

## QUALITY RED WINES: CHANGING THE CONTENT OF ANTHOCYANINS IN THE PROCESS OF RIPENING GRAPES

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**Abstract:** The content of anthocyanins in grape affects the color and sensory properties of red wine. This paper deals with the quality of Cabernet Sauvignon wines examining changes in the content of anthocyanins in the process of ripening grapes. The HPLC results show that the skin extracts obtained from the early defoliation plants showed up to 6 % and corresponding wine up to 24 % increase of the content of total anthocyanins as compared to the extracts from the control grape and corresponding wine samples. Partial early defoliation may be an excellent tool for control of anthocyanins in grapes and may be employed as a management practice as parameter for quality of red wines.

**Key words:** *Vitis vinifera* L. grape, red wine, anthocyanins, defoliation

### Introduction

To obtain quality wine, it is important to know the physicochemical characteristics of the grape at the moment of maturity. The brightness, freshness, aroma and the biochemical quality of wine depend on fruit acidity at harvest. Low concentrations of anthocyanins lead to a lack of color in wine (Almanza et al., 2011). A programmed reduction in leaf is an efficient way of reducing yield, but improving the grape quality (Intrieri et al., 2008).

So far, the influence of microclimate of the quality of grapes and wines is investigated. It was found that the sunlight increases the content of total soluble solids in the plants, such as the sugars and the phenolic compounds, especially anthocyanins and reduced the concentration of malic acid, titratable acidity, the pH value and the potassium content. (Coombe, 1987; Crippen et al., 1986; Guidoni et al., 2008; Kliever et al., 1970; Pastore et al., 2013; Smart et al., 1985; Wolf et al., 1986).

The selective removal of grapevine leaves around berry clusters can improve the quality of ripening fruits by influencing the phenolic content at harvest. The outcome depends strongly on the timing of defoliation, which influences the source-sink balance and the modified microclimate surrounding the berries. Thus, defoliation can be implemented at any time between pre- or full-bloom and veraison.

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The photosynthetic activity of basal leaves at veraison is lower than that of intermediate and apical leaves, so defoliation at this stage has a strong impact on light and temperature exposure. In contrast, the removal of basal leaves before flowering affects the source–sink balance significantly, reducing yields and improving berry quality in many cultivars and vineyard environments (Intrieri et al., 2008; Poni et al., 2009).

Biosynthesis of phenolic compounds is one process most impaired by defoliation in berries but the outcome seems dependent on the timing of defoliation and on the genotype (Cortell et al., 2007; Pereira et al., 2006).

In this work we investigated the content of anthocyanins in Cabernet Sauvignon grape during ripening and corresponding red wines, using HPLC analysis.

### Material and methods

*Chemicals:* Malvidin-3-glucoside chloride was supplied from Sigma Chemical Co. (St. Louis, MO). Acetonitrile, methanol and formic acid, were obtained from Merck (Darmstadt, Germany). The reagents used were of analytical quality.

*Samples:* The Cabernet Sauvignon *Vitis vinifera* grape samples were taken in Rajački vineyard region (Serbia), during growing season 2012.

*Extraction Procedure:* The samples of Cabernet Sauvignon grape skin were extracted with solvent system 50/50 of methanol/water by stirring continuously at room temperature in dark for 30 min. The obtained extracts were centrifuged at room temperature (Tehnica LC-320, Zelezniki, Slovenia) at 4000 rpm for 10 min. Extracts were filtered through a 0.45 µm syringe filter before analysis.

*HPLC analysis:* Anthocyanins were analyzed by direct injection of each sample into an Agilent 1200 chromatographic system. The HPLC-grade solvents used were (A) formic acid/water (5 : 95 v/v) and (B) acetonitrile/formic acid/water (80 : 5 : 15 v/v). The elution profile was as follows: 0 min, 100% A + 0% B; 10 min, 90% A + 10% B; 20 min, 80% A + 20% B; 30 min, 70% A + 30% B; 35 min, 50% A + 50% B; 40 min, 20% A + 80% B. The detection wavelength was 520 nm. Identification and quantification of the anthocyanins were made by means of calibration curve obtained with standard solutions of malvidin-3-glucoside. Results are expressed as mg/g and mg/L sample.

*Statistical analysis:* Three analytical replicates were carried out on each grape sample. Measurements were averaged and results are given as mean ± standard deviation (SD).

### Results and discussion

Biosynthesis of phenolic compounds is dependent from the timing of defoliation of plants (Pastore et al., 2013). Tannins, such as flavan-3-ols, are the most abundant category of soluble polyphenols in grape berries, found predominantly in the hypodermal layers of the skin and the soft parenchyma of the seeds.

Anthocyanins are the second important group of phenolic compounds, which is co-located with tannins in the thick-walled hypodermal cells of the skin of grape. During grape ripening the concentrations of these compounds were changed.

The results of total anthocyanins and malvidin-3-glucoside, determinate by HPLC analysis of Cabernet Sauvignon grape skin extracts and corresponding red wines, treated (early and late defoliation) and untreated (control) from Rogljevačko-Rajački vineyard region (Serbia) during growing season 2012, are shown in Table 1:

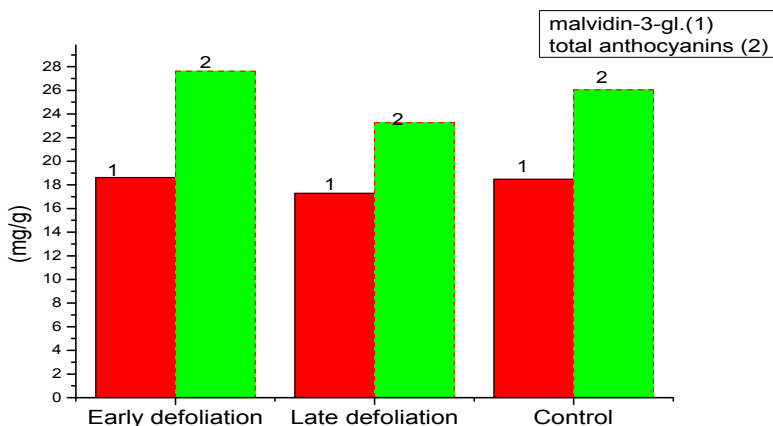
Tabela 1. Sadržaj malvidin-3-glukoyida i ukupnih antocijana određenih HPLC analizom u ekstraktima Kaberne Sovinjon pokožici i vina

Table 1. Content of malvidin-3-glucoside and total anthocyanins, determinate by HPLC analysis in Cabernet Sauvignon skin extracts and wines

	Malvidin-3-gl. u ekstraktu pokožice (mg/g) <i>Malvidin-3-gl. in skin extract (mg/g)</i>	Malvidin-3-gl. u vinu (mg/L) <i>Malvidin -3-gl. in CS wine (mg/L)</i>	Ukupni antocijani u ekstraktu pokožice (mg/g) <i>Total anthocyanins in CS skin extract (mg/g)</i>	Ukupni antocijani u vinu (mg/L) <i>Total anthocyanins in CS wine (mg/L)</i>
Rana defolijacija <i>Early defoliation</i>	18.84 ± 0.03	340.44 ± 0.02	27.62 ± 0.05	444.27 ± 0.01
Kasna defolijacija <i>Late defoliation</i>	17.29 ± 0.09	301.54 ± 0.04	23.27 ± 0.03	362.23 ± 0.03
Kontrola <i>Control</i>	18.47 ± 0.05	324.55 ± 0.03	26.04 ± 0.01	358.17 ± 0.03

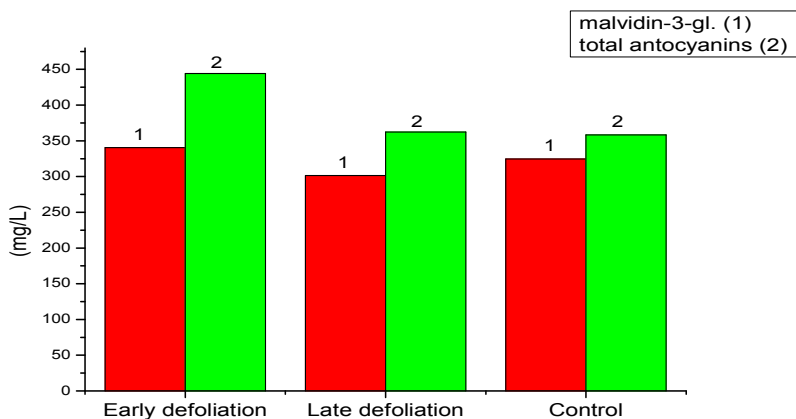
If we compare the results of HPLC analysis of anthocyanins in investigated skin grape extracts obtained from selective removal of grapevine leaves with untreated samples, it can be seen that there is an increase of the content of malvidin-3-glucoside and total anthocyanins. The HPLC results show that the skin extracts obtained from the early defoliation plants showed up to 6 % and corresponding wine up to 24 % increase of the content of total anthocyanins as compared to the extracts from the control grape and wine samples. HPLC analysis, also show that the grape skin extracts obtained from the early defoliation plants showed up to 2 % and corresponding wine up to 5 % increase of the content of malvidin-3-glucoside as compared to the extracts from the control grape and wine samples.

The contents of malvidin-3-glucoside and total anthocyanins of appropriate Cabernet Sauvignon grape and wine samples are shown in Figure 1 and 2:



Graf. 1. Sadržaj malvidin-3-glukoyida i ukupnih antocijana određenih HPLC analizom u ekstraktima Kaberne Sovinjon pokožici (mg/g)

*Graph. 1. Content of malvidin-3-glucoside and total anthocyanins, determinate by HPLC analysis in Cabernet Sauvignon skin extracts (mg/g)*



Graf. 1. Sadržaj malvidin-3-glukozida i ukupnih antocijana određenih HPLC analizom u Kaberne Sovinjon vinima (mg/L)

*Graph. 1. Content of malvidin-3-glucoside and total anthocyanins, determinate by HPLC analysis in Cabernet Sauvignon wines (mg/L)*

Both treatments (early and late defoliation) influenced fruit ripening parameters and hence enological berry traits. Defoliation before flowering caused a slight increase in sugar level and anthocyanins, whereas defoliation at veraison reduced the anthocyanin content and increased the negative impact of sunburn (Pastore et al., 2013). We concluded, also that the early defoliation was much more favorable than the late

defoliation for the biosynthesis of the malvidin-3-glucoside and total anthocyanins in investigated Cabernet Sauvignon samples. This is reflected in the content of the anthocyanins in the examination of appropriate Cabernet Sauvignon wines.

### Conclusion

Based on the HPLC analysis, it was concluded that partial early defoliation may be an excellent tool for control of the biosynthesis of the anthocyanins in grape and may be employed as a management practice in grape-growing that improves biochemical quality of red wine.

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