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## THE ACCUMULATION OF ANTHOCYANINS AND TOTAL PHENOLS DURING RIPENING IN CV PROBUS AND CABERNET SAUVIGNON GRAPE BERRIES IN FRUSKA GORA WINE REGION

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**Summary:** The anthocyanin and total phenols concentrations of *Vitis vinifera* cv Probus (Serbian wine grape variety) and Cabernet Sauvignon, during ripening in Fruska Gora region, were investigated. Phenolic concentration was evaluated by spectrophotometric analysis. Probus had higher anthocyanins concentration in the skin and total phenols per berry. Cabernet Sauvignon did not show big enlargement in anthocyanins content during this period. Contrastingly, anthocyanins content in Probus berry skin was increasing until harvest. Small berries of cv. Probus had colored pulp. There was a decrease in the Cabernet Sauvignon berry weight near the harvest. Optimum moment to harvest Probus was 50 days after veraison. Overall, these two varieties showed differences in anthocyanins accumulation during ripening.

**Key words:** anthocyanin, total phenols, berry weight, Probus, Cabernet Sauvignon

### INTRODUCTION

One of the important and certainly the most visible change, during ripening of red skinned grape varieties is the change in berry colour. Berry colour results from the synthesis and accumulation of group of coloured secondary metabolites called anthocyanins. Starting at véraison, when berries change the colour, anthocyanins accumulate in the berry and their concentration increases during ripening (Kennedy, 2008). An accelerated growth of the berry is among the other changes that occur during this period like berry softening, sugar increase, malate decrease and the change in seed colour (Coombe and Iland, 2004).

Many factors affect anthocyanins accumulation in the berry, including temperature, water regime and variety. González-Neves et al. (2013) found a significant separation between the wines of different varieties, since the colour and composition were largely related to the cultivar and berry ripeness, independent of winemaking

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procedures. Anthocyanins content can decline late in berry development (Anđelković et al., 2013) due to high temperatures (Yamamoto et al., 2010) and water regime (Yamamoto et al., 2010, Romero et al., 2013). Moriet et al. (2005) found that anthocyanins content was lower in the skin of berries grown under high night temperatures, than in the skin of berries grown under low night temperatures.

Based on the sugar and acid content, Cabernet Sauvignon and some other red wine grape cultivars, could be successfully grown in different Serbian localities, including Fruska Gora wine region (Sivev et al., 2012).

Probus is a perspective red grape variety in Serbia which was created in Sremski Karlovci, at the experimental field of the Faculty of Agriculture, University of Novi Sad. It resulted from the cross, 'Cabernet Sauvignon' x 'Kadarka'. This variety has the ability to accumulate high content of coloured compounds in grapes (Cindrić et al., 2000), but anthocyanins and total phenolic content have never been investigated.

The aim of this work was to determine the influence of ripening stage on the anthocyanins and total phenolic contents of Probus and Cabernet Sauvignon in Fruska Gora region.

## MATERIAL AND METHODS

The experiment was conducted during 2014. in Sremski Karlovci at the experimental field of the Faculty of Agriculture, University of Novi Sad (45°10' N, 20°10' E). *Vitis vinifera* cv Probus (Serbian perspective variety for red wine production, cross between Cabernet Sauvignon and Kadarka) and Cabernet Sauvignon, 14 year-old vines, were investigated. Row and vine spacing were 2.8 m (between rows) x 1.6 m (between pairs of vines in a row). Vines were trained to modified Guyot training system (≈14 buds per vine). The vineyard orientation was NE-SW. Two rows (one row per variety) containing about 60 vines each, were selected for the study.

Meteorological data were collected from the meteorological station at the experimental field in Sremski Karlovci and are shown in Table 1.

**Table 1.** Meteorological conditions during 2014. with average values (1994 - 2014)

Period	Precipitation (mm)	Average (mm) (1991-2014)	Temperature (°C)	Average (°C) (1991-2014)
January	44.9	50.0	4.3	1.7
February	12.1	47.1	6.4	3.0
March	61.8	42.9	10.2	7.7
April	69.2	47.3	13.2	12.7
May	265.0	79.2	16.1	17.6
June	47.4	86.4	20.7	20.9
July	170.0	68.3	22.0	22.7
August	52.8	50.2	20.9	22.6
September	116.8	62.0	17.0	17.5
October	65.4	61.0	13.1	12.5
November	15.8	54.2	8.4	7.3
December	64.0	57.2	4.2	2.3
Year	985.3	705.5	13.0	12.4
Vegetation	786.6	415.7	17.6	18.9

Starting at veraison, the samples of 200 berries (3 replicates per variety) were collected every 10 days (14.08.; 24.08.; 03.09.; 13.09.; 23.09.; 03.10.). The berries were collected in plastic bags and stored in the freezer at -20°C. 10 berries were selected from each replication to be weighed (berry weight). The seeds were then separated, weighed, counted, and total phenols extracted in 20 mL of an ethanol:water:hydrochloric acid (70:29:1) solution overnight. Also, the berry skins were separated, weighed and extracted following the same procedure.

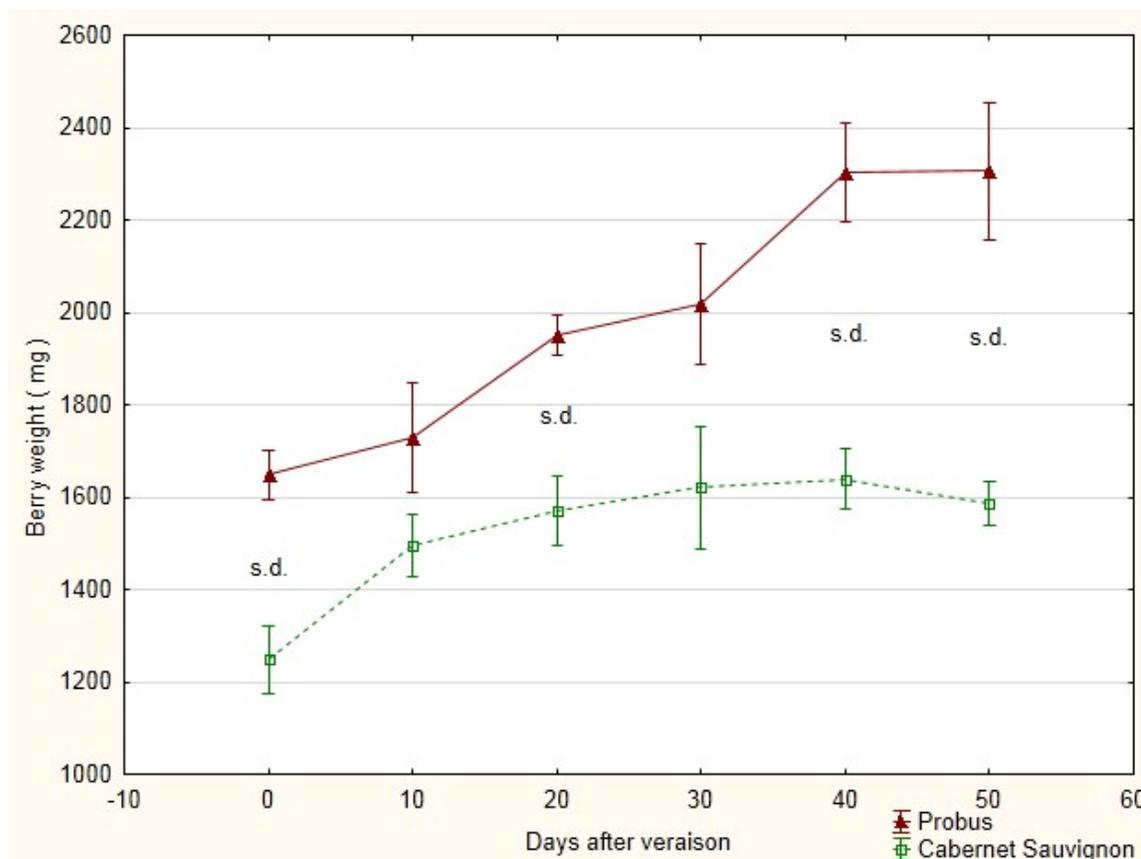
After filtration, total phenolics in the extracts were analyzed according to a modified protocol from Di Stefano et al. (1989): 2.5 mL of water were put in a 10 mL flask and added of 0.5 mL of diluted extract and 0.5 mL of Folin Ciocalteu reagent. After 3-5 minutes 2 mL of 10 % Na<sub>2</sub>CO<sub>3</sub> were added and the flask was filled up to 10 mL with water. After 90 min the absorbance at 700 nm was read at a spectrophotometer (compared with a blank made in the same way, but with water instead of the tissue extract). The total polyphenols were expressed as catechin (mg·L<sup>-1</sup>) concentration and calculated by applying the formula "catechin (mg·L<sup>-1</sup>) = 186.5 x E700 x d", where E700 = absorbance at 700 nm, and d = dilution.

Pigmented grape skin extracts were also analysed concerning the anthocyanin contents after dilution. The absorbance values at 540 nm were converted in concentration values of malvidin-3-O-glucoside through multiplying the absorbance by the coefficient 16.17 and by the dilution factor (Rustioni et al., 2014).

The results were analysed in the computer program STATISTICA 12.

## RESULTS

The changes in berry weight of Probus and Cabernet Sauvignon are shown in Figure 1.

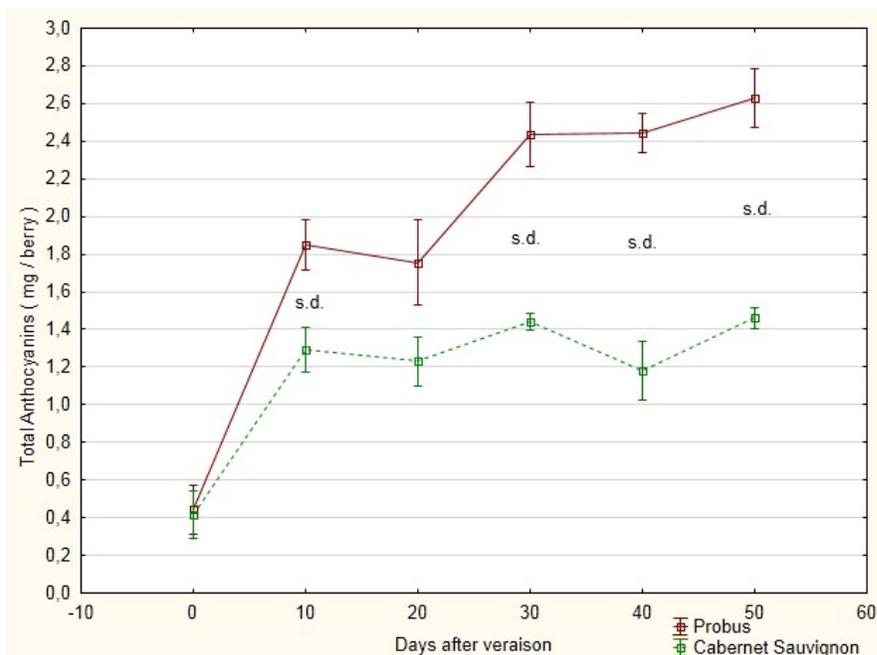


**Figure 1.** Berry weight change during the 2014. growing season  
 Error bars indicate  $\pm$  SEM (standard error of the mean) (N=3)  
 s.d. indicates a significant difference between varieties (T – test ;  $p=0.05$ )

Probus berries reached a maximum berry weight 2305,67 mg at harvest, 50 days after veraison. The maximum berry weight of Cabernet Sauvignon (1639 mg), was observed 40 days after veraison.

Probus had higher average number of seeds per berry (2.1) compare to Cabernet Sauvignon (1.9). Beginning at veraison, and continuing through harvest, the seeds lost their green colour and became progressively browner with time.

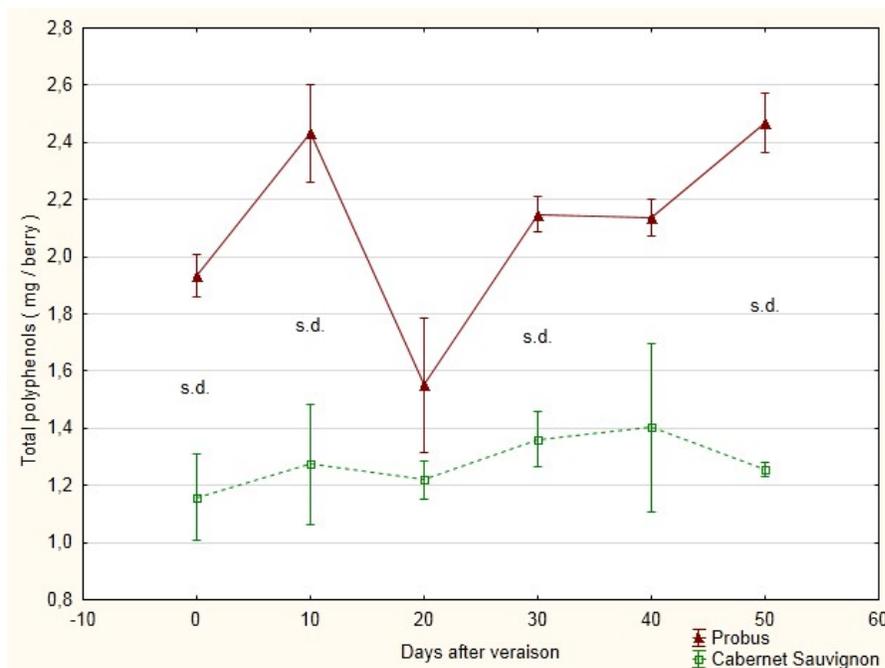
Antocyanins contents in berries during grape ripening are shown in Figure 2.



**Figure 2.**Anthocyanins content (mg / berry) during grape berry ripening  
 Error bars indicate  $\pm$  SEM (standard error of the mean) (N=3)  
 s.d. indicates a significant difference between varieties (T – test ; p= 0.05)

Cabernet Sauvignon accumulated 1.29 mg anthocyanins / berry just 10 days after veraison and after that did not show big increase until harvest. Contrastingly, Probus increased anthocyanins content almost linearly during all ripening phase, accumulated 2.63 mg/l at harvest.

Total phenolic contents in berries during grape ripening are shown in figure 3.



**Figure 3.**Total phenolics content (mg / berry) during grape berry ripening  
 Error bars indicate  $\pm$  SEM (standard error of the mean); (N=3);  
 s.d. indicates a significant difference between varieties (T – test ; p= 0.05)

Probus berries reached a maximum total phenolics content (2,47 mg/berry) at harvest. The maximum total phenolics content in Cabernet Sauvignon (1,26 mg/ berry) was observed 40 days after veraison.

### DISCUSSION

From the 40<sup>th</sup> day after veraison in the Cabernet Sauvignon grape berries there was a slight decrease in total phenols. An increase in total phenol content during grape ripening and then a slight decrease from the 40<sup>th</sup> day after veraison has also been reported by Anđelković (2013). On the other hand, Probus increased its phenolic content almost linearly until harvest. At harvest Probus had a significantly higher total phenolic content than Cabernet Sauvignon. Surprisingly, Probus showed big fluctuations in phenolic content during the observed period. In both varieties there was a decline in total phenolic content from the 10<sup>th</sup> day to the 20<sup>th</sup> day after veraison. Afterwards, the concentrations of total phenols increased in a different way.

Similarly to the total phenolic contents, in both varieties there was a decline in anthocyanin contents from the 10<sup>th</sup> day after veraison to the 20<sup>th</sup> day after veraison. Probus increased anthocyanin content almost linearly during all ripening phase. Started at the 30<sup>th</sup> day after veraison until harvest there were significant differences in anthocyanin contents between Probus and Cabernet Sauvignon. Low anthocyanin content in Cabernet Sauvignon grapes might be attributed to the fact that there was no optimum water regime during ripening phase (Ferrer et al., 2014).

The berry weight of Cabernet Sauvignon was significantly lower compared to Probus. Throughout the middle of ripening phase Cabernet Sauvignon showed relatively constant berry weight. Between 40 days after veraison and harvest the Cabernet Sauvignon berry weight loss occurred. Kennedy et al. (2008) also found a decrease in the berry weight of Pinot Noir near the harvest.

### CONCLUSION

Probus and Cabernet Sauvignon showed different dynamics in anthocyanin accumulation during ripening in Fruška Gora wine region. Anthocyanins and total phenolic content in the skin of Probus cultivar increased from veraison until harvest. On the other hand, Cabernet Sauvignon grape berries did not show a big increase in total phenols. In Northern Serbia climate conditions, Probus showed good characteristics due to its ability to accumulate enough colored compounds. For further research it will be interesting to determine the presence of other chemical compounds in Probus berries.

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**DINAMIKA NAKUPLJANJA ANTOCIJANA I UKUPNIH FENOLA U BOBICAMA SORTI PROBUS I KABERNE SOVINJON TOKOM SAZREVANJA U USLOVIMA FRUŠKE GORE**

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**Izvod:** Cilj rada bio je da se ispita dinamika nakupljanja antocijana i ukupnih fenola u bobicama sorti probus i kaberne sovinjon tokom sazrevanja. Praćen je sadržaj ovih jedinjenja od šarka do berbe, u vremenskom razmaku od 10 dana. Sorte su pokazale različitu dinamiku nakupljanja. Dobijeni rezultati pokazuju da sorta probus nakuplja više bojenih materija od kaberne sovinjona.

**Ključne reči:** antocijani, ukupni fenoli, probus, kaberne sovinjon

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