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Total polyphenolic compounds contents (TPC), total antioxidant activities (TAA) and HPLC determination of individual polyphenolic compounds in selected Moravian and Austrian wines

Research Article

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Abstract: Wine samples (*Grüner Veltliner (GV)* and *Zweigelt (ZW)* from four different geographical regions of Austria and Czech Republic) were analyzed to determine their total phenolic content (TPC) by applying the Folin-Ciocalteau method, total antioxidant activity (TAA) by FRAP (ferric reducing antioxidant power) and DPPH (1,1-diphenyl-2-picryl-hydrazyl) assays, and to identify and quantify eleven phenolic compounds using a HPLC/UV-VIS method.

Keywords: *Phenolic compounds • Wine • Total antioxidant activity • Total phenolic content • HPLC/UV-VIS*

1. Introduction

In recent years, many papers have paid attention to the bioactive compounds, particularly to the antioxidant activity of polyphenolic compounds in food and beverages, due to their positive effect on the human body. As consumers have become more conscious of the health benefits of phenolic compounds and their antioxidant activities via the conventional media, the beverage industries have recognized new marketing opportunities for their products. Therefore, the phenolic compounds and their antioxidant capacity in foods and beverages become an important quality parameter, especially in niche markets concerned with health benefits.

Wine is a widely consumed beverage in the world, with thousands of years of tradition. It is an excellent source of various classes of polyphenols. The phenolic compounds are responsible for the sensory characteristics, particularly color, astringency, bitterness and aroma [1,2]. The phenolic compounds in red wine exhibit a board spectrum of beneficial pharmacological properties, believed to be related to their antioxidative properties. Anti-atherogenic, anti-tumour, anti-ulcer, and anti-inflammatory activities have all been demonstrated by the consumption of red wine and red wine phenolic compounds [3-8]. As one of the winemaking procedures, the phenolic compounds of the wine grape are one of the most important aspects that determine wine quality. A large number of published papers have focused on the essential contributions of phenolic compounds profiles to wine quality and sensory properties [1,2].

The phenolic profiles in wine depend on the phenolic compounds present in the grapes, the extraction parameters, winemaking technologies as well as fermentation temperature, yeast strain, processing enzymes, cap management, and alcohol concentration [9-11]. On the other hand, the phenolic compounds of grapes are affected by many factors such as agro

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technical processes, genetic variation, maturity, climatic and geographical conditions [12-14]. Other factors that influence the extent of phenolic extraction are the molecular weight, size and type of phenolic molecules, the surface area, the concentration gradient, other temperature treatments including grape and must freezing and thermo-vinification, and factors that affect cell permeability, such as pectolytic enzyme selection [15]. Also, the environmental condition (temperature, annual precipitation levels, altitude and geochemical characteristics) can affect the grapes maturation and consequently the concentration of their phenolic compounds.

Many papers dealing with phenolic compounds of wine and grapes and their total antioxidant capacity have been published. However, little attention has been paid to comparing the phenolic compounds of wine grapes from different origins in Moravian wine, as well as comparing the phenolic contents and antioxidant activities of phenolic compounds. Flavonoids, phenolic acids, flavonols and *trans*-resveratrol and other groups of compounds could be key agents of the antioxidant action on the human metabolism pathway, the reason why we are qualifying the wines from a nutritional point of view.

This study determines the total content of phenolics, identifies and quantifies individual phenolic compounds and determines the total antioxidant activity in wine samples collected from four different geographical regions of Austria and Czech Republic (two wineries in Austria – Poysdorf and Grossriedenthal; and two wineries in the Czech Republic - Velké Bílovice and Velké Hostěrádky - Bošovice). The study assesses the influence of different geographical conditions on the phenolic composition and evaluates the relationship between antioxidant potential and the phenolic content of Moravian and Austrian wine.

2. Experimental Procedure

2.1. Instrumentation

A single beam flash scan diode array spectrophotometer covering 330-800 nm Biochrom Libra S6 (Biochrom Ltd, Cambridge, UK) was used for spectrophotometric assays. The UltiMate® 3000 HPLC system consisted of UltiMate 3000 RS pump, UltiMate 3000 RS autosampler, UltiMate 3000 RS column compartment and UltiMate 3000 RS diode array detector (Dionex Co., Sunnyvale, CA, USA). Chromatographic separation was carried out on Supelcosil LC-18-DB column (250×4.6 mm, 5 µm, Supelco, USA) at 30°C by gradient elution with a mobile phase containing solvent A (5% v/v aqueous acetonitrile (ACN) acidified with 0.35 mL trifluoroacetic anhydride (TFAA) and solvent B (50% v/v aqueous acetonitrile acidified with 0.25 mL TFAA). Run time was 30 min and the flow rate was 1 mL min^{-1}.

2.2. Chemicals

Folin-Ciocalteau reagent, gallic acid, 2,4,6-tris-(-2-pyridyl)-s-triazine (TPTZ) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were obtained from Sigma-Aldrich (Steinheim, Germany). A standard solution of DPPH c = 0.20 mol L^{-1} was prepared in methanol. A working DPPH solution was prepared at c = 100 µmol L^{-1} containing an acetate buffer of pH 4.3 with a ratio of 1:2 (DPPH:buffer). Tannin was obtained from Merck KGaA (Darmstadt, Germany). Phenolic reference standards including gallic acid, catechin, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, rutin, cinnamic acid, quercetin and resveratrol were purchased from Aldrich (Zwijndrecht, Belgium). Lab-Scan acetonitrile (ACN) was obtained from POCH A.S. (Gliwice, Poland). Other chemicals and reagents of reagent grade purity were purchased from Penta, Chrudim and/or Lachema, Brno, (both Czech Republic). All solutions were prepared with deionised (DI) water (Aquaosmotic, Tišnov, Czech Republic).

2.3. Sampling

A total of 32 wine samples including 16 white and 16 red wines (all wines from the 2009 vintage) were collected before bottling in Vinopol Ltd. (Velké Bílovice, Czech Republic). Wines were made from *Grüner Veltliner* (GV) and *Zweigelt* (ZW) wine grapes grown in four different geographical regions of Austria and Czech Republic; two wineries in Austria (Poysdorf and Grossriedenthal) and two wineries in the Czech Republic (Velké Bílovice and Velké Hostěrádky - Bošovice). Four localities in mutual distances (more than 40 km) were selected for sampling to minimize the risks posed by weather conditions. The Austrian vineyards of the wineries were located in two districts. Poysdorf is located in Austria's largest wine-growing area called "Weinviertel" (16.650 ha). Grossriedenthal is embedded in the wine-growing area called "Wagram" (2.800 ha). In the Czech Republic, the vineyards are located in the Moravian sub-district called "Velkopavlovická" (5.200 ha). Grapes were harvested in four repetition units (see Fig. 1) from each vineyard; wine samples were made by each repetition grape samples. Table 1 shows a list of analyzed wine samples.

The climatic conditions of the two wine-growing areas in Austria show nonsignificant differences. Poysdorf (225 m above sea level) and Grossriedenthal (277 m above sea level) are affected by the Pannonian climate that is characterized by hot dry summers and

Table 1. List of analysed wine samples

Sample Code*	Type of wine	Vineyards (district)	Wineries
SGV	White	Velkopavlovická	Velké Bílovice
SZW	Red		
PGV	White	Velkopavlovická	Velké Hostěrádky - Bošovice
PZW	Red		
OGV	White	Weinviertel	Poysdorf
OZW	Red		
BGV	White	Wagram	Grossriedenthal
BZW	Red		

** GV – Gruener Veltliner, ZW – Zweigelt, variations 1 – 4 acc. Fig. 1 were collected*

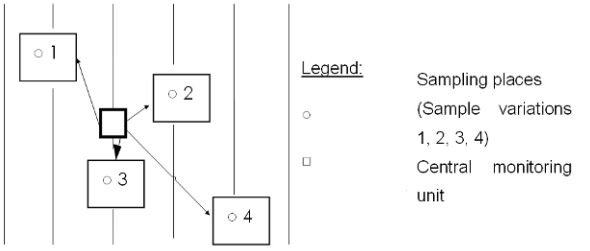

Figure 1. Plan of the experimental fields for sampling from individual experimental vineyards in Czech Republic (S, P) and Austria (O, B).

cold winters. The annual average temperature is 9.1°C and the annual precipitation is 480 mm. The geology of the region shows that Poysdorf (embedded in the Vienna basin) and Grossriedenthal (embedded in the Molasse basin) is characterized by loess and loess-clay. The pedology of the vineyards differs between the two wine-growing areas; in Poysdorf the soil type is a virgin soil and in Grossriedenthal it is a brown soil. In the Czech Republic, the vineyards in Velké Bílovice (185 m above sea level) and Velké Hostěrádky - Bošovice (215 m above sea level) are affected by the continental climate with an occasional influence of Atlantic climate. The annual average temperature is 9.4°C and the annual precipitation is 510 mm. The geology of the wine-growing area is characterized by calcareous clay, marl and sandstone. Virgin soil characterizes the soil type of the vineyards .

2.4. Sampling strategy and wine making procedures

Sample procedures were performed in accordance with the following criteria in different geographical regions of the experimental fields: i) 400 grapevines per experimental field, ii) 4 repetition units, iii) crop age 6 – 25 years, iv) distance between rows 2 – 2.5 m, v) application of full mechanization and triennial depth-loosener, vi) break out of grapevines 8 – 10 t per vintage.

According to a standard winemaking procedure, there is no wood contact in the experimental cellar of Vinopol. After crushing 100 kg of grapes, diammonium phosphate, SO_2 and *Saccharomyces cerevisiae* strain were added to each wine . The grape skin was separated from the juice using standard pressing procedure. Pressed juice was added to the 50-L glass bottles and fermented by standard procedure at 8°C, and the cap was punched down two times per day. Wines were cold-stabilized for several weeks at 8°C and filtered using ceramic filters.

2.5. Methods

2.5.1. Folin-Ciocalteau method

The TPC was determined according to the Folin-Ciocalteau method [16]. Briefly, a 0.025 mL sample was mixed with 1 mL of the 10-fold diluted Folin-Ciocalteau reagent and allowed to stand for 3 min. Then 5 mL of 200 g L^{-1} sodium carbonate (Na_2CO_3) was added, and a final volume was made up to 50 mL with DI water. Each sample was measured spectrophotometrically at 765 nm after 30 min of standing against the blank. Five-point calibration was strictly linear (R^2>0.9999) in the concentration range 0 - 250 mg L^{-1} with tannin as the standard. The regression equation was A = 0.0018 c + 0.0028. The determined values were expressed as tannin equivalents (TE, mg L^{-1}). All samples were analyzed as triplicates. Standards and samples gleaned highly repeatable results.

2.5.2. DPPH radical scavenging activity [17,18]

A mixture of an undiluted sample (0.1 mL) with a 10 mL working DPPH solution was measured immediately at 515 nm against a methanol blank ($A_{C(0)}$). The mixture was then incubated at room temperature and in the dark for 30 minutes and was again measured spectrophotometrically at 515 nm ($A_{A(t)}$). The gallic acid (GA) calibration curve was plotted as a function of the percentage of the DPPH radical scavenging activity. The measurement was compared to the standard calibration curve, and the free radical scavenging activities were expressed as micromoles of gallic acid equivalents (GAE) per milliliter of sample (μmol mL^{-1}). The calibration curve was strictly linear (A = 855.59, c = 16.015, R^2 = 0.9980, where A is absorbance value, c is the concentration of gallic acid in standard solutions) in the concentration interval 0.02 – 0.08 μmol mL^{-1} gallic acid. The μmol mL^{-1} inhibition of the DPPH radical caused by a wine sample were determined according to the following formula: $(A_{C(0)} - A_{A(t)})/A_{C(0)} \times 100$, where $A_{C(0)}$ is the absorbance of the sample at t = 0 min and $A_{A(t)}$ is the absorbance of sample at t = 30 min). All samples were analyzed as triplicates.

2.5.3. Ferric ion reducing antioxidant power (FRAP)

The reducing activity of the samples was determined by the FRAP method [19]. A 0.1 μmol L^{-1} standard solution of gallic acid (GA) was prepared in H_2O. The oxidant in the FRAP assay was prepared by mixing 5 mL of 10 mmol L^{-1} 2,4,6-tripyridyl-s-triazine (TPTZ) in water, 50 mL of acetate buffer pH 3.6, and 5 mL of $FeCl_3 \cdot H_2O$ (20 mmol L^{-1}). A sample (25 μL) was added to the 4 mL reagent. Absorbance was measured spectrophotometrically at 593 nm (A_0min). Then the sample solution was allowed to stand at room temperature and in the dark for 10 min and measured again at 593 nm (A_{10}min). The difference of absorbances ($\Delta A = A_{10}\text{min} - A_0\text{min}$) of the reaction mixture was calculated and related to ΔA of a Fe(II) standard solution. The difference in absorbance ΔA was linearly proportional to the concentration of the antioxidant and indicated increased reducing power. The measurement was compared to a calibration curve of the prepared gallic acid solution, and then final results were expressed as micromoles of gallic acid equivalents (GAE) per milliliter of the sample (μmol mL^{-1}). The calibration curve was strictly linear ($A = 1.0800\ c + 0.0072$, $R^2 = 0.9999$, where A is the absorbance value, c is the concentration of gallic acid in standard solutions) in the concentration interval 0.02 – 0.1 μmol mL^{-1} gallic acid. All samples were analyzed as triplicates.

2.5.4. HPLC analysis of phenolic composition

The individual phenolic compounds were quantified using a HPLC method [20] using gradient elution with the mobile phase containing solvent A (5% v/v aqueous ACN acidified with 0.35 mL trifluoroacetic anhydride (TFAA) and solvent B (50% v/v aqueous ACN acidified with 0.25 mL TFAA). The UV detector was set at 205, 210, 275 and 375 nm. The wine sample was filtered using 0.45 μm pore size Nylon membrane filter 13 mm (FFNN1345-100, Gronus, SMI labHut Ltd., Maisemore, Gloucestershire, UK) using filter devices (Millipore, Bedford, MA, USA) before injecting. The injection volume was 20 μL. Individual phenolic compounds were identified by comparing retention times and UV spectra of the corresponding standard compounds. Data were quantified using the corresponding calibration curves of the individual standard compound.

3. Results and Discussion

In this study, a total of 32 wine samples including 16 white and 16 red wines, which were made from the *Grüner Veltliner* and *Zweigelt* grape varieties, were selected to determine the total phenolic contents (TPC) and the total antioxidant activity (TAA). *Grüner Veltliner* is a variety of white wine grape grown primarily in Austria and in the Czech Republic. *Zweigelt* is a red wine grape variety that is the most widely grown in Austria today.

3.1. Total phenolic contents

The different variations of red and white wine samples were tested for TPC in four sets of analyses. The TPC varied from 218 to 328 mg L^{-1}, averaging 263 mg L^{-1}, for the four white wine samples SGV and from 1182 to 1232 mg L^{-1}, averaging 1216 mg L^{-1}, for the four red wine samples SZW from Velké Bílovice. The total phenolic contents ranged from 268 to 283 mg L^{-1}, averaging 274 mg L^{-1} for PGV samples and from 564 to 729 mg L^{-1}, averaging 651 mg L^{-1} for red wine samples PZW from Velké Hostěrádky - Bošovice. Samples PGV-3 and PZW-3 showed a high content of total phenolics; the same as SGV-3 and SZW-3. It can be assumed that the high reading of the phenolic contents of the grape samples depends on the location of the vineyard where the grapes grew, their shelter from the wind, intensity of sunlight radiation as well as shaded or non shaded clusters and other factors.

In the next set of analyses, wine samples from Austrian regions were tested (four red wines and four white wines). The TPC varied from 260 to 304 mg L^{-1}, averaging 275 mg L^{-1}, for the white wine samples OGV and from 824 to 878 mg L^{-1}, averaging 846 mg L^{-1}, for the red wine samples OZW collected in Poysdorf. The corresponding values from 117 to 210 mg L^{-1}, averaging 179 mg L^{-1}, in white wine BGV, and from 1068 to 1184 mg L^{-1}, averaging 1122 mg L^{-1} TE, in red wine BZW from Grossriedenthal were obtained. In this set, the highest TPC for the red wines was found in OZW-4 and for the white wines was found in OGV-3 samples.

Figs. 2 and 3 show (see also Table 2) the TPC in white and red wine samples. The mean values of the total phenolic contents in white wine samples were determined in the interval from 263 to 275 mg L^{-1} TE, except for the BGV (179 mg L^{-1} TE). Our values for white wines were about 50% higher compared to the results published by Simonetti *et al.* [21] and approximated thevalues published by Komes *et al.* [22], Sáncher-Moreno *et al.* [23], Heinonen *et al.* [24], Jewell [25] and Stevanato *et al.* [26]. The TPC for eight white wines from South Moravia were determined by Stratil *et al.* [27] and values of TPC were approximately 40-60 % lower than our results. Fig. 2 shows the antioxidant activity for four different variations grown in four different vineyards, a total of 16 white wine samples. As shown in this figure, the highest TPC were found for wine samples O (275 mg L^{-1}) grown in Weinviertel, Austria and P (274 mg L^{-1}) grown in Velké Hostěrádky - Bošice, Czech Republic.

The average values of TPC in red wine samples ranged from 651 (PZW) to 1216 (SZW) mg L^{-1} TE. These results were in the range of the previously summarized [27-29] data (824-4059 mg L^{-1}) by Crozier *et al.* [28], except for the PZW samples (564-729 mg L^{-1}). Moreover, TPC in red wine were approximately 40-75% lower compared to the results published by selected authors [21-28,30,31].

The TPC for 16 red wine samples were compared with each other. The results are shown in Fig. 3. The highest TPC were determined for red wine samples S (mean value for four variations, 1216 mg L^{-1}) grown in Velkopavlovická, the Czech Republic and for red wine samples B (mean value for four variations, 1122 mg L^{-1}) grown in Wagram, Austria. However, the red wine samples showed a low content of total phenolics for wine samples O which were grown in Weinviertel, Austria.

Generally, the total phenolic contents of white wine samples were observed at relatively high levels (117-328 mg L^{-1}), but they were still at least 5-times lower than the red wines (564-1216 mg L^{-1}). In the white wine samples, the lowest TCP (117 mg L^{-1}) was determined in wine samples B grown in Wagram, but in contradiction, for red wine samples the high content was found in wine samples B grown in the same region. These results indicated that the geographical origin, average annual temperature, annual levels of precipitation and pedology strongly influenced the wine quality and quantity.

3.2. Antioxidant activity of wines

The total antioxidant activity values of the wine samples, using the DPPH and FRAP methods, are given in Figs. 4 and 5. Wine samples showed high TAA, mainly those with high levels of total phenolic contents. According to the FRAP values determined in white wines, the TAA can be qualified in decreasing order (GAE mmol L^{-1}): OGV-3 (0.50) ~ OGV-4 (0.50) > PGV-3 (0.43) ~ SGV 3 (0.43). These results indicated that OGV and SGV have a higher antioxidant activity than PGV and BGV white wines. The highest antioxidant activity was found in BGV-2 and BGV-1 red wines (3.8-3.5 mmol L^{-1}, *i.e.*, 7 to 11-times higher than that in white wines). The lowest value, less than half of the highest value, was found in PZW-4 red wine (1.4 mmol L^{-1}). The average antioxidant activity values in each vineyard determined by the FRAP method were 0.43 (SGV), 0.4 (PGV), 0.49 (OGV) and 0.38 (BGV) in white wines, moreover, 2.9 (SGV), 1.53 (PGV), 1.73 (OGV) and 3.46 (BGV) mmol L^{-1} in red wines, respectively.

Furthermore, according to the DPPH average values determined in white wines, the total antioxidant activity can be qualified in this decreasing order (GAE mmol L^{-1}): PGV (0.35) > OGV (0.32) > BGV-3 (0.3) > SGV (0.29). These results were in disagreement with the values of the highest antioxidant activity in white wines which were determined by FRAP method. However, results for the highest antioxidant activity in red wine was found in BGV-2 red wine (2.01 mmol L^{-1}, *i.e.*, 5- to 8-times higher than that in white wines). Also,, the lowest value, less than half of the highest value, was found in the PZW and OZW red wine samples (0.67 and 0.68 mmol L^{-1}). These results were in agreement with the values determined by the DPPH method. The mean values of the antioxidant activities in each vineyard determined by the DPPH method were 0.43 (SGV), 0.4 (PGV), 0.49 (OGV) and 0.38 (BGV) mmol L^{-1} in white wines, moreover, 2.9 (SGV), 1.53 (PGV), 1.73 (OGV) and 3.46 (BGV) mmol L^{-1} in red wines, respectively.

It was difficult to compare our values of the TAA with the literature data. The majority of authors have used various methods such as inhibition of lipid oxidation, the ORAC method, the ABTS method, the DPPH method with the evaluation of EC_{50} (the sample concentration necessary to reduce the remaining DPPH by 50%). On the other hand, it is possible to partially evaluate, some of the values determined by the FRAP, DPPH methods using the Trolox standard, *e.g.* Stratil *et al.* [27]. For the DPPH method, values were determined

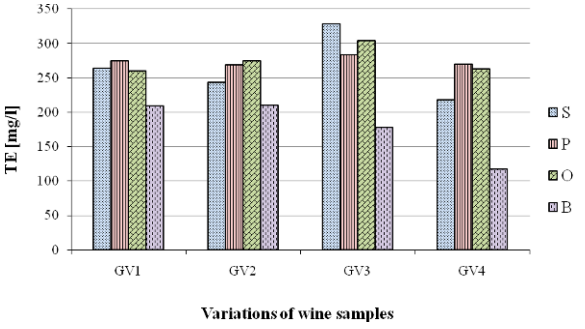

Figure 2. Total phenolic contents in 16 white wine samples from individual experimental vineyards in Czech Republic (S, P) and Austria (O, B). SDs values see Table 2.

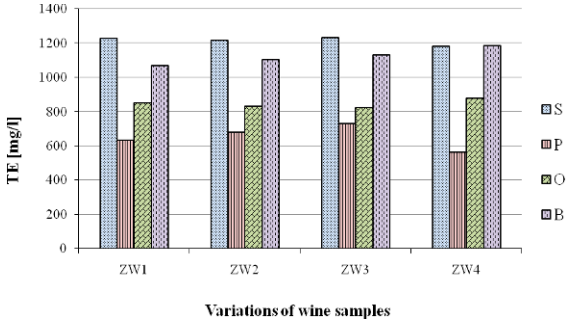

Figure 3. Total phenolic contents in 16 red wines from individual experimental vineyards in Czech Republic (S, P) and Austria (O, B). SDs values see Table 2.

Table 2. Content of phenolic compounds in wine samples determined by HPLC in Gruener Veltliner (GV) and Zweigeltrebe (ZW) fro four vineyards (P, S, O B) in Czech Republic (P, S) and Austria (O, B) collected from 4 different sampling locations in each vineyard measured in triplicates.

Phenolic compounds	Sample No	White wines					Red wines		
		SGV	PGV	OGV	BGV	SZW	PZW	OZW	BZW
Gallic acid	1	5.70 ± 0.00	5.79 ± 0.01	5.53 ± 0.01	5.71 ± 0.02	14.5 ± 0.03	8.93 ± 0.01	14.2 ± 0.02	16.8 ± 0.08
	2	5.66 ± 0.00	5.67 ± 0.31	6.08 ± 0.01	6.01 ± 0.02	14.7 ± 0.01	9.46 ± 0.02	12.9 ± 0.02	17.4 ± 0.04
	3	5.71 ± 0.00	5.80 ± 0.05	5.83 ± 0.01	5.44 ± 0.02	14.2 ± 0.05	9.65 ± 0.08	14.4 ± 0.02	16.8 ± 0.04
	4	5.04 ± 0.31	5.71 ± 0.01	6.19 ±0.01	5.36 ± 1.00	14.2 ± 0.05	9.23 ± 0.01	14.1 ± 0.02	16.9 ± 0.02
	Average	5.52 ± 0.32	5.74 ± 0.06	5.90 ± 0.29	5.63 ± 0.29	14.4 ± 0.23	9.31 ± 0.31	13.9 ± 0.68	16.9 ± 0.30
Catechin	1	5.01 ± 0.03	9.90 ± 0.0	4.63 ± 0.01	6.46 ± 0.07	23.5 ± 14.85	23.5 ± 0.03	25.6 ± 0.04	20.2 ± 0.28
	2	8.08 ± 0.02	10.57 ± 0.01	4.21 ± 0.01	8.38 ± 0.05	38.7 ± 0.01	13.8 ± 0.02	25.0 ± 0.01	22.8 ± 0.05
	3	10.11 ± 0.07	11.44 ± 0.76	6.21 ± 2.23	7.00 ± 0.03	29.7 ± 0.46	24.9 ± 0.47	23.9 ± 0.04	25.1±0.82
	4	6.77 ± 0.02	10.69 ± 0.09	6.79 ± 0.03	6.63 ± 0.04	29.7 ± 0.46	24.1 ± 0.02	24.8 ± 0.03	24.7 ± 0.04
	Average	7.49 ± 2.15	10.65 ± 0.63	5.46 ± 1.23	7.11 ± 0.87	30.4 ± 6.24	21.5 ± 5.21	24.8 ± 0.70	23.2 ± 2.24
Vanillic acid	1	1.02 ± 0.02	-	-	1.02 ± 0.02	3.11 ± 0.02	-	1.68 ± 0.01	1.14 ± 0.01
	2	0.93 ± 0.01	-	0.99 ± 0.03	0.75 ± 0.0	2.71 ± 0.03	1.25 ± 0.02	1.62 ± 0. 0	1.11 ± 0.0
	3	0.72 ± 0.00	-	1.19 ± 0.01	-	2.36 ± 0.00	1.28 ± 0.04	1.68 ± 0.02	1.45±0.02
	4	0.81 ± 0.04	-	-	0.88 ± 0.0	2.36 ± 0.00	1.37 ± 0.01	1.64 ± 0.02	1.09 ± 0.01
	Average	0.87 ± 0.13	-	1.09 ± 0.14	0.88 ± 0.13	2.63 ± 0.35	1.3 ± 0.06	1.65 ± 0.03	1.19 ± 0.16
Caffeic acid*	1	ND	0.14 ± 0.01	0.13 ± 0.01	ND	2.81 ± 0.21	1.40 ± 0.01	2.35 ± 0.01	0.56 ± 0.0
	2	ND	-	0.19 ± 0.02	0.60 ± 0.02	2.64 ± 0.01	0.75 ± 0.01	1.53 ± 0.01	0.49 ± 0.0
	3	-	2.10 ± 0.39	3.58 ± 0.0	0.61 ± 0.01	2.34 ± 0.17	1.68 ± 0.01	2.28 ± 0.01	0.52±0.004
	4	0.05 ± 0.00	-	0.23 ± 0.01	0.34 ± 0.01	2.34 ± 0.17	10.4 ± 0.33	2.13 ± 0.01	4.08 ± 0.01
	Average	-	-	1.03 ± 1.69	0.51 ± 0.15	2.53 ± 0.23	3.6 ± 4.59	2.07 ± 0.37	1.41 ± 1.77
Ferulic acid	1	2.26 ± 0.00	-	2.31 ± 0.13	ND	4.05 ± 0.00	2.40 ± 0.01	2.92 ± 0.02	ND
	2	2.31 ± 0.00	-	-	ND	4.41 ± 0.00	2.40 ± 0.01	2.91 ± 0.01	ND
	3	-	-	-	ND	4.04 ± 0.08	2.42 ± 0.01	2.92 ± 0.15	ND
	4	2.27 ± 0.00	-	-	ND	4.04 ± 0.08	2.42 ± 0.01	2.86 ± 0.01	ND
	Average	2.28 ± 0.02	-	.	-	4.13 ± 0.18	2.41 ± 0.01	2.90 ± 0.02	ND
Sinapic acid	1	2.56 ± 0.01	2.71 ± 0.01	2.58 ± 0.02	2.71 ± 0.08	7.09 ± 0.06	3.65 ± 0.01	5.23 ± 0.05	4.25 ± 0.05
	2	2.48 ± 0.00	2.52 ± 0.01	2.50 ± 0.02	2.62 ± 0.01	9.23 ± 0.04	5.22 ± 0.01	5.28 ± 0.03	5.58 ± 0.01
	3	2.47 ± 0.02	2.59 ± 0.01	2.49 ± 0.14	2.74 ± 0.01	3.94 ± 0.02	3.03 ± 0.00	4.92 ± 0.03	4.18±0.10
	4	2.50 ± 0.00		2.56 ± 0.04	2.63 ± 0.01	7.40 ± 1.20	2.76 ± 0.01	5.36 ± 0.01	4.19 ± 0.05
	Average	2.50 ± 0.04	2.61 ± 0.01	2.53 ± 0.04	2.67 ± 0.05	6.91 ± 0.19	3.66 ± 1.10	5.19 ± 0.19	4.55 ± 0.68
Rutin	1	3.43 ± 0.01	3.29 ± 0.01	3.56 ± 0.01	3.37 ± 0.00	3.95 ± 0.01	10.4 ± 0.08	6.93 ± 0.01	5.34 ± 0.01
	2	3.44 ± 0.01	3.32 ± 0.01	3.46 ± 0.02	3.45 ± 0.01	5.12 ± 0.00	9.49 ± 0.09	3.83 ± 0.01	5.82 ± 0.01
	3	3.64 ± 0.01	3.32 ± 0.01	3.41 ± 0.17	3.41 ± 0.04	3.94 ± 0.02	7.19 ± 0.09	3.56 ± 0.01	6.11±0.02
	4	3.40 ± 0.00	3.31 ± 0.01	3.50 ± 0.01	3.49 ± 0.01	3.95 ± 0.02	8.84 ± 0.04	4.51 ± 1.61	7.25 ± 0.05
	Average	3.47 ± 0.01	3.31 ± 0.01	3.48 ± 0.06	3.43 ± 0.05	4.24 ± 0.58	8.97 ± 1.35	4.51 ± 1.61	6.13 ± 0.81
Resveratrol	1	-	ND	0.93 ± 0.02	0.86 ± 0.01	1.19 ± 0.00	0.84 ± 0.01	1.00 ± 0.01	1.42 ± 0.01
	2	-	ND	ND	0.56 ± 0.48	0.86 ± 0.00	0.85 ± 0.00	0.96 ± 0.02	1.38 ± 0.03
	3	0.96 ± 0.01	-	0.93 ± 0.01	-	0.97 ± 0.14	-	1.05 ± 0.002	1.29±0.01
	4	0.84 ± 0.01	-	0.88 ± 0.03	-	0.97 ± 0.14	-	0.98 ± 0.01	1.32 ± 0.0
	Average	0.90 ± 0.08	-	0.91 ± 0.02	0.71 ± 0.21	0.99 ± 0.13	0.84 ± 0	0.99 ± 0.03	1.35 ± 0.05
Cinnamic acid	1	-	ND	ND	4.90 ± 0.17		ND	ND	
	2	-	ND	ND	4.57 ± 0.09		ND	ND	
	3	-	-	ND	4.66 ± 0.02		ND	ND	
	4	-	ND	ND	4.18 ± 0.57		-	ND	
	Average	-	-	-	4.57 ± 0.29	-	ND	ND	
Quercetin**	1	5.47 ± 0.04	5.43 ± 0.51	5.14 ± 0.32	5.71 ± 0.02	3.63 ± 0.01	3.58 ± 0.00	3.57 ± 0.03	3.65 ± 0.01
	2	5.20 ± 0.14	5.13 ± 0.28	5.01 ± 0.18	6.01 ± 0.02	3.59 ± 0.00	3.57 ± 0.02	3.62 ± 0.02	3.67 ± 0.01
	3	5.02 ± 0.16	9.39 ± 0.61	4.92 ± 0.17	5.44 ± 0.02	2.14 ± 0.00	2.04 ± 0.00	3.56 ± 0.001	3.63±0.003
	4	5.05 ± 0.01	4.63 ± 0.01	4.69 ± 0.09	5.36 ± 1.00	2.14 ± 0.01	3.57 ± 0.00	3.59 ± 0.004	3.69 ± 0.0
	Average	5.18 ± 0.21	6.14 ± 2.18	4.94 ± 0.18	5.63 ± 0.29	2.87 ± 0.84	3.19 ± 0.76	3.58 ± 0.02	3.66 ± 0.02

**caffeic acid – detected at 275 nm, **quercetin - detected at 375 nm, p-coumaric acid was not detected in any sample, ND – not detected*

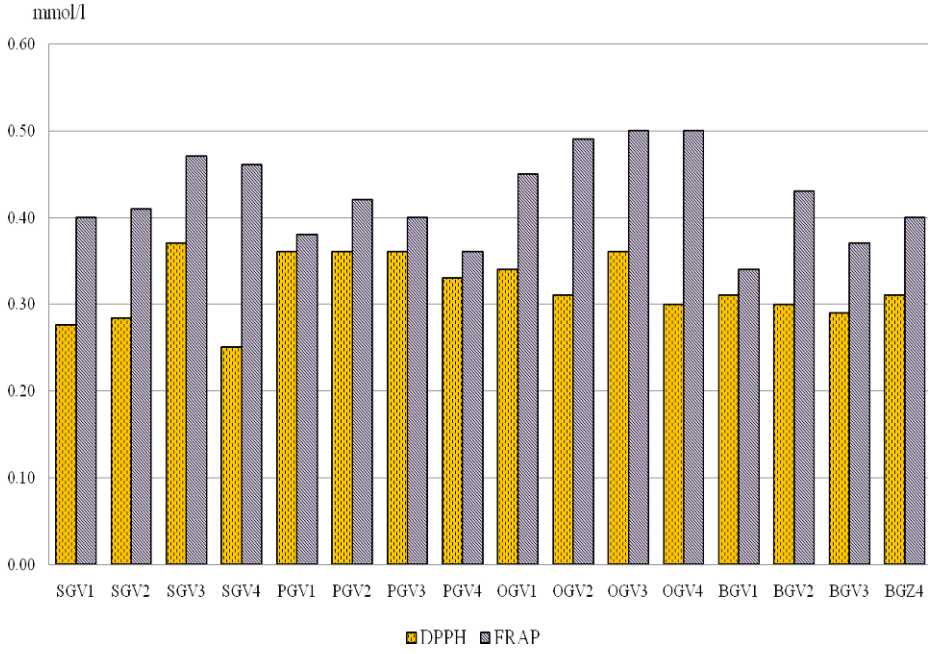

Figure 4. Total antioxidant activities in white wine samples determined by the DPPH and FRAP methods (GAE mmol L^{-1}, SDs see Table 2)

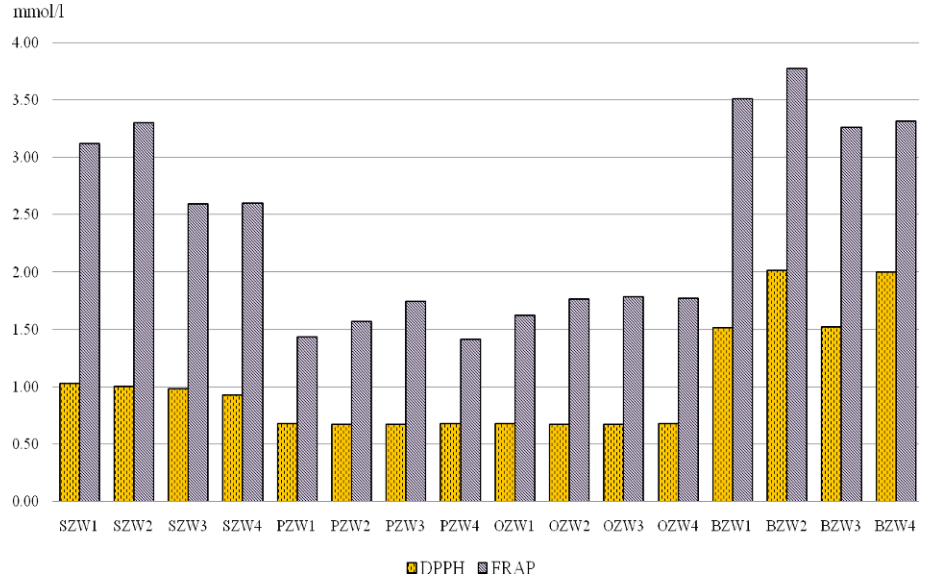

Figure 5. Total antioxidant activities in red wine samples determined by the DPPH and FRAP methods (GAE mmol L^{-1}, SDs see Table 2)

with intervals approximately 0.6-2.9 mmol L^{-1} for white wines and values with interval approximately 3-9 mmol L^{-1} (TE) for red wines. These values were about 3-15 times higher than our values, and, for the FRAP method, our values were lower by 30-90% in red wines, and 50-80% in white wines, respectively [27]. Beer *et al.* [32] presented the values of DPPH in the range from 0.8-1.06 mmol L^{-1} TE for white wines. Our values were lower than one half compared to these values. The differences could be caused by the different standard used in the experiments.

3.3. Individual phenolic content

The following compounds were identified in the wine samples (see Fig. 6): the phenolic acids (*i.e.*, gallic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid and cinnamic acid), catechin, resveratrol, quercetin and rutin. Contents of the individual phenolics in wines from four different wine-producing sub-regions of the Czech Republic and Austria are reported in Table 2.

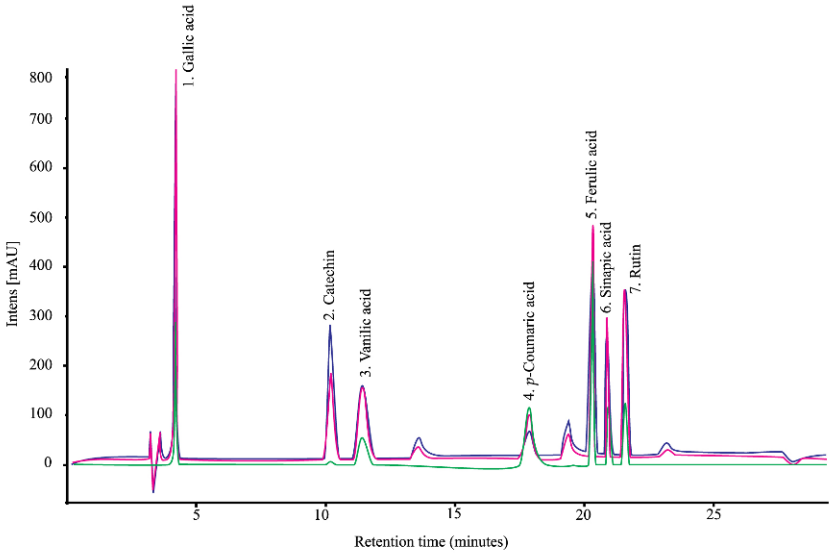

Figure 6. HPLC-UV-VIS DAD chromatogram of mixture of polyphenols standards at 210 nm, Supelcosil LC-18-DB column, 30°C, gradient elution: solvent A (95% (v/v) *acetonitrile* acidified with 0.35 mL TFAA) and solvent B (50% (v/v) aqueous *acetonitrile* acidified with 0.25 mL TFAA), injection volume 10 μL, flow rate of 1 mL min^{-1}.

The results obtained confirmed a variation in the phenolic contents among wines tested due to their different geographical origin. A comparison of these results with literature values was difficult because of the sample preparation and because the chromatographic conditions significantly differed. Moreover, the content data of individual phenolics were limited to a few compounds and samples in this study. However, the obtained results were in agreement with the values reported in literature. Gallic acid was the most abundant phenolic compound (mean 5.69 mg L^{-1}) in white wines; the highest level (6.19 mg L^{-1}) was found in OGV-4 sample from the Weinviertel vineyard, while the lowest amount (5.04 mg L^{-1}) of gallic acid was found in SGV-4 sample from the Velkopavlovická vineyard. Results were compared to the previously published data by Malovaná *et al*. [33], Rastija *et al*. [16], and Komes *et al*. [22], within the concentration range of gallic acid (from 5.16 to 28.3 mg L^{-1}) determined in samples from the Canary Islands, (0.7-8.4 mg L^{-1}) found in samples from Croatia and 2.63 mg L^{-1} from Zagorje, respectively. Gallic acid (mean 13.1 mg L^{-1}) in red wine was 3- to 5-times and 5 times lower than the results published for Turkish and Italian wines, respectively [34,35]. Catechin, with a mean concentration of 7.6 mg L^{-1}, was the second most abundant phenolics in white wines and with 24.5 mg L^{-1} also in red wines; this was from 3- to 10-times higher than the Croatian wines results (mean 2.86 mg L^{-1}) and similar to (mean 25.1 mg L^{-1}) the results of Turkish red wines, respectively [34,35]. The highest amount of vanillic acid was found (in average 2.64 mg L^{-1}) for SZW red wine and the lowest was (average value 0.87 mg L^{-1}) for SGV (white wine). High values of vanillic acid, ranging from 4.66 to 5.22 mg L^{-1}, were detected in some red wines from Turkish regions [34]. Moreover, low values of this acid, ranging from 0.05 to 0.28 mg L^{-1}, were found in Spanish wines [36]. Caffeic acid ranged from 0.01 to 10.4 mg L^{-1} in white and red wines. These results were similar to results in Spain wines (4.09 mg L^{-1}) and Italian red wines (ranged from 2.5 to 17.9 mg L^{-1}), respectively [35,36]. *p*-Coumaric acid and cinnamic acids were detected exceptionally in some samples in much lower amounts, but it was difficult to quantify their exact concentrations. The average values of ferulic acid ranged from 2.28 to 2.31 mg L^{-1} in white wines and 2.41 - 4.13 mg L^{-1} in red ones. These results aligned with the results by Komes *et al*. [22], (ranged from 1.88 to 3.2 mg L^{-1}). Mean concentrations of sinapic acid were 2.55 mg L^{-1} in white wines and 5.07 mg L^{-1} in red wines, respectively. Amounts of rutin and quercetin ranged from 3.29 to 10.4 mg L^{-1} and from 2.04 to 9.39 mg L^{-1} in white and red wines, respectively. Our results were in agreement with values obtained by Malovaná *et al*. [33] and Rastija *et al*. [16]. Resveratrol, a compound with multiple health benefits, was found in all wine samples, except for PGV. Amounts were comparable with the concentration ranges found in the literature [16,37].

The total phenolic content significantly correlated with the antioxidant activity and contents of individual phenolic compounds in the wines investigated (see Fig. 7 as an example) if the spectrum of the phenolics (their relative abundance) and matrix of a sample were similar. For instance, the total contents of

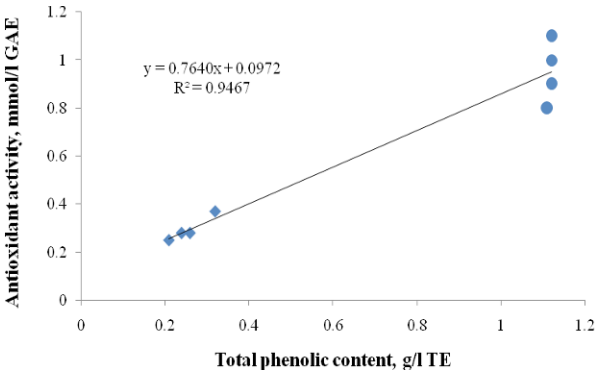

Figure 7. Relationship between the total polyphenolic compounds contents (TPC) and antioxidant activity (TAA) by DPPH method values of SGV and SZW (as an example). (♦) white wine (●) red wine

phenolic compounds and the antioxidant activity (DPPH method) values of S variations grown in Velkopavlovická, P variations grown in Velké Hostěrádky - Bošovice, O variations grown in Weinviertel and B variations grown in Wagram showed a good correlation. These results agree with the relationship between the total phenolic content and the total antioxidant potential values of wines (R^2 = 0.9876) by Minussi *et al*. [30]. Moreover, in SGV and SZW, the total antioxidant activities of wines investigated correlated well with gallic acid (R^2 = 0.95), catechin (R^2 = 0.81), vanillic acid (R^2 = 0.97), ferulic acid (R^2 = 0.94), sinapic acid (R^2 = 0.73), rutin (R^2 = 0.56), quercetin (R^2 = 0.62) and resveratrol (R^2 = 0.39), respectively. In PGV and PZW, the total antioxidant activities of wines investigated well correlated with gallic acid (R^2 = 0.98), catechin (R^2 = 0.74), sinapic acid (R^2 = 0.37), rutin (R^2 = 0.93) and quercetin (R^2 = 0.35), respectively. In OGV and OZW, the total antioxidant activities of wines investigated correlated with gallic acid (R^2 = 0.97), catechin (R^2 = 0.98), vanillic acid (R^2=0.99), caffeic acid (R^2=0.44), rutin (R^2=0.19), quercetin (R^2 = 0.88) and resveratrol (R^2 = 0.22), respectively. In BGV and BZW, the total antioxidant activities of investigated wines correlated with gallic acid (R^2 = 0.95), catechin (R^2 = 0.91), vanillic acid (R^2 = 0.37), sinapic acid (R^2 = 0.91), rutin (R^2 = 0.92), quercetin (R^2 = 0.74) and resveratrol (R^2 = 0.82), respectively. Our results were in agreement with some investigations presented in the literature. According to Frankel *et al*. [38], the relative antioxidant activity of 20 selected Californian wines correlated with total contents of phenolics in wines (R^2 = 0.94), and concentration of gallic acid (R^2 = 0.92), catechin (R^2 = 0.75), quercetin (R^2 = 0.68), caffeic acid (R^2 = 0.63) and rutin (R^2 = 0.50). Sánchez-Moreno *et al*. [39] observed that the free radical-scavenging activity of gallic acid was the highest; tannic acid, caffeic [acid, quercetin and rutin activities were intermediate and ferulic acid and resveratrol were the lowest.

4. Conclusions

The highest TPC values were found for red wine samples (1230 mg L^{-1}) grown in Velkopavlovická, Czech Repuiblic. The lowest TPC value (564 mg L^{-1}) was found for red wine samples grown in Weinviertel (approximately half of the value), Austria.The TPC values of white wine samples were relatively high but several times lower than compared to red wines. The lowest TPC value was found in the white wine grown in Wagram in contrast to the red wine samples from the same region.

The highest TAA by the FRAP method was found in red wines (3.5 - 3.8 mmol L^{-1}, *i.e*., 7 to 11-times higher than that in white wines) while the lowest TAA value (1.41 mmol L^{-1}), lower than half of the highest value, was found in a red wine grown in Velké Hostěrádky - Bošovice. The highest TAA by applying the DPPH method was found in a red wine (2.0 mmol L^{-1}, *i.e*., still 5- to 8-times higher than that found in white wines).

Gallic acid was the most abundant phenolic compound (mean 5.7 mg L^{-1}) in white wines; the highest level (6.2 mg L^{-1}) was found in a white wine sample from Weinviertel, while the lowest amount (5.04 mg L^{-1}) of gallic acid was found in a sample from the Velkopavlovická region. Catechin, with a mean concentration of 7.6 mg L^{-1}, was the second most abundant phenolics in white wines, and with 24.5 mg L^{-1}, also in red wines. The highest concentration of vanillic acid was found (in average 2.6 mg L^{-1}) for red wines and the lowest was (average value 0.9 mg L^{-1}) for SGV white wines. The caffeic acid concentration ranged from 0.01 to 10.4 mg L^{-1} in white and red wines. *p*-coumaric acid and cinnamic acids were detected in some samples in much lower amounts but it was not possible to exactly quantify their concentrations. The average concentrations of ferulic acid and sinapic acid were 2.3 mg L^{-1} and 2.6 mg L^{-1} in white wines and 2.4 - 4.1 mg L^{-1} and 5.1 mg L^{-1} in red wines, respectively. Amounts of rutin and quercetin ranged from 3.3 to 10.4 mg L^{-1} and from 2.0 to 9.4 mg L^{-1} in white and red wines, respectively.

According to the results, it was concluded that i) the total content of phenolics and total antioxidant activity/ total antioxidant capacity and the concentration of phenolic compounds could be a indicator for possible identification of wines' geographical origin, ii) the total contents of phenolic compounds significantly correlated with the antioxidant activity and the contents of individual phenolic compounds if the spectrum of phenolics (relative abundance) and the matrix were similar, iii) the gallic acid was the most abundant compound; tannic acid, caffeic acid, quercetin and rutin activities were intermediate; and the ferulic acid and resveratrol showed the lowest influence on the free radical-scavenging activity.

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