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Determination of phenolic compounds in Moravian wines

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ABSTRACT: Wines were made from *Grьner Veltliner* and *Zweigelt* and cultivars were grown in four different geographical regions of Austria and Czech Republic; two wineries in Austria (Poysdorf, GroЯriedenthal) and two wineries in the Czech Republic (Velkě Bнlovice, Bošovice). Eleven individual phenolics were quantified using a HPLC/UV-VIS method.

Keywords: Moravian wine, phenolics, HPLC/UV-VIS, phenolic acids

INTRODUCTION

The phenols compounds in wine include a large group of several hundred chemical compounds, known as polyphenols that affect the taste, color and mouthfeel of wine. This large group can be broadly separated into two categories such as flavonoids and non-flavonoids. Flavonoids include anthocyanins and tannins which contribute to the color and mouthfeel of the wine. Non-flavonoids include stilbenes sach as resveratrol and compounds derived from acids in wine like benzoic, caffeic and cinnamic acid.

White wine contains significantly lower amounts of total polyphenols compared with red wines, mainly hydroxycinnamic acids, hydroxybenzoic acids and flavan-3-ols [1]. As a material for winemaking, the phenolic compounds of wine grape are one of the most important aspects determining wine quality. A large number of published works have focused on the essential contributions of phenolic profiles to wine quality and sensory properties [2, 3].

The phenolic profiles in wine depend on the phenols contained in the grapes, the extraction parameters, yeast strain, processing enzymes, cap management, and alcohol concentration [4,5], while the phenolic compounds of grapes are affected by many factors such as genetic variation, maturity, climatic and geographical conditions [6,7]. Other factors that influence the extent of phenolic extraction are the molecular weight, size and type of phenolic molecules, the surface area for the concentration gradient, other temperature treatments including grape and must freezing and thermovinification, and factors that affect cell permeability [8].

In this study some flavonoids, phenolic acids, flavonols and resveratrol were determined. These compounds could be key agents of the antioxidant action on the human metabolism pathway, the reason why we wanted to obtain indication to qualify the wine from a nutritional point of view. Also, the environmental condition (temperature, rainfall/humidity, high above sea level and geochemical characteristics) can affect the wine maturation and consequently the concentration of its phenolic compounds.

We have investigated phenolic compounds of Moravian wines from four different geographical regions of Austria and Czech Republic.

Many researches about phenolic compounds of wine and grapes and antioxidant capacity of wine have been published. However, little attention has been paid to comparison on phenolic compounds of wine grapes from different origin in Moravian wine.

EXPERIMENTAL

Sampling. Total of 8 wine samples including 4 white and 4 red were collected. All wines were made from *Grьner Veltliner* and *Zweigelt* and cultivars were grown in four different geographical regions, two wineries in Austria (Poysdorf, Groβriedenthal) and two wineries in the Czech Republic (Velkė Bĭlovice, Bošovice).

Wine making techniques. Grapes for each wine according to a standard procedure of Vinopol, Ltd., Velkй Bнlovice, Czech Republic. After crushing, di-ammoniumphosphate and *Saccharomyces cerevisiae* strain were added. Fermentation was carried out at 8°C, and the cap was punched down two times per day. The skin was separated from the juice using standard pressing procedure. Pressed juice was taken in 50 l glass bottles and at 8°C by standard procedure was fermented. Wines were cold-stabilized for several weeks, filtered using ceramic filters.

HPLC analysis of phenolic compounds. Individual phenolic compounds present in wines were separated and quantified using a HPLC method [9, 10] with fluorescence detection. Chromatographic separation was carried out on Supelcosil LC-18-DB (16096-001 58335-c46) column (250 x 4.6 mm, 5 µm, Supelco, USA) at 30°C temperature. Elution was carried by using a gradient procedure with a mobile phase containing solvent A and solvent B.

Solvent A (mobile phase A) was 950 ml Distilled water (dH_2O), 50 ml acetonitrile and 0.35 ml trifluoroacetic anhydride (TFAA). Solvent B (mobile phase B) was 500 ml dH_2O, 500 ml acetonitrile and 0.25 ml TFAA. Run time was 30 min and the flow rate was 1µl/min. The UV detector was set at 205, 210, 275 and 375 nm. Wine sample was filtered using 0.45 µm pore size Nylon membrane filter 13 mm (FFNN1345-100, Gronus, SMI- labHut Ltd.

Table 1. List of analyzed wine samples

Code	Type of wine	Sample code	Vineyards	Wineries
S	White	GV	Velkopavlo vickб	Velkй Bнlovice
	Red	ZW		
P	White	GV	Velkй Hostěrбdky	Bošovice
	Red	ZW		
O	White	GV	Weinviertel	Poysdorf
	Red	ZW		
B	White	GV	Wagram	GroЯriede nthal
	Red	ZW		

Maisemore Gloucester, UK) using filter devices (Millipore) before injecting into column. Injection volume was 20 µl.

RESULTS AND DISCUSSION

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 present in wines were separated and quantified using a HPLC method with fluorescence detection. Contents of the determined phenolics show in tables 2-3.

Table 2. Content of phenolic compounds in white wine samples, mg/l

№	Phenolic compounds	SGV	PGV	OGV	BGV
1	Gallic acid	5.52 ± 0.32	5.74 ± 0.06	5.90 ± 0.29	5.63 ± 0.29
2	Catechin	7.49 ± 2.15	10.65 ± 0.63	5.46 ± 1.23	7.11 ± 0.87
3	Vanillic acid	0.87 ± 0.13	ND	1.09 ± 0.14	0.88 ± 0.13
4	Caffeic acid*	0.05 ± 0.0	1.12 ± 1.38	1.03 ± 1.69	0.51 ± 0.15
5	*p*-Coumaric acid	ND	ND	NC	ND
6	Ferulic acid	2.28 ± 0.02	ND	2.31 ± 0.0	ND
7	Sinapic acid	2.50 ± 0.04	2.57 ± 0.09	2.53 ± 0.04	2.67 ± 0.05
8	Rutin	3.47 ± 0.1	3.31 ± 0.01	3.48 ± 0.06	3.43 ± 0.05
9	Resveratrol	0.9 ± 0.08	ND	0.91 ± 0.02	0.71 ± 0.21
10	Cinnamic acid	ND	ND	ND	ND
11	Quercetin*	5.47 ± 0.4	6.14 ± 2.18	4.94 ± 0.18	4.57 ± 0.29

Caffeic acid* – detected on the 275 nm, Quercetin* - detected on the 375 nm, ND – not detected

The results confirmed a variation in the phenolics content of wines due to their different geographical origin.

Table 3. Content of phenolic compounds in red wine samples, mg/l

№	Phenolic compounds	SZW	PZW	OZW	BZW
1	Gallic acid	14.4 ± 0.23	9.31 ± 0.31	13.9 ± 0.68	16.9 ± 0.3
2	Catechin	30.4 ± 6.24	21.5 ± 5.21	24.8 ± 0.70	23.2 ± 2.24
3	Vanillic acid	2.63 ± 0.35	1.3 ± 0.06	1.65 ± 0.03	1.19 ± 0.16
4	Caffeic acid*	2.53 ± 0.23	3.6 ± 4.59	2.07 ± 0.37	1.41 ± 1.77
5	*p*-Coumaric acid	ND	ND	ND	ND
6	Ferulic acid	4.05 ± 0.00	2.41 ± 0.01	2.90 ± 0.02	ND
7	Sinapic acid	7.09 ± 0.06	3.66 ± 1.1	5.19 ± 0.19	4.55 ± 0.68
8	Rutin	3.95 ± 0.01	8.97 ± 1.35	4.51 ± 1.61	6.13 ± 0.81
9	Resveratrol	1.19 ± 0.00	0.84 ± 0	0.99 ± 0.03	1.35 ± 0.05
10	Cinnamic acid	ND	ND	ND	ND
11	Quercetin*	3.63 ± 0.01	3.19 ± 0.76	3.58 ± 0.02	3.66 ± 0.02

Caffeic acid* – detected on the 275 nm,Quercetin* - detected on the 375 nm, ND – not detected

The data on content of determined phenolics were limited to a few compounds and samples in this study. However, the ranges that obtained were in agreement with the values reported in available literature. Gallic acid was the most abundant phenolic compound (mean 5.69 mg/l) in white wines; the highest level (5.9 mg/l) was found in OGV sample from Weinviertel vineyard, while the lowest amount (5.52 mg/l) of gallic acid was found in SGV sample from Velkopavlovickб vineyard.

Results were compared to the previous results by Malovanб *et al*., Rastija *et al*., and Komes *et al*., within the concentration range of gallic acid (from 5.16 to 28. 3 mg/l) determined in samples from the Canary Island, (0.7-8.4 mg/l) found in samples from Croatia and 2.63 mg/l from Zagorje, respectively [11,12,13]. Gallic acid (mean 13.6 mg/l) in red wine was from 3 times to 5 times and from the same to 5 times lower than results published in Turkish wines and Italian wines, respectively [14,15]. Catechin, with mean concentration 7.6 mg/l, was the second most abundant phenolics in white wines and with 24.5 mg/l also in red wine, which was ranged from 3 times to10 times higher than result in Croatian wines (mean 2.86 mg/l) and similar to (mean 25.1 mg/l) result in Turkish red wines, respectively [12,14]. The highest amount of vanillic acid was found (2.64 mg/l) for SZW (red wine) and the lowest was (0.87 mg/l) for SGV (white wine). High values of vanillic acid, ranges from 4.66 to 5.22 mg/l, were detected in some red wines from Turkish regions [14]. Moreover, low values of this acid, ranging from 0.05 to 0.28 mg/l, were found in Spanish wines [16]. Caffeic acid ranged from 0.01 to 10.4 mg/l in white and red wines. These results were similar to results in Spain wines (4.09 mg/l) and Italian red wines (ranged from 2.5 to 17.9 mg/l) [16,15]. *p*-Coumaric acid and cinnamic acids were detected in some samples in much lower amounts but it was not possible to quantify their concentrations exactly. The average values of ferulic acid were in the range from 2.28 to 2.31 mg/l in white wines and 2.41-4.13 mg/l in red wines, respectively. These results agreed with results of Komes *et al*., (ranged from 1.88 to 3.2 mg/l) [13]. Mean concentrations of sinapic acid were 2.55 mg/l in white wines and 5.07 mg/l in red wines, respectively. Amounts of rutin and quercetin ranged from 3.29 to 10.4 mg/l and from 2.04 to 9.39 mg/l in white and red wines, respectively. Our results were in agreement with values obtained by Rastija *et al*. and Malovanб *et al*. [12, 11]. The resveratrol , a compound with multiple health benefits, was found in all wine samples, except of PGV, and amounts were comparable with the concentration range found in the literature [12,17].

CONCLUSIONS

The results of the study showed that the most abundant phenolic compounds were gallic acid, catechin, vanillic acid, ferulic acid, sinapic acid, rutin and quercetin in the analysed wines. Gallic acid, catechin were the highest; caffeic acid, quercetin and rutin activities were intermediate and ferulic acid and resveratrol were showed the lowest influence to the free radical-scavenging activity in the Moravian wines.

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