PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF SLOVAK VARIETAL WINES OF MUSCAT TYPE

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ABSTRACT

Grapes and wine are important sources of antioxidants in human diet. Phenolic substances contained in grapevine berries belong to an important group of natural substances that get into wine in the process of wine making. Polyphenols and flavonoids are primarily accountable for the colour and taste of the wine, they affect the perception of bitterness and acerbity. They also have antioxidant properties, thus have a positive effect on human health. Health benefits of polyphenolic substances from wine may be associated with a wide range of biological processes. Thanks to the development of modern analytical methods, which is constantly being researched in terms of the content of antioxidants, and its importance to human health. Sixteen Slovak white wines of Muscat type produced in different geographical regions were analysed in this study. The object of this work was to determine total polyphenol content and total flavonoid content, and to evaluate antioxidant effects of quality wines of Muscat varieties produced in Slovakia. Antioxidant activity, total polyphenol content, and total flavonoid content of particular wines is described in the study. Studied characteristics were analysed by UV-VIS spectrometry method. Muscat wines showed weak to high antioxidant activity, ranging from 25.2% inhibition of DPPH to 67.7% inhibition of DPPH. Average antioxidant activity was 38.7% inhibition of DPPH. Total polyphenol content in the Muscat type varietal wines varied from 262.1 GAE. to 568.3 GAE. Average total polyphenol content was 382.13 GAE. The content of total flavonoids in Muscat type varietal wines ranged from 24.8 mg CE. to 169.1 mg CE. Average total flavonoid content was 100.5 mg CE.

INTRODUCTION

Wine is a complex mixture obtained by complete or partial fermentation of grape must (Cheynier, 2010). It contains more than a thousand substances, some of which have not been precisely analysed yet. Most of these substances come from berries of the grape vine, others are formed during processing. Some substances are partially or completely degraded during processing (Dominé, 2005). The extraction of phenolic compounds depends on the ripening of berries, and these substances subsequently give wine its color and flavour. In white wines, with gentle processing of grapes, their content changes up to 300 mg.L−1. In red wines, content of polyphenolic compounds is up to 450 mg.L−1 (Míchlovský, 2014). Phenols are compounds of great importance for viticulture and winemaking. There is a significant difference between varieties of red and white wines in the content of phenolic substances, and their composition in grapes and wine (Pavlović, 2011). Phenolic substances are significant to both white and red wines. In white wines, higher levels of polyphenols are mostly undesirable, as they can contribute to excessive bitterness and also to the tendency of the wine to brown when it is exposed to air. In red wines, they contribute to the bitterness, astringency, and other organoleptic properties, such as color of the wine (Waterhouse, 2003). These substances affect the taste and appearance of the wine, in particular colour, bitterness, acerbity, absorption of oxygen and the aging process of the must or the wine (Steidl, 2010). The extraction of phenolic compounds in the process of winemaking has important effect on the colour and taste of the wine (Jiang, Zhang, 2012).

The phenolic composition of wine is affected by the composition of grapes, which is influenced by many aspects, such as cultivar, viticultural practices and environmental conditions, and various techniques, and several reactions occurring during the process of wine making (Sacchí, Biston, Adams, 2005). Viticultural practices, such as canopy management, yield regulation, irrigation, and harvest time, can influence the content and composition of different flavonoids, e.g. anthocyanins, proanthocyanidins and flavonols (Downey, Dokouzian, Kristic, 2006). Dumitriu (2015) proved that nanomaterials could decrease the total phenolic content in wines.

Vermerris and Nicholson (2006) define phenolic substances as compounds having one or more hydroxyl groups attached directly to the aromatic ring formed by benzene. They comprise of approximately 8000 compounds (Kabera et al., 2014). There is no consensus regarding how phenolic compounds should be classified. Most classifications of phenolics are based on their chemical structure. In this sense, we can classify them in four different ways: 1. Flavonoids ad non-flavonoids, 2. by the amount of acidic rings, 3. the carbon skeleton, and 4. by the basic chemical structure, which is most widely used (Santana-Gálvez, Jacobo-Velázquez, 2018). Phenolic compounds mainly identified in wine are hydroxycinnamic and hydroxybenzoic acids, flavonols, flavonones, flavones, flavanones, stilbenes, and anthocyanins (Monagas, 2005). From a nutritional point of view, grapes and wine are good food sources of phenolic compounds (Cueva et al., 2017). The most significant phenolics found in the human nutrition are phenolic acids, flavonoids, and tannins (Vuolo, Lima, Junior, 2019).

Lately, dietary polyphenols have drawn attention because of their ability to scavenge free radicals, chelate metal and regulate digestive enzymes, (Rasouli, Farzaei, Khodarahimi, 2017). A number of health benefits have been linked to phenolic compounds, besides antioxidant effects. Studies shown, that polyphenols also have anti-inflammatory effect, anti-bacterial and anti-thrombotic activity (Rechner, Kroner, 2005). They also have positive effects on the composition and function of the human microflora (Cueva et al., 2010). Phenolic compounds found in wine may prevent or defer the development of gastric diseases caused by inflammation and oxidative stress. Moreover, wine polyphenols acts as prebiotics (Blasi et al., 2014). They and their metabolites interact with epithelial cells, and by controlling the microbial composition of intestines contribute to the maintenance of gastrointestinal health. Nutritional polyphenols also act as substrates for intestinal microflora. (Hervert-Hernandez, 2013).
Goni, 2011). Phenolic compounds have been shown to have positive bactericidal and antioxidant properties, as well as beneficial effects to the health and protection of the organism (Ribéreau-Gayon et al., 2006). Due to the potential health benefits for human nutrition, studies have significantly increased in recent years, including the development of analytical methods for the determination and measurement of phenolic acids from food and beverages (Robbins, 2003). Analysis of phenolic compounds in grapes and wine may offer particular biomarkers that could help to better evaluate the chemical evolution of grapes during growth and maturation, as well to make progress in the wine authentication by developing and applying new or advanced control methods (Niculescu, 2018).

Polyphenols extracted from wine and analysed by 1H NMR are a good marker for variety, geographical origin, and vintage of wines (Downey, 2016).

The aim of this study was to determine and evaluate chosen properties (total polyphenol content, total flavonoid content, and antioxidant activity) and their mutual correlations in Slovak varietal wines of Muscat type, of various Slovak vineyard areas origin.

### Materials and Methods

#### Chemicals and instruments

The chemicals used for analysis were: Folin-Ciocalteau reagent, monohydrate of gallic acid p.a., anhydrous natrium carbonate p.a., aluminum chloride p.a., sodium nitrite p.a., sodium hydroxide p.a., 35%, catechin hydrate 98%, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical p.a., methanol p.a. All analysed parameters – total polyphenol content, total flavonoid content and antioxidant activity in wines were analysed using UV/VIS spectrophotometry (spectrophotometer Shimadzu UV/VIS-1240, Shimadzu, Japan).

#### Samples

Analysed bottled, Slovak varietal wines of Muscat type, namely Moravian Muscat, Muscat Ottonel and Yellow Muscat and their characteristics are described in Table 1. Wine samples of origin in various Slovak vineyard areas were purchased in retail network, to provide that analysed samples of wine would have the same characteristics as wines that are consumed by customers.

### Table 1 Characteristics of analysed wine samples

| Sample | Variety       | Producer              | Vineyard area | Vintage | Wine type |
|--------|---------------|-----------------------|---------------|---------|-----------|
| MM1    | Moravian Muscat | Chateau Topoľčianky   | SSWR          | 2015    | sweet     |
| MM2    | Moravian Muscat | Golruz                | LCWR          | 2016    | semi sweet|
| MM3    | Moravian Muscat | Vinkova               | LCWR          | 2016    | semi dry  |
| MM4    | Moravian Muscat | Vinkor                | NWR           | 2017    | dry       |
| MM5    | Moravian Muscat | Vinis Winery          | SSWR          | 2017    | dry       |
| MM6    | Moravian Muscat | Chateau Topoľčianky   | NWR           | 2016    | semi dry  |
| MM7    | Moravian Muscat | Sommelier Select      | SSWR          | 2015    | dry       |
| MO1    | Muscat Ottonel | Vino Chudy, s.r.o.    | NWR           | 2016    | dry       |
| MO2    | Muscat Ottonel | Matyšák               | SSWR          | 2014    | dry       |
| MO3    | Muscat Ottonel | Chateau Pezinok       | LCWR          | 2015    | semi sweet|
| MO4    | Muscat Ottonel | Chateau Pezinok       | LCWR          | 2017    | semi sweet|
| MO5    | Muscat Ottonel | Vino Nichita          | NWR           | 2017    | semi dry  |
| MY1    | Yellow Muscat | Tokaj&CO, s.r.o.      | WRoT          | 2016    | semi sweet|
| MY2    | Yellow Muscat | Zlatý strápec - Anna Nagyová | WRoT | 2013 | semi sweet |
| MY3    | Yellow Muscat | J&J Ostožovič        | WRoT          | 2015    | semi dry  |
| MY4    | Yellow Muscat | Terra Wylak           | SSWR          | 2017    | semi dry  |

Legend: SSWR – South Slovakian Wine Region, LCWR – Little Carpathians Wine Region, NWR – Nitra Wine Region, WRoT – Wine Region of Tokaj

### Sample analysis

#### Determination of total polyphenol content

Total polyphenol content (TPC) was evaluated by modified method of Singleton and Rossi (1965). We used 20% solution of Na₂CO₃, Folin-Ciocalteau reagent and distilled water. We pipetted 1 mL of wine sample into 50 mL volumetric flask and diluted it with 25 mL of distilled water. Then, we added 2.5 mL Folin-Ciocalteau reagent to diluted mixture, and after 3 minutes, we added 1.5 mL of 20% solution of Na₂CO₃. Then we filled the sample with distilled water to volume 50 mL, and after mixing, left at the laboratory temperature for 2 hours. We prepared the blank and calibration solutions of gallic acid by the same procedure. We measured absorbance of samples solutions against blank solution at 765 nm. The content of total polyphenols 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 evaluated by aluminum chloride method (Chang et al., 2002). We used 5% solution of NaNO₂, 10% solution of AlCl₃, solution of NaOH and distilled water. We added 1 mL of wine sample and 4 mL of deionized water to 10 mL volumetric flask. 5 min after adding 0.3 mL of 5% sodium nitrite, we added 0.6 mL of 10% aluminum chloride. Then we added 2 mL of sodium hydroxide with concentration 1 mol.L⁻¹ to the reaction mixture after 6 min incubation. The final volume was immediately made up to 10 mL with deionized water. We measured absorbance of the solution at 510 nm against blank solution. The content of total flavonoids in wine samples was calculated as amount of catechin equivalent (CE) in mg per 1 litre of wine.

#### Determination of antioxidant activity

Antioxidant activity (AA) was evaluated by method of Brand-Williams et al. (1995) using of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. 3.9 mL of DPPH solution was pipetted into cuvette. We measured absorbance of DPPH solution at 515.6 nm, and then added 0.1 mL of wine sample, stirred and waited for 10 minutes. After 10 minutes, we measured absorbance 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.

All chemical analyses were performed as four parallels.

### Statistical analysis

MS Excel 2016 and XLSTAT were used to perform statistical analysis. To obtain statistically significant information about the differences between the tested samples, nonparametric Kruskal-Wallis test was conducted (Addinsoft, 2014).

### Results and Discussion

All studied parameters –total polyphenol content, total flavonoid content and antioxidant activity of muscat type wines are described in Table 2, 3, 4.
Total polyphenol content in analysed wines variety Moravian Muscat (MM) ranged from 226.8 mg GAE.L⁻¹ to 468.6 mg GAE.L⁻¹. Average TPC in all wines variety MM was 330.2 mg GAE.L⁻¹. Mráz (2017) reported lower average TPC in analysed wines variety MM – 145.7 mg GAE.L⁻¹. Kývalová (2013) reported higher average value of TPC in analysed Czech wines variety MM – 547.5 mg GAE.L⁻¹. Snopěk (2019) studied TPC in Czech white wines (including MM) and their changes during storage. He reported that average TPC of wines variety MM was 291.7 GAE.L⁻¹. After 12 months, average TPC was 260.75 GAE.L⁻¹. These results show decrease in TPC during storage. Total polyphenol content in analysed wines variety Muscat Ottonel (MO) ranged from 311.5 mg GAE.L⁻¹ to 406.1 mg GAE.L⁻¹. Average content of TPC in all wines variety MO was 344.8 mg GAE.L⁻¹. Mítě et al. (2010) reported that average TPC in varietal wines variety MO from Serbia is 270.2 mg GAE.L⁻¹. Lachman et al. (2004) reported lower average TPC in analysed Czech wines variety MO – 267.0 mg GAE.L⁻¹. Total polyphenol content in analysed wines variety Yellow Muscat (YM) ranged from 308.7 mg GAE.L⁻¹ to 568.3 mg GAE.L⁻¹. Average TPC in all wines variety YM was 471.4 mg GAE.L⁻¹. Bajčan et al. (2018) reported lower average TPC in analysed wines variety YM – 420.5 mg GAE.L⁻¹. Rugovská (2018) reported higher average TPC in wines variety YM – 525.6 mg GAE.L⁻¹. Lugasi and Hovari (2003) reported that average TPC in wines variety YM from Hungary is 250 mg GAE.L⁻¹. This is almost half of the value we determined. Our study showed that average TPC in wines variety between wines of same variety. Bajčan et al. (2017) analysed Slovak white wines variety Welschriesling and Chardonnay, and reported that their average TPC is 303.2 mg GAE.L⁻¹ and 356.5 mg GAE.L⁻¹ respectively. Spálovská et al. (2012) analysed Slovak white wines, and reported that their average TPC range from 299 mg GAE.L⁻¹ to 407 mg GAE.L⁻¹. Starčko et al. (2008) reported that average TPC in Slovak white wines (including MM and MO) is 270 mg GAE.L⁻¹. More studies of TPC in Slovak white wines (Nedelják, 2013; Vasková, 2013; Štefančová 2014) reported average TPC in range from 305.6 mg GAE.L⁻¹ to 365.7 mg GAE.L⁻¹. According to the average value of TPC an order for wines by variety could be as following: Yellow Muscat (471.38 mg GAE.L⁻¹) > Muscat Ottonel (434.8 mg GAE.L⁻¹) > Moravian Muscat (330.2 mg GAE.L⁻¹). According to the average value of TPC an order for wines by vineyard area could be as following: wines from WRO (525.6 mg GAE.L⁻¹) > wines from SSWR (355.46 mg GAE.L⁻¹) > wines from NWR (340.5 mg GAE.L⁻¹) > wines from LCWR (301.18 mg GAE.L⁻¹). We found statistically significant difference between TPC of Muscat Ottonel and TPC of Yellow Muscat (p < 0.0001, α = 0.05), and between TPC of Moravian and TPC of Yellow Muscat (< 0.0001, α = 0.05). We found statistically significant difference between TPC of wines from WRO and LCWR, and between TPC of wines from WRO and SSSR, and WRO and NWR (< 0.0001, α = 0.05). Total flavonoid content in analysed wines variety Moravian Muscat ranged from 24.8 to 64.0 mg CE.L⁻¹. Average TPC in all wines variety MM was 42.8 mg CE.L⁻¹. Mráz (2017) reported that average TPC in analysed wines variety MM is 52.0 mg CE.L⁻¹, which is higher value compared to average value of TFC in our samples. There are not many studies analysing TFC of Moravian Muscat, mainly because it is relatively new variety, mostly grown in Czech Republic and Slovakia. Total flavonoid content in analysed wines variety Moravian Muscat Ottonel ranged from 42.2 to 77.7 mg CE.L⁻¹. Average TPC in all wines variety MO was 50.4 CE.L⁻¹. Bleiziffer et al. (2017) reported that average TFC in Serbian wines variety MO is 37.5 mg CE.L⁻¹, which is slightly lower value than ours. Total flavonoid content in analysed wines variety Yellow Muscat ranged from 43.8 mg CE.L⁻¹ to 169.1 mg CE.L⁻¹. Average TPC in all wines variety YM was 100.5 mg CE.L⁻¹. Rugovská (2018) reported that average TFC in wines variety YM is 99.1 mg CE.L⁻¹. Bajčan et al. (2018) reported lower average TPC in analysed wines variety YM – 83.0 mg CE.L⁻¹. Our study showed that average TFC in wines varieties between wines of same variety. Bajčan et al. (2017) analysed Slovak white wines variety Welschriesling and Chardonnay, and found out their average TPC is 51.9 mg CE.L⁻¹ and 60.1 mg CE.L⁻¹ respectively. More studies of TFC in Slovak white wines (Nedelják, 2013; Vasková, 2013; Štefančová 2014) reported average TFC in range from 38.8 mg CE.L⁻¹ to 67.4 mg CE.L⁻¹. According to the average value of TFC an order for wines by variety could be as following: Yellow Muscat (100.5 mg CE.L⁻¹) > Muscat Ottonel (50.4 mg CE.L⁻¹) > Moravian Muscat (42.8 mg CE.L⁻¹) According to the average value of TFC an order for wines by vineyard region could be as following: wines from WRO (119.4 mg CE.L⁻¹) > wines from SSSR (50.6 mg CE.L⁻¹) > wines from VR (45.6 mg CE.L⁻¹) > wines from LCWR (39.9 mg CE.L⁻¹). We found statistically significant difference between TFC of wines of Muscat Ottonel and TPC of Yellow Muscat (p < 0.0001, α = 0.05), and between TFC of Moravian Muscat and TFC of Yellow Muscat (p < 0.0001, α = 0.05). We found statistically significant difference between TPC of wines from LCWR and SSSR, and between TPC of wines from WRO and LCWR, and WRO and NWR (p < 0.0001, α = 0.05).
Antioxidant activity (AA) in analysed wines variety Moravian Muscat ranged from 27.9 % inhib. of DPPH (0.317 mmol Trolox·L⁻¹) to 67.0 % inhib. of DPPH (0.787 mmol Trolox·L⁻¹). Average value of AA in all wines variety MM was 38.7 % inhib. of DPPH (0.443 mmol Trolox·L⁻¹). Critová, 2016 reported lower average AA in wines variety MM – 29.95 % inhib. of DPPH.

Antioxidant activity in analysed wines variety Muscat Ottonel ranged from 33.6 % inhib. of DPPH (0.318 mmol Trolox·L⁻¹) to 40.7 % inhib. of DPPH (0.461 mmol Trolox·L⁻¹). Average value of AA in all wines variety MO was 38.6 % inhib. of DPPH (0.437 mmol Trolox·L⁻¹). Bleiziffer et al. (2017) reported higher average AA in analysed wines variety MO – 43.9 % inhib. of DPPH (0.542 mmol Trolox·L⁻¹). Antioxidant activity in analysed wines variety Yellow Muscat ranged from 43.9 % inhib. of DPPH (0.498 mmol Trolox·L⁻¹) to 67.4 % inhib. of DPPH (0.793 mmol Trolox·L⁻¹). Average value of AA in all wines variety YM was 55.1 % inhib. of DPPH (0.635 mmol Trolox·L⁻¹). Bajćan et al. (2018) reported that average AA in analysed wines variety YM is 47.2 % inhib. of DPPH (0.542 mmol Trolox·L⁻¹). Rugová (2018) reported that average AA in wines variety YM is 47.2 % inhib. of DPPH. Eftimović (2016) reported higher average AA in wines variety YM – 63.8 % inhib. of DPPH. Bajćan et al. (2017) analysed Slovak white wines variety Welschriesling and Chardonnay, and found out their average AA is 35.0 % inhib. of DPPH and 43.3 % inhib. of DPPH respectively. More studies of AA in Slovak white wines (Nedeljac, 2013; Vasková, 2013; Štefanová 2014) reported average AA in samples in range from 35.75 % inhib. of DPPH to 50.3 % inhib. of DPPH.

According to the average value of AA an order for wines could be as following: Yellow Muscat (55.1 % inhib. of DPPH) > Moravian Muscat (38.7 % inhib. of DPPH) > White Muscat (35.0 % inhib. of DPPH) > Welschriesling (34.3 % inhib. of DPPH) > Chardonnay (33.5 % inhib. of DPPH).

The study of phenolic substances in wine is subject to constant development. There are many studies regarding antioxidant activity in red wines. Until recently, the prevailing opinion was that the production of white wine does not have beneficial effects on the human health, in terms of antioxidant content. White wines have also been shown to have antioxidant activity and health benefits.

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