potential of vine

varieties. According to our results, the highest bunch mass was recorded for Evita cultivar (386 g and 450 g in 2015 and 2016, respectively), while the lowest values were achieved for Čokot Zemun (70 g and 124.25 g in 2015 and 2016, respectively). Evita variety is particularly distinguished by bunch length (14 cm and 16.2 cm in 2015 and 2016, respectively), the average mass of all the berries in a bunch (372 g and 441.0 g in 2015 and 2016, respectively) and weight of berry skin obtained from 100 berries (11.99 g and 13.23 g in 2015 and 2016, respectively). In the two-year research period, Čokot Zemun had the lowest values of the average mass of berry skin from 100 berries (6.11 and 5.24 g) as well as other indicators of the mechanical composition of grapes and berries (Table 1). Variations of presented parameters of mechanical analysis are also under the influence of the year. The values for some of them for the Prokupac variety were higher in 2015 (bunch length, % of the stem in the bunch, % of mesocarp in bunch, % of mesocarp in berries). On the other hand, Evita and Čokot Zemun varieties had higher values of the mechanical composition of grapes and berries parameters in 2016.

The qualitative parameters of grape juice-must are also shown in Table 1. The highest sugar content was recorded for Prokupac variety (22.8 %), followed by Čokot Zemun with 22.3 % and Evita with 21.8 %. All tested varieties had a higher total acid content in the second year of research. The higher content of total acids expressed as tartaric acid in grape juice in the second year of investigation, is a consequence of greater rainfall and slightly poorer temperature conditions during ripening in the autumn 2016. The pH values had the same variation trend.

2.2. Anthocyanin profile

Among the phenolic compounds which have been frequently used as chemical markers the ratio of accumulated anthocyanins is closely connected with genetic characteristics of the

grapevine variety.[5] On the other hand, although conditions such as climate, and physical and chemical characteristics of soil significantly influence the content of individual anthocyanins, numerous papers pointed out that these environmental and agronomical conditions possess a greater effect on anthocyanins concentration rather than on their relative distribution.[5] In this study, we have analysed anthocyanin profile of three Serbian cultivars: Prokupac (clone 41/6), Čokot Zemun and Evita. LC-MS and HPLC-DAD analyses of grape skin methanolic extracts showed significant qualitative and quantitative differences in anthocyanin compounds of investigated varieties.

2.2.1. Qualitative and quantitative analysis

As presented in Table 2, LC-MS analysis detected 17 anthocyanin compounds in grape skin extract obtained from Čokot Zemun variety. Peaks 1-7 correspond to mono- and di-glucosides of anthocyanidin compounds in grape (malvidin, delphinidin, peonidin and petunidin); peak 8 to pyranoanthocianin vitisin A; peaks 9 and 13 to acetic acid-acylated anthocyanin compounds; peaks 14 to caffeoil acylated malvidin; peaks 10-12 and 15-17 to *p*-coummarates of delphinidin, petunidin, peonidin and malvidin. Samples prepared from other varieties (Evita and Prokupac) contained lower number of anthocyanins. In Evita samples 16 compounds were detected, with the exception of Mv-3,5-diglucoside compared to Čokot Zemun variety.

Using HPLC-DAD technique 12 anthocyanins were quantified in the investigated samples. In Figure 1 it can be seen that good separation of anthocyanin compounds (monoglucosides and corresponding acetyl and coumaroyl derivatives) was achieved. The highest concentration of total anthocyanins calculated using HPLC-DAD technique was obtained for Evita variety (33.75 mg g^{-1}), while the lowest concentration of total anthocyanins was determined in Prokupac variety (clone 41/6) and it was almost 10 fold lower (2.70 mg g^{-1}) (Table 3). To

facilitate the comparison of anthocyanin profiles of the wines, the anthocyanin concentrations were normalized, namely, the compounds were expressed as percentages of total anthocyanin content (calculated by HPLC-DAD).

The predominant anthocyanin compound in all of the investigated samples was Mv-3-*O*-glucoside with the content ranging from 1.8 mg g^{-1} in Prokupac clone 41/6 to 15.62 mg g^{-1} in Evita variety. This is in agreement with a general perception that malvidin-3-*O*-glucoside is a major grape anthocyanin and it is demonstrated for the number of other grape varieties such as Cabernet Franc, Merlot and Pinot Noir.[10] Having in mind the percentage of Mv-3-*O*-glucoside in the sum of total anthocyanins (calculated using HPLC technique) the situation is slightly different. Obviously, the percentage of this substance was highest in Prokupac (51.10 %), while in varieties Evita and Čokot Zemun this percentage was lower (48.1 % and 35.17 %, respectively).

In investigated varieties, Df-3-*O*-glucoside and Pn-3-*O*-glucoside were also present, while cyanidin derivatives were not detected. This fact suggests high activity of two enzymes 3'-*O*-methyltransferase and flavonoid 3'-*O*-hydroxylase which are responsible for the conversion of cyanidine into peonidin and delphinidin, respectively.[11] The content of Df-3-*O*-glucoside was highest in Čokot Zemun and Evita varieties, while in Prokupac clone this content was lower. The same pattern was recorded for Pn-3-glucoside. In Čokot Zemun variety the concentration of non-acylated derivatives decreased in the following order: Mv-3-glucoside > Df-3-glucoside > Pe-3-glucoside > Pn-3-glucoside > Vitisin A. In the samples obtained from Evita variety the decreasing order was the following: Mv-3-glucoside>Pn-3-glucoside>Df-3-glucoside>Pt-3-glucoside. Of special interest is the finding of the presence of non-acylated anthocyanin diglucoside compounds in sample „A" (Čokot Zemun). In this sample, high amount of Mv-*O*-3,5-diglucoside (20.43 %) and Pn-*O*-3,5-diglucoside (14.19 %) was also found. In other investigated samples diglucoside derivatives have not been found. Although

more stable compared to monoglucoside derivatives, diglucosides are less colored and more sensitive to browning.[12] They are typical for hybrid vines and are mainly demonstrated in grapes such as *V. amurensis*, *V. riparia*, *V. rupestris*.[13] Still, not every hybrid contains this color type. In *V. vinifera* varieties 3, 5-*O*-diglucosides are usually not present or can be found only in traces. According to previous results, detectable amounts of Mv-3,5-diglucoside were previously reported in *V. vinifera* variety Cabernet-Sauvignon, as well as in skin extracts of Merlot, Cabernet Franc, Shiraz, Sangiovese, Pinot Noir and Prokupac grapes originating from Serbia.[14] Based on the high content of this compound in Čokot Zemun we can assume that it can be a result of the presence of species other than *V. vinifera* in its pedigree.

2.2.2. Classification of anthocyanin compounds

All of the identified anthocyanins were grouped according to two main parameters:

a) distribution of anthocyanidin groups (delphinidin, petunidin, peonidin and malvidin)

b) acylation pattern (non-acylated, acetylated and coumaroylated compounds). Results of these classifications are presented in Tables 3, 4 and 5.

In all of the investigated samples, malvidin group was the dominant one, followed by peonidins, delphinidins and petunidins. The exception was sample Čokot Zemun where the following order was observed: malvidins>delphinidins>peonidins>petunidins. The highest content of malvidins was recorded in Evita sample and the lowest was detected in Prokupac 41/6. The relative percent of malvidins ranged from 72.5 % (sample Čokot Zemun) to 88.8 % (41/6 Prokupac clone).

With respect to classification based on acylation pattern, it is clear that nonacylated compounds were the dominant group in all of the investigated samples (Table 2). Content of this group of compounds ranged from 55.2 % in Prokupac variety to 81.1 % in Čokot Zemun

variety. This group of compounds was followed by coumaroylated derivatives with a range from 15.3 % (Čokot Zemun variety) to 33.0 % (Evita variety). The presence of acylated anthocyanins may be very important for wine color since they participate in intra/molecular co/pigmentation processes, thus increasing the intensity of the wine color.[15] Also, non-acylated anthocyanins are more sensitive to oxidation reactions in berries, while acylated ones represent a more stable form of anthocyanins to oxidative effects increasing that way color stability.[16] According to some authors,[7,17] the percentage of acylated anthocyanins and in particular, those in form of acetates, are those that most contribute to varietal differences. In sample obtained from Prokupac clone 41/6 significantly higher content of acetylated derivatives was recorded compared to samples obtained from varieties Čokot Zemun and Evita. Some grape varieties such as Pinot noir completely lacks acylated anthocyanins.[18]

2.3. Anthocyanin ratios

The precursor in anthocyanins biosynthesis is cyanidin-3-O-glucoside. Under the influence of enzymes 3-*O*-hydroxylase and 3-*O*-methyltransferase compounds, delphinidin-3-*O*-glucoside and 3-O-methyltransferase are formed.[3] The occurrence and the activities of enzymes engaged in the mentioned metabolic pathway are in high correlation with the grape genetic structure.[6] Based on this correlation it can be concluded that the content of anthocyanin compounds can be used for the determination of vine cultivars.

According to the measured contents of individual anthocyanin compounds several anthocyanin ratios were calculated for investigated samples and presented in Table 4. The ratio of malvidin and peonidin derivatives (∑Mv/∑Pn**)** sums point towards the activity of two enzymes flavonoid-3'-hidroxylase and O-dihydroxyphenyl-O-transferase. This ratio corresponds to the ratio of disubstituted (cyanidin and peonidin derivatives) and trisubstituted

(delphinidin, petunidin and malvidin derivatives) anthocyanins and it directly reflects enzyme activity.[6]

The second important ratio is the ratio of sums of coumaroylated and acetylated anthocyanin derivatives (∑Coumaroyl/∑Acetil.).[17,19]

Determined anthocyanin coefficient for investigated cultivars are presented in Table 4. The highest level of ∑Df/∑Pn coefficient is determined for Čokot Zemun cultivar (1.1), while in other cultivars it ranged from 0.2 to 0.9. These results showed that the activity of hydrolases and methyltransferases is higher in Čokot Zemun cultivar and as a result higher content of delphinidin and peonidin is obtained in this compared to other investigated cultivars.

The highest value of ∑Mv/∑Pn is obtained in Prokupac clone (29.5). The lowest value is obtained for cultivar Čokot Zemun (7.5).

According to results obtained in this study, malvidin and its derivatives were dominant anthocyanin compounds in all of the tested samples. The coefficient that represents the ratio of the sums of coumaroylated and acetylated derivatives is also calculated and presented in the Table 4. This coefficient reflected the activity level of acetyl and coumaroyl transferase and is important for grape variety characterization. Previous studies showed that the formation of acetic esters and *p*-coumaric esters seems to be independent of one other based on remarkable variability of the ratio between two ester types in different varieties.[20] For instance, Pinot noir cultivar has no acylated anthocyanins,[8] while they were very abundant in Cabernet-Sauvignon.[6] For cultivars Evita and Čokot Zemun values of this coefficient were 7.4 and 4.3, respectively. These values are significantly higher compared to values obtained for Vranec cultivar (2.4).[8] According to literature data, the value of this coefficient is significant when it is higher than 3.0. Nunez et al.[6] suggested that values of this coefficient can be used for characterization and separation of cultivars Graciano, Cabernet-Sauvignon and Tempranilo (1.9, 0.3 and 5.5, respectively). Having in mind results obtained in our study,

anthocyanin coefficients ∑Mv/∑Pn and ∑Coumaroyl/∑Acetil can be considered as chemical markers for cultivars Evita and Čokot Zemun.

3. CONCLUSION

Data reported in this study represent a certain contribution to a database of mechanical properties and chemical composition of grape varieties originating from Balkan. During different climatic conditions (influence of the year) that prevailed during 2015 and 2016, examined varieties reacted differently which can be seen through parameters of mechanical analysis. Some of them were higher in 2015 (bunch length, % of the stem in a bunch, % of mesocarp in bunch, % of mesocarp in berries, % sugar, alcohol content vol%) for Prokupac variety, while for Evita and Čokot Zemun these parameters were higher in 2016. Also, the results of the mechanical analysis were under the influence of variety. Evita variety had the highest values for most of thr investigated parameters, while Čokot Zemun had significantly lower values of the mechanical composition of grapes and berries in the two-year testing period. All varieties had a higher total acid content in the second year of research, pH values of grape juice-must had the same variation trend.

Analysis of anthocyanin compounds showed that high content of coumaroyl derivatives was recorded in Čokot Zemun and Evita compared to Prokupac variety. Also, a significantly higher content of monoglucosides was found in Čokot Zemun sample. Also, high content of diglucosides derivatives was exhibited for Čokot Zemun variety. We can assume that it can be the result of the presence of species other than *V. vinifera* in its pedigree.

Experimental Section

Chemicals

Formic acid, hydrochloric acid and methanol were purchased from Zorka Pharma, Serbia, while sodium hydroxide from Superlab, Serbia. All reagents were of analytical grade. Ultrapure water used in analyses was generated by the MiliQ system (Milipore, Bedford, USA). HPLC grade acetonitrile was purchased from Sigma-Aldrich (Steinheim, Germany). All of the standard anthocyanin compounds were purchased from Extrasynthese (Cedex, France).

Grape samples

Vitis vinifera L. cultivars Prokupac, Evita and Čokot Zemun tested in this study have been grafted and planted on the Faculty of Agriculture experimental vineyard „Radmilovac", Serbia during the research period (2015–2016). Prokupac is an autochthonous Serbian variety. Evita is an interspecies hybrid of the fourth generation, created by the crossing of Clinton, Black Hamburg and Prokupac varieties. This variety is created at Faculty of Agriculture University of Belgrade and recognized in 1991. Čokot Zemun is a variety which is by its ampelotechnical and economic-technological characteristics the closest to interspecies hybrid of the fourth generation-Saibel hybrid. Collecting and identification of plant material were performed by Professor dr Nebojša Marković and Professor dr Zoran Pržić. Sampling of leaves and grape clusters for ampelographic description was performed in the each year of investigation during the flowering phenophase and the grape ripening phenophase. In order to confirm with certainty the originality of the examined varieties, their identification (ampelographic description) was performed according to the valid standard norms described in the OIV descriptor list for grape and Vitis species, International list of vine varieties (Prokupac No. 2833; Evita No. 1101 and Seibel No . 3211) and their synonyms and IPGRI Descriptors for grapevine (*Vitis* spp.). [21-23]

For investigated varieties grapes were harvested at the technological stage of ripening. Technological maturity of the grapes refers to the moment when berries are most suitable for processing. For the tested table grape variety (Evita), technological maturity occurs before full maturity, since table grapes are harvested and used for fresh consumption. This stadium is characterized with a slightly higher content of total organic acids by which an enhanced feeling of freshness is achieved. For tested wine varieties (Čokot Zemun and Prokupac), technological maturity usually overlaps with their full maturity when there is no further accumulation of sugar and organic acids in the berry.

Grape samples of three investigated cultivars were collected and their analysis was performed at the laboratory of the Faculty of Agriculture University of Belgrade and Institute for Medicinal Plants. Ten clusters per 10 vines of each variety separately were collected. Berries were harvested in late summer at full maturity (phenolic maturity) and immediately frozen until extraction and chemical analysis. Full maturity (phenolic maturity was determined visually, based on the degree of synthesis of the color matter of epidermis and seeds mechanical structure through a change in its color and the level of presence of sclerenchyma cells.

Mechanical analysis of grape bunches and berries was done according to Prostoserdov.[24] Bunches were measured for their weight, length and width, and rachis (pedicel) from each berry was carefully cut off with scissors so that as little mesocarp as possible was left on the stem. The number of berries per bunch was also determined and berry mass per bunch and mass of stems were measured on analytical balance. After that from each variety, 100 berries were randomly selected for mechanical analysis and after measuring the mass of berries, berry skin and seeds were separated. Mass of seeds and skin of 100 berries was measured on an analytical balance, and the number of seeds in 100 berries was determined by counting. Other parameters were obtained by computation.

Ripening parameters are shown through the content of accumulated sugar in grape juice-must and total acids content. Sugar content was determined by physicochemical methods using refractometer and Oeshle mostwage, values were calculated using Dujardin-Salleron tables. The total acid content was determined by titration with n/4 NaOH.

The first year of investigation was characterized by warm weather with higher values of temperature sums and longer dry intervals, especially during the autumn months at the time of grape ripening. The second year was characterized by large temperature fluctuations, especially during the spring and autumn months. The vegetation period was characterized by a large amount of precipitation followed by slightly higher observed mean daily temperatures, both during the summer and autumn months. It can be concluded that during the first year of testing, more favorable conditions for normal development and maturation of the vine prevailed.

Extraction of anthocyanins from grape skin

Grapes (around 15 kg) were peeled with the help of scalpel and the skins were dried in an oven at 40 °C for 24 h (until constant weight). Dried grape skins (350-600 g depending of variety) were crushed in mortar, homogenised and stored at -20 °C until further analysis. The extraction of 1g of grape skin was performed with 10 mL of solvent consisting of 1 % (w/v) HCl in methanol in ultrasound bath (bath power 35 W, continuous mode at frequency of 40 kHz, Maget, Bela Palanka, Serbia) for 30 min. The extracts were filtered through 0.45 μm syringe and analysed for the content of anthocyanin compounds.

LC-MS analysis of anthocyanin compounds

The identification of anthocyanin compounds was done using 6210 Time-of-Flight LC–MS system (Agilent Technologies, Santa Clara, California, USA) connected to an Agilent 1100

Series HPLC instrument (Agilent Technologies, Waldbronn, Germany), with a degasser, a binary pump, an autosampler, a thermostated (40 ºC) column compartment equipped with a Zorbax SB-Aq column (5 µm, 4.6 mm × 250 mm) and a diode-array detector, via ESI interface. The mobile phase (0.8 mL/min) consisted of water containing 10 % formic acid (v/v) and 3 % (v/v) acetonitrile (A) and acetonitrile containing 40 % water (v/v) and 10 % formic acid (v/v) (B) was used for the separation of compounds under following conditions: 0–15 min, 6–30 % B; 15–30 min, 30–50 % B; 30–35 min, 50–60 % B, 35–41 min, 60–6 % B, 41–46 min, 6 % B. Injection volume was 10 µL. Spectral data from all the peaks were accumulated in the range of 190–900 nm and chromatograms were recorded in the same range. Full scan mass spectra were measured between 100 and 1500 *m/z* in positive ion mode. For electrospray ionization positive ESI ionization mode has been applied with the following conditions: capillary voltage 4000 V, fragmentor voltage 140 V, skimmer voltage 60 V, OCT RF voltage 250 V. For drying and evaporation nitrogen was used (pressure 45 psi, temperature 350 °C, flow rate 12 L min^{-1}). Using these parameters eluted compounds were detected as $[M]^+$ signals in positive ion mode.

A personal computer running MassHunter Workstation software was used for data acquisition and processing.

HPLC-DAD analysis of anthocyanin compounds

Analyses of phenolic compounds from the aqueous and organic fractions were performed using HPLC Agilent 1200 Series with UV–Vis diode-array detector (DAD) for multi-wavelength detection. The aqueous fraction was separated on a Zorbax SB-Aq column (250mm×4.6 mm, 5µm) according to the Compendium of International Methods OIV.15.[25] A gradient consisting of solvent A (H_2O/HCOOH/CH_3CN, 87:10:3, v/v/v) and solvent B (H_2O//HCOOH/CH_3CN, 40:10:50, v/v/v) was applied at a flow rate 0.8 mL min^{-1} as follows:

6 to 30 % B linear in 0 to15 min, 30 to 50 % B linear in 15 to 30 min, 50 to 60 % B linear in 30 to 35min, and 60 to 6 % B linear in 35 to 41 min. The column was thermostated at 40 °C. Fifty microlitres of wine, previously filtered through a 0.45 μm membrane, was injected onto the column. Identification was possible by monitoring the anthocyanins at 520 nm and by comparing their spectra and retention times with those of commercial standards. For external standard calibration standard solutions were prepared in different concentrations and quantification of individual anthocyanin compounds was performed using a five-point regression curve.

Statistical analysis

The means and standard deviations were calculated using IBM SPSS Statistics 2.0, Chicago, IL, USA. For comparison of the means, one-way ANOVA and Tukey's post-hoc test were applied at the 95% significance level. Differences were considered statistically significant if the p-value was less than 0.05.

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Author Contribution Statement

Nebojsa Menkovic conceived and designed the research. Jelena Zivkovic contributed to data analysis and manuscript drafting and performed HPLC analysis of the samples. Milka Jadranin performed LC/MS analysis of the samples. Danijel Sokolovic and Katarina Savikin contributed to the revision of the article. Zoran Przic and Nebojsa Markovic performed

agronomic field experiments, statistical analysis of data and mechanical analysis of investigated samples.

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Table 1. Bunch and berry mechanical composition of Prokupac, Evita and Čokot Zemun

Varieties/Clon	**Prokupac-41/6**		**Evita**		**Čokot Zemun**	
Year	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
Bunch lenght (cm)	14.20	13.80	14.00	16.20	9.94	11.80
Bunch width (cm)	10.60	7.80	14.20	13.60	8.00	6.60
Bunch mass (g)	295.00	200.00	386.00	450.00	70.00	124.25
Average berries number in bunch	100.60	79.40	126.80	157.20	23.60	61.00
Bunch stem mass (g)	12.00	10.00	14.00	9.00	8.80	5.26
Berries mass (g)	283.00	190.00	372.00	441.00	62.00	118.99
Mass of 100 berries (g)	276.00	274.00	308.00	296.00	274.00	252.69
Mass 100 seeds (g)	2.82	2.43	2.97	3.10	3.64	4.46
Mass of bery skin from 100 berries (g)	9.65	7.62	11.99	13.23	6.11	5.24
Mass of seeds from 100 berries (g)	5.12	4.71	5.90	6.31	9.22	9.42
Average seed number in 100 berries (g)	201.00	196.00	195.00	205.00	255.00	212.00
Average mass of one berry (g)	2.76	2.74	3.08	2.96	2.74	2.52
Average mass of berry skin of one berry (g)	0.09	0.07	0.12	0.13	0.06	0.05
Average mass of seeds of one berry (g)	0.05	0.04	0.05	0.06	0.09	0.09
Average number of seeds in one berry (g)	2.01	1.96	1.95	2.05	2.55	2.12
Average mass of 100 seeds (g)	2.54	2.40	3.02	3.07	3.61	4.44
Average mass of one seed (g)	0.02	0.02	0.03	0.03	0.03	0.04
Average mass of masocarp in 100 berries (g)	261.23	261.67	290.11	276.46	253.67	238.03
Average mass of all berries in bunch (g)	283.00	190.00	372.00	441.00	61.20	118.99
Average mass of berry skin in one bunch (g)	9.70	6.05	15.20	20.79	1.74	3.19
Average mass of seeds in one bunch (g)	5.15	3.74	7.48	9.91	2.63	5.74
Average mass of mesocarp in one bunch (g)	268.14	180.21	349.31	410.28	56.81	110.04
Average number of seeds in one bunch (g)	202.20	155.62	247.26	322.26	72.93	129.32
% of stem in bunch	4.06	5.00	3.62	2.00	12.57	4.23
% of berries skin in bunch	3.29	3.02	3.93	4.62	2.49	2.57
% of seeds in bunch	1.74	1.87	1.93	2.20	3.76	4.62

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% of mesocarp in bunch	90.89	90.10	90.49	91.17	81.16	88.56
% of berries skin in berry	3.49	2.78	3.89	4.47	2.23	2.07
% of seed in berries	1.85	1.71	1.91	2.13	3.36	3.72
% of mesocarp in berries	94.64	95.50	94.19	93.39	94.40	94.19
Berry indicator	34.10	39.70	32.85	34.93	40.85	49.09
Indicator of berry weight composition	27.07	34.34	24.19	20.89	42.33	45.42
The bunch skeleton	7.35	8.02	7.56	6.62	15.06	6.80
Hard residue of bunch	9.10	9.89	9.50	8.82	13.83	11.43
Indicator of cluster mass composition	23.58	19.00	26.57	49.00	6.95	22.62
Bunch structure indicator	9.98	9.10	9.52	10.33	4.30	7.74
			Quality parameters			
% of sugar	22.8	20.2	19.2	21.8	22.3	20.8
Total acid content (g L^{-1})	7.1	7.6	7.5	8.7	7.1	7.4
Glycoacidometric index	3.2	1.71	2.56	2.5	3.12	1.82
pH	3.2	3.5	3.3	3.4	3.2	3.6

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Table 2. Anthocyanin compounds identified using LC-MS-DAD technique in grape skin extract of Čokot Zemun variety

Compounds		**Retention time, $t_{R\ (min)}$**	**Molecular ion $[M]^+$ (m/z)**	**Spectral characteristics λ_{max}(nm)**
1.	Delphinidin-3-glucoside	10.73	465	278, 346, **524**
2.	Peonidin-3,5-diglucoside	12.50	625	248, 277, **513**
3.	Malvidin-3,5-diglucoside	13.56	655	274, 348, **526**
4.	Petunidin-3-*O*-glucoside	14.12	479	276, 346, **528**
5.	Peonidin-3-*O*-glucoside	16.07	463	278, **520**
6.	Malvidin-3-*O*-glucoside (*cis*)	16.68	439	276, 350, **528**
7.	Malvidin-3-*O*-glucoside (*trans*)	17.42	439	278, 348, **526**
8.	Vitisin A	18.21	561	302, 372, **510**
9.	Petunidin-3-*O*-acetilglucoside	22.03	521	246, 282, **530**
10.	Delphinidin-3-*O*-(6-*O*-*p*-coumaroyl) glucoside	22.69	611	246, 282, **530**
11.	Peonidin-3-*O*-(6-*O*-*p*-coumaroyl)-5-*O*-diglucoside	23.64	771	246, 280, **530**
12.	Malvidin-3-*O*-(6-*O*-*p*-coumaroyl) -5-*O*-diglucoside	24.39	801	248, 278, **530**
13.	Malvidin-3-*O*-(6-*O*-acetyl) glucoside	26.10'	535	248, 278, 346, **532**
14.	Malvidin-3-*O*-(6-*O*-*p*-caffeoyl) glucoside	26.43'	655	248, 282, **532**
15.	Peonidin-3-*O*-(6-*O*-*p*-coumaroyl) glucoside	26.48'	609	282, **526**
16.	Malvidin-3-*O*-(6-*O*-*p*-coumaroyl) glucoside (*cis*)	29.55'	639	248, 282, **532**
17.	Malvidin-coumaroyl-3-glucoside (*trans*)	30.21'	639	282, **530**

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Table 3. The content of anthocyanins (mg g^{-1}) in skin extracts of cultivars Čokot Zemun, Evita and Prokupac (clone 41/6) (n=3*)

№	Compound	$t_{R\ (min)}$	Čokot Zemun	Evita	41/6
			mg g^{-1}	mg g^{-1}	mg g^{-1}
1.	De-3-*O*-gl	11.47	1.98±0.04[a]	1.65±0.02[b]	0.04±0.00[c]
2.	Pn-3,5-*O*-digl	13.19	1.08±0.03[a]	0.03±0.00[b]	nd
3.	Mv-3,5-digl	14.46	5.27±0.15	nd	nd
4.	Pt-3-*O*-gl	15.00	nd	nd	nd
5.	Pn-3-*O*-gl	16.99	1.08±0.04[a]	1.82±0.06[b]	0.10±0.00[c]
6.	Mv-3-*O*-gl	18.42	9.07±0.31[a]	15.62±0.64[b]	1.38±0.04[c]
7.	Vitisin A	19.37	0.53±0.01[a]	nd	0.05±0.00[b]
8.	De-3-*O*-(6-*p*-coumaroyl)-gl	23.87	0.23±0.01[a]	0.55±0.01[b]	0.03±0.00[c]
9.	Mv-3-*O*-(6-*p*-coumaroyl)-5-*O*-digl	25.47	0.49±0.00[a]	0.41±0.00[a]	0.05±0.00[b]
10.	Mv-3-*O*-(6-*O*-acetyl)-gl	27.36	0.91±0.03[a]	1.85±0.06[b]	0.38±0.01[c]
11.	Pn-3-*O*-(6-*O*-*p*-coumaroyl)-gl	30.50	0.36±0.02[a]	0.75±0.03[b]	0.07±0.00[c]
12.	Mv-coumaroyl-3-*O*-gl	31.46	2.42±0.08[a]	9.92±0.09[b]	0.50±0.02[c]

De-delphinidin, Pn-peonidin, Mv-malvidin, Pt-petunidin, gl-glucoside; [a,b,c] – different values in the same row indicate a significant difference among varieties according to Tukey's test, $p<0.05$; * - values are given as average values obtained for three different samples.

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Table 4. Anthocyanin coefficients obtained for investigated vine cultivars

Anthocyanin ratios	Čokot Zemun	Evita	Prokupac (41/6)
∑Mv/∑Pn	7.48	11.46	29.51
∑Coumaroyl/∑Acetyl	4.34	7.37	1.71
∑Mv+∑Pt+∑Df/∑Pn	9.32	11.89	30.51
∑Df/∑Pn	1.08	0.71	0.87
∑Pt/∑Pn	0.76	0.42	0.12

De-delphinidin, Pn-peonidin, Mv-malvidin, Pt-petunidin, gl-glucoside

Figure caption:

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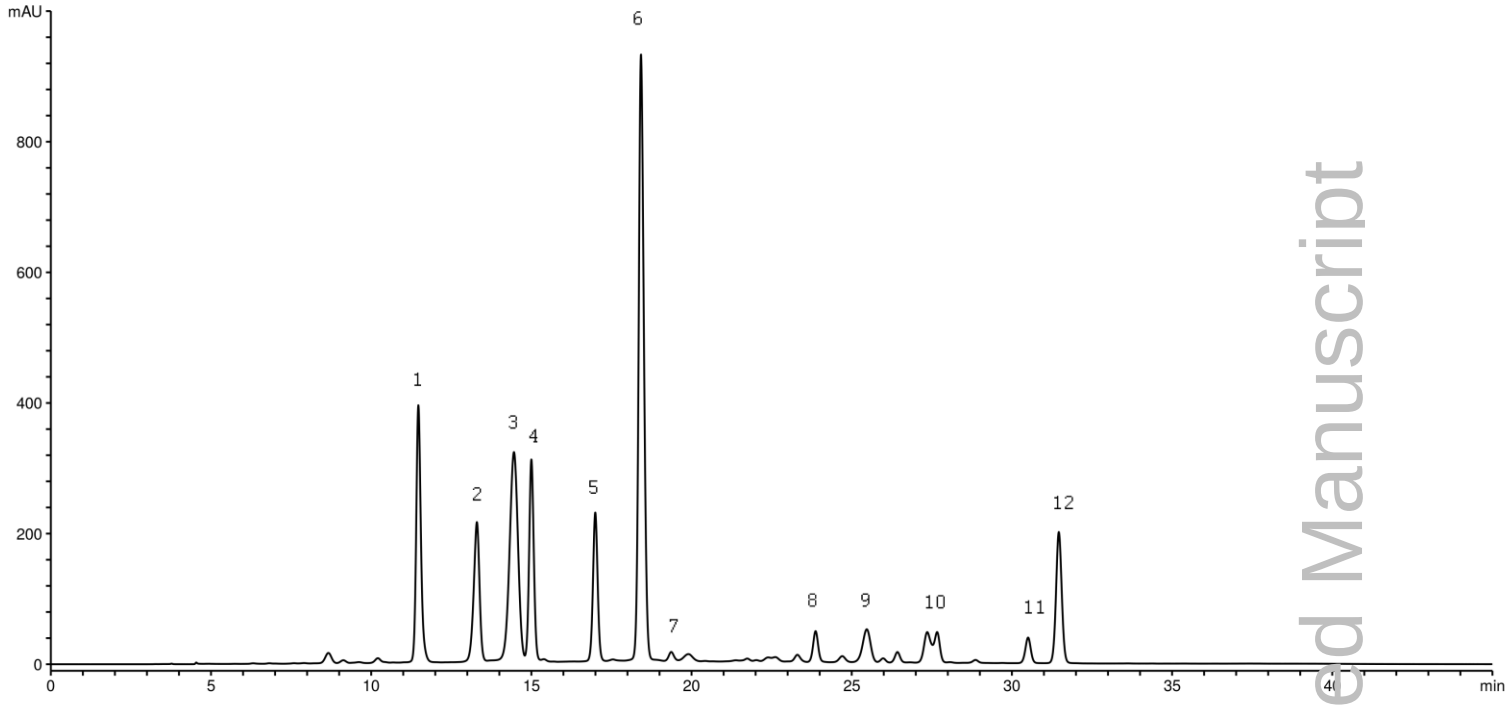

Fig 1. HPLC chromatogram of Čokot Zemun skin extract recorded at 520 nm. Peak identification: (1) De-3-*O*-gl, (2) Pn-3,5-*O*-digl, (3) Mv-3,5-digl, (4) Pt-3-*O*-gl, (5) Pn-3-*O*-gl, (6) Mv-3-*O*-gl, (7) Vitisin A, (8) De-3-*O*-(6-*p*-coumaroyl)-gl, (9) Mv-3-*O*-(6-*p*-coumaroyl)-5-*O*-digl, (10) Mv-3-*O*-(6-*O*-acetyl)-gl, (11) Pn-3-*O*-(6-*O*-*p*-coumaroyl)-gl, (12) Mv-coumaroyl-3-*O*-gl