INFLUENCE OF SELECTIVE REMOVAL OF GRAPEVINE LEAVES ON QUALITY OF RED WINE

UTICAJ SELEKTIVNOG UKLANJANJA LISTOVA VINOVE LOZE NA KVALITET CRVENOG VINA

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ABSTRACT

The selective removal (defoliation) of grapevine leaves around berry clusters can improve the quality of ripening grape by influencing the phenolic content at harvest. In this work we investigated the influence of partial defoliation on the phenolic composition and antioxidant activity of Vitis vinifera L. Cabernet Sauvignon grape, grown in Serbia and its wine. The results show significant difference between treated (early and late defoliation) grape samples and untreated control samples. The skin extracts of Cabernet Sauvignon grape obtained from the early defoliation plants showed to 88.75 % increase of total phenol content and to 12.70 % increase of antioxidant activity in 2012 grown season, as compared to the extracts from the control grape samples. This is reflected in the values of total phenols and the antioxidant capacity of appropriate wine samples. Partial early defoliation may be an excellent tool for yield control and quality grape and he employed as a management practice as parameter for quality of red wine.

Key words: Vitis vinifera L. grape, defoliation, phenolic composition, antioxidant activity, quality red wine.

REZIME

Mikroklima utiče na mnoge osobine bobice voća, kao na primer sunčeva svetlost povećava nivo ukupnih rastvorljivih materija, šećera, fenolnih jedinjenja, a smanjuje kiselost, pH, nivo kalijuma itd. Tanini, kao što su flavon-3-oli su najzastupljenija kategorija rastvorljivih polifenola u bobici grožđa i nalaze se pre svega u hipodermalnim slojevima pokožice i delovima semena. Antocijanini su druga grupa značajnih fenolnih jedinjenja koja su locirana zajedno sa taninima u debelim zidovima hipodermalnih celija pokožice grožđa. Tokom zrenja grožđa koncentracija ovih jedinjenja se menja. Selektivno utklanjanje (defolijacija) listova vinove loze oko bobičastog klastera može poboljšati kvalitet grožđa u toku zrenja, utičući na sadržaj fenolnih jedinjenja. Defolijacija može da bude u bilo kojem periodu pre cvetanja ili u punom cvetanju. U ovom radu ispitivan je uticaj delimične defolijacije na fenolni sastav i antioksidativnu aktivnost Vitis vinifera L. Cabernet Sauvignon grožđa i njegovog vina iz Srbije. Antioksidativna aktivnost grožđa i vina je određena slobodnom inhibitorom DPPH testom. Rezultati pokazuju značajnu razliku između tretiranih (rana i kasna defolijacija) uzoraka grožđa i netretiranih kontrolnih uzoraka. Ekstrakt pokožice Cabernet Sauvignon grožđa koje je bilo tretirano sa ranom defolijacijom su pokazali veće vrednosti ukupnog sadržaja fenolnih jedinjenja do 88,75% i antioksidativne aktivnosti do 12,70% u 2012. sezoni, u poređenju sa ekstraktima iz kontrolnih uzoraka. Ovo se reflektuje u dobijenim vrednostima ukupnog fenolnih jedinjenja i antioksidativnog kapaciteta u odgovarajućih uzoraka vina. Parcijalna rana defolijacija može da bude odličan način za kontrolu prinosa i kvaliteta grožđa i da se koristi u menadžmenskoj praksi kao parametar za kvalitet crvenog vina.

Ključne reči: Vitis vinifera L. grožđe, defolijacija, fenolni sastav, antioksidativna aktivnost, kvalitet crvenog vina.

INTRODUCTION

Some investigators found that microclimate influences many berry quality properties, as well as sunlight increases the level of total soluble solids (sugars) and phenolic compounds and reduced titratable acidity, malic acid, pH and potassium levels in fruit (Coombe, 1987; Crippen et al., 1986; Guidoni et al., 2008; Kliever et al., 1970; Pastore et al., 2013; Smart et al., 1985; Voća et al., 2010; Wolf et al., 1986).

Polyphenols play an important role in human health, such as lowering of human low-density lipoprotein, as anti-inflammatory, antimicrobial and anti-aging effects and also play a preventing role from cardiovascular diseases (Lacopini et al., 2008; Katalinić et al., 2010; Radovanović et al., 2011).

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.

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 (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 (Pereira et al., 2006; Pastore et al., 2013).

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 all depend on fruit acidity at harvest. Low concentrations of anthocyanins lead to a lack of color in wine (Almazanta et al., 2011). A programmed reduction in leaf is an efficient way of reducing yield and improving grape quality (Intrieri et al., 2008).

In this work we investigated the influence of partial defoliation on the skin phenolic composition and antioxidant activity of Cabernet Sauvignon Vitis vinifera L. grapevine variety and its wine.
MATERIAL AND METHOD

Chemicals

Gallic acid, caffeic acid, quercetin, malvidin-3-glucoside chloride and 2,2’-diphenyl-1-picrylhydrazyl (DPPH) free radical were supplied from Sigma Chemical Co. (St. Louis, MO). Acetone, ethanol, methanol, acetic acid, and hydrochloric 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 Rogljevacko-Ražački vineyard region (Serbia), during growing season 2012.

Partial removal of the first five leaves of Cabernet Sauvignon cluster (early defoliation) was done on 08 June, and late defoliation was done on 02 August 2012. Grape harvest was done on 15 September 2012. The analysis of grape juce were determined following parameters ripeness of the grapes: the sugar content 26.6 % and the total acids 8.3 g/L (early defoliation) and the sugar content 25.0 % and the total acids 7.9 g/L (late defoliation).

The Cabernet Sauvignon wine samples were produced in “Vimmid” winery from Negotin.

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 and then centrifuged at room temperature (Tehnica LC-320, Zelezniki, Slovenia) at 4000 rpm for 10 min the supernatants from three extraction procedures. Extracts were filtered through a 0.45 μm syringe filter before analysis.

Determination of phenolic composition

Total phenols, hydroxycinnamoyl tartaric acids and flavonols in selected extract samples were determined spectrophotometrically (Agilent 8453 UV/VIS spectrophotometer, Santa Clara, CA, USA), with determine method (Mazza et al., 1999). The absorbance at 280 nm was used to determine total phenolic content (gallic acid was used as standard), at 320 nm to determine total phenols, and at 360 nm to determine flavonols (quercetin was used as standard). Results were expressed as miligrams gallic acid equivalents (total phenols), caffeic acid equivalents and quercetin equivalents per g of sample.

Determination of antioxidant activity

Antioxidant activity (AA) test grape samples was determined by using DPPH free radical scavenging assay (Radovanović et al., 2010). This antioxidant method is based on the measurement of DPPH colour loss due to the changes in absorbance at 517 nm, caused by the reaction of DPPH with the test sample. The DPPH-scavenging activity of each sample was calculated from the decrease in absorbance according to the following relationship:

\[
\text{AA} (\%) = \left[1 - \left( \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \right) \right] \times 100
\]

where: 
- \( A_{\text{sample}} \) is the absorbance of control (8.0 x 10^-5 M methanol solution of DPPH),
- \( A_{\text{blank}} \) is the absorbance of sample
- \( A_{\text{control}} \) is the absorbance of the sample with the same concentration of DPPH free radical as in control.

The antioxidant activities of investigated extracts were expressed as median efficient concentrations (EC50).

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

The phenols represent the third most abundant constituent in grapes after carbohydrates and fruit acids (Mazza et al., 1999).

The phenolic composition in grape varies widely and is usually determined by several factors, such as: the variety of grape and conditions under which they was grown (soil, geographical location, light exposure, temperature, sun exposure of the clusters, location of growth, ripening time) and other factors.

Biosynthesis of phenolic compounds is dependent from the timing of defoliation of plants (Pereira et al., 2006; 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.

It is found that the total extractable phenolic compounds in grape are present at only about 10 % or less in pulp, 60-70 % in the seeds and 28-35 % in the skins (Shi et al., 2003).

The results of phenolic analysis of Cabernet Sauvignon grape skin samples, treated (early and late defoliation) and untreated samples (control) from Rogljevacko-Ražački vineyard region (Serbia) during growing season 2012 and their antioxidant activity are shown in Tables 1:

**Table 1. Phenolic composition (mg/g DW) and antioxidant activity (AA), EC50 (gm/DW) of Cabernet Sauvignon (CS) skin extracts, grown season 2012.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Control Early defolitation</th>
<th>Late defoliation</th>
<th>Early defoliation</th>
<th>Late defoliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td>4.08±0.13</td>
<td>3.71±1.12</td>
<td>1.34±1.11</td>
<td>1.46±0.16</td>
</tr>
<tr>
<td>Flavonols</td>
<td>3.97±1.15</td>
<td>3.17±0.18</td>
<td>2.01±0.11</td>
<td>1.46±0.16</td>
</tr>
<tr>
<td>Antho-cyanins</td>
<td>0.28±0.05</td>
<td>0.25±0.07</td>
<td>0.21±0.01</td>
<td>0.21±0.01</td>
</tr>
<tr>
<td>AA g/mg</td>
<td>2.52±0.05</td>
<td>2.53±0.03</td>
<td>3.23±0.09</td>
<td>3.23±0.09</td>
</tr>
</tbody>
</table>

If we compare the spectrophotometric results of analysis of phenolic composition in all investigated skin grape extracts obtained from selective removal of grapevine leaves with untreated samples can be seen that there is an increase of all phenolic compounds.

Also, the results show that treated grape skin extracts have stronger antioxidant activity (2.95 and 2.93 g/mg) compared to control grape skin samples (3.23 g/mg).

The skin extracts of Cabernet Sauvignon grape obtained from the early defoliation plant showed to 88.75 % and late defoliation to 63.59 % increase of total phenol content. The antioxidant activity of skin grape extracts obtained from treated...
the early defoliation plant showed to 12.70 % and late defoliation to 9.30 % increase of antioxidant activity in 2012 grown season, as compared to the extracts from the control grape samples.

The phenolic profile and antioxidant activity of appropriate Cabernet Sauvignon wine samples, prepared from these treated and untreated grape samples, growing season 2012 are shown in Table 2:

<table>
<thead>
<tr>
<th>Cabernet Sauvignon (CS) wine samples</th>
<th>Early defoliation</th>
<th>Late defoliation</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenols</td>
<td>1902.51±1.11</td>
<td>1648.35±1.35</td>
<td>1147.91±1.21</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td>381.98±0.07</td>
<td>338.94±1.05</td>
<td>272.85±1.15</td>
</tr>
<tr>
<td>Flavon-ols</td>
<td>306.38±1.11</td>
<td>278.97±1.23</td>
<td>247.87±0.25</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>33.48±0.14</td>
<td>28.71±0.18</td>
<td>26.41±0.22</td>
</tr>
<tr>
<td>AA, mL/mg</td>
<td>42.62±0.11</td>
<td>44.63±0.13</td>
<td>48.62±0.18</td>
</tr>
</tbody>
</table>

Also, if we compare the results of phenolic composition in all investigated wine samples obtained from selective removal of grapevine leaves with untreated samples can be seen that there is an increase of all phenolic compounds. The Cabernet Sauvignon wine obtained from the early defoliation grape showed to 65.74 % and late defoliation to 43.60 % increase of total phenol content.

The antioxidant activity of wine samples obtained from treated the early defoliation grape showed to 12.34 % and late defoliation to 8.21 % increase of antioxidant activity in 2012 grown season, as compared to the extracts from the control grape samples.

The obtained results shown that the selective removal of Cabernet Sauvignon grapevine leaves can improve the quality of ripening plant by influencing its phenolic composition and antioxidant activity at harvest.

It is clear that the early defoliation was much more favorable than the late defoliation for the biosynthesis of phenolic compounds in investigated grape samples. This is reflected in the concentration of phenolic compounds and the values of antioxidant capacity obtained in the examination of appropriate wines.

The results show also that the concentrations of investigated phenolic compounds in grape skin extracts and wine samples are in excellent correlation with their antioxidant activity. The correlation constants are in the range of 0.926 to 0.9979 (Table 3):

<table>
<thead>
<tr>
<th>Correlation</th>
<th>(A) R ± SD</th>
<th>(B) R ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenols vs AA</td>
<td>0.954±0.172</td>
<td>0.999±0.009</td>
</tr>
<tr>
<td>Phenolic acids vs AA</td>
<td>0.994±0.064</td>
<td>0.998±0.288</td>
</tr>
<tr>
<td>Flavonols vs AA</td>
<td>0.954±0.176</td>
<td>0.988±0.653</td>
</tr>
<tr>
<td>Anthocya-nins vs AA</td>
<td>0.909±0.239</td>
<td>0.926±1.631</td>
</tr>
</tbody>
</table>

On the other hand, significant differences were also found between the concentrations of total phenols, phenolic acids, flavonols and total anthocyanins of investigated grape skin extracts. It is clear that the greatest increase was noticed in the concentration of flavonol and at least of concentration of anthocyanins.

Cabernet Sauvignon wines obtained from the early defoliation grapevine showed for 65.74 % increase of total phenols, for 40.0 % increase of phenolic acids, for 23.61 % increase of flavonols and for 26.77 % increase of anthocyanins compared with the concentrations of same phenolic compounds obtained in the Cabernet sauvignon wines of the control samples.

Then, Cabernet Sauvignon wines obtained from the late defoliation grapevine showed for 43.60 % increase of total phenols, for 24.22 % increase of phenolic acids, for 12.55 % increase of flavonols and for 8.71 % increase of anthocyanins compared with the concentrations of same phenolic compounds obtained in the Cabernet sauvignon wines of the control grape samples.

CONCLUSION

According to the results, we can conclude that the selective removal (defoliation) of Cabernet Sauvignon grapevine leaves affected of the phenolic composition and antioxidant activity of grape and wine. Based on the spectrophotometric analysis, early defoliation allowed achieving highest content of the phenolic compounds in the berry skin and highest their antioxidant activity, while late defoliation made to lower content of phenolic compounds and antioxidant activity compared to untreated samples of grape.

This is reflected in the concentration of phenolic compounds and the value of antioxidant activity obtained in the examination of appropriate wines.

Partial early defoliation may be an excellent tool for yield control and may be employed as a management practice in grape-growing that improves biochemical quality of red wine.

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