# Antioxidant Activity and Total Polyphenol Content of Different Varieties of Grape from the Small Carpathians Wine Region of Slovakia

**Eva Ivanišová[1*], Attila Kántor[1], Miroslava Kačániová[2]**

*[1]Slovak University of Agriculture in Nitra, Department of Technology and Quality of Plant Products, Faculty of Biotechnology and Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*
*[2]Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*

**Abstract**
The aim of the present study was to determine antioxidant profile (antioxidant activity, total polyphenol, phenolic acid, flavonoid and in red varieties also anthocyanin content) in two red grape varieties (Dornfelder, Blaufränkisch) and ten white varieties (Sauvignon Blanc, Welschriesling, Weisser Riesling, Irsai Oliver, Pinot Blanc, Palava, Müller-Thurgau, Grűner Veltliner, Feteasca Regala and Chardonnay) produced in the Small Carpathians wine region of Slovakia in the year 2018. The antioxidant activity tested by DPPH method was the highest in Dornfelder variety: 0.73±0.03 mg TEAC/g fresh matter (TEAC – Trolox equivalent antioxidant activity). The highest content of total polyphenol was detected in Dornfelder and Feteasca Regala (2.36 ±0.07 mg GAE/g and 0.86 ±0.13 mg GAE/g fresh matter; GAE – gallic acid equivalent), respectively. The highest total flavonoid content was found in Dornfelder and Blaufränkisch (1.29 ±0.09 mg QE/g and 0.34 ±0.02 mg QE/g fresh matter; QE – quercetin equivalent) and the highest total phenolic acid content in Dornfelder and Feteasca Regala samples (1.23 ±0.18 mg CAE/g and 0.41±0.05 mg CAE/g fresh matter; CAE – caffeic acid equivalent). The higher level of total anthocyanins was determined in Dornfelder (3.96±0.11 mg/g fresh matter) in comparison with Blaufränkisch. The obtained results confirmed that the grape is a good source of compounds with antioxidant activity.

**Keywords**: *Vitis vinifera* L., fresh matter, flavonoids, phenolic acids, DPPH method.

## 1. Introduction

Grapes of *Vitis vinifera* L. are one of the most cultivated fruit crops in the world. Grapes, and in particular red grape products, are rich in bioactive compounds especially polyphenols. Phenolic compounds have drawn attention not only because of their important role in the development of grape products, but also for their beneficial health effects [1]. Several studies proved that health promoting properties of grape phenolics are due to their ability to act as antioxidants by a free radical-scavenging mechanism (both *in vivo* and *in vitro*) and metal ion chelation [2]. Grapes contain high level of antioxidants which have been shown to exert beneficial effects on health. For instance, polyphenolic compound in grapes are known to lower oxidative stress, to modulate the inflammatory cascade, to reduce the oxidation of LDL cholesterol and to induce protection against atherothrombotic episodes including myocardial ischemia and inhibition of platelet aggregation [3]. Slovakia is proud with more than three-thousand-year-old tradition of viticulture and wine

*Corresponding author: Eva Ivanišová, tel.: +421376414421, E-mail: eva.ivanisova@uniag.sk

production. Small Carpathians wine region is the largest consisting of 12 vineyard zones with territory of 120 vineyard villages. The vineyards located on plains or slight slope at elevation of 145-260 m.a.s.l. [4]. Agro-ecological condition of this area provides great conditions for growing the excellent quality grapes.

The aim of this study was to determine antioxidant activity, total polyphenols, flavonoids, phenolics acid content in selected varieties of grape from the Small Carpathians wine region of Slovakia.

## 2. Materials and methods

### 2.1 Biological materials

The two kinds of red grape (Dornfelder, Blaufränkisch) and ten kinds of white grape varieties (Sauvignon Blanc, Welschriesling, Weisser Riesling, Irsai Oliver, Pinot Blanc, Palava, Müller-Thurgau, Grűner Veltliner, Feteasca Regala and Chardonnay) were purchased from private producer in the Small Carpathians wine region located in Vrbové in 2018 (188 m.a.s.l.).

### 2.2 Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

### 2.3 Sample preparation

An amount of 1 g of sample was extracted with 20 ml of 80% ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids, phenolic acids). Extraction was carried out in triplicate.

### 2.4 Radical scavenging activity – DPPH method

Radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [5]. The sample (0.4 ml) was mixed with 3.6 ml of DPPH solution (0.025 g DPPH in 100 ml methanol). Absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10-100 mg/L; $R^2$=0.989) was used as the standard and the results were expressed in mg/g Trolox equivalents.

### 2.5 Total polyphenol content

Total polyphenol content samples was measured by the method of Singleton and Rossi [6] using Folin-Ciocalteu reagent. 0.1 ml of each sample was mixed with 0.1 ml of the Folin-Ciocalteu reagent, 1 ml of 20% (w/v) sodium carbonate, and 8.8 ml of distilled water. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25-300 mg/L; $R^2$=0.998) was used as the standard and the results were expressed in mg/g gallic acid equivalents.

### 2.6 Total flavonoid content

Total flavonoids were determined using the modified method of Willett, [7]. 0.5 ml of sample was mixed with 0.1 ml of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 ml of 1 M potassium acetate and 4.3 ml of distilled water. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.5-20 mg/L; $R^2$=0.989) was used as the standard and the results were expressed in mg/g quercetin equivalents.

### 2.7 Total phenolic acid content

Total phenolic acids content was determined using method of Farmakopea Polska [8]. A 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 M hydrochloric acid, 0.5 ml Arnova reagent (10% $NaNO_2$ +10% $Na_2MoO_4$), 0.5 ml of 1 M sodium hydroxide (w/v) and 0.5 ml of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1 – 200 mg/L , $R^2$ = 0.999) was used as a standard and the results were expressed in mg/g caffeic acid equivalents

### 2.8 Total anthocyanin content

Anthocyanin content in red varieties was measured according to the method of Fuleki and Francis [9] with modifications (Lee et al., 2005). For pH 1.0, a sample (0.4 ml) was diluted with 0.025 M of potassium chloride (3.6 ml). For pH 4.5, a sample was diluted (0.4 ml) with 0.4 M of sodium acetate. The absorbance of sample was measured at 520 nm and 700 nm against the blank reagent (distilled water). The concentration (mg/g) of total anthocyanins was calculated according to the following formula and expressed as cyanidin-3-glucoside (Cy-3-glc) equivalent:

A[mg/g] = (A*Mw*1000)/( ε*L),

where A is the absorbance difference =($A_{520}$–$A_{700}$)pH 1.0 – ($A_{520}$–$A_{70}$0)pH 4.5; $M_W$ is the molecular weight of Cy-3-glc=449.2 g/mol; ε is the extinction coefficient of Cy-3-glc=1700 cm/mol; L the absorption; path length in cm=1.

### 2.9 Statistical analysis

All experiments were carried out in triplicate and the results reported are the results of those replicate determinations with standard deviations. Correlation coefficients were calculated by CORR analysis (P ≤0.05) [10].

## 3. Results and discussion

### 3.1. Radical scavenging activity – DPPH method

Results showed that all tested samples exhibited antioxidant activity (Table1). The highest activity was determined in Dornfelder variety (0.73 mg TEAC/g FM) followed by Feteasca regala variety (0.66 mg TEAC/g FM). The strong antioxidant activity of Dornfelder variety was found in study of Liang et al. [11]. These authors used for testing ORAC (oxygen radical absorbance capacity) and PSC method (peroxyl radical scavenging capacity). Their obtained results were several times higher that activity of this variety in our study – ORAC: 14.79 ±1.49 mg vitamin C equivalent/g fresh matter; PSC: 4.04 ±0.81 mg vitamin C equivalent/g fresh matter. Similarly, Gharwalová et al. [12] identified stronger antioxidant activity with DPPH method in variety Dornfelder from conventional vineyards with average value of 44.76 ±0.4% inhibition. Dornfelder belongs to black-blue table grape varieties and is very popular for winemaking around the world. Antioxidant activity is very strongly influenced by agroecological condition, grape origin, variety and the detection method.

**Table 1.** Antioxidant activity of evaluated grape varieties

| Sample | DPPH [mg TEAC/g FM] |
|---|---|
| Dornfelder | 0.73 ±0.03 |
| Blaufränkisch | 0.59 ±0.03 |
| Sauvignon Blanc | 0.26 ±0.06 |
| Welschriesling | 0.21 ±0.03 |
| Weisser Riesling | 0.21 ±0.01 |
| Irsai Oliver | 0.08 ±0.01 |
| Pinot Blanc | 0.21 ±0.02 |
| Palava | 0.32 ±0.04 |
| Müller-Thurgau | 0.17 ±0.01 |
| Grűner Veltliner | 0.28 ±0.03 |
| Feteasca Regala | 0.66 ±0.01 |
| Chardonnay | 0.29 ±0.04 |

mean ± standard deviation; TEAC – trolox equivalent antioxidant capacity; FM – fresh matter

### 3.2. Total polyphenol, flavonoid, phenolic acid and anthocyanin content

Results of total polyphenol, flavonoid and phenolic acid content are presented in Table 2. The highest total polyphenols was determined in variety Dornfelder (2.36 mg GAE/g FM) following by Feteasca Regala (0.86 mg GAE/g FM) and Blaufränkisch (0.84 mg GAE/g FM). Red grape varieties usually contain the higher amounts of polyphenols. The higher content of total polyphenols in colored grapes than green-yellow grapes is attributed to grape skin the anthocyanins [13]. Total anthocyanin content which was detected in our study in red varieties ranged from 1.59 ±0.09 mg/g (Blaufränkisch) to 3.96 ±0.11 mg/g (Dornfelder). Anthocyanins from red grapes and also wines had shown antioxidative, antiangiogenesis, anticancer, antidiabetes, improved visual health, anti-obesity, antimicrobial, and neuroprotection effect to human body [14].

The highest total flavonoids was determined in variety Dornfelder (1.29 mg QE/g FM) following by Blaufränkisch (0.34 mg QE/g FM) and Feteasca Regala (0.26 mg QE/g FM). In study of Liang et al. [13] which analyzed 24 grape varieties the total flavonoid content in colored grape varieties was ~4.63 ±0.57 mg CE/g while in white grape varieties ~2.69 ±0.66 mg CE/g (catechin equivalent). Our results had similar tendency – in red varieties was detected generally higher values of total flavonoids with compare to white varieties.

The highest total phenolic acids was determined in variety Dornfelder (1.23 mg CAE/g FM) following by Feteasca Regala (0.41 mg CAE/g FM) and Blaufränkisch (0.34 mg CAE/g FM). According to Soyollkham et al. [15] in grape as well in wine is dominant from phenolic acid gallic

acid followed by ferulic, sinapic, vanillic and caffeic acids

Statistically strong correlation ($P \leq 0.05$) was observed in our study between antioxidant activity by DPPH method and between total polyphenol content ($r^2=0.848$), total flavonoid content ($r^2=0.722$) and phenolic acid content ($r^2=0.829$).

**Table 2.** Total polyphenol, flavonoid and phenolic acid content in evaluated grape varieties

| Sample | Total polyphenols [mg GAE/g FM] | Total flavonoids [mg QE/g FM] | Total phenolic acids [mg CAE/g FM] |
|---|---|---|---|
| Dornfelder | 2.36 ±0.07 | 1.29 ±0.09 | 1.23±0.18 |
| Blaufränkisch | 0.84 ±0.12 | 0.34 ±0.11 | 0.34 ±0.02 |
| Sauvignon Blanc | 0.35 ±0.02 | 0.19 ±0.03 | 0.10 ±0.01 |
| Welschriesling | 0.17 ±0.01 | 0.14 ±0.01 | 0.07 ±0.01 |
| Weisser Riesling | 0.19 ±0.04 | 0.13 ±0.01 | 0.03 ±0.01 |
| Irsai Oliver | 0.24 ±0.02 | 0.15 ±0.02 | 0.08 ±0.01 |
| Pinot Blanc | 0.13 ±0.01 | 0.11 ±0.01 | 0.04 ±0.00 |
| Palava | 0.26 ±0.02 | 0.21 ±0.02 | 0.05 ±0.01 |
| Müller-Thurgau | 0.13 ±0.02 | 0.12 ±0.02 | 0.03 ±0.01 |
| Grűner Veltliner | 0.25 ±0.03 | 0.19 ±0.02 | 0.07 ±0.02 |
| Feteasca Regala | 0.86 ±0.13 | 0.26 ±0.05 | 0.41 ±0.05 |
| Chardonnay | 0.35 ±0.02 | 0.19 ±0.03 | 0.11 ±0.01 |

mean ± standard deviation; GAE – gallic acid equivalent; QE – quercetin equivalent; CAE – caffeic acid equivalent; FM – fresh matter

## 4. Conclusions

Results showed that grape varieties are interesting source of bioactive compounds in our diet. Antioxidant activity was the highest in variety Dornfelder and Feteasca regala. In these samples was also detected the highest amount of total polyphenols and phenolic acids. High biological activity was also observed in our study in variety Blaufränkisch. Biological activity can be strongly influenced by agroecological condition, grape origin, variety and the detection method.

## Acknowledgements

This work was supported by grant VEGA 1/0411/17.

## References

1. Burin, V.M., Facäo, L.D., Gonzaga, L.V., Fem, R., Rosier, J.P., Bordignon-Luiz, K. Colour, phenolic content and antioxidant activity of grape juice. Food Science and Technology, 2010, 30, 1027-1032.
2. De Oliviera, D.N., Sartor, S.B., Damário, N., Gollűcke, A.P.B., Catharino, R.R. Antioxidant activity of grape products and characterization of components by electrospray ionization mass spectrometry. Journal of Food Measurement and characterization, 2014, 8, 9-14.
3. Ky, I., Lorrain, B., Kolbas, N., Crozier, A., Teissedre, P.L. Wine by-products: Phenolic characterization and antioxidant activity evaluation of grapes and grape pomace from six different French grape varieties. Molecules, 2014, 19, 482-506.
4. Malík, F. Wine of Small Carpathians. Marenčín PT: SK. 2005. ISBN 8089218075. 212 pp.
5. Sánchés-Moreno, C., Larrauri, A., Saura-Calixto, F. A procedure to measure the antioxidant afficiency of polyphenols. Journal of Science and Food Agriculture, 1998, 76, 270-276.
6. Singleton, V.L., Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic. phosphotungstic acid reagents. American Journal of Enology and Agricultural, 1965, 144-158
7. Willet, W.C. Balancing life-style and genomic research for disease prevention. Science, 2002, 292, 695-698.
8. Farmakopea Polska, 1999. The Polish Farmaceutical Society [online] s.a. [cit.2017-01-25] Available:http://www.ptfarm.pl/?pid=1&language=en.
9. Fuleki, T., Francis, F.J. Quantitative methods for anthocyanins. Determination of total anthocyanin and degradation index for cranberry juice. Journal of Food Science, 1968, 33, 78–83.
10. SAS 2009. Users Guide Version 9. 2. SAS/STAT (r) SAS Institute Inc. Cary, NC, USA.
11. Liang, Z., Cheng, L., Zhong, G.Y., Liu, R.H. Antioxidant and antiproliferative activities of twenty-four *Viitis vinifera* grapes. Plos One, 2014, 9, 1-10.
12. Gharmalová, L., Hutár, D., Masak, J., Kolouchová, I. Antioxidant activity and phenolic

content of organic and conventional wine cane extracts. Czech Journal of Food Science, 2018, 36, 289-245.
13. Liang, Z., Owens, CH., Zhong, G.Y., Cheng, L. Phenolic profiles in the ripe berries of *Vitis vinifera* germplasm. Food Chemisty, 2011, 129, 940-950.
14. Khoo, H.E., Azlan, A., Tang, S.T., Lim, S.M. Anthocyanidins and anthocyanins: colored pigment as food, pharmaceutical ingredients, and the potential 16.
health benefits. Food and Nutrition Research, 2017, 61, 1-21.
15. Soyollkham, B., Valášek, P., Fišera, M., Fic, V., Kubáň, V., Hoza, I. Total phenolic compounds content (TPC), total antioxidant activities (TAA) and HPLC determination of individual polyphenolic compounds in selected Moravian and Austrian wines. Central European Journal of Chemistry, 2011, 9, 677-687.