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ANTIOXIDANT PROPERTIES, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT OF THE SLOVAK WHITE WINES – WELSCHRIESLING AND CHARDONNAY

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ABSTRACT
The biologically active compounds in wines, especially phenolics, are responsible for reduced risk of developing chronic diseses (cardiovascular diseases, cancer, diabetes, Alzheimer disease, *etc*.), due to their antioxidant activities. Twenty six Slovak white wines, produced from different geographical origins, were examined in this study. The antioxidant activity, total phenolic and flavonoid contents of two types monovarietal wines – Welschriesling and Chardonnay were evaluated. All three mentioned parameters were determined by UV-VIS absorption spectrometry. The results showed that both types of Slovak white wines were high in polyphenols (average content was 303.2 mg GAE.L^{-1} in Welschriesling, resp. 355.6 mg GAE.L^{-1} in Chardonnay) and flavonoids (average content was 51.9 mg CE.L^{-1} in Welschriesling, resp. 60.1 mg CE.L^{-1} in Chardonnay), as well as a high antioxidant activity (average value was 35.0% inhibition of DPPH in Welschriesling, resp. 43.3% inhibition of DPPH in Chardonnay), comparable to the wines produced in other regions in the world. Among the white wines, Chardonnay had higher content of total polyphenols, as well as flavonoids and higher values of antioxidant activity. Our results confirmed very strong linear correlations between all three analysed parameters (TPC, TFC and AA): TPC and TFC (r = 0.818), AA and TPC (r = 0.699), resp. TFC and AA (r = 0.693).

Keywords: antioxidant activity; flavonoid; polyphenol; white wine

INTRODUCTION
The terms antioxidant and free radical are popular expressions used by nutritionists, scientists and general public. Free radicals are chemical species, compounds and/or elements with one or two unpaired electrons in their outer layer, which can be created in a multiple ways. They can be exogenic (e.g. ultraviolet radiation, xenobiotics and infections) or endogenic **(Andzi Barhé and Feuya Tchouya, 2014)**. A lack of antioxidant or an overproduction of free radicals can lead to an imbalance between the oxidant and antioxidant system **(Guerci et al, 2001; Puitha et al., 2005)**. Oxidative stress is involved in several illnesses, including diabetes **(Huang et al., 2004)**, atherosclerosis, Alzheimer´s disease and Parkinsons´s disease **(Drobek-Slowik and Karczewicz, 2007)**. The provision of antioxidants through diet is a simple means to reduce the development of illnesses brought on by oxidative stress **(Zafra-Stone et al., 2007)**.

Wine is an alcoholic fermented beverage with considerable amounts of phytochemicals **(Gresele et al., 2011)**. Wine is very rich source of polyphenols, such as catechins, epicatechins, quercetin, rutin, myricetin, anthocyanins, fenolics acids (gallic acid, caffeic acid, *p*-coumaric acid, etc.), trans-resveratrol and many others polyphenols and compounds. Many of these componds have been reported to have multiple biological activities, including cardioprotective, anti-carciogenic, antiviral and antibacterial properties **(King et al., 2006; Santos-Buelga and Scalbert, 2000; Špakovská et al., 2012)**. To date, over 3000 flavonoids have been identified. These can mainly be found in the pigments in flowers or in leaves **(Marfak, 2003)**. Flavonoids are primarily known for their anti-oxidative **(Bruneton, 1999)**, vasculoproprotectrice **(Vitor et al., 2004)**, anti-inflammatory **(Chen et al., 2008)** and anti-diabetic **(Marfak, 2003)** properties. Currently, chemoprevention is being used in medicine as a new strategy to prevent cancers. Natural phytochemicals, including wine and/or grape polyphenols, appear to be very promising substances to block, reverse, retard or prevent the process of carcinogenesis **(Russo, 2007)**.

The total amount of polyphenols in wines has been estimated to range from 200 to 6000 mg.L^{-1} **(Quideau et al., 2011)**. The highly variable level of phenolic compounds in wine is due to differences in grape variety and source as well as processing. Wine polyphenols have been reported to be bioavailable in several studies

(Nardini et al., 2009; Vitaglione et al., 2005). These compounds are directly related to the quality of wines, so phenolic analysis can be used as an effective tool in characterizing defferent wines. Many factors can influence the phenolic composition if wines, including grape variety and the technology applied **(Mulero et al., 2011)**.

The purpose of this study was to determine and evaluate chosen properties (the content of total polyphenols, content of total flavonoids and antioxidant activity) and their mutual correlations in white wines – Welschriesling (typical Central European variety) and Chardonnay (most famous world variety), originated from 3 most important Slovak vineyard areas.

MATERIAL AND METHODOLOGY

Chemicals and instruments

All analysed parameters – content of total polyphenols, content of total flavonoids and antioxidant activity 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., aluminium chloride p.a., sodium nitrite p.a., sodium hydroxide p.a., 35%, catechin hydrate 98%, methanol p.a., 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical p.a.

Samples

Analysed, bottled, white, especially dry wines Welschriesling (WR), resp. Chardonnay (CH) and their characteristics are mentioned in Table 1 and 2. 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).

Methods

Antioxidant activity determination

Antioxidant activity (AA) was assessed by method of **Brand-Williams et al. (1995)** using of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the nonradical form DPPH-H **(Chanda and Dave, 2009)**. This transformation results in a color change from purple to yellow, which is measured spectrophotometrically. Briefly, 0.1 mL of white wine was added to 3.9 mL of a $6x10^{-5}$ $mol.L^{-1}$ solution of DPPH in methanol. A control sample containing the same volume of water instead of extract was used to measure the maximum DPPH absorbance. After the reaction had been allowed to take place in the dark for 10 min, the absorbance at 515.6 nm was recorded to determine the concentration of remaining DPPH. The percentage inhibition of the initial concentration of DPPH radical was calculated as: % inhibition = [(ADPPH - Awine)/ADPPH] x 100. The results were also expressed as Trolox equivalent antioxidant capacity using calibration curve method.

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 total flavonoid content

Total flavonoid content (TFC) was assessed by aluminium chloride method **(Chang et al., 2002)**. 1 mL of wine sample and 4 mL of deionised water were added to a 10 mL volumetric flask. 5 min after adding 0.3.mL of 5% sodium nitrite, 0.3 mL of 10% aluminium chloride was added. 2 mL of sodium hydroxide with cincentration 1 $mol.L^{-1}$ was added to the reaction mixture after 6 min incubation. The final volume was immediately made up to 10 mL with deionised water. The absorbance of the solution was measured at 510 nm against blank solution.

Table 1 Characteristics of analysed Welschriesling wine samples.

Sample	Producer	Vineyard area	Vintage	Quality
WR-1	Villa Víno Rača, Bratislava	Little Carpathian	2011	quality
WR-2	Malokarpatská vinohrad. spol., Pezinok	Little Carpathian	10/2012[d]	quality
WR-3	Víno Matyšák, Pezinok	Little Carpathian	2011	quality
WR-4	Víno Jano, Limbach	Little Carpathian	10/2014[d]	quality
WR-5	Virex, Nesvady	South Slovak	5/2011[d]	quality
WR-6	VVD, Dvory nad Žitavou	South Slovak	2011	quality
WR-7	Vitis Pezinok / Hubert J.E. Sereď	South Slovak	2012	quality
WR-8	Víno Matyšák, Pezinok	South Slovak	2013	quality
WR-9	Víno Nitra, Nitra	Nitra	2011	quality
WR-10	Vinárske závody Topoľčianky, Topoľčianky	Nitra	2011	quality
WR-11	Vinárstvo Šintavan, Šintava	Nitra	2010	cabinet
WR-12	Vinárstvo Trnovec, Nitra	Nitra	2013	quality

[d] – date of bottling (unknown vintage).

Table 2 Characteristics of analysed Chardonnay wine samples.

Sample	Producer	Vineyard area	Vintage	Quality
CH-1	Mrva & Stanko, Trnava	Little Carpathian	2010	late harvest
CH-2	Villa Víno Rača, Bratislava	Little Carpathian	2011	quality
CH-3	Chateau Zumberg, Pezinok	Little Carpathian	2012	quality
CH-4	Víno Matyšák, Pezinok	Little Carpathian	2013	quality
CH-5	Vitis Pezinok / Hubert J.E. Sereď	South Slovak	2011	quality
CH-6	Víno Nitra, Nitra	South Slovak	2011	quality
CH-7	Vinárske závody Topoľčianky, Topoľčianky	South Slovak	2012	late harvest
CH-8	Chateau Modra, Modra	South Slovak	2011	select harvest
CH-9	Víno Velkeer 1113, Veľký Kýr	South Slovak	2014	late harvest
CH-10	Vinárstvo Trnovec, Nitra	Nitra	2011	select harvest
CH-11	Vinárske závody Topoľčianky, Topoľčianky	Nitra	2012	quality
CH-12	Ivan Czakó, Nit. Hrnčiarovce	Nitra	2011	late harvest
CH-13	Peter Belan, Mojmírovce	Nitra	2013	late harvest
CH-14	PD Mojmírovce, Mojmírovce	Nitra	2012	select harvest

The content of total flavonoids (TF) in wine samples was calculated as amount of catechin equivalent (CE) in mg per 1 litre of wine.

Statistical analysis

Statistical analysis were 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 flavonoids and antioxidant activity of the slovak wines Welschriesling, resp. Chardonnay are described in Table 3 and Table 4.

Total polyphenol content in analysed Welschriesling wine samples was in the range from 247.5 to 388.1 mg GAE.L^{-1}. Average content of TP was 303.2 mg GAE.L^{-1}. The results are very similar to results reported by **Slezák (2007)**, who found out the content of TP in slovak wines – Welschriesling in range from 250 to 361 mg GAE.L^{-1} (average value was 304.6 GAE.L^{-1}). Other (foreign) scientists **(Li et al., 2009; Lužar et al., 2016; Ma et al., 2014)** analyzing TPC in WR wines reported also very similar results (219 – 445 mg GAE.L^{-1}). **Ivanova et al. (2010)** and **Stratil et al. (2008)** reported much lower average value of TPC (205, resp. 97 mg GAE.L^{-1}) in WR wines. On the other hand, **Sato et al. (1996)** found out much higher phenolic content (721 mg GAE.L^{-1}) in one wine sample of Welschriesling. According to the average value of TPC an order for wines – Welschriesling 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 (at significance level p = 0.05) between TPC in wines – Welschriesling made in various vineyard areas in Slovakia.

Total polyphenol content in analysed Chardonnay wine samples was in the range from 280.2 to 475.1 mg GAE.L^{-1}. Average content of TP was 355.6 mg GAE.L^{-1}. Our results are very similar to results reported by **Chircu Brad et al. (2012)** and **Ma et al. (2014)**, who found out the content of TP in wines – Chardonnay in range from 275 to 454 mg GAE.L^{-1}. Other scientists **(Frankel et al., 1995; Li et al., 2009; Minussi et al., 2003** and **Stratil et al., 2008)** analyzing TPC in CS wines reported much lower average

Table 3 The content of total polyphenols (TPC), content of total flavonoids (TFC) and antioxidant activity (AA) in analysed wines Weschriesling.

Wine sample	TPC (mg GAE.L^{-1} ±SD)	TFC (mg CE.L^{-1} ±SD)	AA (% inhib. ±SD)	AA (mmol Trolox.L^{-1} ±SD)
WR-1L	327.2 ±2.5	58.9 ±0.5	33.0 ±1.0	0.375 ±0.012
WR-2L	272.3 ±9.4	44.2 ±1.1	28.2 ±0.7	0.321 ±0.009
WR-3L	289.6 ±2.3	44.5 ±3.0	29.9 ±0.7	0.340 ±0.009
WR-4L	247.5 ±6.5	42.4 ±1.8	44.2 ±0.5	0.501 ±0.006
Average LCVA	***284.2 ±38.7^{a}***	***47.5 ±8.0^{a}***	***33.8 ±7.8^{a}***	***0.384 ±0.087^{a}***
WR-5S	317.6 ±4.7	61.1 ±2.0	35.5 ±0.5	0.403 ±0.006
WR-6S	275.0 ±14.2	45.6 ±3.5	29.5 ±0.7	0.335 ±0.009
WR-7S	292.7 ±2.4	44.0 ±1.1	30.3 ±1.1	0.344 ±0.014
WR-8S	310.7 ±6.4	44.7 ±1.1	33.9 ±0.7	0.385 ±0.009
Average SSVA	***299.0 ±20.7^{b}***	***48.9 ±8.3^{b}***	***32.3 ±2.9^{b}***	***0.367 ±0.023^{b}***
WR-9N	342.3 ±9.7	55.0 ±1.1	32.7 ±0.9	0.371 ±0.011
WR-10N	388.1 ±11.8	83.7 ±0.5	42.5 ±1.6	0.482 ±0.020
WR-11N	293.7 ±3.1	49.4 ±0.4	41.6 ±0.8	0.472 ±0.010
WR-12N	281.2 ±8.7	49.6 ±3.4	38.8 ±0.5	0.440 ±0.006
Average NVA	***326.3 ±52.0^{c}***	***59.4 ±16.7^{c}***	***38.9 ±4.8^{b}***	***0.441 ±0.054^{b}***
Total average	**303.2 ±37.3**	**51.9 ±11.8**	**35.0 ±5.5**	**0.397 ±0.062**

Note: $^{a-c}$ 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.

Table 4 The content of total polyphenols (TPC), content of total flavonoids (TFC) and antioxidant activity (AA) in analysed wines Chardonnay.

Wine sample	TPC (mg GAE.L^{-1} ±SD)	TFC (mg CE.L^{-1} ±SD)	AA (% inhib. ±SD)	AA (mmol Trolox.L^{-1} ±SD)
CH-1L	376.7 ±2.5	57.3 ±0.8	42.1 ±1.1	0.477 ±0.014
CH-2L	314.9 ±4.7	42.3 ±0.5	34.5 ±0.9	0.391 ±0.011
CH-3L	321.2 ±5.2	67.5 ±3.3	47.3 ±0.5	0.537 ±0.006
CH-4L	324.8 ±6.2	65.2 ±3.4	44.0 ±0.3	0.499 ±0.004
Average LCVA	***334.4 ±30.0^a***	***58.1 ±12.2^a***	***42.0 ±6.2^a***	***0.476 ±0.071^a***
CH-5S	288.0 ±4.6	47.9 ±1.6	31.9 ±0.8	0.362 ±0.010
CH-6S	357.4 ±2.5	50.9 ±0.5	32.5 ±1.5	0.369 ±0.019
CH-7S	475.1 ±4.8	92.5 ±0.5	76.3 ±1.9	0.924 ±0.023
CH-8S	389.3 ±5.3	92.3 ±1.3	47.5 ±0.8	0.540 ±0.010
CH-9S	377.9 ±5.0	64.4 ±1.4	39.4 ±0.7	0.447 ±0.009
Average SSVA	***377.5 ±76.2^b***	***69.6 ±19.2^b***	***45.5 ±19.1^b***	***0.528 ±0.242^b***
CH-10N	371.2 ±4.7	52.5 ±1.2	40.3 ±0.5	0.457 ±0.006
CH-11N	435.4 ±2.5	82.3 ±1.1	48.4 ±3.2	0.550 ±0.040
CH-12N	280.2 ±4.8	35.9 ±0.5	32.8 ±1.8	0.372 ±0.022
CH-13N	353.3 ±9.6	47.4 ±1.0	45.3 ±0.8	0.514 ±0.010
CH-14N	313.2 ±3.7	43.2 ±1.9	44.1 ±0.4	0.500 ±0.005
Average NVA	***350.7 ±66.7^c***	***52.3 ±20.0^c***	***42.2 ±6.7^c***	***0.479 ±0.077^c***
Total average	**355.6 ±54.6**	**60.1 ±18.2**	**43.3 ±11.1**	**0.496 ±0.140**

Note: $^{a-c}$ 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.

value of TPC (119 – 258 mg GAE.L^{-1}) in CH wines.

According to the average value of TPC an order for wines – Chardonnay could be as following: wines from South Slovak VA >wines from Nitra VA >wines from Little Carpathian VA. Gained results also did not exert statistically significant differences (at significance level *p* = 0.05) between TPC in wines – Chardonnay made in various vineyard areas in Slovakia.

Total flavonoid content in analysed Welschriesling wine samples varied from 42.4 to 83.7 mg CE.L^{-1}. Average content of TF was 51.9 mg CE.L^{-1}. Similar results (42.5 – 89.7 mg CE.L^{-1}) were reported by **Ivanova et al. (2010)**, **Li et al. (2009)** and **Ma et al. (2014)**. According to the average value of TFC an order for wines – Welschriesling 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 (at significance level *p* = 0.05) between TFC in wines – Welschriesling made in various vineyard areas in Slovakia.

Total flavonoid content in analysed Chardonnay wine samples was in the range from 35.9 to 92.5 mg CE.L^{-1}. Average content of TF was 60.1 mg CE.L^{-1}. Our results are in agreement with the data of **Li et al. (2009)**, **Ma et al. (2014)** and **Mitic et al. (2010)** who found out TFC in range 31.0 – 94.2 mg CE.L^{-1}. **Chircu Brad et al. (2012)** determined lower values of total flavonoid content in Chardonnay wines which varied from 16.7 to 63.7 mg CE.L^{-1}. On the other hand, **Lee and Rennaker (2007)** found out much higher TFC (85.5 – 249 mg CE.L^{-1}) in Chardonnay wine samples. According to the average value of TFC an order for wines – Chardonnay could be as following: wines from South Slovak VA >wines from Little Carpathian VA >wines from Nitra VA. Gained results also did not exert statistically significant differences (at significance level *p* = 0.05) between TFC in wines – Chardonnay made in various vineyard areas in Slovakia.

Antioxidant activity in analysed Welschriesling wine samples was in range 28.2 – 44.2% inhibition of DPPH (0.321 – 0.501 mmol Trolox.L^{-1}). Average value of AA was 35.0% inhibition of DPPH (0.397 mmol Trolox.L^{-1}). Our data are in agreement with the results published by **Li et al. (2009)** and **Ma et al. (2014)** who determined values of AA in the range 0.26 – 0.602 mmol Trolox/L. Slightly lower values of AA reported **Slezák (2007)**, who found out AA in slovak wines – Welschriesling in the range from 20.11 to 41.95% inhibition of DPPH (average value was 30.9% inhibition of DPPH). On the basis of value of AA for wines – Welschriesling an order could be as following: wines from Nitra VA >wines from Little Carpathian VA >wines from South Slovak VA. Gained results exert statistically significant differences between values of antioxidant activity in wines made in South Slovak VA and AA in wines made in Nitra VA.

Antioxidant activity in analysed Chardonnay wine samples was in range 31.9 – 76.3% inhibition of DPPH (0.362 – 0.924 mmol Trolox.L^{-1}). Average value of AA was 43.3% inhibition of DPPH (0.496 mmol Trolox.L^{-1}). Similar data (49.1 – 74.7% inhibition of DPPH, resp. 0.082 – 0.87 mmol Trolox.L^{-1}) were reported by **Chircu Brad et al. (2012)**, **Li et al. (2009)**, **Ma et al. (2014)** and **Stratil et al., 2008)**. On the basis of value of AA for wines – Chardonnay an order could be as following: wines from South Slovak VA >wines from Nitra VA >wines from Little Carpathian VA. Gained results also did not exert statistically significant differences (at significance level P = 0.05) between values of AA in wines – Chardonnay made in various vineyard areas in Slovakia.

The results showed that both types of Slovak white wines were high in polyphenols and flavonoids, as well as a high antioxidant activity, comparable to the wines produced in other regions in the world. Similar conclusions were reported by **Sák et al. (2013)** and **Špakovská et al. (2012)** who analysed Slovak white and red wines.

In order to investigate the mutual relations between analyzed parameters, the linear regressions were obtained. The statistical evaluation of the obtained results confirmed very highly significant correlations at significance level p <0.001 between all 3 studied parameters: TPC and TFC (r = 0.818), AA and TPC (r = 0.699), resp. TFC and AA (r = 0.693). This was consistent with many other articles in the literature, which also reported a very high degree of correlation between the total phenols, total flavonoids and antioxidant properties of wines **(Chircu Brad et al., 2012; Li et al., 2009; Ma et al., 2014 and Minussi et al., 2003)**.

CONCLUSION

The phenolic, resp. flavonoid content and antioxidant properties of white wines (Welschriesling and Chardonnay) made in 3 most important Slovak vineyard areas was evaluated in the present study. All 3 studied parameters of analysed Slovak wines – total polyphenol content, total flavonoid content and antioxidant activity are comparable to the wines produced in other regions in the world. Slovak white wines – Welschriesling and Chardonnay have high content of healthy useful phenolic compounds and high antioxidant activity. The results didn´t showed statistically significant differences for all 3 studied parameters (except AA in Welschriesling) in wines made in different vineyard areas in Slovakia. On the basis of statistical evaluation of our results we can state that statistically very highly significant correlations were demonstrated between all 3 parameters (TPC, TFC and AA).

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