Influence of bunch morphology on quality of wines produced from clones of grape variety Prokupac

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Abstract: Wine quality depends mainly on the characteristics of the grape it is made of, and one of the attributes affecting wine composition is cluster and berry morphology. The aim of this study was to represent variability of the morphological characteristics between different clones of the autochthonous grape variety Prokupac and to perform chemical evaluation of wines obtained from them. Total phenolic content was generally low and it ranged from 33.0 to 114.5 mg GAE/100 mL. Six main anthocyanin compounds including malvidin as the main anthocyanidin were detected. Malvidin 3-O-glucoside was the most abundant anthocyanin with concentration of 59.8 to 101.7 µg/mL. Clones 43/5 and 43/4 yielded highest quality wines. According to the results, clonal selection makes a significant difference in Prokupac wine quality. On the other hand, there is a minor dependance of wine quality parameters to variation in morphological attributes of clusters and berries (bunch weight, proportion of stem, berry and seed weight, skin, pulp and seed weight per berry).

Keywords: anthocyanins; clonal selection; malvidin 3-O-glucoside.

INTRODUCTION

Clonal selection is considered a very important tool for grapevine genetic improvement.1 For Vitis vinifera L. (Vitaceae), clones could be selected aiming for better grape quality attributes, stronger wine aroma and coloration, as well as genetic resistance to main pests and diseases. Clones from one grape variety can differ in their productive traits and their ability to produce wines with different organoleptic characteristics. It has been shown that some clones have the cap-
acity to produce wines with distinct color, aromatic profile and phenolic content.\textsuperscript{2,3}

The main polyphenols present in the wine are phenolic acids, stilbenes, flavonols, dihydroflavonols, anthocyanins, flavanol monomers (catechins) and flavanol polymers – proanthocyanidins.\textsuperscript{4} As the phenolic acids are largely present in the berry pulp, anthocyanins and stilbenes in the berry skin, and other polyphenols (catechins, proanthocyanidins and flavonols) in the berry skin and seeds, the proportion of the different polyphenols in wines could vary according to the type of vinification.\textsuperscript{5} Total phenolic content, combined with total anthocyanin, proanthocyanidin and tannin content, are important parameters to be considered for the quality of the produced wine.\textsuperscript{6} The quantity and composition of these secondary metabolites greatly depend on genetic factors, but their amount could also be affected by environmental factors and cultural practices, as well as berry morphology.\textsuperscript{7} It is widely believed by grape growers that smaller berries and lower yield produce higher quality wines due to a higher proportion of skin and seed derived compounds.\textsuperscript{8} Although wine composition can be manipulated by changing berry size, growers need to balance this with the need for a high yield from well-sized berries on their vines.

The southern area of Serbia has a well established tradition of viticulture and winemaking since the dominant soil types and climatic conditions of the region are very beneficial for the cultivation of vines.\textsuperscript{9} Prokupac is a Serbian autochthonous red wine variety, mostly spread throughout southern and central parts of Serbia, as well as in Macedonia and Bulgaria. It is characterized by the strong vigor and big yielding capacity. Its bunch could be classified as medium large, cylindrical or conical in shape, bearing medium compact, round or slightly oval berries with dark blue epidermis.\textsuperscript{10} Prokupac wine is refreshing and nicely red colored.

The objective of this study was to represent the morphological variability among clones of the autochthonous variety Prokupac and make chemical evaluation of the wines obtained from them. Major groups of compounds were evaluated in total amounts, \textit{i.e.}, total phenolics, total anthocyanins, total proanthocyanidins, and the identification and quantification of individual anthocyanins in Prokupac wines was done. The influence of grape berry morphological parameters on the wine composition, especially anthocyanin extractability was also determined.

EXPERIMENTAL

\textit{Wine grape sample preparation}

Prokupac clones tested in this study have been allocated in vineyards cca. 100 years old from southern and central parts of Serbia. During research period (2010–2013), 26 clones were grafted and planted on Faculty of Agriculture experimental field Radmilovac, Serbia.
Ministry of Agriculture of Serbia recognized 12 clones as technologically superior compared to the standard variety.

Morphological analysis within each clone was performed by standard Prostoserdov method\(^{11}\) on 10 clusters per 10 vines separately. Firstly, weight of bunches was measured. Rachis (pedicel) from each berry was carefully separated, so that as little as possible mesocarp was left on the stem. Berry mass per bunch as well as mass of stems per bunch was measured using analytical balance. Mass of seeds and skin of 100 berries was measured using analytical balance, and number of seeds in 100 berries was determined by counting. Other parameters shown in Table 1 were obtained by calculation.

Microvinification was performed in the laboratory of Faculty of Agriculture, University of Belgrade. Harvesting was done manually. Grape crashing was done using mechanical press and crushed grapes during microvinification were stored in 10 L container. To crushed grapes 0.1 g/1 kg of sulphur was added. For microvinification process, selected *Saccharomyces cerevisiae* red wine yeast-BDX Lalemand was used (2.5 g/10kg of crushed grape). Each clone was tested in triplicate. Microvinification ending was detected by Oechsle mostwage when 0°Oe of sugar in must was indicated. Wine was stored in 1 L glass bottle prior to analysis.

**Determination of total phenolics**

The concentration of total phenolic compounds in wine was estimated spectrophotometrically using slightly modified Folin–Ciocalteu method.\(^{11}\) Two hundred microliters of wine (5 mg/mL 50% EtOH) were added to 1 mL of diluted Folin–Ciocalteu reagent (1:10). After 4 min, 800 μl of sodium carbonate (75 g/L) were added. The absorbance was measured after 2 h of incubation at room temperature, at 765 nm. Gallic acid (0–100 mg/mL) was used for calibration of a standard curve. The results were expressed as milligrams of gallic acid equivalents per gram of dry weight of fraction (mg GAE/g DW).

**Determination of total anthocyanins**

Total anthocyanin content was analyzed according to the procedure described in European Pharmacopoeia 6.0. using slight modifications.\(^{12}\) Wine samples were diluted (1:5) in a solution of hydrochloric acid in methanol (0.1 vol. %). The absorbance was measured at 528 nm, with 0.1% v/v solution of hydrochloric acid in methanol as the compensation liquid. The percentage content of anthocyanins was calculated as \(A \times 5000/718m\) (\(A\) = absorbance at 528 nm; 718 = specific absorbance of cyaniding 3-glucoside chloride at 528 nm; \(m\) = mass of the wine to be examined in grams) and expressed as cyaniding 3-glucoside chloride. The results were presented as percentages.

**Determination of total proanthocyanidins**

The content of total proanthocyanidin compounds in the samples was determined spectrophotometrically using \(p\)-dimethylaminocinnamaldehyde (\(p\)-DMACA) reagent with slight modifications.\(^{13}\) One hundred μL of wine samples were mixed with 80 μL of \(p\)-DMACA reagent, 2 mL of methanol, and a drop of glycerol. After 7 min, the absorbance was measured at 640 nm. The content of proanthocyanidins in the samples was presented as milligrams of catechin equivalents per 100 mL of sample (mg CE/100 mL).

**LC–MS analysis**

LC–MS analysis was performed on an Agilent MSD TOF coupled to an Agilent 1200 series HPLC. Wine samples were separated on Zorbax SB-Aq column (250 mm×4.6 mm, 5μm). A gradient consisting of solvent A (10 % formic acid in water) and solvent B (acetonitrile) was applied at a flow rate of 1 mL min\(^{-1}\) as follows: 0–1 min, 1–7 % B; 1–4 min, 7 %
HPLC-DAD analysis

Analysis of anthocyanins was done using HPLC Agilent 1200 Series with UV–Vis DAD for multiwavelength detection. Wine samples were separated on Zorbax SB-Aq column (250 mm×4.6 mm, 5μm) according to the Compendium of International Methods OIV.\textsuperscript{14} Mobile phase consisting of solvent A (H\textsubscript{2}O/HCOOH/CH\textsubscript{3}CN, 87:10:3 volume ratio) and solvent B (H\textsubscript{2}O/HCOOH/CH\textsubscript{3}CN, 40:10:50 volume ratio) was applied at a flow rate of 0.8 mL/min according to following gradient program: 6 to 30% B linear from 0 to 15 min, 30 to 50% B linear from 15 to 30 min, 50 to 60% B linear from 30 to 35 min, and 60 to 6% B linear from 35 to 41 min. The injection volume was 50 μL, and the column was thermostated at 40 °C. Identification was possible by monitoring anthocyanins at 520 nm and by comparing their spectra and retention times with those of commercial standards. Quantification was done using calibration curves of authentic standards.

Statistical analysis

Statistical analysis was carried out using the software package Statistica, v. 7.0. Data are presented as the mean values ± standard deviation from three independent measurements. The variation in chemical parameters was analyzed using one-way analysis of variance (ANOVA) and differences between clones were estimated with Duncan test ($p < 0.05$). Correlations were considered statistically significant, if the $p$-value was less than 0.05. Hierarchical cluster analysis was applied for finding relatively homogenous clusters of cases based on measured characteristics. Differences between classes were tested with average Euclidean distances using the ward method. Results of hierarchical clustering process were represented as dendrogram. Relationship between individual anthocyanins and total compounds were determined by principal component analysis (PCA).

RESULTS AND DISCUSSION

Morphological characteristics of Prokupac clones

Some of the viticultural characteristics that make distinct clones include berry and cluster morphology as well as final yield. The same characteristics are also widely recognized as factors influencing the winegrape quality\textsuperscript{15}. There are numerous factors, besides genetics that can affect berry size and the composition of grapes and wines like water status, cultural practices or annual weather conditions.\textsuperscript{16} In our study, since all the samples were grown under the same conditions, the differences in morphological parameters were mainly the outcome of genetic factors. The results of basic morphological characteristics for the tested Prokupac clones are presented in Table I. Although not statistically significant,
minor differences were observed among clones for investigated morphological parameters. Clone 42/2 had the lowest bunch weight (145.30 g) and clone 41/1 the
<table>
<thead>
<tr>
<th>Clone</th>
<th>Bunch weight, g</th>
<th>Proportion of stem, %</th>
<th>Proportion of berries, %</th>
<th>Seed weight, g</th>
<th>Berry weight, g</th>
<th>Skin weight/berry, g</th>
<th>Seeds weight/berry, g</th>
<th>Pulp weight/berry, g</th>
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</thead>
<tbody>
<tr>
<td>40/5</td>
<td>206.15±67.38</td>
<td>4.80±2.44</td>
<td>95.20±2.44</td>
<td>0.03±0.00</td>
<td>2.41±0.33</td>
<td>0.16±0.07</td>
<td>0.04±0.01</td>
<td>2.22±0.27</td>
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<td>187.22±48.35</td>
<td>4.23±1.15</td>
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<td>0.03±0.01</td>
<td>2.00±0.44</td>
<td>0.10±0.04</td>
<td>0.04±0.01</td>
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<td>205.32±12.74</td>
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<td>41/1</td>
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<td>172.08±32.63</td>
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<td>0.06±0.02</td>
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<td>212.50±73.61</td>
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<td>42/2</td>
<td>145.30±60.83</td>
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<td>1.91±0.60</td>
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<td>0.04±0.01</td>
<td>1.76±0.58</td>
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<td>170.70±38.22</td>
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<td>2.14±0.50</td>
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<td>0.04±0.02</td>
<td>2.26±0.32</td>
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<td>191.30±36.44</td>
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<td>96.21±1.14</td>
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<td>184.70±76.02</td>
<td>3.59±1.06</td>
<td>94.66±4.04</td>
<td>0.03±0.01</td>
<td>2.41±0.34</td>
<td>0.09±0.02</td>
<td>0.04±0.02</td>
<td>2.27±0.31</td>
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<td>43/7</td>
<td>193.12±45.33</td>
<td>3.63±1.05</td>
<td>96.37±1.05</td>
<td>0.03±0.00</td>
<td>2.38±0.32</td>
<td>0.11±0.02</td>
<td>0.06±0.02</td>
<td>2.20±0.30</td>
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</table>
highest (224.78 g). The bunch structure is defined through percent of bunch stem (rachis) and percent of berries in a bunch. Clone 42/2 also had the highest proportion of bunch stem (5.48%), but the lowest proportion of berries (94.52%) in a bunch. On the contrary, clone 43/2 had the lowest proportion of stem (3.08%) and the highest proportion of berries (96.92%). The seed mass was uniform (0.03 g) for most of the samples with the exception of clone 41/3 that was slightly larger (0.04 g). In relation to the values of berry structural composition expressed as skin, seed and pulp weight, the highest values were recorded for skin of clone 40/5, seeds of clones 40/8 and 43/7 and pulp of clones 40/8 and 41/3. According to the results, Prokupac berries are comprised of approximately 5% skin, 2.5% seed and 97.5% flesh. Generally, the proportion of skin to seed is smaller than in Cabernet Sauvignon grapevines where relative skin mass varied between 15 and 20% and relative seed mass between 4 and 6%\textsuperscript{17} or in the Chardonnay variety where skin proportion in berry weight was 16%\textsuperscript{18}. For the increasing berry size, the ratio of seed weight/berry weight decreased, while we have not observed any pattern for skin weight/berry mass ratio.

Wine phenolic composition

After microvinification process, produced wine samples of the investigated clones were further analysed using spectrophotometric methods.

Phenolic compounds are of considerable oenological cultivar potential and play a key role in estimating the quality of wine. The amount of total phenolics in the wines obtained from 13 clones of autochthonous grape variety Prokupac are presented in Table II. The values ranged from 33.0 (clone 40/8) to 114.5 (clone 43/4) mg GAE/100 mL and statistically significant ($F = 55.09, p < 0.001$) difference was noticed between tested clones. Previously, a significant difference in total phenolic content between wines produced from Cabernet Sauvignon clones 169 and 685 cultivated in Brazil was reported by Burin et al.\textsuperscript{2}

The phenolic composition is primarily dependent of the grape cultivar and oenological practices, and for young red wines it ranges between 100 and 500 mg/100 mL.\textsuperscript{19} According to previous results,\textsuperscript{20} among the wines produced from different cultivars (Merlot, Cabernet Sauvignon, Pinot Noir and Prokupac) the lowest phenolic content was found in the wine from the native cultivar Prokupac. Depending on the applied winemaking technology, total phenolic content in Prokupac wines varied from 54.4–115.9 mg GAE/100 mL, which is in accordance with our results. In Balkan countries, the most similar results of total phenolic contents in red wines compared to our Prokupac clone wines reported Kallithraka et al.\textsuperscript{21} for Greek red wines, where the values ranged from 62.2–320.0 mg GAE/mL. Šeruga et al.\textsuperscript{22} reported slightly greater values (101.2–326.4 mg GAE/mL) for Croatian red wines.
### TABLE II. Phenolic compounds (µg ml⁻¹) in the wine samples obtained from 13 clones of Serbian autochthonous grape variety Prokupac; data are presented as means ± SD, n = 3; F: significant at p < 0.001. TP – total phenolics, TA – total anthocyanins, TPR – total proanthocyanidins, D 3-O-G: delphinidin 3-O-glucoside, P 3-O-H: peonidin 3-O-hexoside, M 3-O-G: malvidin 3-O-glucoside, PE 3-O-(6-O-A) H: peonidin 3-O-(6-O-acetyl)hexoside, M 3-O-(6-O-A) H: malvidin 3-O-(6-O-acetyl)hexoside, M 3-O-(6-O-C) H: malvidin 3-O-(6-O-coumaroyl)hexoside. Means followed with different letters are significantly different at p < 0.05.

<table>
<thead>
<tr>
<th>Clone</th>
<th>TP (mg GAE/100 ml)</th>
<th>TA×10ⁿ</th>
<th>TPR (mg catechin/100 ml)</th>
<th>D 3-O-G</th>
<th>P 3-O-H</th>
<th>M 3-O-G</th>
<th>PE 3-O-(6-O-A)</th>
<th>M 3-O-(6-O-A)</th>
<th>M 3-O-(6-O-C)</th>
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<tr>
<td>40/5</td>
<td>112.71±9.51ᵃ</td>
<td>14±2ᵃ</td>
<td>67.52±2.11ᵃ</td>
<td>1.96±0.06ᵇ</td>
<td>1.74±0.05ᵇ</td>
<td>73.10±3.22ᵇ</td>
<td>2.08±0.07ᵇ</td>
<td>9.58±0.22ᵇ</td>
<td>6.61±0.18ᵇ</td>
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<td>40/8</td>
<td>33.05±1.52ᵇ</td>
<td>6±0.0ᵇ</td>
<td>20.41±2.09ᵇ</td>
<td>2.43±0.07ᵇ</td>
<td>1.94±0.05ᵈ</td>
<td>60.90±2.95ᵇ</td>
<td>2.20±0.09ᵇ</td>
<td>16.42±0.73ᵇ</td>
<td>8.68±0.21ᵇ</td>
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<tr>
<td>41/1</td>
<td>92.63±2.54ᵈ</td>
<td>12±1ᵈ</td>
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<td>3.23±0.07ᵃ</td>
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<td>101.70±4.81ᵈ</td>
<td>2.47±0.06ᵈ</td>
<td>19.38±1.11ᵉ</td>
<td>13.02±0.60ᵇ</td>
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<td>84.40±4.10ᵈ</td>
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<td>69.90±2.74ᵇ</td>
<td>1.99±0.02ᵇ</td>
<td>9.22±0.34ᵈ</td>
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<td>2.03±0.05ᵇ</td>
<td>73.70±2.02ᵇ</td>
<td>2.39±0.04ᵇ</td>
<td>11.76±0.54ᵈ</td>
<td>7.06±0.10ᵇ</td>
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<td>108.42±8.01ᵇ</td>
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<td>50.41±1.18ᵇ</td>
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<td>1.99±0.06ᵇ</td>
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<td>93.00±4.02ᵈ</td>
<td>3.82±0.09ᵇ</td>
<td>20.21±1.20ᵇ</td>
<td>12.02±0.38ᵇ</td>
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</table>
The principal source of red color in wine comes from its anthocyanin content. In grape berries anthocyanins are found mainly in the skin, while pulp contains little or no anthocyanins. Since anthocyanins are primarily responsible for the red wine color, they were extensively studied in order to identify the environmental impacts and genetic effects on their synthesis in the berry skin and accumulation in the wine.\textsuperscript{23–25} Also, they were used for chemotaxonomic purposes with the goal of classifying red-grape varieties and the red wines made from them.\textsuperscript{26} The amounts of total anthocyanins in wines obtained from 13 clones of autochthonous grape variety Prokupac are presented in Table II. Same as for the total phenolics, statistically significant ($F = 2.58, p < 0.05$) difference among total anthocyanins content between wines produced from different clones was reported. The smallest amount of total anthocyanins (0.006 \%) was measured in the clone 40/8 while the most value (0.015 \%) was noticed in the clone 43/4 where the highest level of total phenolic content was also observed.

Tannins in the wine, which are mainly responsible for its bitterness and astringency are composed of proanthocyanidins localized in the grape skin and seeds. While skin proanthocyanidins are larger polymers contained primarily of epigallocatechin, seed proanthocyanidins are smaller molecules with higher proportion of galloylated subunits.\textsuperscript{16} The amounts of total proanthocyanidins in the wines obtained from investigated Prokupac clones are presented in Table II. The total proanthocyanidin content of red wines averaged at 17.5 mg/100 mL,\textsuperscript{27} which is significantly lower compared to Prokupac wines investigated in this study. The smallest amount was recorded in the clone 40/8 (20.41 mg catechin/100 mL) which also had the lowest amount of total phenolics. The highest amount (80.11 mg catechin/100 mL) was observed in clone 43/5, one of the richest in total phenolics. Same as in the case of total phenolics and total anthocyanins, statistically significant ($F = 142.93, p < 0.001$) difference was noticed for total proanthocyanidins among tested wine samples. Significant correlation was found between total phenolics and total anthocyanins as well as between total anthocyanins and total proanthocyanidins.

**HPLC analysis**

The anthocyanins were tentatively identified using LC/MS and since the standard compounds were not available, the concentrations of detected compounds were expressed as aglycones. Six main anthocyanin compounds (Table II) were identified and malvidin was the main anthocyanidin present. Actually, malvidin 3-O-glucoside (the most abundant anthocyanin ranging from 59.8–101.7 µg/mL), malvidin 3-O-(6-O-acetyl)hexoside and malvidin 3-O-(6-O-coumaroyl)hexoside were found. Previous results showed that those are the predominant anthocyanins for *Vitis vinifera* wines.\textsuperscript{28,29} Moreover, in our investigation, other anthocyanins were also detected: delphinidin 3-O-glucoside, peoni-
3-O-hexoside and peonidin 3-O-(6-O-acetyl)hexoside. In the tested wine samples the group of anthocyanin monoglycosides represented the highest proportion of anthocyanins varying from 67.90 (clone 43/5) to 83.10 % (clone 42/2), followed by the acetyl derivatives varying from 11.79 (clone 43/7) to 21.46 % (clone 43/5) and p-coumaroyl glucosides at 6.21 (clone 41/6) to 10.64 % (clone 43/5). Although in red wines of different grape varieties derivatives of cyanidin were detected,30,31 our Prokupac wine samples did not contain that aglycon or its derivatives.

Variation in the amount of individual phenolics in wines obtained from the same grape variety were noticed by other authors. For example, Van Leeuw et al.32 showed large variability in the levels of individual phenolic compounds as well as in antioxidant capacity in 38 different wine samples obtained from clones of four main grape varieties.

The typical concentration of free anthocyanins in full bodied red wines is approximately 50 mg/100 mL, but in some cases could be higher than 200 mg/100 mL.33 For our tested wine samples the content of total anthocyanins calculated by HPLC analysis ranged from 78.30 (clone 43/7) to 143.80 (clone 40/6).

Hierarchical cluster analysis was also employed in order to investigate the relationship between the samples and produce a tree diagram.34 Based on mean values for the investigated chemical parameters (TP, TPR, TA and contents of individual anthocyanin compounds) we designed a dendrogram of phenotypic differences for the examined Prokupac clones. As proximity measures of average linkage between groups, we used Euclidean distances. Two distant clusters were detected (Fig. 1). Five clones were classified in cluster I (41/1, 41/2, 40/6, 43/5 and 43/4), while cluster II containing 8 clones can be divided into two subgroups (subgroup I – 40/5, 42/2, 43/6, 41/6, 43/7 and subgroup II – 40/8, 41/3 and 43/2). The minimum distance was observed between clones 40/5 and 42/2. According to cluster analysis regarding morphological characteristics of the tested clones, these two samples were very distant.

In order to determine any relation among the investigated samples and to identify the variables that could discriminate among clones, principal component analysis (PCA) was also performed (Fig. 2). The obtained results showed that from nine principal components, the first two having eigenvalues greater than one are sufficient to explain 85.02 % of the total variability observed. The first principal component presented 58.19 % of the variation and was mostly due to the content of detected individual anthocyanins, which were positively correlated, while the second principal component accounted for 26.83 % of the variation and was mostly due to the content of total phenolics, total proanthocyanidins and total anthocyanins. The wines grouped on the right side of the diagram (obtained from clones 40/5, 40/8, 41/6, 42/2, 43/2, 43/6 and 43/7) were characterized by lower levels of individual anthocyanins. The vertical separation obs-
erved along PC2 is caused by lower content of total phenolics, total anthocyanins and total proanthocyanidins in wines obtained from clones 40/8, 41/1, 41/3, 41/6, 43/2 and 41/2.

![Cluster dendrogram of the wines obtained from 13 clones of Serbian autochthonous grape variety “Prokupac” based on their chemical characteristics.](image1)

![Scatterplot of the first two principal components (PC1 vs. PC2) for Prokupac wine samples.](image2)

**Correlation analysis**

The grape berries typically contain non-flavonoid compounds in the pulp and flavonoid compounds in the skin, seeds and stem.35
The results of correlation analysis showed that there is a poor response of quality parameters to variation in morphological parameters. Total phenolics did not show significant correlation to investigated morphological parameters. This is in accordance with the results obtained by Poni et al. They showed that the size of a grape berry has no influence on the wine composition. On the other hand, Walker et al. found a difference in the anthocyanin concentration and wine color only for the smallest size class.

Anthocyanins are located in the skin cells, in free form inside the vacuoles. They become diluted by sap released from the berry flesh upon crushing. In our study, low correlation was found between berry weight and total anthocyanin content in the tested wines, as well as between the skin weight per berry and the total anthocyanin content. Such low correlation could be explained by the fact that during maceration, anthocyanins are remained in the berry skin due to mechanical properties of skin cell walls and low extractability.

Romero-Cascales et al. found a higher concentration of anthocyanins in smaller Monastrel berries compared to bigger ones, but these two berry categories were from different localities. Similarly, wine made from smaller berries had higher concentration of anthocyanins and tannins compared to the wine obtained from intermediate or large berries. Also, Gil et al. found that total phenolic index, anthocyanins, hydroxycinnamic acids as well as the stilbene concentration increase when berry size decreases. In the same study, the amount of individual anthocyanins obtained by HPLC did not show any specific trend for the wines made from different sized berries. On the other hand, for Cabernet Sauvignon the relationship between berry size and fruit composition parameters (including total anthocyanins) was not direct, Calderon-Orellana et al. Matthews and Nuzzo concluded that viticultural practices used to control yield in a vineyard may be more important than the yield or berry size values per se in determining the quality of the resulting grapes and wines.

Although the majority of flavan-3-ol monomers in wine come from the seeds, low correlation was observed between the seed weight per berry and proanthocyanidin content in the wine. Due to their chemical composition, skin and pulp cell walls may adsorb proanthocyanidins. Cell walls are composed of 90% polysaccharides and 10% structural proteins. Due to their hydroxyl groups, aromatic and glycosidic oxygen atoms, cell wall polysaccharides are capable of forming hydrogen bonds and establishing hydrophobic interactions with some molecules, proanthocyanidins among them.

**CONCLUSION**

According to our results, clonal selection makes a significant difference in Prokupac wine quality. Clones 43/5 and 43/4 were marked as those from which wines with the highest quality (in terms of total phenolics, total anthocyanins and
total proanthocyanidins) were obtained. No significant differences in berry morphology were observed. Very different differences in the quality of Prokupac wines were noted that were obviously not related to morphological parameters of the Prokupac clones.

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