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Antioxidant activity and total phenol content of white wine Žilavka

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Abstract: It is already well known that wine consist of different compounds with strong antioxidant activity. Among them, most common ones are different phenol compounds generally separated in two major groups; flavonoids and nonflavonoids. In this paper we determined total phenol concentration and antioxidant activity of Herzegovinian white wines. Eighteen commercially available white wines made from autochthonous grape varieties Žilavka (vintage 2011) were analyzed. Total phenol content was determined spectrophotometrically according to the Folin-Ciocalteu method using gallic acid as a standard. Two distinct methods were used to assess the antioxidant activity of tested wines: spectrophotometric monitoring of *2,2-diphenyl-1-picryl*hydrazyl (DPPH•) free radical scavenging activity and Briggs-Rauscher (BR) oscillating reaction method.

Total phenol concentration in wine samples varied from 249.3 ±SDmgL^{-1} to 801. ±SDmgL^{-1} expressed as mg of gallic acid equivalent per liter of wine, determined from a standard calibration curve. Similar antioxidant activity was obtained by both performed methods. The antioxidant capacity obtained by DPPH• method ranged from 28.8%±SD to 70.2%±SD. In some cases, the results obtained using both, DPPH• and BR methods, confirmed the fact that wines with higher total phenol content have stronger antioxidant activity.

INTRODUCTION

Antioxidants are synthesized or natural substances that may prevent or delay some types of cell damage by donating electron or hydrogen atom to reactive free radical (Kinsella, Frankel, German et al, 1993), (Pryor, 1991). Antioxidants are found in many foods, including fruits, vegetables and wine. A lot of wine ingredients, including several hundred different phenols, possess strong antioxidant activity and thus are common research topic. The phenolic content in wine refers to large group of chemical compounds that affect the taste, color and taste of wine. These compounds include phenolic acids, stilbenoids, flavonols, dihydroflavonols, anthocyanins, flavanol monomers (catechins) and flavanol polymers (proanthocyanidins) (Kennedy, Matthews and Waterhouse 2012).

Žilavka is native grape variety from the region of Herzegovina. The variety of Žilavka gives quality white wine very often with the addition of 15% of Krkošija and Bena, which are autochthonous varieties of the region of Herzegovina as well.

In the present study, we determined total phenol concentration (according to the Folin-Ciocalteu method) and antioxidant activity using DPPH• (*2,2-diphenyl-1-picryl-hydrazyl*hydrate) radical scavenging and Briggs-Rauscher (BR) oscillating reaction methods in Herzegovinian white wines made from domestic grape Žilavka.

To this date, there has been no research paper on the antioxidant activity and phenol content of white Herzegovinian wine in the literature.

EXPERIMENTAL

Chemicals

All solutions were prepared using analytical-reagent grade substances and Milli-Q deionized water (18.2 MΩ·cm).

Potassium iodate, ethanol, sulphuric acid, hydrogen peroxide, malonic acid, starch, manganese sulfate monohydrate and Folin-Ciocalteau reagent were purchased from Kemika d.d. Zagreb, Croatia. DPPH• reagent and gallic acid were purchased from Fluka Chemie GmbH.

Wine samples

For this study, we selected eighteen controlled geographic origin wines (Table 1), commercially available and widely consumed. All samples were kindly supplied by sixteen private cellars. Wines were stored at room temperature in dark space until analysis. Analysis were performed in June, 2014. All samples were analyzed in triplicates.

Table 1: Description of analyzed Žilavka white vine samples (vintage 2011).

Cod	Winery/Cellar	Location
1	Hepok Ljubuški	Ljubuški
2	Hercegovina produkt	Čitluk
3	V. Sivrić	Međugorje
4	Ostojić	Čitluk
5	Keža	Ljubuški/Studenac
6	Džajo	Ljubuški
7	Buntić	Ljubuški
8	Škegro	Ljubuški
9	Begić *	Ljubuški
10	Begić	Ljubuški
11	Rebac	Čapljina/Trebižat
12	Ereš	Mostar/Sretnice
13	Brkić	Čitluk
14	AG Međugorje	Međugorje
15	Čitluk**	Čitluk
16	Čitluk	Čitluk
17	Zadro	Čapljina
18	Andrija	Čitluk, Paoča

* Vintage 2008;

** Vineyard on special lime stone ground, so-colled *Stone wine*

Apparatus

Shimadzu UV mini-1240 (Shimadzu, Kyoto, Japan) UV-Vis spectrophotometer equipped with a cell (Hellma, Müllheim, Germany) of 10 mm optical path was used. Spectrophotometric data acquisition and control of measurement were achieved by coupling detector with personal computer and using UVmini-1240 data manager software and plug-in memory card with kinetics program both from Shimadzu (Shimadzu, Kyoto, Japan).

Total phenol concentration

Total phenol concentration was determinated spectrophotometricaly by Folin-Ciocalteau method. After adding Folin-Ciocalteau reagents in wine samples, colored product was formed. Folin-Ciocalteau reagent is phosphowolfram and phosphomolybdic acid mixture. During oxidation reaction, phenol groups are oxidized toquinone which are blue colored. Absorption of the resulting solution was measured at 765nm (Amerine and Ough , 1988).

Working solutions were prepared by mixing 0.25 mL of sample (pre-diluted 1:10), 15 mL deionized water and 1.25 mL of Folin-Ciocalteau reagent in 25 mL volumetric flask. One minute after, 3.75mL of sodium carbonate (Na_2CO_3, w=20%) soloution was added and flask was made up to the volume with deionized water. Solutions were stored for two hours in dark space prior the measurements of absorbance at 765nm.

Calibration curve was created using gallic acid as a standard. Seven stock solutions of gallic acid were prepared in the concentration range from 0 to 1000 mg L^{-1}.The volume of 0.25 mL of each solution was trasnfered in 25 mL volumetric flask and treated according total phenols determination method. The results are reported as a gallic acid equivalent γ(GAE) mgL^{-1}.

2,2-diphenyl-1-picrylhydrazyl (DPPH•) antioxidant assay

DPPH• free radical scavenging activity is the basis of a used antioxidant assay. DPPH• free radical method is an antioxidant assay based on electron-transfer reaction that produces a violet solution in methanol (Huang, Ou and Prior, 2005). This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless methanol solution. The use of the DPPH• assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry. Decreas of DPPH• solution absorbance was measured for 30 minutes at 517nm.

For this method DPPH• methanol solution was used (c=6×10^{-5} mol L^{-1}).The volume 1.5 mL of DPPH• solution (absorption≈1) and 25 µL of wine sample was pipetted into the spectrophotometric cuvette. Decreas of absorption was measured for 30 minutes at 517 nm (Yen and Duh, 1994). The percent inhibition (%I) of DPPH• radical by the wine samples was calculated according to equation:

$$\% I = [(A_0-A_t)/A_0]\times 100$$

where A_0 is the absorbance of DPPH• solution without antioxidant at the beginning of measurement (t = 0) and A_t is the absorbance of DPPH• solution containing antioxidant at the end of measurement (t = 30 min).

Briggs-Rauscher (BR) oscillating reaction method

BR oscillating reactions occur as series of reactions which cause color changes in specific time intervals (colorless-yellow-dark blue) (Cervellati, Höner, Furrow et al, 2001). BR oscillating system consists of the iodination and oxidation of an organic substrate (malonic acid) by acidic iodat in the presence of hydrogen peroxide and with the Mn^+ ion as catalyst. The antioxidant leads to immediate cessation of oscillation, and after the so-called inhibition time, the oscillatory behavior is regenerated (De la Rosa, Alvarez-Parilla and Gonzalez-Aguilar, 2010). Duration of reaction cessation is directly affected by type and amount of added antioxidant.

Three solutions were prepared:
1.The iodate solution, 0.2 mol L^{-1},was prepared by dissolving 4.28 g potassium iodate in 0.45 mL concentrated sulfuric acid and diluted with deionized water up to 100 mL.
2. Hydrogen peroxide *w* (H_2O_2)*=15%* (fresh prepared).
3.The mixture with resulting concentration of 0.15 mol L^{-1} for malonic acid, 0.02 molL^{-1} solution of manganese sulfate was prepared by transferring appropriate amount of these substances in 100 mL volumetric flask and dissolving it in 50 mL deionized water. In this mixture, starch solution (0.03% resulting concentration) was also added, and finely diluted up to nominal volume with deionized water. Five milliliters of each of colorless solutions were mixed. Diluted wine samples (1:10) were added to 15 ml of an active, well-stirred (300 r.p.m.) BR mixture, after the third oscillation. Different volumes of diluted wine samples were added: 0.10, 0.25, 0.50 and 0.75 mL, respectively. Inhibition time was measured e.g., till dark-blue color occurs again. Results are expressed graphically as volume and inhibition time linear dependence (Marković and Talić, 2013). The obtained slope of regression line is measure of antioxidant activity; where steeper slope refer to stronger antioxidant activity.

RESULTS AND DISCUSSION

In this paper wide range of phenol concentrations (mean value of 425.23 mgL^{-1}GAE) in selected wine were reported. Measured concentrations of phenolic compounds are presented in Table 2 and results are expressed as γ(GAE) mgL^{-1}. Highest total phenol concentration value was measured in Žilavka produced by Winery Buntić, 801.9 ± 4.0 mgL^{-1} GAE, and the lowest was in Žilavka produced by Winery Škegro 175.0 ± 1.9mgL^{-1} GAE. According to literature (Mitić, Obradović, Grahovac et al, 2010), obtained phenol concentration for Žilavka compared to other researches' results (white wine from Croatia, Greece, Spain, Italy, Czech Republic), who used the same Folin-Ciocalteau methods, are much higher.

Table 2: Phenol content of white wine sample

Cod	Total phenols γ (GAE), mg L^{-1} ±SD
1	376.8 ±2.2
2	623.3 ± 4.1
3	515.6 ± 3.7
4	249.3 ± 2.6
5	310.5 ± 2.0
6	340.6 ± 1.9
7	801.9 ± 4.0
8	175.0 ± 1.9
9	525.9 ± 3.0
10	324.1 ± 2.3
11	395.5 ± 3.1
12	523.9 ±5.0
13	436.4 ± 3.3
14	467.0 ± 1.5
15	419.0 ± 5.1
16	388.3 ±3.8
17	305.2 ± 2.7
18	479.0 ± 2.0

Antioxidant activity was measured by two distinct methods. Results reported by DPPH• method are presented in Table 3. The greatest value of DPPH• radical scavenging, measured at thirtieth minute is in wine number 9; Žilavka produced by Winery Begić (83.1%) while the lowest value is measured in wine number 17; Žilavka produced by Winery Vina Zadro (23.4%). Other researches (Katalinic, 2004) reported slightly weaker antioxidant activity (39.0%–60.2%).
The differences between our values and the published results could be primarily affected by the nature of the analyzed wines, i.e. by their actual contents of phenolic compounds. On the other hand it is difficult to confront our values of antioxidant activity with the literature data, since majority of authors used various methods such as the inhibition of lipid oxidation, DPPH• method with the evaluation of EC50 (the sample concentration necessary to reduce the remaining DPPH• by 50%), and ORAC method (Oxygen Radical Absorbance Capacity) (Stratil, Kubáň and Fojtová, 2008).
Table 3 represent results obtained by Briggs -Rauscher oscillation reactions method. There is a linear relationship between volume of wine added in reaction mixture and time of inhibition. Line slope was created for each sample and compared, higher slope values (steeper slope) represent stronger antioxidant activity (Prenesti, Toso and Berto, 2005).
Greatest antioxidant activity value was measured in wine produced by Winery Buntić (slope=1516.8) which also contain greatest total phenol concentration (801.9 ± 4.0 mg L^{-1} GAE). Weakest antioxidant activity was measured in wine produced by Winery Čitluk (slope = 143.37).

Table 3: Phenol content of white wine sample

Cod	(% I) DPPH*	BR**
1	28.8 ± 0.4	178.37
2	63.0 ± 1.3	443.34
3	47.2 ±1.4	490.61
4	42.8 ± 3.2	231.43
5	31.1 ± 2.0	179.39
6	35.7 ± 0.9	276.12
7	49.2 ± 6.5	1516.80
8	37.4 ± 5.6	393.10
9	83.1 ± 1.6	169.80
10	64.9 ± 5.2	1120.50
11	35.9 ± 3.6	849.50
12	56.2 ± 3.6	1237.10
13	37.3 ± 0.2	793.14
14	37.9 ± 0.1	404.60
15	46.9 ± 1.0	311.02
16	37.1 ± 5.3	143.37
17	23.4 ± 1.4	216.84
18	47.5 ± 1.2	732.65

*Inhibition of DPPH radical ± SD
****** The obtained slope of regression line

No significant correlation between antioxidant activity determinated by DPPH• and BR reaction method was found. This is due to different antioxidant mechanism involved in used methods.
Some literature findings report high correlations for white wine between the used assays (Mitić et al 2010, Katalinić 2004, Hua et al 2009). However, our results are in agreement with those obtained by Fotakis et al. This

research group obtained a weak correlation for white wine as well (Fotakis et al, 2012).
Obviously, there is need for identification of phenols becauce structure and nature of these compounds is also very important for antioxidant activity.

CONCLUSIONS

Samples of white wine Žilavka analyzed in this paper shoved extremely high total phenol concentration and antioxidant activity, compared to other researchers results. The weak correlation between phenol content of all tested wine and antioxidant activity indicate need for further qualitative analysis of phenols and different assays of antioxidant activity.

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Summary/Sažetak

Brojna istraživanja potvrđuju kako različita vina posjeduju snažan antioksidacijski učinak. Pripisujemo ga fenolnim spojevima koje čine dvije skupine; flavonoidi i neflavonoidi. U ovom radu određivana je koncentracija fenola i antioksidacijski učinak u bijelom vinu sorte Žilavka iz područja Hercegovine. Analizirano je osamnaest komercijalno dostupnih vina - berba 2011. Koncentracija ukupnih fenola određena je spektrofotometrijski pomoću Folin-Ciocalteau reagensa koristeći galnu kiselinu kao standard. Dvije različite metode su korištene za procjenu antioksidacijskog učinka analiziranih vina. Metoda redukcije 2,2-difenil-1-picrilhidrazil (DPPH$^{\bullet}$) slobodnog radikala te metoda Briggs-Rauscher (BR) oscilirajućih reakcija.Dobivene koncentracije ukupnih fenola su u rasponu od 249,3 ±SD mgL^{-1} do 801, ±SD mgL^{-1} galne kiseline po litru vina, što je značajno više od dobivenih vrijednosti drugih istraživača za bijela vina. Slične vrijednosti antioksidacijskog učinka dobivene su prema obje korištene metode. Antioksidacijski učinak prema metodi redukcije slobodnog radikala DPPH$^{\bullet}$ je u rasponu od 28,8%±SD do 70,2%±SD. Rezultati dobiveni za pojedine uzorke vina upućuju kako vina sa većim sadržajem fenola imaju snažniji antioksidacijski učinak.