PHENOLIC PROFILES OF RIESLING WINES ORIGINATING FROM DIFFERENT TERROIRS OF THE CZECH REPUBLIC

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'Riesling' belongs to the main grapevine varieties cultivated in the Czech Republic in cool climate viticultural conditions. In this study, 34 varietal Riesling wines from five wine-growing regions in the Czech Republic were investigated. Seventeen phenolic compounds belonging to hydroxybenzoic and hydroxycinnamic acids, stilbenes and flavan-3-ols were analysed by means of HPLC. The study proved that the majority of the assessed phenolic compounds is not influenced by vintage year but only by geographical origin. The obtained analytical parameters were evaluated for this purpose by CVA (canonical variate analysis). The following phenolic compounds may be used to identify the geographical origin of Riesling wines: *p*-coutaric acid, *trans*-resveratrol, *cis*-resveratrol, (+)-catechin and (-)-epicatechin. Most of these phenolic compounds have their origin in grapes and thus have a close relation to the geographical origin.

Keywords: wine, authenticity, terroir, phenolic compounds, chemometric

Phenolprofile von Riesling-Weinen verschiedener Terroirs der Tschechischen Republik. 'Riesling' gehört zu den im kühlen Weinbauklima der Tschechischen Republik angebauten Hauptrebsorten. In dieser Studie wurden 34 sortenreine Riesling-Weine aus fünf Weinbaugebieten der Tschechischen Republik untersucht. Siebzehn phenolische Verbindungen, die zu den Hydroxybenzoe- und Hydroxyzimtsäuren, den Stilbenen und den Flavan-3-olen gehören, wurden mittels HPLC analysiert. Die Studie zeigte, dass die Mehrheit der untersuchten phenolischen Verbindungen nicht durch den Jahrgang, sondern nur durch die geographische Herkunft beeinflusst wird. Die erhaltenen analytischen Parameter wurden zu diesem Zweck mittels CVA (kanonische Korrelationsanalyse) ausgewertet. Zur Identifizierung des geographischen Ursprungs von Riesling-Weinen können folgende Phenolverbindungen verwendet werden: *p*-Coutarsäure, *trans*-Resveratrol, *cis*-Resveratrol, (+)-Catechin und (-)-Epicatechin. Die meisten dieser phenolischen Verbindungen haben ihren Ursprung in den Trauben und daher eine enge Beziehung zum geographischen Ursprung.

Schlagwörter: Wein, Authentizität, Terroir, Phenolverbindungen, chemometrisch

Grapes and wine are a very complex mixture of several hundred various chemical compounds which can also be described as primary and secondary metabolites. Generally, metabolites in wine are mainly influenced by climate, soil, vineyard management, variety and vinification. The identification of metabolites in wine which are responsible for discrimination and differentiation is necessary for the assessment of terroir influence through materializing the scientific correlation between the terroir factors and the wine metabolites (Hong, 2011).

Metabolomics therefore plays an important role in the improvement of agricultural systems and of plant-based foods. Metabolomic studies aim to capture snapshots of the metabolome and relate the observed metabolic profiles to physiological states that may be of genetic or environmental origin. Geographical origin can be an important attribute of commercial plant-derived foods as it gives an indication of quality, phytochemical composition, the amount of phytochemical variation expected and appeals to certain values of consumers. Additionally, where regions produce crops with specific terroir, being able to classify geographical origin is a way of protecting the reputation and value of specific products as is specially recognized in the European Union under "Protected Designation of Origin" and "Protected Geographical Indication" classifications (McGhie and Rowan, 2012).

According to the definition of the OIV (Organisation Internationale de la Vigne et du Vin) the terroir is a concept which refers to an area in which collective knowledge of the interactions between environment and applied vitivinicultural practices develops, providing distinctive characteristics for products originating from this area. Terroir includes specific soil, topography, climate, landscape characteristics and biodiversity features.

Analytical chemistry constantly plays a more and more significant role in winemaking. Chemical analysis of wine is therefore a basic tool for providing wine safety and origin. Phenolic compounds belong to the most significant group of secondary metabolites in grapes and wine (Ali et al., 2010) and can be successfully used for the wine authenticity assessment, as these compounds are characteristic for the type of wine and can provide information on its geographical origin (Andreu-Navarro et al., 2011).

Phenolic compounds occurring in grapes and wine can be divided into non-flavonoids and flavonoids. These compounds influence wine colour as well as sensory traits, especially astringency and bitterness in a significant way.

Non-flavonoids include hydroxycinnamates, hydroxybenzoic acids and stilbenes (Rentzsch et al., 2009; Cheynier et al., 2010). Phenolic acids (hydroxybenzoic and hydroxycinnamic acids) are important phenolic compounds in white wines and are dominant antioxidants. Hydroxycinnamic acids influence the colour and the flavour of wine in a significant way, they are thus the main phenolic compounds in white wines where they can serve as oxidation substrates and precursors of browning. Hydroxybenzoic acids are a minor group of phenolic compounds present in wine.

Among the significant flavonoids in grapes and wine are flavan-3-ols, flavonols, and, in the case of red varieties, also anthocyanines. Flavan-3-ols are the abundant class of flavonoids and are found mainly in skin and seeds of grape berries (Waterhouse, 2002).

Phenolic compounds can be used as chemometric markers of geographical origin. Phenolic compounds in wines with different geographical origin indicate that the accumulation of phenolic compounds in grapes and wines is significantly influenced by terroir (Rastija et al., 2009; Jaitz et al., 2010; Li et al., 2011).

Generally, differentiation and classification of wine samples on the basis of their chemical composition, geographical origin, variety or quality is one of the basic applications of chemometric methods in enology (De Villiers et al., 2005).

The Czech Republic with its natural and climatic conditions belongs to the wine-making countries with cool climate viticulture. Vineyards in the Czech Republic cover the area of approximately 20 000 ha and the history of grapevine cultivation on Czech territory dates back to the period of Roman invasions in the 2nd century AD.

'Riesling' belongs to the typical varieties for cool climate viticulture and in the Czech Republic it is cultivated on an area of 1266 ha in all wine-growing subregions and is the fourth-most cultivated grapevine variety.

The aim of this work is to characterize 34 varietal wines made from Riesling grapes of identical quality in late harvest (Spätlese) category from 5 different regions in the Czech Republic on the basis of the content of phenolic compounds, i. e. hydroxybenzoic acids, hydroxycinnamic acids, stilbenes and flavan-3-ols, and further, with the use of multivariate statistical analysis, to find phenolic compounds, which may enable differentiation of wines according to their geographical origin.

MATERIALS AND METHODS

SAMPLING

34 commercial varietal Riesling wines were sampled from five wine-growing regions in the Czech Republic. All wines had been produced over the years 2006 to 2008. Four wine samples originated from the wine-growing region of Mikulov, 9 from Slovácko, 3 from Velké Pavlovice, 12 from Znojmo, and 6 from the Litoměřice region. All wine samples were from grapes of identical quality in late harvest (Spätlese) category. Wines were made in 12 different wineries in the Czech Republic, each made one sample from their region per year. One company from Mikulov region made only a sample from the vintage 2006 and afterwards put an end to collaboration. Winemaking technology was identical in each winery and typical for Riesling wines in the Czech Republic. Harvested grapes were destemmed and crushed without maceration. Pressing was done using pneumatic presses with almost the same settings in each winery. Dosage of 100 g/hl of bentonite was used for sedimentation of must. Fermentation was carried out using the commercial yeast strain CEG (Uvaferm, Fredericia, Denmark). Fermentation and ageing of wine took place in 3000 l stainless steel tanks. After the end of fermentation, the young wine was racked and treated with sulphur dioxide to the content of 40 mg/l free SO_2. Thereafter, the wine was bottled.

DETERMINATION OF PHENOLIC COMPOUNDS

In all wine samples the following phenolic compounds were analysed: hydroxybenzoic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid), hydroxycinnamic acid (caffeic acid, caftaric acid, *p*-coumaric acid, *p*-coutaric acid, ferulic acid, fertaric acid,), stilbenes (*trans*-resveratrol, *cis*-resveratrol, *trans*-piceid, *cis*-piceid) and flavan-3-ols ((+)-catechin, (-)-epicatechin).

HPLC ANALYSIS

Concentrations of the individual phenolic compounds were determined by a method with direct injection of a sample as described below. The wine samples were centrifuged at 3000 g for 6 min and diluted with ratio 1:1 by 100 mM HClO4.

The chromatographic system Shimadzu LC-10A (Shimadzu, Kyoto, Japan) consisted of two pumps LC-10ADvp (Shimadzu, Kyoto, Japan), a column thermostat with manual injection valve, a DAD detector SPD-M10Avp (Shimadzu, Kyoto, Japan) and a personal computer running the chromatographic software LC solution (Shimadzu, Kyoto, Japan). The chromatographic separations were performed on a column Alltech Alltima C18 (3 µm, 3 × 150 mm; Grace, Deerfield, USA) equipped with a guard column (3 × 7.5 mm ID) filled with the same sorbent. The temperature of separations was 60 °C. The mobile phases were the following: A = 15 mM $HClO_4$ and B = 15 mM $HClO_4$, 10% MeOH, 50 % ACN. The gradient programme is described in Table 1, with a flow rate of 0.6 ml/min.

The total length of the analysis was 43 minutes and the regeneration time was 4 minutes. Data were recorded in the range of wavelength 200 to 520 nm.

The detection wavelength was 200 nm for (+)-catechin and (-)-epicatechin, 260 nm for vanillic, protocatechuic and *p*-hydroxybenzoic acid, 275 nm for gallic and syringic acid, 285 nm for *cis*-piceid and *cis*-resveratrol, 310 nm for *p*-coumaric acid and its derivatives, *trans*-piceid and *trans*-resveratrol, 325 nm for caffeic acid.

Table 1: HPLC gradient programme for phenolic compounds

Time (min)	15 mM HClO₄ (%)	15 mM HClO₄, 10% MeOH, 50% acetonitrile (%)
0.00	96	4
20.00	72	28
30.00	58	42
35.00	40	60
38.00	0	100
40.00	0	100
40.01	100	0
41.00	96	4
43.00	96	4

REAGENTS AND STANDARD SOLUTIONS

Acetonitrile (ACN) and methanol (MeOH) were of HPLC supergradient purity. Vanillic acid, protocatechuic acid, *p*-hydroxybenzoic acid, gallic acid, syringic acid, *p*-coumaric acid, *trans*-resveratrol, *trans*-piceid, caffeic acid, ferulic acid, (+)-catechin, (-)-epicatechin and perchloric acid were obtained from Sigma-Aldrich (St. Louis, USA). Other used chemicals were at least of analytical grade and were obtained from local suppliers (Lachema-Penta, Brno, Czech Republic).

A stock standard solution was prepared by accurately weighing 10 mg of each phenol in a 25 ml volumetric flask. The standard was dissolved in 10 ml of acetonitrile and brought up to volume with distilled water.

Cis-resveratrol was obtained by exposing the *trans*-res- veratrol standard solution to direct UV light for 10 minutes. The source of UV light was the fluorescent tube Philips Ultraviolet TUV 30W/G30 T (Philips, Rosemont, USA). The sample was placed directly below the tube in a sealed quartz cell. Concentration of *cis*-resveratrol was expressed as a decrease in the concentration of *trans*-resveratrol (71 % conversion).

HPLC METHOD VALIDATION

Commercially available phenolic compounds were determined by using calibration curves of the relevant standards. Esters of tartaric acid with hydroxycinnamic acids for which no standards are available were quantified using the calibration curves belonging to the basic acids from which they were derived. Calibration curves of commercially available substances were obtained for the concentration normally present in wine (approximately 0.1 to 50 mg/l). Table 2 shows the retention times, detection wavelengths, detection and quantification limits (3 and 10 times baseline noise, resp.), response factors, correlation factors and the variation coefficients obtained in the consecutive analysis of 6 standards (1 mg/l). The concentration of each substances' sample are averages of three parallel analyzes.

Table 2: Retention times, detection wavelengths, detection and quantification limits, response factors, correlation factors and the variation coefficients for HPLC method

Compound	Retention time (min)	Detection wavelengtht (nm)	Detection limit (mg/l)	Quantification limit (mg/l)	Responce factor	Correlation factor (R^2)	Variation coefficient (%)
gallic acid	2.88	275	0.010	0.034	1.018E-05	0.9961	3.51
protocatechuic acid	5.41	260	0.008	0.025	7.583E-06	0.9991	1.83
p-hydroxybenzoic acid	8.50	260	0.005	0.017	5.121E-06	0.9966	2.54
vanilic acid	12.03	260	0.007	0.025	7.387E-06	0.9962	2.71
syringic acid	14.24	275	0.010	0.030	9.517E-06	0.9972	1.98
caffeic acid	12.44	325	0.005	0.017	5.030E-06	0.9979	1.54
caftaric acid	8.74	325					
p-coumaric acid	16.76	310	0.003	0.011	3.423E-06	0.9963	2.31
p-coutaric acid	11.84	310					
ferulic acid	20.30	325	0.005	0.016	4.774E-06	0.9937	2.64
fertaric acid	14.68	325					
trans-resveratrol	28.27	310	0.001	0.004	1.082E-06	0.9922	2.09
cis-resveratrol	31.79	285	0.004	0.014	4.194E-06	0.9952	3.02
trans-piceid	22.31	310	0.004	0.014	4.337E-06	0.9983	2.17
cis-piceid	26.83	285	0.013	0.043	1.296E-05	0.9912	3.26
(+)-catechin	10.76	200	0.002	0.007	2.094E-06	0.9970	2.74
(-)-epicatechin	15.14	200	0.002	0.007	2.226E-06	0.9951	2.94

STATISTICAL DATA TREATMENT

The obtained data were processed with respect to wine-growing regions and expressed by mean values and standard deviations. The use of one-way analysis of variance (ANOVA) and a LSD test at p < 0.05 with the aim to find the influence of a region and vintage was a further step.

For finding markers for the geographical origin, the application of chemotaxonomic statistical analysis methods is suitable. Then the multivariate chemometric methods were used, followed by canonical variate analysis (CVA) as a supervised learning technique for differentiation of wines to groups on the basis of the wine-growing regions and finding markers which show a significant discrimination value.

Canonical discriminant analysis is a dimension-reduction technique related to PCA and canonical correlation. Given a nominal classification of variables together with several interval variables, canonical discriminant analysis derives canonical variables that explain the inter-class variation in the same way that principal components summarize total variation (MORENO-ROJAS et al., 2010). Afterwards, the contents of individual phenolic compounds were compared by a Pearson correlation with the aim to find mutual dependencies.

All statistical analyses were performed with the use of the statistical program UNISTAT (Unistat, Brno, Czech Republic).

RESULTS AND DISCUSSION

EVALUATION OF HYDROXYBENZOIC ACIDS IN VARIETAL RIESLING WINES

Gallic acid and protocatechuic acid are hydroxybenzoic acids with a close relation to grapes as they occur mainly in the pulp of berries. Because of the strong influence of viticultural conditions on berry characteristics, it can be said that these acids have a close relation to the place of grapevine cultivation – terroir.

Gallic acid, p-hydroxybenzoic acid, protocatechuic acid, syringic acid, salicylic acid and vanillic acid can be then identified in wine. The level of hydroxybenzoic acids in wine shows a great variability in dependency on grapevine variety and growing conditions (terroir) (RENTZSCH et al., 2009), which was also confirmed in this study. Among hydroxybenzoic acids occuring in wine in the highest concentrations are gallic acid, protocatechuic acid and p-hydroxybenzoic acid. In our study, this is true for gallic and protocatechuic acids. Table 3 shows mean values, standard deviations and analysis of variance of hydroxybenzoic acids in wines from different regions.

Table 3: Mean values, standard deviations and analysis of variance of hydroxybenzoic acids and hydroxycinnamic acids in Riesling wines from the Czech Republic

| Chemical parameters (mg/l) | Region | | | | | Effect | |
	Mikulov (n=4)	Slovácko (n=9)	Velké Pavlovice (n=3)	Znojmo (n=12)	Litoměřice (n=6)	Region F	Year F
gallic acid	2.84±3.01	2.32±2.50	2.86±1.31	2.05±1.38	2.07±0.64	0.22 n.s.	3.47 *
protocatechuic acid	1.63±0.37a	2.73±0.49b	3.32±0.43b	2.72±0.95b	2,44±0.52ab	2.97*	1.34 n.s.
p-hydroxybenzoic acid	0.39±0.14	0.68±0.18	0.75±0.14	0.63±0.36	0.87±0.41	1.66 n.s.	3.32 *
vanillic acid	0.84±0.51b	0.79±0.49b	0.54±0.30ab	0.71±0.36b	0.06±0.02a	4.07***	4.63 **
syringic acid	0.23±0.08ab	0.29±0.07b	0.23±0.07ab	0.16±0.05a	0.25±0.12b	4.14***	0.56 n.s.
caffeic acid	3.15±1.47a	9.21±9.27b	5.74±4.42ab	2.21±0.55a	2.11±0.62a	3.03*	0.68 n.s.
caftaric acid	15.19±7.52a	29.66±13.63b	18.12±7.89ab	23.84±3.78ab	40.03±12.05c	5.42***	1.20 n.s.
p-coumaric acid	1.66±0.89ab	2.58±1.27b	2.79±1.73b	1.44±0.70a	1.60±0.38ab	2.62*	1.15 n.s.
p- coutaric acid	1.54±0.43a	3.37±1.44bc	2.08±1.58ab	3.12±0.79bc	4.18±0.85c	4.56***	1.35 n.s.
ferulic acid	0.55±0.11	0.74±0.29	0.72±0.24	0.48±0.17	0.60±0.15	2.40 n.s.	2.32 n.s.
fertaric acid	2.83±1.59	4.00±0.49	3.48±0.39	3.35±0.56	3.95±0.72	2.42 n.s.	1.15 n.s.

*Significant for p < 0.05, ** Significant for p < 0.01, *** Significant for p < 0.001. Data followed by different letters in the same row are significantly different by LSD test at p < 0.05. n.s. not significant.

The highest mean content was found for protocatechuic acid (2.60 mg/l), followed by gallic acid (2.29 mg/l). In the case of protocatechuic acid, the influence of the region on its content was proved.

The content of hydroxybenzoic acids found in Riesling wines from the Czech Republic is significant, as in German 'Riesling' wines from the vintages 1989 to 1998 amounts of main hydroxybenzoic acids (gallic acid, protocatechuic acid) were below detection level (Pour Nikfardjam et al., 2007). A similar content of main hydroxybenzoic acids was also found in white wines from South Africa where the content of gallic acids varied in the range of 2.0 to 4.7 mg/l, however, the content of protocatechuic acid was lower (De Villiers et al., 2005). A similar content of gallic acid in 'Riesling' wines was also found in wines from the Ontario region in Canada (Soleas et al., 1997). Lower contents of gallic acid (1.34 mg/l) were also found in 'Riesling' wines from Brasil (Ballus et al., 2012) and, on the contrary, higher contents of gallic acid (4.2 to 45.5 mg/l) and protocatechuic acid (5.2 to 13.5 mg/l) were found by assessment of 'Riesling' wines from the vintages 1892 to 1921 from the Rheingau region (Dietrich et al., 2004). In Croatian 'Riesling' wines lower contents of gallic acid (0.5 mg/l), but significantly higher contents of protocatechuic acid (18.49 mg/l) were found. Protocatechuic acid is considered to be the main hydroxybenzoic acid in Riesling wines (Komes et al., 2007). Despite the significantly lower content, protocatechuic acid also showed to be the main hydroxybenzoic acid for 'Riesling' wines from the Czech Republic. The third-most significant hydroxybenzoic acid in 'Riesling' wines was p-hydroxybenzoic acid (0.67 mg/l). Vanillic acid with the mean content of 0.62 mg/l and syringic acid (mean content 0.23 mg/l) were of minor representation in the studied wines. The content of these hydroxybenzoic acids was significantly influenced by the region.

The content of some hydroxybenzoic acids, namely gallic acid, p-hydroxybenzoic acid and vanillic acid was also influenced by the vintage year. Dominant hydroxybenzoic acids in 'Riesling' wines are gallic acid and protocatechuic acid. The content of these acids exceeds almost in all cases the values for 'Riesling' wines from other regions.

EVALUATION OF HYDROXYCINNAMIC ACIDS IN VARIETAL RIESLING WINES

Hydroxycinnamic acids are the main phenolic compounds in white wines. They originate mostly from hydroxycinnamic tartaric esters present in grapes. Among the main hydroxycinnamic acids, p-coumaric acid, caffeic acid and ferulic acid are mostly present in grapes (Cheynier et al., 2010). The profile of hydroxycinnamic acids studied in 'Riesling' wines from different regions in the Czech Republic is shown in Table 3.

Caftaric acid with a mean content of 26.71 mg/l also showed to be the most represented hydroxycinnamic acid in 'Riesling' wines from the Czech Republic. High content of caftaric acid (110.8 mg/l) also was found in Riesling wines from Ontario (Nagel et al., 1979). The content of caftaric acid in German Riesling wines was lower (11.2 mg/l) (Dietrich et al., 2004). Caftaric acid is however considered to be one of the most significant phenolic compounds in 'Riesling' wines (Ritter et al., 1994). The content of caftaric acid in all studied wines is very significantly influenced by the region and not influenced by the vintage year. The highest content was found in wines from the Litoměřice region (40.03 mg/l) that significantly differs from other regions. On the other side is Mikulov region with the lowest content (15.19 mg/l) of caftaric acid. Caffeic acid is also significantly represented in 'Riesling' wines from the Czech Republic. Mean content of caffeic acid in all studied wines is 4.47 mg/l. A similar content of caffeic acid was also found in 'Riesling' wines from Ontario region (3.79 mg/l) (Soleas et al., 1997). On the contrary, lower content was found in 'Riesling' wine from Brasil (Ballus et al., 2012). The content of caffeic acid in the Czech Republic was influenced by the region and not influenced by the vintage year. The significantly highest content of caffeic acid was found in the Slovácko region (9.21 mg/l) that exceeded all values for Riesling wines published before. Among hydroxycinnamic acids, caftaric acid has the most significant representation which is almost 50 % of total hydroxycinnamic acids in wine (Rentzsch et al., 2009), this was confirmed by this study.

Another important hydroxycinnamic acid in 'Riesling'

wines is *p*-coutaric acid with the mean content of 3.10 mg/l. The content of p-coutaric acid is very significantly influenced by the region of wine origin and is not influenced by the vintage year. This hydroxycinnamic acid significantly separated the regions of Litoměřice (4.18 mg/l) and of Mikulov (1.54 mg/l). In German wines from Rheingau region (Ritter et al., 1994), a similar content of *p*-coutaric acid as in Mikulov region was found. Significantly higher content of *p*-coutaric acid in Riesling wines was found by Nagel et al. (1979). p-coutaric acid can be a suitable marker for geographical origin of Riesling wines.

Hydroxycinnamic acids in Riesling wines were also studied in German wines from the vintages 1989 to 1998 (Pour Nikfardjam et al., 2007). In comparison with the studied wines from the Czech Republic, significantly lower contents of caffeic acid, caftaric acid and *p*-coutaric acid were found in German wines.

In Riesling wines from the Czech Republic, a relatively high content of fertaric acid (3.58 mg/l) was found. The content of fertaric acid was however not influenced by region as it was in the case of ferulic acid (0.60 mg/l). The content of ferulic acid was lower in comparison with Croatian Riesling wines (Komes et al., 2007) and also with wines from the Ontario region where ferulic acid was found to be a phenolic compound of the highest content (Soleas et al., 1997). The last hydroxycinnamic acid with a relation to the region is *p*-coumaric acid. Mean content of p-coumaric acid was 1.91 mg/l. This value is comparable also with German Riesling wines (Pour Nikfardjam et al., 2007).

Among hydroxycinnamic acids, it is possible to find markers of authenticity of the geographical origin what will be further verified by chemometric statistical methods.

EVALUATION OF STILBENES IN VARIETAL RIESLING WINES

Stilbenes are synthesized by several species within the family of *Vitaceae* including *Vitis vinifera* L.. Resveratrol exists in two isomer forms (*cis-* and *trans-*). 3-O-β-D-glu-cosides of cis- and trans-resveratrol are called piceids (Rentzsch et al., 2009). Table 4 shows mean values, standard deviations and analysis of variance of stilbenes in Riesling wines from different regions in the Czech Republic.

The most significant compound of the group of stilbenes with a proved relation to a region was *trans*-resveratrol. Mean content of *trans*-resveratrol in the studied 'Riesling' wines was 0.28 mg/l. The highest content of *trans*-resveratrol was found in wines from Znojmo (0.37 mg/l) and Litoměřice (0.34 mg/l) regions. High content of *trans*-resveratrol in wines from Litoměřice region in 'Riesling' wines fully corresponded to the findings which stated that the content of resveratrol in grapes most probably reflects the climatic conditions during the period of vegetation and fungal pressure which is higher in cold and humid conditions in the northern part of the Czech Republic (Melzoch et al., 2001). The values of *trans*-resveratrol in wines from the Czech Republic correspond too with the values for wines from Germany. The concentration of *trans*-resveratrol in white German wines ranged from 0 to 0.7 mg/l with the mean value of 0.3 mg/l (Pour Nikfardjam et al., 1999).

EVALUATION OF FLAVAN-3-OLS IN VARIETAL RIESLING WINES

The major flavan-3-ol monomers found in grapes and wine include (+)-catechin, (-)-epicatechin and (-)-epi-catechin-3-O-gallate. In varietal Riesling wines from the Czech Republic, the content of (+)-catechin and (-)-epicatechin was evaluated (Table 4).

In 'Riesling' wines evaluated in this study, a higher content of (+)-catechin than of (-)-epicatechin was found. The mean content of catechin was 3.28 mg/l. A similar content of (+)-catechin was found in Brasilian 'Riesling' wines (2.96 mg/l) (Ballus et al., 2012) or also in German 'Riesling' wines (2.5 mg/l) (Ritter et al., 1994). The content of (+)-catechin was highly significantly influenced by the region and not influenced by the vintage year. The highest content of (+)-catechin was found in wines from Znojmo region (4.52 mg/l). The mean content of (-)-epicatechin in the studied wines was 1.26 mg/l. The content of (-)-epicatechin found in Czech wines was comparable with German Riesling wines (0.9 mg/l) (Pour Nikfardjam et al., 2007) but significantly lower in comparison with 'Riesling' wines from the Rheingau region (6.1 mg/l) (Ritter et al., 1994) and also with Croatian 'Riesling' wines (6.73 mg/l) (Komes et al., 2007). The content of (-)-epicatechin was also very

Table 4: Mean values, standard deviations and analysis of variance of stilbenes and flavan-3-ols in Riesling wines from the Czech Republic

Chemical parameters (mg/l)	Region					Effect	
	Mikulov (n=4)	Slovácko (n=9)	Velké Pavlovice (n=3)	Znojmo (n=12)	Litoměřice (n=6)	Region F	Year F
*trans-*resveratrol	0.12±0.04a	0.21±0.06a	0.25±0.15ab	0.37±0.25b	0.34±0.20b	2.70*	0.94 n.s.
*cis-*resveratrol	0.08±0.05b	0.22±0.09a	0.22±0.22a	0.14±0.06ab	0.27±0.23a	2.70 *	0.19 n.s.
*trans-*piceid	0.06±0.03	0.12±0.08	0.11±0.14	0.12±0.08	0.14±0.08	0.54 n.s.	0.78 n.s.
*cis-*piceid	0.13±0.09	0.26±0.18	0.28±0.37	0.15±0.06	0.23±0.16	1.07 n.s.	5.90 **
(+)-catechin	2.94±1.50ab	2.04±0.74a	2.65±1.34ab	4.52±1.72b	3.21±1.51ab	4.19***	1.02 n.s.
(-)-epicatechin	0.57±0.35a	0.42±0.16a	0.70±0.61ab	2.03±1.04c	1.75±0.91bc	7.34***	0.98 n.s.

*Significant for $p < 0.05$, ** Significant for $p < 0.01$, *** Significant for $p < 0.001$. Data followed by different letters in the same row are significantly different by LSD test at $p < 0.05$. *n.s.* not significant.

significantly influenced by the region of wine origin. The highest content was found, similarly as for (+)-catechin, in the Znojmo region (2.03 mg/l).

On the basis of these results it can be assumed that the studied flavan-3-ols which are very significantly influenced by the geographical origin and not influenced by the year of vintage can thus represent suitable markers for the geographical origin of wine.

CORRELATION BETWEEN THE EVALUATED PARAMETERS

Among hydroxybenzoic acids significant correlations were found between protocatechuic acid and *p*-hydroxybenzoic acid (r = 0.6726).

Hydroxycinnamic acids are formed within the phenylpropanoid pathway which begins by transformation of phenylalanin aminoacid to *trans*-cinnamic acid, then to *p*-coumaric acid and further spectrum of hydroxycinnamic acids. *P*-coumaric acid is a precursor of vanillic acid, caffeic acid and subsequently other phenolic compounds (IRITI and FAORO, 2010). The mutual formation within the phenylpropanoid pathway is also evident by significant correlations. Significant correlations were found between *p*-coumaric acid and caffeic acid (r = 0.6774) and between *p*-coumaric acid and ferulic acid (r = 0.6856). A highly significant correlation also exists between (+)-catechin and (-)-epicatechin (r = 0.8967).

CHEMOMETRIC ANALYSIS FOR EVALUATION OF GEOGRAPHICAL ORIGIN

The phenolic profile of grapes and wines has a distinctive relation to a variety and is genetically controlled. On the other hand, environmental effects such as geology, soil type and climatic factors can also influence the phenolic profile. For wine authenticity assessment in dependence on the geographical origin, it is therefore ideal to use varietal wines, similarly as in this study.

Canonical variate analysis is often used for analysis of wines on the basis of their geographical origin, e. g. wines from South Italy (GALGANO et al., 2008) or Italian red wines (GALGANO et al., 2011). Canonical variate analysis (CVA) was therefore used for the determination of chemical markers which are also most important for the wine differentiation on the basis of geographical origin in this study.

Were excluded from the evaluation with CVA compounds which do not show a significant effect of terroir on their content with ANOVA, like in studies by GALGANO et al. (2008) and GALGANO et al. (2011). From the CVA therefore were excluded these phenolic compounds: *p*-hydroxybenzoic acid, ferulic acid, fertaric acid, *trans*-piceid, cis-piceid.

The resulting explained variance represents 69.38 % for CV1 and 18.13 % for CV2 which is 87.51 % of total variation. The evaluation of standardized canonical discriminant coefficients for the individual CVs indicates which phenolic compounds are suitable for differentiation of Riesling wines on the basis of their geographical origin (Table 5).

Table 5: Standardized canonical discriminant functions for geographical origin-based differentiation

Chemical parameters	CV1	CV2
protocatechuic acid	-1.1169	-1.2710
vanillic acid	-0.0376	-0.0690
syringic acid	-0.6817	-0.4806
caffeic acid	-0.3097	0.3153
caftaric acid	0.6545	-0.9679
p-coumaric acid	-0.7824	-0.1463
p- coutaric acid	-2.0274	1.6007
trans-resveratrol	-1.6885	-1.9862
cis-resveratrol	2.7804	1.4541
(+)-catechin	3.1449	-0.8953
(-)-epicatechin	-0.9961	2.0477

The canonical function 1 (CV1) loading of discriminant analysis indicated that *cis*-resveratrol and (+)-catechin in a positive way, and *p*-coutaric acid in a negative way were the most important discriminating variables among the terroir-using canonical components. The canonical function 2 (CV2) loading of discriminant analysis indicated that *cis*-resveratrol, (-)-epicatechin and *p*-coutaric acid in a positive way, and (+)-catechin in a negative way were the most important discriminating variables among the terroir-using canonical components. This analysis is successful in its designating function that separates the 'Riesling' wines on the basis of their geographical origin. By the evaluation of Croatian wines, it was found that the samples from Central and Southern Dalmatia have the highest content of *trans*-resveratrol, whereas the wines from Istria have a low content. *Trans*-resveratrol was proved to be one of the markers of geographical origin (Rastija et al., 2009), which was also confirmed by this study with Riesling wines.

Trans-resveratrol and *cis*-resveratrol also participated in discrimination of Italian red wines on the basis of their geographical origin (Galgano et al., 2011). For the discrimination of Greek red wines according to their geographical origin, compounds from the group of flavan-3-ols such as (+)-catechin and (-)-epicatechin (Makris et al., 2006) were also used. It was also possible to use *trans*-resveratrol and (+)-catechin for the discrimination of varietal Cabernet Sauvignon wines on the basis of their geographical origin (Li et al., 2011). These results confirm that the compounds from the group of stilbenes are useful markers for the differentiation of the geographical origin of white wines (Rastija et al., 2009)

as well as red wines (Galgano et al., 2011; Makris et al., 2006; Li et al., 2011), which was also confirmed in this study with varietal 'Riesling' wines from the Czech Republic. Similarly, as in this study, (-)-epicatechin was found as a marker for the geographical origin for Chardonnay wines in Australia (Bellomarino et al., 2009). On the contrary, by comparison of Italian and Spanish wines, especially hydroxybenzoic acids were found as markers of the geographical origin, which was not confirmed in this study. Furthermore, gallic acid was stated as a discriminant parameter of the geographical origin, e. g. for Italian and Spanish wines (Andreu-Navarro et al., 2011). The importance of gallic acid as a discriminant parameter was not confirmed in this study.

The composition of phenolic compounds in wines can be significantly influenced by the vintage year (Ali et al., 2011). None of the compounds which were found as discriminant parameters of the geographical origin in this study was however influenced by the vintage year, which emphasizes their suitability as markers for geographical origin. The composition of phenolic compounds and metabolic fingerprint of wines can be used for their classification on the basis of the geographical origin (Li et al., 2011), which was also confirmed by this study.

A graphic presentation of the studied wines defined by the first two canonical variates (CVs) is shown in Figure 1 (based on CVs of all chemical parameters from Table 5), which allows a good differentiation of wines according to the individual regions.
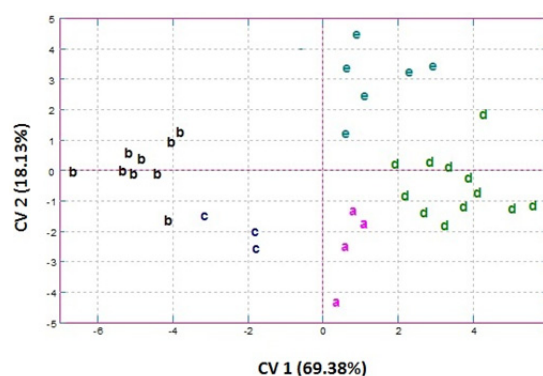

Fig. 1: Canonical variate analysis for first (CV1) and second (CV2) canonical variate of wines from different terroirs: a (Mikulov), b (Slovácko), c (Velké Pavlovice), d (Znojmo), e (Litoměřice)

CV 1 separates well the regions a (Mikulov), d (Znojmo) and e (Litoměřice) which are mainly characterized on the basis of *trans*-resveratrol and (-)-epicatechin from the regions b (Slovácko) and c (Velké Pavlovice) which are mainly characterized by coutaric acid. CV 2 then separates very well the region a (Mikulov) and c (Velké Pavlovice) which are mainly characterized by (+)-catechin from the region e (Litoměřice) which is mainly characterized by *cis*-resveratrol and (-)-epicatechin. With the use of canonical variate analysis, we succeeded in classifying 100 % of all studied wines correctly.

From the view of using phenolic compounds as possible markers for the geographical origin, their relation to grapes and thus to vineyard terroir is important. From the group of hydroxycinnamic acids, *p*-coutaric acid was found as a marker for the geographical origin for its close relation to grapes and terroir. *p*-coutaric acid (*trans-p*-coumaryoltartaric acid) as one of hydroxycinnamic acids which originates within the phenylpropanoid pathway, occurs naturally in grapes.

From the group of stilbenes, *trans*-resveratrol and *cis*-resveratrol were proved as markers for the geographical origin in this study. *Trans*-resveratrol, *trans-* and *cis*-piceid were identified in grapes (Bavaresco et al., 2007). Cis-resveratrol is not present in grapes, but only in wine (WATERHOUSE, 2002), which results from the fact that *cis*-resveratrol is formed by photochemical isomerization of the *trans*-form to the cis-form during the winemaking process (ALI et al., 2010).

Significant markers of the geographical origin in varietal Riesling wines are (+)-catechin and (-)-epicatechin in this study. Flavan-3-ols, the group to which these compounds belong, represent the most significant group of flavonoids in grapes.

There are many environmental factors which influence the formation, transport and accumulation of these compounds in grapes (BRAIDOT et al., 2008). To the main factors of terroir expression there belong soil, climate and variety and mutual effects of these factors (VAN LEEUWEN, 2010). This is why just these parameters, very closely related to the origin of grapes and terroir, were chosen as markers for the geographical origin of wines in this study.

CONCLUSION

Assessment of a wide spectrum of varietal 'Riesling' wines in relation to the geographical origin of wine is one of the first studies of that type in the Czech Republic. Analysis of the phenolic profile of 34 varietal 'Riesling' wines by the use of chemometric methods proved that it is possible to use phenolic compounds to discriminate wines on the basis of their geographical origin. Analysis of phenolic compounds using the HPLC method and, subsequently the CVA method were able to differentiate 100 % of the studied wines on the basis of wine-growing regions in the Czech Republic. To the compounds which are applicable for wine discrimination on the basis of the geographical origin, there belong hydroxycinnamic acid (p-coutaric acid), flavan-3-ols ((+)-catechin, (-)-epicatechin) and stilbenes (*trans*-resveratrol and *cis*-resveratrol).

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