THE INFLUENCE OF THE BERRY SIZE ON THE SKIN ANTHOCYANINS CONTENT OF SOME BLACK WINE VARIETIES

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ABSTRACT

The influence of the berry size on the skin anthocyanins content of the black wine grape varieties Cabernet sauvignon (clone 169), Merlot (clone 348) and Pinot noir (clone 115) was studied. Research was conducted in vineyard of Experimental estate "Radmilovac" and in the laboratory of Faculty of Agriculture, University of Belgrade. The aim of the research was the determination of an anthocyanin of malvidin-3-glucoside on the berry skin. For all three varieties, berries were separated into three categories: small, medium and large. Based on the results, the goal was to compare the berry skin anthocyanin content of different sizes of berries. Determination of fertility coefficients, yield indicators, analysis of the composition and structure of clusters and berries and the content of sugars and total acids were performed regularly. The obtained results on the content anthocyanin of malvidin-3-glucoside were expressed in mg/g skin fresh weight. Varieties Cabernet sauvignon and Pinot noir had an expected result, meaning that the highest anthocyanin content was recorded in the smallest berries (diameter < 7.5 mm). For the Merlot variety, the highest anthocyanin content was observed in the medium berries (diameter 7.6 – 10 mm). The lowest anthocyanin content in varieties Cabernet sauvignon and Merlot was obtained in the largest berries (diameter > 10.1 mm), which was the expected result, while in the Pinot noir variety the lowest anthocyanin content was registered in the medium category (diameter 7.6 – 10 mm). Variety Cabernet sauvignon (clone 169) showed the highest anthocyanins content (average 6.871 mg/g fresh skin weigh), followed by Merlot variety, clone 348 (average 4.61 mg/g fresh skin weigh), whereas the lowest anthocyanin content was observed in Pinot noir, clone 115 (average 4.05 mg/g fresh skin weigh).

Keywords: variety, clone, berry, berry skin, anthocyanins.

INTRODUCTION

Irrespective of cultivar characteristics, total polyphenols and their qualitative profiles are influenced by interactions of environmental factors and cultivation techniques, such as irrigation, pruning, canopy management, row orientation, etc. (Poni *et al.*, 2006;Chorti *et al.,* 2010;). Furthermore, these factors may also affect berry size and modify proportions of skin, flesh and seed in the grapevine berry. The influence of berry size on grape composition and hence on the quality of red wine is the subject of much debate.Authors have shown the relationship between berry weight and the

contents of components determining must quality (Roby and Matthews, 2004; Santesteban and Royo, 2006). However, according to Roby *et al.,* (2004), the berry content of all solutes increased approximately in proportion to the increase in berry size, and other researchers have reported that other factors besides the size of the berry, such as temperature, cultivation techniques, rainfall, etc., are involved in grape composition (Walker *et al.* (2005) and Matthews & Nuzzo (2007) concluded that the resultant winemaking traits of large or small berries depend more on the factor modifying the weight of the berry (variety, water deficit, etc.) than on the size itself.

Variety is one of the major factors determining the difference in size and composition of the berry as a result of specific genetic characteristics associated with growth and the relative proportion of the components (flesh, seeds and skin) and their relationships (Nicolas et al., 2013) Genetics determines composition and has an influence on the ability of the variety to accumulate compounds, on the way in which photosynthetic products are distributed within the plant and on their influence on secondary metabolism (Sadras *et al.,* 2008) Weight and number of seeds have a direct impact on total berry weight due to the hormonal regulation exerted by the seeds on cell proliferation and expansion (Ristic and Iland, 2005). The relationships between the various components provide information on the oenological potential of the wine grape (Attia *et al.,* 2010; Chaves *et al.,*2007; Barbagallo *et al.,* 2011).

MATERIALS AND METHODS

The study was imposed during 2017 at the Experimental estate “Radmilovac” Faculty of Agriculture, University of Belgrade, Serbia. The experiment was established in experimental vineyard located in Belgrade vineyard region (44^{o} 45´ 24,66´´ N, 20^{o} 34´ 54,50´´ E, altitude 153 m). Three black wine grape varieties: Cabernet sauvignon (clone 169), Merlot (clone 348) and Pinot noir (clone 115) was studied. In all cases the rootstock was Kober 5BB. The vines were spacing 3m x 1m, with rows oriented N-S, a canopy height of 0.9 m, and spur (leaving two buds) and canes (leaving 10 buds) pruned, and durig the resaerch, were applied standard cultivation techniques. The experiment was laid out with 9 plants of each variety. Yield was determined at harvest.Total yield per vine and the number of clusters per vine was recorded. Yield was expressed in kg/vine.

The composition and structure of clasters and berries were done using a method described by Žunić and Garić (2010). Three samples (replicates) of 5 bunches were randomly collected and analized from each variety.

The content anthocyanin of malvidin-3-glucoside were expressed in mg/g skin fresh weight and was determinated a method described by Iland et al. (1996). The harvest took place at "technological maturity" of grapes cv. Pinot noir, Merlot and Cabernet sauvignon, respectively. After harvest, berries was separated into three categories: small (diameter < 7.5 mm), medium (diameter 7.6 – 10 mm) and large (diameter > 10.1 mm). For each category and each variety, 120 berries per sample were selected. Samples were frozen end keeped until further analysis.

New samples for average 50 berry skins were created: skins were removed from frozen beeries. first making a small cut to the skin with a scalpel, then carefully peeling it from the berry. Skin was immersed in liquid nitrogen prior to grind to a fine powder using a ceramic vessel. 1 g homogenized berry skin powder was taken and moved into penicillin bottle and extracted with 10 mL of ethanol solution. Bottles were closed with rubber stopper and left for one hour with shaking every 10 minutes. After extraction period, extract was decanted to separate from precipitate and keeped in the fridge. 200µl of extracted sampe used and added 3,8ml 1 mol HCl. Tubes left on dark place for minimum 3h (not longer than 24h). After that, samples was analyzed. Total skin anthocyanins were determined by spectrophotometry and absorbance read at 540 nm. From these values, total anthocyanins content was calculated using the formula:

$$\mathrm{AC(mg/g)} = \frac{\mathrm{A520}x\mathrm{DF}x10{,}5\ \mathrm{(ml)x1000}}{500x100x\mathrm{homogenate\ mass\ (g)}}$$

AC – anthocianin content
A520 – spectrophotometric values at absorbance at 540 nm
DF – dilution factor (at 1 mol HCl):20
10,5 ml - standard volume of extract
500 – apsorption of 1% solution malvidine-3-glucoside
homogenate mass: 0,95-1,05g

RESULTS AND DISCUSION

Yield parameters

Merlot variety had the highest yield and average yield per vine was 4,84 kg. Yield was varied from 3,48 to 5,54kg/ vine. Average yield per vine of variety Cabernet sauvignon was varied from 1,37 to 4,18kg, average 2,81kg/vine. The lower yield was obteined at cv. Pinot noir. Average yield was 1,33 kg/vine, and varied from 0,58 do 2,12 kg/vine. Ranković-Vasić (2013) reported that aveage yield of cv. Pinot noir was 1,61-1,79 kg/vine. In this study, average yield was lower (1,33kg/vne).

Table 1. Yield of grapes per plants (kg)

Vine number	Cultivars		
	Pinot noir (clone 115)	Metlot (clone 348)	Cabernet Sauvignon (clone 169)
1	2,04	5,54	2,17
2	0,58	4,98	4,18
3	2,12	5,06	2,47
4	1,05	5,46	1,37
5	1,32	4,79	3,21
6	0,88	3,48	2,88
7	-	5,21	2,90
8	-	5,14	2,90
9	-	3,93	3,24
average	**1,33**	**4,84**	**2,81**

Cv. Merlot (Clone 348) had significantly higher yied conpareing with others cultivars. Our result show that achieved yield per vine cv.Cabernet sauvignon was 2,81kg and this walue is higher than results reported by authors Vukosavljević and Garić (2015)–1,885 kg/vine and Pržić (2014) (1,89kg) and significantly higher than Paprić et al. (2008).

Bunch and berry structure

Variety Pinot noir was characterized by the smallest bunch size paremeters. Average bunch mass was 84g. % of bunch stem was 4,33%, and % of berries 95,65% (5,42 % of skin, 2,68% of seeds and 87,56 % of pulp. Bunch length varied from 6,6 to 12.9 cm (average 9,05 cm); width varied from 5,5 to 12,5 cm (average 8,13 cm); weight varied from 58 to 175 g (average 116,8 g).

Bunch and berry structure

Table 2.

Parameter	Cultivar		
	Pinot noir	Merlot	Cabernet sauvignon
Bunch composition			
Bunch mass (g)	116,8	221	190
Bunch length (cm)	9,05	17,15	16,07
Bunch width (cm)	8,13	11,03	9,8
Number of berries per bunch	113,66	198,93	149,06
Total weight of beeries in bunch (g)	111,26	213,46	182,73
Weight of rachis (g)	5,53	7,53	7,26
Berry composition			
Mass of 100 berries (g)	90,66	103,66	122,66
Skin weight of 100 berries (g)	4,07	4,51	7,29
Seed weight of 100 berries (g)	5,003	3,87	3,89
Seed number of 100 berries	197	145,66	141
Weight od 100 seeds (g)	2,53	2,69	2,78
Weight of one berry (g)	0,906	1,03	1,22
Skin weight of one berry (g)	0,04	0,044	0,072
Seed weight of one berry (g)	0,049	0,038	0,038
Seed number of one berry	1,97	1,45	1,41
Weight od one seed (g)	0,025	0,026	0,027
Pulp mass of 100 berries (g)	81,59	95,27	111,47
Skin mass of bunch (g)	4,61	8,99	10,83
Seed mass of bunch (g)	5,73	7,71	5,76
Pulp mass of bunch (g)	100,91	196,76	166,14
Seed number of bunch	225,96	289,85	209,46
Average berry length (mm)	11,78	10,31	9,98
Average berry width (mm)	10,28	10,38	9,85

Bunch structure			
% of bunch stem	4,74	3,42	3,81
% of berry	95,25	96,57	96,17
% of berry skin	3,94	4,06	5,71
% of seed	4,92	3,49	3,03
% of pulp	86,37	89,003	87,42
Berry structure			
% of skin	4,51	4,35	5,94
% of seed	5,51	3,74	3,16
% od pulp	89,96	91,89	90,87

Average weight of bunch stem (rachis) was 5,53 g, and average weight all berries per bunch was 111,26 g. Number of berries per bunch varied from 47 to 197 berries. Compared to other observed varieties, Pinot noir had the highest values of berry diameter (11,78 mm length and 10,28 mm width). Skin weight was 4,07 g, seed weight was 5,003g, and berry pulp weight was 81,59g. Bunch structure is expressed in percentages of bunch stem (rachis) and berries in a bunch. Variety Pinot noir had 4,74% of bunch stem, 95,25% of berries in a bunch and 3,94% of berry skin. The lowest % of pulp in bunch was recorded in variety Pinot Noir (86,37%), but this variety characterized by highest % of seeds (4,92%). Berry structural composition expressed in percentages of berry skin, berry pulp and seeds and variety Pinot noir had the following valuess: 4,51% of berry skin; 5,51% seeds and 89,96% berry pulp respectively. Vlaue of % of bunch stem was higher and value of %of berries was lower than results reported by Rankovic-Vasic (2013).

Variety Merlot was characterized by the biggest bunch size parameters. Bunch length varied from 12 to 22,33 cm (average 17,15 cm); width varied from 6,7 to 14,9 cm (average 11,03 cm); weight varied from 82 to 322 g (average 221 g). Average weight of bunch stem (rachis) was 7,53 g and average weight all berries per bunch was 213,46 g. Number of berries per bunch varied from 92 do 300 berries (average 198,93 berries). Skin weight was 4,51 g, seed weight was 5,003g, and berry pulp weight was 95,27 g. Compared to other observed varieties, Merlot had medium values of berry diameter (10,31 mm length and 10,38 mm width), but also had the lowest % of bunch stem (3,42%) and lowest % of berry skin (4,35%). Bunch structural parameters was: 89,003% of pulp; 4.06% of skin: 3,49% of seed. Other berry structural composition parameters had the following values: 3,74 % seeds and 91,89 % berry pulp. Results of structural analysis bunch and berries show that average bunch mass, % of bunch stem, average berry number was higher values

than it reported by Pajovic et al. (2009), while all other parameters were lower.

Variety Cabernet sauvignon: bunch length varied from 11,4 to 20,3 cm (average 16,07 cm), width varied from 5,9 to 14,8 cm (average 9,8 cm), weight varied from 135 to 262 g (average 190 g). Average weight of bunch stem (rachis) was 7,26 g, and average weight all berries per bunch was 182,73 g. Number of berries per bunch varied from 47 to 197 berries (average 149,06 berries). Skin weight was 4,07 g, seed weight was 5,003g, and berry pulp weight was 81,59g. Compared to other observed varieties, Cabernet sauvignon was characterized by the highest berry weight (1,22 g) and skin weight (7,29 g), but smallest berry diameter (9,98 x 9,85 mm). Bunch structure: 3,81%. of bunch stem; 96,17% of berries in a bunch; 5,71% of berry skin; 3,03% of seed and 87,42% of pulp in bunch was recorded in variety Cabernet sauvignon. Compared to other observed varieties, Cabernet sauvignon had the highest % of berry skin (5,94%). This parameter had positive impact on high anthocyanin content, and our result confirm that. Other berry structural composition was: 3,16% seeds and 90,87% berry pulp.

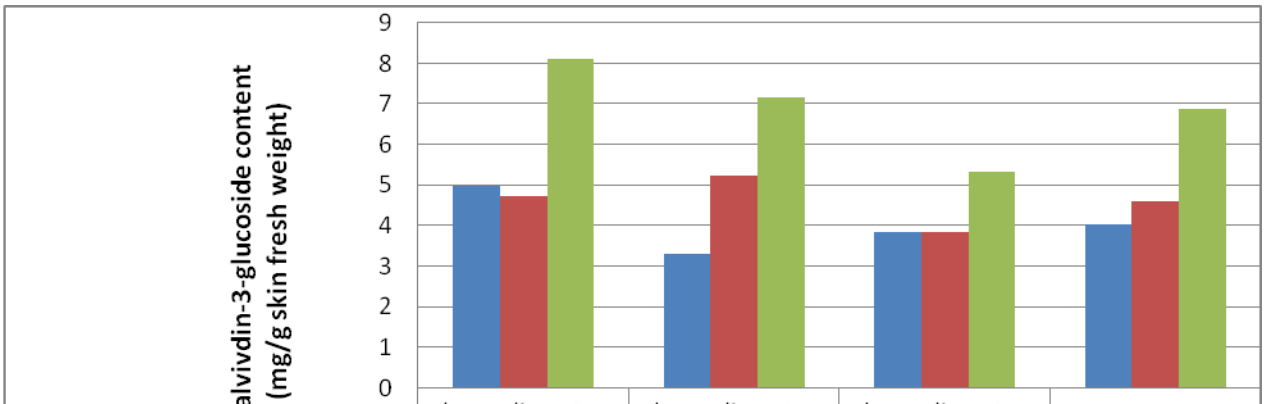
Values of the structural analysis bunch and bunch mass, number and mas of berries in the bunch, bunch stem mass, pulp mass in 100 berries, % of pulp in berries was higher; mass of 100 berries, number of seeds in 100 berries and % of bunch stem was accoeding to, while for other parameters results was lower than results reported by Markovic et al. (2011).

Anthocyanin content in berry skin

Quantification of berry skin anthocyanin content was the major part of this paper. Content was established in skin of three categories of berry size: small, medium and large. According to other authors decreasing berry size increase anthocyanin content, cause higher proportion of skin in total berry weight. Our results shown same or similar tendency (Figure 1).

The highest anthocyanin content was found in the small berry category 4,98 mg/g, but Cabernet sauvignon, Pinot noir had higher content malvidin-3-glucoside in large (3,846 mg/g) then medium (3,324 mg/g) berry category. Compared to the other observed varieties, Pinot noir had the lowest average content of malvidin-3-glucoside (4,05 mg/g skin fresh weight).

Variety Merlot, different to other, the highest anthocyanin content had in sample of medium category (5,229 mg/g). The lowest content was recorded in large (3,851 mg/g), and medium content (4,75 mg/g) was recorded in small category. Average content of malvidin-3-glucoside of Merlot was (4,61 mg/g skin fresh weight). Cabernet sauvignon variety had the highest average values of malvidin-3-glucoside content (6,871 mg/g skin fresh weight).



	berry diameter <7,5mm	berry diameter 7,6 - 10mm	berry diameter >10,1mm	average
Pinot noir (clone 115)	4,98	3,324	3,846	4,05
Merlot (clone 348)	4,75	5,229	3,851	4,61
Cabernet sauvignon (clone 169)	8,106	7,166	5,342	6,871

Figure 1. Skin anthocyanin content observed varieties influenced by berry size

The highest anthocyanin content was found for the small berry category and values were decrese incrising the berry size (8,106 mg/g small category; 7,166 mg/g medium category and 5,342 mg/g large category). Observing average content of anthocyanins, the highest values was recorded et cv. Cabernet sauvignon, then Merlot, and the lowest content had Pinot noir. Results were in accordance with Liu et al. (2015). Moyer et al. (2002) suggests. that the amount of skin or surface area increases as berry size decreases; leading to the general observation that smaller fruit contain more anthocyanins per unit volume in plant species whose pigments reside exclusively in the skin. Cabernet sauvignon show expected results, the higher anthocyanin content was from small berry category (< 7,5mm), and anthocyanin content decreased increasing berry size. Similar to Cabernet sauvignon, Pinot noir had the highest anthocyanin content in small berry category (4,98 mg/g), but different to same variety, Pinot noir had higher content of malvidine-3-o-glucoside in large berries (3,846mg/g) than small berries(3,324 mg/g).

CONCLUSION

Compearing yield of grapes, cv. Merlot had significantly higher yield in regard to others cultivars. Parameters of banch and berry structure significantly varied among cultivars. The lowest anthocyanin content recorded in large category of berries (> 10,1 mm) of cv. Cabernet sauvignon and Merlot. The highest anthocyanin content had cv. Cabernet sauvignon, clone 169 (average 6,871 mg/g FW of skin), than cv. Merlot, clone 348 (average 4,61 mg/g FW of skin). The lowest anthocyanin content had cv. Pinot noir, clone115 (average 4,05 mg/g FW of skin).

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