



## PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF MONOVARIETAL RED WINES PRODUCED IN LIMBACH REGION

*Daniel Bajčan*<sup>1\*</sup>, *Silvia Čéryová*<sup>1</sup>, *Tomáš Tóth*<sup>1,2</sup>

**Address:** <sup>1</sup>Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

<sup>2</sup>Institute of Chemistry, Centre of excellence for white-green biotechnology, Slovak Academy of Sciences, Trieda A. Hlinku 2, 949 76, Slovak Republic

\* Corresponding author: bajcan@gmail.com

---

### ABSTRACT

Chosen antioxidant properties as content of total polyphenols, content of total anthocyanins and antioxidant activity of the red wines, originating from winery region Limbach, were determined. Three types of mono-varietal wines – Cabernet Sauvignon, Blaufränkisch and St. Laurent, originating from 3 local producers were analysed. Total polyphenols content was determined with phenol Folin-Ciocalteu's reagent, total anthocyanins content was determined by pH differential method and antioxidant activity was determined using DPPH stable free radical. All three parameters were determined by UV-VIS absorption spectrometry.

The determined total polyphenol contents in observed wines were within the interval 1908 – 3276 mg gallic acid.l<sup>-1</sup>, total anthocyanin contents within the interval 96 – 334 mg.l<sup>-1</sup> and antioxidant activity within the interval 65.5 – 84.1 %. This result indicates high antioxidant properties of the Limbach's wines. The differences between total polyphenol contents of the wines originating from different producers were not statistically significant. However, we found significant differences between results of the rest analysed parameters (total anthocyanins content and antioxidant activity) of the wines originating from different producers. The differences between results of all analysed properties of the wines of different variety were not statistically significant.

**Keywords:** polyphenols, anthocyanins, antioxidant activity, red wine, Limbach

---

## INTRODUCTION

Recent reports about discussed theme by scientific teams, scientific disciplines, as well as by medicine – moderate drinking of wine appears to be related to beneficial effects on human health. Grape wine has been for thousands years the most wide spread and the most popular cultural beverage in the world. It is also considered to be an important source of antioxidants among beverages and its antioxidant effects are comparable and in many cases even higher than by fresh fruit juices (Šindler, 2011). Flavonoids and other polyphenolic compounds are very investigated group of antioxidants in wines exerting bio-protective effects and have strongly positive influence on human health. Their effect is remarkable especially in reducing of many chronic, cardiovascular and carcinogenic diseases (Slezák, 2007). Phenolic compounds are part of almost all foodstuffs. They are very heterogeneous group of compounds, from which some act as aromatic, or gustatory compounds, natural colours, or exert special biological effects and rank among natural antioxidants, natural toxic components of foodstuffs, or defence compounds (phytoalexins). The group of polyphenols comprises of large and diversified scale of compounds, from simple phenolic acids to highly polymerised tannins (Timoracká et al., 2008).

In general, red wines contain more phenolic compounds than white wines, what is determined by winemaking technology. Grapes's skins are removed before fermentation by white wines making process (Beer et al., 2006). Content of polyphenols in wines is affected not only by grape variety, growing locality climatic conditions, but also by procedures by winemaking: length of contact of wine must with skins, mixing, temperature, pH value, SO<sub>2</sub> content, and content of alcohol (Villano et al., 2006; Lachman and Šulc, 2006).

Village Limbach is an old vineyard locality in south-eastern part of Little Carpathians. This municipality is a part of vineyard subregion Pezinok that belong to Little Carpathian vineyard area. Although Limbach is known by its traditional white wine - Green Silvaner (Ruman, 2006), its red wines with origin in mentioned village have also high quality.

The aim of our study was to analyse red wines from famous winery locality Limbach. Selected antioxidant properties: total content of phenolic compounds, total content of anthocyanins, and antioxidant activity of three most popular and bestselling, varietal, red wines

in Slovakia - Blaufränkisch, Cabernet Sauvignon and Saint Laurent were analysed. Bottled, quality wines made by three local producers were prepared for analyses.

## MATERIAL AND METHODS

Analysed, bottled, quality red wines and their characteristics are reported in Table 1. Samples of wine were gained as gifts from wine producers and all wines were from vintage year 2009. Samples of wine were kept until analyses (app. 2 weeks after distribution) in horizontal position, by temperature 20 ° C, without light irradiation.

Total content of polyphenols, total content of anthocyanins and antioxidant activity in wines were determined by UV-VIS spectrometry (spectrophotometer Shimadzu UV/VIS-1240 - *Shimadzu, Japonsko*). Content of total polyphenols (TP) was determined by method of **Faitová et al. (2003)**. Aliquot 1 cm<sup>3</sup> of wine was pipetted into 50 cm<sup>3</sup> volumetric flask and diluted 5 cm<sup>3</sup> with distilled water. 2.5 cm<sup>3</sup> Folin-Ciocalteu reagent was added into diluted mixture and after 3 minutes 7.5 cm<sup>3</sup> 20 % of aqueous solution Na<sub>2</sub>CO<sub>3</sub> was added. Then the sample was filled up to 50 cm<sup>3</sup> with distilled water and after mixing left to stay at laboratory temperature for 2 hours. With the same procedure blank and calibration solutions of gallic acid were prepared. Absorbance of sample solutions was read against blank at 765 nm. Total content of polyphenols in wines was expressed as amount of gallic acid (GA) in mg per 1 litre of wine.

Modified pH differential method of **Lapornik et al. (2005)** was used for assessment of total anthocyanins (TA). Principle of this method is reducing of pH value of the sample on values 0.5 to 0.8 associated with transformation of color of all anthocyanins on red colored flavilium cation. Into two test tubes 1 cm<sup>3</sup> of wine was pipetted and 1 cm<sup>3</sup> of 0.01 % HCl in 80 % ethanol was added. Afterwards, into first test tube 10 cm<sup>3</sup> of 2 % HCl was added and into second test tube 10 cm<sup>3</sup> of McIlvain reagent with pH = 3.5 was added. Absorbance of both solutions was measured after mixing at 520 nm against blank. Total content of anthocyanins was calculated from differences of absorbance values of sample solutions and expressed as amount of anthocyanins in mg per 1 litre of wine.

Antioxidant activity (AA) was assessed by method of **Brand-Williams et al. (1995)** using of DPPH (2,2-difeny-1-picrylhydrazyl) radical. Absorbance was read at 515.6 nm and antioxidant activity was expressed as % inhibition of DPPH (quantitative ability of selected component to remove in exact period certain amount of DPPH radical).

All analyses were performed in four parallels.

**Table 1** Characteristics of analysed wines

Sample	Producer	Variety	SO <sub>2</sub> content in mg.l <sup>-1</sup>	Wine type <sup>A</sup>	Alcohol content in %
C1	Vinárstvo	Cabernet Sauvignon	23	semi dry	12.2
F1	Michal Sadloň,	Blaufränkisch	15	dry	12.1
S1	Limbach	Saint Laurent	24	dry	12.8
C2	Limbašské	Cabernet Sauvignon	37	dry	12.4
F2	vinohradnícke	Blaufränkisch	28	semi dry	13.1
S2	družstvo, Limbach	Saint Laurent	26	dry	12.5
C3		Cabernet Sauvignon	31	dry	11.7
F3	Víno JANO, Limbach	Blaufränkisch	38	dry	12.0
S3		Saint Laurent	13	dry	11.7

Legend: Wine type<sup>A</sup> – by the residual sugar content

## RESULTS AND DISCUSSION

Total content of polyphenols in tested wines was in range 1908 - 3276 mg GA.l<sup>-1</sup>. According to average value of TP content the order according to producers of analysed wines could be as following: Limbašské vinohradnícke družstvo > Vinárstvo Michal Sadloň > Víno Jano. According to average content value of TP the order of analysed wines according to variety could be as following: Saint Laurent > Blaufränkisch > Cabernet Sauvignon. Obtained results did not exert statistically significant differences (at significance level P = 0.05) between total content of polyphenols in wines made by different producers and as well as between wines of different varieties.

Our results of TP content in Blaufränkisch were slightly higher than those published by Slezák (2007) and Čižmárová (2009) who found out that the content of TP in wines made in Little Carpathian vineyard area was in range 1646 - 2290 mg GA.l<sup>-1</sup>. Average value of TP content in Blaufränkisch was 1854 mg GA.l<sup>-1</sup>. Our results of TP content in Saint Laurent were diverse from those reported by Slezák (2007) and Čižmárová (2009), who determined remarkably higher contents of TP in wines - Saint Laurent, i.e. in range from 2890 to 4246 mg.l<sup>-1</sup>. Average content value of TP was 3600 mg GA.l<sup>-1</sup>.

**Table 2** The content of total polyphenols (in mg gallic acid  $\cdot\text{l}^{-1}$ ), content of total anthocyanins (in mg  $\cdot\text{l}^{-1}$ ) and antioxidant activity (% of DPPH inhibition) in wines

Sample	Content TP <sup>B</sup>	Content TA <sup>B</sup>	AA <sup>B</sup>
C1	2080 ± 26	183 ± 26	81.5 ± 1.8
F1	2171 ± 27	96 ± 27	77.4 ± 2.2
S1	2421 ± 24	165 ± 24	78.1 ± 1.9
<b>Average – Sadloň</b>	<b>2224 ± 202</b>	<b>148 ± 51.4</b>	<b>79.0 ± 2.4</b>
C2	2197 ± 41	328 ± 41	65.5 ± 2.4
F2	2947 ± 50	267 ± 50	75.3 ± 2.9
S2	3276 ± 49	334 ± 49	71.0 ± 1.7
<b>Average – LVD</b>	<b>2807 ± 678</b>	<b>310 ± 39.6</b>	<b>70.6 ± 5.8</b>
C3	2237 ± 4	151 ± 4	80.1 ± 2.5
F3	1908 ± 19	161 ± 19	84.1 ± 1.8
S3	2105 ± 64	204 ± 44	80.5 ± 1.1
<b>Average – Jano</b>	<b>2083 ± 194</b>	<b>172 ± 31.3</b>	<b>81.6 ± 2.4</b>
<b>Average – Cab. Sauvignon</b>	<b>2171 ± 93</b>	<b>221 ± 105</b>	<b>75.7 ± 9.5</b>
<b>Average – Blaufränkisch</b>	<b>2342 ± 614</b>	<b>174 ± 101</b>	<b>78.9 ± 5.2</b>
<b>Average – St. Laurent</b>	<b>2601 ± 692</b>	<b>234 ± 100</b>	<b>76.5 ± 5.6</b>

Legend: <sup>B</sup> – the contents of TP (TA) and values of AA are expressed as arithmetic average ± standard deviation

Total content of anthocyanins in tested wines was in range 96 – 334 mg  $\cdot\text{l}^{-1}$ . According to average content value of TA the order of analysed wines according to producers could be as following: Limbašské vinohradnicke družstvo > Víno Jano > Vinárstvo Michal Sadloň. According to average value of TA the order of analysed wines according to variety could be as following: Saint Laurent > Cabernet Sauvignon > Blaufränkisch. Gained results exerted statistically significant differences (at significance level  $P = 0.01$ ) between total content of anthocyanins in wines made by Limbašské vinohradnicke družstvo and total content of anthocyanins in wines made by other two producers (Vinárstvo Michal Sadloň and Víno Jano). Our results did not show statistically significant differences (at significance level  $P = 0.05$ ) between total content of anthocyanins in wines of different varieties.

Our results of TA content in Blaufränkisch were remarkably lower than those published by Slezák (2007) and Čižmárová (2009), who assessed TA content in range 216 - 445 mg  $\cdot\text{l}^{-1}$  in wines gained from Little Carpathian vineyard area. The average content value of TA in Blaufränkisch was 368 mg  $\cdot\text{l}^{-1}$ . Similarly, as by Blaufränkisch, when wine - Saint Laurent was evaluated, our results were considerably lower than those referred by Slezák (2007) and Čižmárová (2009), who determined in wines - Saint Laurent content of TA in range 553 – 630 mg  $\cdot\text{l}^{-1}$ . The average value of TA content was 599 mg  $\cdot\text{l}^{-1}$ .

Antioxidant activity in tested wines was in range 65.5 - 84.1 % inhibition of DPPH. According to average value of AA the order of analysed wines of different producers could be as following: Víno Jano > Vinárstvo Michal Sadloň > Limbašské vinohradnícke družstvo. This order is contrary to the order by comparing of total polyphenols content. Similar fact (inverse dependence of antioxidant activity on total polyphenols content) was found out also by other analyses (Tóth *et al.*, 2010). According to value of AA the order for analysed wines of different varieties could be as following: Blaufränkisch > Saint Laurent > Cabernet Sauvignon. Gained results exerted statistically significant differences (at significance level  $P = 0.05$ ) only between AA values in wines made by Limbašské vinohradnícke družstvo and AA in wines made by company Víno Jano. Results did not show statistically significant differences (at significance level  $P = 0.05$ ) between values of AA in wines of different varieties.

Our gained results of AA values in Blaufränkisch were similar to those mentioned in works of Slezák (2007) and Čížmarová (2009), who evaluated values of AA in wines produced in Little Carpathian vineyard area in range 77.3 - 79.5 % inhibition of DPPH. Average value of AA in Blaufränkisch was 78.7 % inhibition of DPPH. When evaluating wine - Saint Laurent, our results were in agreement with those obtained by Slezák (2007) and Čížmarová (2009), who determined values of AA in St. Laurent's wine in range 75.1 - 76.5 % inhibition of DPPH. The average value of AA was 75.6 % inhibition of DPPH.

## CONCLUSION

Red wines are important source of flavonoids, anthocyanins, and many other polyphenolic compounds. In addition, regular and moderate consumption of wine, in suitable doses has highly positive effect on human health and is recommended also by doctors. Our work was focused on determination of total polyphenols and anthocyanins content, as well as antioxidant activity of three most important varietal red wines - Cabernet Sauvignon, Blaufränkisch and Saint Laurent. Quality wines, bottled and produced in famous wine region Limbach, from 3 local wine producers, were analysed. Total polyphenols content in selected wines was in range from 1908 to 3276 mg gallic acid.l<sup>-1</sup>. The highest content of polyphenols was assessed in red wines from producer Limbašské vineyard enterprise and when evaluating the variety - wine Saint Laurent appeared as the one with the highest content of total polyphenols. The lowest content of phenolic compounds were evaluated in wines from producer Víno Jano and in wine Cabernet Sauvignon with regard to variety. Total content of

anthocyanins in tested wines was in range 96 - 334 mg.l<sup>-1</sup>. The highest content of anthocyanins that by the considerable rate affected the sensory properties of wine, were determined in wines from producer Limbašské vinohradnícke družstvo. From varietal wines the highest content of anthocyanins was determined in St. Laurent's wine. The lowest content of anthocyanins were evaluated in wines from producer Vinárstvo Michal Sadloň and in Blaufränkisch with regard to variety. Antioxidant activity in analysed wines was in range 65.5 – 84.1 % inhibition of DPPH. The highest values of antioxidant activity were determined in wines with the lowest content of total polyphenols (wines of producer Víno Jano) and contrary the lowest values of antioxidant activity were recorded in wines with the highest content of polyphenols (wines from Limbašské vinohradnícke družstvo). When evaluating varietal wines, the lowest antioxidant activity was recorded by wine - Cabernet Sauvignon and contrary the highest one by wine - Blaufränkisch. The results revealed statistically significant differences between monitored antioxidant properties (total content of anthocyanins and antioxidant activity) in wines made by some producers. In summary we can state that that wines made in region Limbach, belonging to Little Carpathian vineyard area, contain high amounts of phenolic substances and have strong antioxidant effect, and thus could compete, with regard to qualitative parameters, with wines from abroad.

**Acknowledgments:** This work was funded by Grant Agency VEGA no. 1/0030/09. This contribution is also the result of the project implementation: Centre of excellence for white-green biotechnology, ITMS 26220120054, supported by the Research & Development Operational Programme funded by the ERDF.

## REFERENCES

- BEER, D. – JOUBERT, E. – MARAIS, J. – MANLEY, M. 2006. Unravelling the total antioxidant capacity of Pinotage wine: contribution of phenolic compounds. In *J. Agric. Food Chem.*, vol. 54, 2006, no. 8, p. 2897-2905
- BRAND-WILLIAMS, W. – CUVELIER, M. E. – BERSET, C. 1995. Use of a free radical method to evaluate antioxidant activity. In *Lebensmittel Wissenschaft und Technologie*, vol. 28, 1995, no. 1, p. 25–30
- ČIŽMÁROVÁ, M. 2009. Antioxidant a antiradical activity of chosen types of wine. In *Dissertation work*. Nitra: SUA, 2009, 168 p.

- FAITOVÁ, K. – LACHMAN, J. – PIVEC, V. – HEJTMÁNKOVÁ, A. – DUDJAK, J. – LACHMAN, J. – ŠULC, M. 2006. Phenolics and antioxidant activity of wines during the winemaking process. In *Control Applications in Post – Harvest and Processing Technology* (Proceeding of the work of the International Scientific Conference). Potsdam: Leibniz-Institut für Agrartechnik Potsdam-Bornim, 2006, 9 p.
- LAPORNIK, B. – PROŠEK, M. – WONDRA, A.G. 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. In *J. Food Eng.*, vol. 71. 2005, no. 3, p. 214-222
- RUMAN, T. In LIPKA, F. et al. 2006. Practical guide of the slovak wines. Bratislava: Belimex s.r.o. 2006, p. 10 ISBN 80-89272-02-9
- SLEZÁK, F. 2007. Conservation of the antioxidant elements in wines occurring Little Carpatian region: research report. Modra: Biocentrum Modra a VÚP Bratislava, 2007. 19 p.
- ŠINDLER, Z. 2011. Phenolic compounds in red wines and their antioxidant effects. In *Bachelor work*. Nitra: SAU, 2011, 44 p.
- ŠULC, M. 2003. Differences in polyphenol and resveratrol content among bottles of tramin wine of the same production series. In *Vitamins 2003 – Natural antioxidants and free radicals* (Proceeding of the work of the International Scientific Conference), Pardubice: University Pardubice, 2003, p. 167-170 ISBN80-7194-549-8
- TIMORACKÁ, M – VOLLMANNOVÁ, A. – BAJČAN, D. 2008. Analysis of polyphenolic compounds in plant material. In *Quality Food*, vol. 8, 2008, no. 2, p. 14-17
- TÓTH, T. – BAJČAN, D. – VOLLMANNOVÁ, A. – TREBICHLASKÝ, P. - LAHUČKÝ, L. – MIŠŠÍK, J. 2011. The phenolic content and antioxidant activity of varietal slovak red wines. In *Chemické listy*, vol. 105, 2011, special no., p. 1029
- VILLANO, D. – FERNÁNDEZ-PACHÓN, M. S. – TRONCOSO, A. M. – GARCÍA-PARRILLA, M. C. 2006. Influence of enological practices on the antioxidant capacity and total polyphenols. In *Food Chem.*, vol. 95, 2006, no. 3, p. 394-404