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COLOUR, PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF THE SLOVAK ALIBERNET RED WINE SAMPLES

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ABSTRACT

Sixteen Alibernet wine samples, originated from three most important Slovak vineyard regions, were evaluated spectrophotometrically for the content of total polyphenols, content of total anthocyanins, antioxidant activity and wine colour density. The determined total polyphenol contents in observed wines ranged from 2141 to 4274 mg gallic acid per liter (average content was 3057 mg gallic acid/L) and total anthocyanin contents ranged from 73.4 to 791.2 mg/L (average content was 403.4 mg/L). Determined values of antioxidant activity were within the interval 54.8 – 86.8% (average value was 74.5%) and the values of wine colour density varied between 0.944 and 4.592 (average value was 2.317). The statistical evaluation of the obtained results confirmed only weak linear correlation between total polyphenol content and total anthocyanin content, however strong linear correlations between total anthocyanin content and antioxidant activity, resp. wine colour density were observed. Our results also confirmed very highly significant linear relationship between wine colour density and total polyphenol content, resp. antioxidant activity and between antioxidant activity and total phenolic content.

Keywords: Polyphenols, anthocyanin, antioxidant activity, red wine, Alibernet

INTRODUCTION

In recent years healthy effects of moderate consumption of wine have been discussed by scientific as well as laic community. Wine is considered to be an important source of antioxidants among beverages in human nutrition **(Faitová *et al.*, 2004)**. Drinking of wine has a lot of positive effects on health of human population, while wine becomes the part of healthy life style. More than 500 various compounds (saccharides, acids, tannins, vitamins, phenolic compounds…) are present in wine. Polyphenolic compounds are very monitored and discussed group of antioxidants in wine that exert bio-protective effects and have strongly positive effect on human health. Their importance is in reducing of cardiovascular and carcinogenic diseases incidence **(Slezák, 2007)**.
Phenolic compounds, as secondary metabolites, rank among phytochemicals groups, and have physiological and morphological importance for plants. From the standpoint of structure phenolic compounds consist of minimally one aromatic ring with one or more substitution –OH groups and form numerous and subsequently diversified group of substances including substances from simple phenolic to polymerized phenolic compounds. This is the reason why they are called polyphenols. Plant polyphenols are amorphous substances occurring almost in all plants, especially in leaves, flowers, seeds, fruits, in pathologic formations, as well as in products of plant origin, honey, propolis and wine. They are present mainly in form of conjugates of saccharides molecules in plants, with one or more phenolic molecules or they occur in form of functional derivatives, such as esters or metylesters **(Balasundram *et al.*, 2006; Vollmanová *et al.* 2009; Timoracká, 2010)**.
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)**.
Variety Alibernet originated from crossing of varieties *Alicante Bouchet* x *Cabernet Sauvignon* in Ukraine in 1950. Alibernet name is derived from the names of two parental varieties. High harvest, higher acids content, lower sugar contents and very high dye content is typical for this grape variety. Must has significant Cabernet taste **(Pospíšilová, 2003)**. Alibernet is little widespread variety in Europe and is grown mainly in warmer locations in Slovakia and South Moravia. In Slovakia, Alibernet grown at about 5.8% of the areas planted with blue grapevine varieties and it is the fourth most cultivated blue variety after Blaufränkisch, St. Laurent and Cabernet Sauvignon **(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 wines – Alibernet, originated from different Slovak vineyard areas.

MATERIAL AND METHODS

Chemicals and instruments

All analysed parameters – total polyphenol content, total anthocyanin content, antioxidant activity and wine colour density in wines were analyzed by 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., Trolox (pure)

Samples

Analysed, bottled, red, especially quality and dry wines Alibernet 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).

Sample analysis

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. Total polyphenol (TP) content in wines was calculated as amount of gallic acid equivalent (GAE) in mg per 1 litre of wine.
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 HCl solution to values 0.5 – 0.8 associated with the transformation of all anthocyans to red colored flavilium cation. The total anthocyanin (TA) content was calculated from the difference absorbance values of both solutions and expressed as the amount of anthocyanins in mg/L of wine.
Antioxidant activity (AA) was assessed by method of **Brand-Williams *et al.* (1995)** using of DPPH (2,2-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) and also as Trolox equivalent calculated from calibration curve.

Table 1 Characteristics of analysed Alibernet red wine samples

Sample	Producer	Vineyard area	Vintage	Quality
A-LC-1	Vitis Pezinok, s.r.o. / Hubert J.E. Sereď	Little Carpathian	2011	quality
A-LC-2	Malokarpatská vinohradnícka spol., a.s., Pezinok	Little Carpathian	2011	quality
A-LC-3	Víno Sabo, Vrbové	Little Carpathian	2011	quality
A-LC-4	JM Vinárstvo Doľany, Doľany	Little Carpathian	2009	grape selection
A-SS-1	Vinárske závody Topoľčianky, s.r.o., Topoľčianky	South Slovak	2010	quality
A-SS-2	Víno Nitra, s.r.o., Nitra	South Slovak	2011	quality
A-SS-3	VVD, Dvory nad Žitavou	South Slovak	2011	quality
A-SS-4	Virex, s.r.o., Nesvady	South Slovak	2011	quality
A-SS-5	Chateu Modra, a.s., Modra	South Slovak	2010	late harvest
A-SS-6	Villa Víno Rača, a.s., Bratislava	South Slovak	2011	quality
A-SS-7	Vinárske závody Topoľčianky, s.r.o., Topoľčianky	South Slovak	2009	late harvest
A-N-1	Vinárske závody Topoľčianky, s.r.o., Topoľčianky	Nitra	2007	quality
A-N-2	VINIDI, s.r.o., Bratislava	Nitra	2008	late harvest
A-N-3	Vinárske závody Topoľčianky, s.r.o., Topoľčianky	Nitra	2012	quality
A-N-4	Vinárstvo Trnovec, Nitra	Nitra	2012	grape selection
A-N-5	Muráni-Víno Čajkov, s.r.o., Čajkov	Nitra	2012	quality

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 length glass cells.

All chemical analyses were performed as four parallels.

Statistical analysis

Statistical analysis was performed using the software Statistica 6.0 (StatSoft) 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 red wines Alibernet are described in Table 2.
Total polyphenol content in analysed wine samples was in the range from 2064 to 4274 mg GAE/L. Average content of TP was 3057 mg GAE/L. The average content of total polyphenols in wines – Alibernet is much higher than we found out in the other three major Slovak varietal red wines Blaufränkisch - 2003 mg GAE/L, Saint Laurent - 2297 mg GAE/L and Cabernet Sauvignon - 2407 mg GAE/L **(Bajčan *et al.*, 2012a,b)**. Our results are slightly lower than results reported by **Slezák (2007)** and **Čižmárová (2009)**, who found out the content of TP in slovak wines - Alibernet in range from 2865 to 5340 mg GAE/L. According to the average value of TPC an order for wines could be as following: wines from Nitra VA > wines from South Slovak VA > wines from Little Carpathian VA. Gained results did not exert statistically significant differences ($P < 0.05$) between TPC in wines made in various vineyard areas in Slovakia.
Total anthocyanin content in analysed wine samples was in the range from 73.4 to 791 mg/L. Average content of TA was 422 mg/L. The average TAC in wines Alibernet is significantly higher than we found out in the other three major Slovak varietal red wines Blaufränkisch – 266.1 mg/L, St. Laurent – 264 mg/L and Cabernet Sauvignon – 209.3 mg/L **(Tóth *et al.*, 2011; Bajčan *et al.*, 2012b)**. Our results of TAC in wines Alibernet cannot be compared with other authors, because they used different methods for the determination of TA. According to the average value of TAC an order for wines could be as following: wines from Nitra VA > wines from Little Carpathian VA > wines from South Slovak VA. Our results also did not exert statistically significant differences between TAC in wines made in various vineyard areas in Slovakia.
Antioxidant activity in analysed wine samples was in range 54.8 – 86.8% inhibition of DPPH (resp. 0.627 – 1.101 mmol Trolox/L). Average value of AA was 74.5% inhibition of DPPH (resp. 0.903 mmol Trolox/L). The average value of AA in Alibernet wines is a slightly lower than we found out in the other three major Slovak red wines Blaufränkisch – 83.3%, St. Laurent – 81.2% and Cabernet Sauvignon – 78.8% inhibition of DDPH **(Bajčan *et al.*, 2012a,b)**. **Slezák (2007)** and **Čižmárová (2009)** reported lower values of AA and they found out AA in slovak wines - Alibernet in range from 61 to 65.1% inhibition of DPPH. On the basis of value of AA an order could be as following: wines from South Slovak VA > wines from Little Carpathian VA > wines from Nitra VA. Gained results did not exert statistically significant differences between values of antioxidant activity in wines made in various vineyard areas in Slovakia.
Wine colour density in analysed wine samples was in range from 0.944 to 4.592. Average value of WCD was 2.317. The average value of WCD in wines Alibernet was much higher than we found out in the other three major Slovak varietal red wines Blaufränkisch – 1.110, St. Laurent – 1.224 and Cabernet Sauvignon – 1.364 **(Tóth *et al.*, 2011; Bajčan *et al.*, 2012b)**. This work is the first study monitoring WCD in Slovak wines Alibernet so we can´t compare our data with other local scientists. According to the average value of WCD an order for wines could be as following: wines from Nitra VA > wines from Little Carpathian VA > wines from South Slovak VA. Gained results did not exert statistically significant differences between values of WCD in wines made in various vineyard areas in Slovakia.

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)	TAC (mg/l)	AA (%)	AA (mmol Trolox/l)	WCD
A-LC-1	2141 ± 23	262 ± 4.0	83.2 ± 1.4	1.038 ± 0.017	1.374 ± 0.026
A-LC-2	3218 ± 23	509 ± 6.1	65.2 ± 1.4	0.762 ± 0.017	3.514 ± 0.034
A-LC-3	3052 ± 45	791 ± 14.1	74.4 ± 2.8	0.895 ± 0.035	2.482 ± 0.021
A-LC-4	2409 ± 61	73.4 ± 1.4	78.7 ± 0.7	0.962 ± 0.008	1.502 ± 0.015
Average LCVA	***2705 ± 523***^{a}	***409* ± 349**^{a}	***75.4 ± 8.7***^{a}	***0.914 ± 0.134***^{a}	***2.218* ± 1.039**^{a}
A-SS-1	2929 ± 23	416 ± 6.3	80.9 ± 0.5	0.999 ± 0.006	1.782 ± 0.021
A-SS-2	2064 ± 23	218 ± 4.2	86.8 ± 0.9	1.101 ± 0.011	0.944 ± 0.014
A-SS-3	3834 ± 23	664 ± 7.0	63.3 ± 1.1	0.736 ± 0.014	3.781 ± 0.012
A-SS-4	2847 ± 45	441 ± 5.5	80.3 ± 0.5	0.989 ± 0.006	1.779 ± 0.008
A-SS-5	2618 ± 70	192 ± 3.7	82.7 ± 0.6	1.029 ± 0.007	1.436 ± 0.009
A-SS-6	2487 ± 70	458 ± 5.3	78.8 ± 0.8	0.964 ± 0.010	1.862 ± 0.019
A-SS-7	3895 ± 45	318 ± 5.3	69.2 ± 1.0	0.818 ± 0.012	2.815 ± 0.025
Average SSVA	***2953 ± 677***^{b}	***387* ± 175**^{b}	***77.4 ± 8.7***^{b}	***0.948* ± 0.135**^{b}	***2.057* ± 1.051**^{b}
A-N-1	3158 ± 92	92.8 ± 5.9	78.4 ± 0.5	0.958 ± 0.006	1.629 ± 0.016
A-N-2	4066 ± 23	322 ± 4.2	61.6 ± 1.5	0.714 ± 0.019	3.309 ± 0.024
A-N-3	3013 ± 23	328 ± 6.1	76.3 ± 0.5	0.924 ± 0.006	2.015 ± 0.018
A-N-4	2908 ± 23	635 ± 5.6	76.9 ± 0.6	0.934 ± 0.007	2.260 ± 0.023
A-N-5	4274 ± 68	732 ± 7.1	54.8 ± 1.4	0.627 ± 0.017	4.592 ± 0.015
Average NVA	***3484 ± 587***^{c}	***422* ± 275**^{c}	***69.6 ± 10.1***^{c}	***0.831* ± 0.142**^{c}	***2.761* ± 1.274**^{c}
Total average	**3057 ± 668**	**403 ± 219**	**74.5 ± 9.0**	**0,903 ± 0.134**	**2.317 ± 1.019**

Legend: LCVA – Little Carpathian vineyard area, SSVA – South Slovak vineyard area, NVA – Nitra vineyard area.
Values of TPC, TAC, AA and WCD are expressed as arithmetic average ± standard deviation,
$^{a-c}$ Values with the same letters denote significant differences (P<0.05) among vineyard areas.

In order to investigate the mutual relations between analyzed parameters, the linear regressions were obtained. The statistical evaluation of the obtained results confirmed only weak linear correlation between TPC and TAC (r = 0.447, P < 0.1). This is not in agreement with our previous study **(Bajčan *et al.*, 2012b)**, where we found out strong linear correlation (r = 0.542, P < 0.01) between TPC and TAC in red wines - Cabernet Sauvignon. **Cioroi and Musat (2007)** also reported very strong correlation between TPC and TAC (r = 0.739 and 0.771) in red wines. The correlations between TAC and AA (r = -0.532), resp. TAC and WCD (r = 0.660) were strong at significance level P < 0.01. Other authors **(Burin *et al.*, 2010; Cioroi and Musat, 2007; Balík *et al.*, 2008)** also found out very strong linear correlations between TAC and AA in wines and grape juices. The statistical evaluation of the obtained results confirmed very strong correlations between AA and TPC (r = -0.917, P < 0.001), WCD and TPC (r = 0.887, P < 0.001), resp. WCD and AA (r = -0.979, P<0.001). Similary very strong correlations between AA and TPC found out other researchers **(Burin *et al.*, 2010; Cioroi and Musat, 2007; Kondrashov *et al.*, 2009; Balík *et al.*, 2008)**. Very strong correlations between WCD and TPC, resp. WCD and AA in red wines (Cabernet Sauvignon) we also reported in our previous study **(Bajčan *et al.*, 2012b).**

CONCLUSION

In the present study we showed that Slovak red wines – Alibernet had high content of healthy useful polyphenols (average value of TPC 3057 mg GAE/L), high antioxidant activity (average value of AA 74.5% inhibition of DPPH), high content of total anthocyanins (average value of TAC 403 mg/L) and extremely intensive red colour (average value of WCD 2.317). The results did not showed statistically significant differences for all four studied parameters (TPC, TAC, AA and WCD) in wines made in three different vineyard areas in Slovakia. On the basis of statistical evaluation of our results we can state, that there are statistically highly significant or very highly significant linear correlations between all studied parameters (excepting correlation between total phenolic content and total anthocyanin content) of Alibernet wines.

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