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ANTIOXIDANT ACTIVITY, PHENOLIC CONTENT AND COLOUR OF THE SLOVAK CABERNET SAUVIGNON WINES

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ABSTRACT

Antioxidants are specific substances that oxidize themselves and in this way they protect other sensitive bioactive food components against destruction. At the same time, they restrict the activity of free radicals and change them to less active forms. Grapes and wine are a significant source of antioxidants in human nutrition. One of the most important group occuring in grapes and wines are polyphenols. Many of phenolic compounds have been reported to have multiple biological activities, including cardioprotective, anti-inflammatory, anti-carcinogenic, antiviral and antibacterial properties attributed mainly to their antioxidant and antiradical activity. Therefore, it is important to know the content of polyphenols and their antioxidant effects in foods and beverages. Twenty-eight Cabernet Sauvignon wine samples, originated from different Slovak vineyard regions, were analyzed using spectrophotometry for the content of total polyphenols, content of total anthocyanins, antioxidant activity and wine colour density. Determined values of antioxidant activity in observed wines were within the interval 69.0 – 84.2% inhibition of DPPH (average value was 78.8% inhibition of DPPH) and total polyphenol content ranged from 1,218 to 3,444 mg gallic acid per liter (average content was 2,424 mg gallic acid.L^{-1}). Determined total anthocyanin contents were from 68.6 to 430.7 mg.L^{-1} (average content was 220.6 mg.L^{-1}) and values of wine colour density ranged from 0.756 to 2.782 (average value was 1.399). The statistical evaluation of the obtained results did not confirm any linear correlations between total polyphenol content, resp. total anthocyanin content and antioxidant activity. The correlations between total polyphenol content and total anthocyanin content, resp. the content of total anthocyanins and wine colour density were strong. The results confirmed very strong correlations between wine colour density and total polyphenol content, resp. antioxidant activity.

Keywords: polyphenol; antioxidant activity; anthocyanin; red wine; Cabernet Sauvignon

INTRODUCTION

Phenolic compounds are the most abundant secondary metabolites present in the plant kingdom. They possess a common structure comprising an aromatic benzene ring with one or more hydroxyl substituents. They represent a large and diverse group of molecules including two main families: the flavonoids based on common C6-C3-C6 skeleton and the non-flavonoids. In plant, they play a role in growth, fertility and reproduction and in various defence reactions to protect against abiotic stress like UV-light or biotic stresses such as predator and pathogen attacks. They also constitute basic components of pigments, essences and flavors **(Weisshaar and Jenkins 1998; Winkel-Shirley, 2002)**. Recent interest, however, in food phenolics has increased greatly because of the antioxidant and free radical-scavenging abilities associated with some phenolics and their potential effects on human health **(Bravo, 1998)**. Many of phenolic compounds (resveratrol, quercetin, rutin, catechin, proanthocyanidins) have been reported to have multiple biological activities, including cardioprotective, anti-inflammatory, anti-carcinogenic, antiviral and antibacterial properties attributed mainly to their antioxidant and antiradical activity **(Lorrain et al., 2013)**.

Grapes and grape products (mainly wines and juices) are a rich source of phenolic compounds. From the clue of "French paradox", polyphenolics from grapes and red wines attracted the attention of scientists to define their chemical composition and quantity **(Urpi-Sarda et al., 2009)**. Globally, red wines contain more phenolic compounds than white wines. It is caused by the technology of winemaking, when making white wines the grapes' skin is removed before fermentation **(Beer et al., 2006)**. The total polyphenols in wine besides variety of grapes, locality of growing, climatic conditions, are affected also by procedure of winemaking: length of contact of stum with grapes's skin, mixing, temperature, content of SO_2, pH value, content of alcohol etc. **(Villano et al., 2006; Lachman and Šulc 2006)**.

Cabernet Sauvignon (CS) is perhaps best known, most popular and one of most cultivated blue grapevine varieties in the world. This variety gives a lower harvest, wines are full-bodied, higher acids and polyphenols content (tannins and dyes) and excellent aging potential. Variety has traditionally mixing with other blue sort to achieve overall softer feel and a more balanced wine taste. Colder climate of Central Europe often makes the aroma of Slovak Cabernets with flavour of green pepper and grass denouncing the lack of ripeness of the grapes. Cabernet Sauvignon is grown mainly in southwestern France, where this variety spread around the world (northern Italy, USA, South Africa, Australia, South America). In Slovakia, CS grown at about 13% of the areas planted with blue grapevine varieties and CS is the third most cultivated blue variety after Blaufränkisch and St. Laurent **(Ďurčová, 2011**; **Šajbidorová, 2012)**.

The purpose of this study was to determine and evaluate chosen antioxidant and sensory properties (the content of total polyphenols, content of total anthocyans, antioxidant activity and wine colour density) and their mutual correlations in red wine samples – Cabernet Sauvignon, originated from different Slovak vineyard areas.

MATERIAL AND METHODOLOGY

Chemicals and instruments

All analysed parameters – total polyphenol content, total anthocyanin content, antioxidant activity and wine colour density in wines were analyzed using UV/VIS spectrophotometry (spectrophotometer Shimadzu UV/VIS – 1240, *Shimadzu*, *Japan*). The chemicals used for all analysis were: Folin-Ciocalteau reagent, monohydrate of gallic acid p.a., anhydrous natrium carbonate p.a., citric acid p.a., dodecahydrate of disodium hydrogen phosphate, 35% hydrochloric acid p.a., ethanol p.a., methanol p.a., 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical p.a.

Samples

Analysed, bottled, red, especially quality and dry wines

Table 1 Characteristics of analysed Cabernet Sauvignon wine samples.

Sample	Producer	Vineyard area	Vintage	Quality
LC-1	Vitis Pezinok / Hubert J.E. Sereď	Little Carpathian	2008	quality
LC-2	Bočko Víno, Šenkvice	Little Carpathian	2008	quality
LC-3	VPS, Pezinok	Little Carpathian	2010	quality
LC-4	Víno Jano, Limbach	Little Carpathian	2009	quality
LC-5	Villa Víno Rača, Bratislava	Little Carpathian	2013	quality
SS-1	Vitis Pezinok / Hubert J.E. Sereď	South Slovak	2007	quality
SS-2	Villa Víno Rača, Bratislava	South Slovak	2008	quality
SS-3	Víno Matyšák, s.r.o., Pezinok	South Slovak	2010	quality
SS-4	VINIDI, s.r.o., Bratislava	South Slovak	2008	late harvest
SS-5	Vinárske závody Topoľčianky	South Slovak	2010	quality
SS-6	Hubert J.E., Sereď	South Slovak	2007	quality
SS-7	Malokarpatská vinohrad. spol., Pezinok	South Slovak	2009	quality
N-1	Víno Nitra, Nitra	Nitra	2009	quality
N-2	Chateau Modra, Modra	Nitra	2009	late harvest
N-3	Vinárske závody Topoľčianky	Nitra	2006	quality
N-4	Vinárske závody Topoľčianky	Nitra	2009	quality
N-5	Víno Nitra, Nitra	Nitra	2009	quality
N-6	Mrva a Stanko, Trnava	Nitra	2011	grapes selection
ES-1	J&J Ostrožovič, Veľká Tŕňa	East Slovak	2009	quality
ES-2	PD Vinohrady, Choňkovce	East Slovak	2008	late harvest
ES-3	PD Vinohrady, Choňkovce	East Slovak	2007	grapes selection
ES-4	Pivnica Tibava, Tibava	East Slovak	2008	quality
ES-5	Pivnica Tibava, Tibava	East Slovak	2009	quality
CS-1	Agro Movino, Veľký Krtíš	Central Slovak	2009	quality
CS-2	Agro Movino, Veľký Krtíš	Central Slovak	2010	quality
CS-3	Agro Movino, Veľký Krtíš	Central Slovak	2011	grapes selection
CS-4	Agro Movino, Veľký Krtíš	Central Slovak	2011	quality
CS-5	L. Korcsog, Korvinum, Rykynčice	Central Slovak	2011	late harvest

Cabernet Sauvignon (CS) and their characteristics are mentioned in Table 1. Wine samples with origin in various Slovak vineyard areas (VA) were purchased in retail network, to provide that analysed samples of wine would have the same properties as wines that are consumed by common consumers (properties of wine affected by various factors, such as period and conditions of storage or distribution of wine).

Antioxidant activity determination

Antioxidant activity (AA) was assessed by method of **Brand-Williams et al., (1995)** using of DPPH (1,1-diphenyl-1-picrylhydrazyl) radical. Absorbance was read at 515.6 nm and antioxidant effectiveness was expressed as % inhibition of DPPH (quantitative ability of tested compound to remove in certain period a part of DPPH radical).

Determination of total polyphenol content

Total polyphenol content (TPC) was determined by modified method of **Singleton and Rossi (1965)**. 0.1 mL of wine sample was pipetted into 50 mL volumetric flask and diluted with 5 mL of distilled water. To diluted mixture 2.5 mL Folin-Ciocalteau reagent was added and after 3 minutes 7.5 mL of 20% solution of Na_2CO_3 was added. Then the sample was filled with distilled water to volume 50 mL and after mixing left at the laboratory temperature for 2 hours. By the same procedure the blank and calibration solutions of gallic acid were prepared. Absorbance of samples solutions was measured against blank at 765 nm. The content of total polyphenols (TP) in wines was calculated as amount of gallic acid equivalent (GAE) in mg per 1 litre of wine.

Determination of total anthocyanin content

Total anthocyanin content (TAC) was assessed by modified pH differential method of **Lapornik et al., (2005)**. The principle of this method is reduction of the pH of wine samples with hydrochloric acid to values 0.5 – 0.8 associated with the transformation of all anthocyans to red colored flavilium cation. The content of total anthocyanins (TA) was calculated from the difference absorbance values of both solutions (origin and acidified) and expressed as the amount of anthocyans in mg per 1 liter of wine.

Determination of wine colour density

Wine colour density (WCD) was assessed by method of **Sudrand (1958)** as the sum of the absorbance at 420 nm and 520 nm. The absorbance of the wine samples was measured in 0.2 cm path lenth glass cells.

All analyses were performed as four parallels.

Statistical analysis

Statistical analysis was performed using the software Statistica 6.0 (StatSoft, Czech Republic) and the results were evaluated by analysis of variance ANOVA.

RESULTS AND DISCUSSION

All studied parameters – the content of total polyphenols, the content of total anthocyanins, antioxidant activity and wine colour density of the Slovak wines Cabernet Sauvignon are described in Table 2.

Antioxidant activity in analysed wine samples was in range 69.0 – 84.2% inhibition of DPPH. Average value of AA was 78.8% inhibition of DPPH. The average value of AA in Cabernet Sauvignon wines is a slightly lower than we found out in the other two major Slovak red wines Blaufränkisch – 83.3% and St. Laurent – 81.2% inhibition of DDPH **(Bajčan et al., 2012)**, but slightly higher compared to Slovak Alibernet wine samples – 74.0% inhibition of DPPH **(Bajčan et al., 2015)**. Similar results of AA reported **Slezák (2007)** and **Špakovská et al., (2012)**, who found out AA in Slovak wines – Cabernet Sauvignon in range from 71.6 to 90.9% inhibition of DPPH. On the basis of value of AA an order could be as following: wines from Little Carpathian VA > wines from East Slovak VA > wines from Central Slovak VA > wines from Nitra VA > wines from South Slovak VA. Gained results did not exert statistically significant differences (at significance level $p = 0.05$) between values of antioxidant activity in wines made in various vineyard areas in Slovakia.

Total polyphenol content in analysed wine samples was in the range from 1,218 to 3,444 mg GAE.L^{-1}. Average content of TP was 2,424 mg GAE.L^{-1}. The average content of total polyphenols in wines - Cabernet Sauvignon is a little higher than we found out in the other two major Slovak varietal red wines Blaufränkisch – 2,003 mg GAE.L^{-1} and St. Laurent – 2,297 mg GAE.L^{-1} **(Bajčan et al., 2012)**. On the other hand, average content of TP in Slovak Cabernet Sauvignon wines was much lower than we determined in Alibernet wines – 3,057 mg GAE.L^{-1} **(Bajčan et al., 2015)**. The results are similar to results reported by **Slezák (2007)** and **Špakovská et al., (2012)**, who found out the content of TP in Slovak wines – Cabernet Sauvignon in range from 2,150 to 3,102 mg GAE.L^{-1}. Other (foreign) scientists **(Kondrashov et al., 2009; Burin et al., 2010**; **Yoo et al., 2011)** analyzing TPC in CS wines reported also very similar results (1,453 – 3,589 mg GAE.L^{-1}). **Cliff et al., (2007)** reported much lower average value of TPC (1,055 mg GAE.L^{-1}) in CS wines originated in British Columbia, Canada what is probably due to cold weather and lack of mature grapes. According to the average value of TPC an order for wines could be as following: wines from Central Slovak VA > wines from South Slovak VA > wines from Nitra VA > wines from Little Carpathian VA > wines from East Slovak VA. Gained results exerted statistically significant differences (at significance level $p = 0.05$) between TPC in wines made in East Slovak VA and TPC in wines made in Central Slovak VA, resp. South Slovak VA.

Total anthocyanin content in analysed wine samples was in the range from 68.6 to 430.7 mg.L^{-1}. Average content of TA was 220.6 mg.L^{-1}. The average TAC in wines Cabernet Sauvignon is significantly lower than we found out in the other three Slovak varietal red wines Blaufränkisch – 266.1 mg.L^{-1}, St. Laurent – 264 mg.L^{-1} and Alibernet – 403 mg.L^{-1} (**Bajčan et al., 2015; Tóth et al., 2011)**. According to the average value of TAC an order for wines could be as following: wines from Central Slovak VA > wines from Nitra VA > wines from Little Carpathian VA > wines from South Slovak VA > wines from East Slovak VA. Gained results exerted statistically significant differences between TAC in wines made in

East Slovak VA and TAC in wines made in Central Slovak VA, resp. Nitra VA.

Wine colour density in analysed wine samples was in range from 0.756 to 2.782. Average value of WCD was 1.399. The average value of WCD in wines Cabernet Sauvignon is a little higher than we found out in the other two major Slovak varietal red wines Blaufränkisch – 1.110 and St. Laurent – 1.224 **(Tóth et al., 2011)**. But on the other hand, average value of WCD in Slovak Cabernet Sauvignon wines was much lower than we determined in Alibernet wines – 2.317 **(Bajčan et al., 2015)**. This is the first study monitoring WCD in Slovak wines Cabernet Sauvignon, so we can´t compare our data with other scientists. The results are little higher to results reported by **Poiana et al., (2007)**, who found out WCD in Romanian wines - Cabernet Sauvignon in range from 0.708 to 1.474 (average value – 1.206).

According to the average value of WCD an order for

Table 2 The content of total polyphenols (TPC), content of total anthocyanins (TAC), antioxidant activity (AA) and wine colour density (WCD) in analysed wines.

Sample	**TPC mg GAE.L^{-1}**	**TAC Mg.L^{-1}**	**AA %**	**WCD**
LC-1	2,206 ±22	82.5 ±2.7	82.9 ±2.7	1.059 ±0.006
LC-2	1,926 ±23	246.3 ±3.7	79.1 ±3.3	1.182 ±0.004
LC-3	2,667 ±46	246.9 ±4.2	82.1 ±3.8	0.896 ±0.011
LC-4	2,237 ±117	151.1 ±5.3	80.1 ±2.5	1.177 ±0.015
LC-5	2,642 ±30	282.4 ±2.8	79.8 ±0.8	1.449 ±0.012
Average LCVA	***2,336 ±308[a]***	***201.8* ±85.8[a]**	***80.8 ±1.6[a]***	***1.153* ±0.237[a]**
SS-1	2,215 ±46	68.6 ±3.1	79.5 ±2.6	1.137 ±0.021
SS-2	2,267 ±46	208.2 ±1.6	81.8 ±1.4	1.064 ±0.009
SS-3	2,966 ±46	292.7 ±2.8	77.1 ±2.6	1.385 ±0.012
SS-4	2,634 ±22	206.4 ±7.4	75.9 ±1.7	1.608 ±0.008
SS-5	2,886 ±22	330.8 ±7.7	76.6 ±2.0	1.861 ±0.008
SS-6	3,365 ±22	111.8 ±7.4	73.5 ±3.8	1.927 ±0.019
SS-7	2,118 ±44	152.3 ±2.5	80.5 ±2.5	1.053 ±0.015
Average SSVA	***2,636 ±461[b]***	***195.8* ±97.0[b]**	***77.8 ±3.1[b]***	***1.434* ±0.323[b]**
N-1	1,632 ±69	103.9 ±0.7	81.1 ±1.7	1.096 ±0.006
N-2	2,747 ±44	330.0 ±2.1	76.2 ±2.0	1.801 ±0.014
N-3	2,513 ±46	272.1 ±5.6	80.0 ±3.9	1.426 ±0.018
N-4	2,885 ±68	293.5 ±3.5	69.0 ±1.8	2.782 ±0.023
N-5	2,628 ±23	162.6 ±6.7	84.2 ±4.0	1.076 ±0.005
N-6	2,798 ±43	363.3 ±8.4	77.4 ±1.0	1.968 ±0.021
Average NVA	***2,534 ±495[c]***	***254.2* ±102.5[c]**	***78.0 ±3.2[c]***	***1.691* ±0.674[c]**
ES-1	1,270 ±23	147.5 ±3.8	71.2 ±4.8	1.066 ±0.011
ES-2	2,206 ±22	84.9 ±3.2	81.9 ±1.2	1.159 ±0.010
ES-3	2,268 ±44	77.3 ±2.1	79.9 ±1.3	1.105 ±0.017
ES-4	2,230 ±22	120.6 ±5.3	83.8 ±2.0	0.888 ±0.009
ES-5	1,218 ±23	111.8 ±2.5	78.7 ±2.7	0.756 ±0.008
Average ESVA	***1,838 ±451[bd]***	***108.4* ±30.2[cd]**	***79.1 ±5.4[d]***	***0.995* ±0.173[bd]**
CS-1	2,409 ±23	236.8 ±3.1	79.9 ±1.7	1.175 ±0.004
CS-2	2,359 ±46	421.2 ±23.9	82.4 ±2.9	1.509 ±0.016
CS-3	3,444 ±91	430.7 ±9.5	74.4 ±3.1	2.095 ±0.022
CS-4	2,873 ±46	341.1 ±5.6	78.8 ±3.7	1.805 ±0.023
CS-5	2,275 ±31	299.8 ±4.9	78.6 ±1.1	1.667 ±0.017
Average CSVA	***2,672 ±502[d]***	***345.9* ±83.3a[d]**	***78.8 ±3.4[e]***	***1.650* ±0.397[d]**
Total average	**2,424 ±537**	**220.6 ±106.4**	**78.8 ±3.7**	**1.399 ±0.483**

NOTE: Values of TPC, TAC, AA and WCD are expressed as arithmetic average ±standard deviation.

[a-e] Values with the same letters denote significant differences ($p < 0.05$) among vineyard areas.

LCVA – Little Carpathian vineyard area, SSVA – South Slovak vineyard area, NVA – Nitra vineyard area, ESVA – East Slovak vineyard area, CSVA – Central Slovak vineyard area.

wines could be as following: wines from Nitra VA > wines from Central Slovak VA > wines from South Slovak VA > wines from Little Carpathian VA > wines from East Slovak VA. Gained results exerted statistically significant differences (at significance level $p = 0.05$) between WCD in wines made in East Slovak VA and WCD in wines made in Central Slovak VA, and South Slovak VA.

In order to investigate the mutual relations between analyzed parameters, the linear regressions were obtained. The statistical evaluation of the obtained results did not confirm any linear correlations between TPC and AA, resp. TAC and AA (r = -0.255, resp. r = -0.279) at significance level p <0.1. This is not in the agreement with the study of **Burin et al., (2010)**, **Kondrashov et al., (2009)** and **Balík et al., (2008)** who found out very strong linear correlations between TPC, resp. TAC and AA in wines and grape juices. Explanation lies in the differences in the methodology of AA determination. The correlations between TPC and TAC (r = 0.542), resp. TAC and WCD (r = 0.600) were highly significant at significance level p <0.01. **Cioroi and Musat (2007)** reported stronger correlation between TPC and TAC (r = 0.739 and 0.771) in red wines. The statistical evaluation of the obtained results confirmed very highly significant correlations at significance level p <0.001 between WCD and TPC, resp. WCD and AA (r = 0.697, resp. r = - 0.714).

CONCLUSION

Slovak red wines – Cabernet Sauvignon have high antioxidant activity (average value 78.8% inhibition of DPPH), high content of healthy useful phenolic compounds (average value of TPC 2,424 mg GAE.L^{-1}), moderate value of TAC (average value 220.6 mg.L^{-1}) and good colour (average value of WCD 1.399). The results showed statistically significant differences for 3 studied parameters (TPC, TAC and WCD) in wines made in some vineyard areas in Slovakia. On the basis of statistical evaluation of our results, statistically significant correlations were demonstrated between wine colour density and other 3 parameters (TPC, TAC and AA), resp. between TPC and TAC.

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