Authentication of Riesling Wines from the Czech Republic on the Basis of the Non-flavonoid Phenolic Compounds

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Abstract

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Eighteen non-flavonoid phenolic compounds comprising hydroxybenzoic acids, hydroxycinnamates, and stilbenes were analysed in 43 monovarietal wines originated from five wine-growing regions in the Czech Republic. The non-flavonoid phenolic compounds in wine were analysed by a HPLC method. The methods of multivariate statistical analysis were used for the wine discrimination on the basis of the geographical origin. The canonical discriminant analysis (CDA) proved the possibility to discriminate wines according to their provenance on the basis of the following parameters: protocatechuic acid, *p*-hydroxybenzoic acid, caftaric acid, *p*-coutaric acid, *trans*-resveratrol, and *cis*-resveratrol. On the basis of statistical analyses, 95.4% of the wine samples were correctly classified. The results therefore indicate that the non-flavonoid phenolic compounds can be used to discriminate the geographical origin of white wines.

Keywords: high-performance liquid chromatography; multivariate analysis; wine; terroir; chemotaxonomy

The grapevine (*Vitis vinifera* L.) is the most widespread cultural plant in the world. The planting location is situated between 52° northern and 40° southern latitude. Wine, the main product made from grapes, has a very close relation to its geographical origin. The terms of authenticity and traceability are therefore very important for the wine producers and consumers.

Wine ranks among the food products which are often subject to falsification from the point of view of the variety or geographical origin. The authenticity of food products has also a significant value for the consumers. It is especially important for wine which is influenced by many factors related to a certain wine-making region: variety, soil and climate, technology of wine production. There are strict rules for defining the geographical origin of wine according to the region in many wine-growing countries (Cynkar *et al*. 2010).

In the Czech Republic, the progressive transition of wine authenticity based on grape variety towards the authenticity based on geographical origin is under way emphasising thus the importance of this study. Particularly wine is a product with a very close relation to its terroir. The wine origin has been considered to be the quality indicator and wine consumers often require information on the provenance and prefer it (Atkin & Johnson 2010). Generally, the differentiation and classification of wine samples on the basis of their chemical composition, geographical origin, variety, or quality belong to the basic applications of chemometric methods in enology (de Villiers *et al*. 2005).

Wines have been classified on the basis of their geographical origin according to the profile of phenolic compounds (Galgano *et al*. 2011; Li *et al*. 2011; Soto Vázques *et al*. 2011), aromatic compounds (Green *et al*. 2011), amino acids (Bouloumpasi *et al*. 2002), mineral elements (Paneque *et al*. 2010; Pérez-Trujillo *et al*. 2011), isotope ratios (Adami *et al*. 2010), or a combination of mineral elements and isotope ratios (Dutra *et al*. 2011).

Phenolic compounds are significant secondary plant metabolites which are found in grape skin, seed, and pulp of the berry and are extracted into wine (La Torre *et al*. 2006). They are a group of secondary metabolites with various chemical structures and functions, and are formed during the physiological plant growth or in a response to different forms of environmental stress (Naczk & Shadidi 2004). Phenolic compounds can be successfully used for wine authenticity assessment as they are characteristic for the type of wine and can provide information on geographical origin (Andreu-Navarro *et al*. 2011). Gambelli and Santaroni (2004), Pereira *et al*. (2006), Rastija *et al*. (2009), and Li *et al*. (2011) observed differences in the concentration of phenolic compounds in wines of different geographical origins.

The wines from four Austrian regions can be differentiated on the basis of their geographical origins with the use of phenolic compounds, preventing thus the wine falsification (Jaitz *et al*. 2010).

Phenolic compounds are among of the most significant qualitative parameters. They are divided into two main groups: non-flavonoids and flavonoids. In the case of white grapes and wines, the group of non-flavonoid phenols is more significant. Non-flavonoids involve hydroxybenzoic acids, hydroxycinnamates, and stilbenes (Li *et al*. 2009; Rentzsch *et al*. 2009; Cheynier *et al*. 2010). Hydroxycinnamic acids in wine originate from hydrolysis of hydroxycinnamic and tartaric esters during the fermentation (Cheynier *et al*. 1986). Hydroxycinnamic acids in wine cause its browning, they are oxidation substrate and contribute to the bitter taste in wine. Hydroxybenzoic acids are a minor group of phenolic compounds contained in wine (Waterhouse 2002). Stilbenes are a subgroup of phenolic compounds naturally present in various plants, but grapes and wine are the most significant dietetic sources of these compounds.

More analytical methods are applicable to the identification and determination of phenolic compounds, but high-performance liquid chromatography is the most used one (Fanzone *et al*. 2010). For the wine authenticity assessment, chemical analysis and chemometric methods are used. Wine is composed of a large amount of compounds, and some of them can serve as chemometric markers for the determination of the geographical origin of wine. Methods of multivariate analysis comprised of principal component analysis (PCA) and canonical discriminant analysis (CDA) are therefore very often used for differentiation of the origin and authentication of wines (Makris *et al*. 2006; Pour Nikfardjam *et al*. 2006; González-Neves *et al*. 2007; Rodriguez *et al*. 2011).

There is a relative lack of information published in relation to the comparison of individual non-coloured phenolic compounds in wines made from one cultivar but from different wine-growing areas (Li *et al*. 2011).

The Czech Republic belongs with its natural and climatic conditions among the wine-growing states of "cool climate viticulture" and its vineyards cover the area of approximately 20 000 ha. Riesling belongs among the typical varieties for "cool climate viticulture" and the same applies to the Czech Republic as well. Riesling is cultivated on the area of 1266 ha in all wine-growing regions and it is the fourth most cultivated variety of grapevine in the Czech Republic.

The aim of this study is to characterise 43 monovarietal wines from five wine-growing regions in the Czech Republic on the basis of the concentration of non-flavonoid phenolic compounds, i.e. hydroxybenzoic acids, hydroxycinnamates, and stilbenes and, in addition, with the use of multivariate statistical analysis and among the non-flavonoid phenolics to find markers which enable to differentiate wines on the basis of their geographical origins, i.e. individual wine-growing regions.

MATERIAL AND METHODS

Wines. 43 commercial monovarietal wines of the variety Riesling were sampled in five wine-growing regions in the Czech Republic. All wines were produced over the years 2005–2008. Eight wines come from the 2005 vintage, 11 from 2006, 13 from 2007, and 11 from 2008. Seven wine samples originated from the wine-growing region of Mikulov, 12 from Slovácko, 3 from Velké Pavlovice, 13 from Znojmo, and 8 from the Litoměřice region.

Determination of non-flavonoid phenolic compounds. In all wine samples of the variety Riesling, the following non-flavonoid phenolics were analysed: hydroxybenzoic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid), hydroxycinnamates (caffeic acid, caftaric acid, *p*-coumaric acid, *p*-coutaric acid, ferulic acid, fertaric acid, ethyl caffeate, *p*-coumaric acid ethylester, ferulic acid ethylester) and stilbenes (*trans*-resveratrol, *cis*-resveratrol, *trans*-piceid, *cis*-piceid).

HPLC analysis. The concentrations of the individual phenolic compounds were determined using the method with direct injection of the sample (Kumšta *et al*. 2012). The method is described here briefly. The samples of wine were centrifuged at 3000 *g* for 6 min and diluted with 100 mmol/l $HClO_4$ in a ratio of 1:1. The chromatographic system Shimadzu LC-10A consisted of two pumps LC-10ADvp, a column thermostat with manual injection valve, a DAD detector SPD-M10Avp, and a personal computer running the chromatographic software LC solution (all 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 *i.d*) filled with the same sorbent. The temperature of the separations was 60°C. The mobile phases were the following: A = 15 mmol/l $HClO_4$ and B = 15 mmol/l $HClO_4$, 10% methanol (MeOH), 50% acetonitrile (ACN). The gradient programme is described in Table 1, with a flow rate of 0.6 ml/minute. The total length of one analysis was 43 min and the regeneration time was 4 minutes. The data were recorded in the range of 200–520 nm. The detection wavelength was 260 nm for vanillic, protocatechuic, and *p*-hydroxybenzoic acids, 275 nm for gallic and syringic acids, 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 and its derivatives, and ferulic acid and its derivatives. The derivatives of hydroxycinnamic acids were calibrated on basic acids from which they were derived.

Reagents and standard solutions. Acetonitrile (ACN) and methanol (MeOH) were 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, and perchloric acid were obtained from Sigma-Aldrich (St. Louis, USA). Other chemicals used were at least of analytical grade and were obtained from local suppliers (Lachema-Penta, Brno, Czech Republic).

Table 1. HPLC gradient programme for non-flavonoid phenolic compounds

Time (min)	A (%)	B (%)
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

A = 15 mmol/l $HClO_4$; B = 15 mmoll $HClO_4$, 10% methanol, 50% acetonitrile

The stock standard solutions were prepared by accurately weighing about 10 mg of each phenol in a 25 ml volumetric flask. The standards were dissolved in 10 ml of acetonitrile and made up to volume with distilled water.

cis-Resveratrol was obtained by exposing the *trans*-resveratrol 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 under the tube in a sealed quartz cell. The concentration of *cis*-resveratrol was expressed as a decrease in the concentration of *trans*-resveratrol (71% conversion). Esters of hydroxycinnamic acids with ethanol were obtained by direct esterification. About 100 mg of each acid were dissolved in 10 ml of absolute ethanol and 0.5 g P_2O_5 was then added. The resulting mixture was incubated overnight at 60°C, and then 5% $NaHCO_3$ was carefully added until the gas ceased to development. The esters were obtained by twice repeated extraction with 5 ml of diethylether. The extracts were dried with anhydrous $MgSO_4$ followed by evaporation of the solvent in a stream of nitrogen.

Statistical analysis. The data obtained were processed according to the wine-growing regions and expressed by mean values and standard deviations. The use of one-way 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. Then the multivariate chemometric methods were used as a supervised learning technique for the differentiation of wines to groups on the basis of the wine-growing regions and finding markers which show a significant discrimination value. When using the multivariate statistical analysis, it is necessary to designate suitable variables for the classification of the samples first. In order to achieve this aim, the elimination of redundant variables using Principal Component Analysis (PCA) is suitable for these purposes. The Principal components are orthogonal, and each principal component is a linear combination of the original variables. Canonical discriminant analysis (CDA) was subsequently performed using variables which

showed higher significance in PCA assessment. Afterwards, the concentrations of the individual non-flavonoid phenolic compounds were compared by a Pearson correlation with the aim to find mutual dependencies.

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

RESULTS AND DISCUSSION

Evaluation of hydroxybenzoic acids profile

The most frequently present hydroxybenzoic acids in wine are gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, syringic acid, salicylic acid, and vanillic acid (Table 2). Among them, gallic acid is present at the highest concentrations (Rentzsch *et al*. 2009).

The most significant hydroxybenzoic acids present in the studied wines were gallic and protocatechuic acids. The mean concentration of gallic acid was 2.38 mg/l (in the range of 1.91–2.86 mg/l). However, the influence of the region on gallic acid concentration was not proved. Another significant hydroxybenzoic acid is protocatechuic acid with the average concentration of 2.67 mg/l (in the range of 1.93–3.32 mg/l). The concentration of protocatechuic acid was the highest one of all hydroxybenzoic acids involved in this study, which was in line with the statement by Komes *et al*. (2007), who found protocatechuic acid to be the main hydroxybenzoic acid of Riesling wines. Similar concentrations of the main hydroxybenzoic acids were also found in white wines from South Africa where the concentration of gallic acids varied in the range of 2.0–4.7 mg/l, however, the concentration of protocatechuic acid was lower (de Villiers *et al*. 2005).

The concentration of protocatechuic acid is influenced by the geographical origin and resembles those of other studied hydroxybenzoic acids (*p*-hydroxybenzoic acid, vanillic acid, syringic acid). It was found by means of ANOVA that *p*-hydroxybenzoic acid is the only one acid whose concentration is influenced by vintage. Therefore the acids, whose concentration is not influenced by vintage, can be considered potential markers of the geographical origin of wine.

The concentration of hydroxybenzoic acids found in Riesling wines from the Czech Republic is relatively significant, as in German wines of cv. Riesling vintage 1989–1998 only non-detectable amounts of the main hydroxybenzoic acids (gallic acid, protocatechuic acid, and syringic acid) were found (Pour Nikfardjam *et al*. 2007).

Evaluation of hydroxycinnamates profile

Hydroxycinnamates significantly influence the colour and flavour of wine. They are primarily found in cell vacuoles of the skin and pulp in the form of tartaric esters (Table 3).

The concentration of none of the studied hydroxycinnamates is influenced by vintage, and, therefore, it is possible to use it successfully for the assessment of the wine authenticity according to its geographical origin. Among the hydroxycinnamic acids, caftaric acid is the most significantly represented one and its concentration reaches 50% of the total hydroxycinnamic acids concentration (Rentzsch *et al*. 2009), which was also confirmed by this study in which the concentration of caftaric acid was in the range of 18.63–40.57 mg/l. The

Table 2. Mean values, standard deviations and analysis of variance of hydroxybenzoic acids in the Riesling wines from the Czech Republic (in mg/l)

	Region					Effect	
	Mikulov	Slovácko	Velké Pavlovice	Znojmo	Litoměřice	region (F)	year (F)
Galic acid	2.46 ± 2.63	2.39 ± 2.16	2.86 ± 1.31	1.91 ± 1.41	2.30 ± 0,68	ns	ns
Protocatechuic acid	1.93^{a} ± 0.64	2.80^{b} ± 0.55	3.32^{b} ± 0.43	2.67^{b} ± 0.93	2.64^{ab} ± 0.62	2.62*	ns
p-Hydroxybenzoic acid	0.48^{a} ± 0.21	0.71^{ab} ± 0.17	0.75^{ab} ± 0.14	0.63^{a} ± 0.34	0.93^{b} ± 0.37	2.68*	5.54**
Vanillic acid	0.85^{b} ± 0.45	0.65^{b} ± 0.40	0.54^{ab} ± 0.30	0.69^{b} ± 0.35	0.07^{a} ± 0.02	5.76***	ns
Syringic acid	0.22^{ab} ± 0.10	0.29^{b} ± 0.06	0.23^{ab} ± 0.07	0.16^{a} ± 0.05	0.24^{b} ± 0.10	4.40**	ns

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; data followed by different letters in the same row are significantly different by *LSD* test at $P < 0.05$; ns – not significant; F – calculated Fischer's F

Table 3. Mean values, standard deviations and analysis of variance of hydroxycinnamic acids in the Riesling wines from the Czech Republic (in mg/l)

	Region					Effect	
	Mikulov	Slovácko	Velké Pavlovice	Znojmo	Litoměřice	region (F)	year (F)
Caffeic acid	$3.07^{a} \pm 1.09$	$7.69^{b} \pm 8.39$	$3.41^{ab} \pm 0.93$	$2.27^{a} \pm 0.57$	$2.34^{a} \pm 0.68$	2.78*	ns
Caftaric acid	$18.63^{a} \pm 7.47$	$27.90^{b} \pm 12.64$	$19.46^{ab} \pm 6.50$	$22.50^{ab} \pm 6.03$	$40.57^{c} \pm 10.23$	6.80***	ns
p-Coumaric acid	1.77 ± 0.79	2.37 ± 1.16	2.79 ± 1.73	1.46 ± 0.68	1.75 ± 0.46	ns	ns
p-Coutaric acid	$1.79^{a} \pm 0.44$	$3.39^{cd} \pm 1.26$	$2.08^{ab} \pm 1.58$	$2.97^{bc} \pm 0.93$	$4.09^{d} \pm 0.74$	6.05***	ns
Ferulic acid	$0.69^{ab} \pm 0.22$	$0.75^{b} \pm 0.27$	$0.72^{ab} \pm 0.24$	$0.48^{a} \pm 0.16$	$0.65^{ab} \pm 0.20$	2.81*	ns
Fertaric acid	3.19 ± 1.38	3.76 ± 0.81	3.48 ± 0.39	3.25 ± 0.64	3.97 ± 0.70	ns	ns
Ethyl caffeate	1.23 ± 1.14	1.13 ± 0.93	1.10 ± 0.29	0.53 ± 0.30	0.49 ± 0.34	ns	ns
p-Coumaric acid ethylester	$0.46^{bc} \pm 0.47$	$0.46^{c} \pm 0.42$	$0.41^{abc} \pm 0.07$	$0.15^{a} \pm 0.08$	$0.15^{ab} \pm 0.14$	2.72*	ns
Ferulic acid ethylester	0.05 ± 0.02	0.11 ± 0.18	0.08 ± 0.03	0.05 ± 0.04	0.04 ± 0.01	ns	ns

*$P < 0.05$, $P < 0.01$, ***$P < 0.001$; data followed by different letters in the same row are significantly different by *LSD* test at $P < 0.05$; ns – not significant; F – calculated Fischer's F

high significance of this acid for the differentiation of the individual wine-growing regions in the Czech Republic was also proved by ANOVA. A significantly lower concentration of caftaric acid (8.8–14.3 mg/l) was found in white wines from South Africa (De Villiers *et al*. 2005).

Another significantly represented compound is *p*-coutaric acid with the concentration in the range of 1.79–4.09 mg/l. This acid also shows highly provable differences between the regions.

In view of the concentrations found in white Riesling wines, it is possible to consider significant also the concentrations of caffeic acid (2.34–7.69 mg/l) and fertaric acid (3.19–3.97 mg/l). However, the concentration of fertaric acid in the studied wines is not influenced by the region.

A similar evaluation of hydroxycinnamic acids profile can be found for Riesling wines from the region of Rheingau (Ritter *et al*. 1994). Similar concentrations were found for caftaric acid (mean value 30.2 mg/l), and lower concentration for coutaric (2.5 mg/l) and caffeic acids (2.3 mg/l), respectively. Therefore, it is possible to conclude that the profile of these hydroxycinnamic acids is influenced by the region. Hydroxycinnamic acids in the Riesling variety wines were also studied in German wines of 1989–1998 vintages (Pour Nikfardjam *et al*. 2007). In comparison to the studied wines from the Czech Republic, significantly lower concentrations of caffeic, caftaric, and *p*-coutaric acids were found in German wines.

Evaluation of stilbenes profile

Stilbenes also belong to non-flavonoid phenolic compounds as well. Stilbenes are a very significant group of phenolic compounds in grapes and wines. Resveratrol and its derivates also are the main phytoalexins produced by grapevine. Grapevine phytoalexins belong to the group of stilbenes, the structure of which is based on *trans*-resveratrol (Table 4).

Table 4, Mean values, standard deviations and analysis of variance of stilbenes in the Riesling wines from the Czech Republic (in mg/l)

	Region					Effect	
	Mikulov	Slovácko	Velké Pavlovice	Znojmo	Litoměřice	region (F)	Year (F)
trans-Resveratrol	$0.15^{a} \pm 0.07$	$0.19^{a} \pm 0.07$	$0.25^{ab} \pm 0.15$	$0.35^{b} \pm 0.25$	$0.32^{ab} \pm 0.17$	2.64*	n.s.
cis-Resveratrol	0.09 ± 0.05	0.18 ± 0.11	0.22 ± 0.22	0.13 ± 0.07	0.22 ± 0.22	ns	n.s.
trans-Piceid	0.09 ± 0.06	0.11 ± 0.07	0.11 ± 0.14	0.12 ± 0.08	0,15 ± 0.08	ns	n.s.
cis-Piceid	0.18 ± 0.09	0.23 ± 0.16	0.28 ± 0.37	0.14 ± 0.07	0.20 ± 0.14	ns	6.62***

*$P < 0.05$, $P < 0.01$, *** $P < 0.001$; data followed by different letters in the same row are significantly different by *LSD* test at $P < 0.05$; ns – not significant; F – calculated Fischer's F

The most significant compound from the group of stilbenes is *trans*-resveratrol which also proved the relation to the geographical origin in this study. The highest concentration of *trans*-resveratrol was found in the wines from the region of Znojmo (0.35 mg/l) and, on the contrary, the lowest concentration was found in the wines from the Mikulov region (0.15 mg/l). The wines of cv. Riesling in the Czech Republic were also investigated by Faitová *et al*. (2004) by the analysis of 76 samples of wine. The concentration of *trans*-resveratrol in the samples from the Bohemian wine-growing region ranged from 0.033 mg/l to 0.421 mg/l with the mean value of 0.117 mg/l. The concentration of *trans*-resveratrol in the samples from the Moravian wine-growing region ranged from 0.033 mg/l to 0.875 mg/l with the mean value of 0.123 mg/l. The wines from cv. Riesling originated from the Czech Republic were also analysed by Šmidrkal *et al*. (2001), the results ranging from 0.2 mg/l to 0.8 mg/l of *trans*-resveratrol. Particularly, the results of *trans*-resveratrol concentration in the wines from the regions Velké Pavlovice (0.25 mg/l), Litoměřice (0.32 mg/l), and Znojmo (0.35 mg/l) are similar to the findings of these authors.

Correlation between the evaluated parameters

A significant positive correlation was found between the protocatechuic and *p*-hydroxybenzoic acids (Pearson coefficient = 0.74, $P < 0.05$).

Among the hydroxycinnamates, several significant correlations dependencies were proved. The most significant correlation was found between ethyl caffeate and *p*-coumaric acid ethylester (Pearson coefficient = 0.91, $P < 0.01$). Significant correlations were also found between caffeic acid and ferulic acid ethylesters (Pearson coefficient = 0.76, $P < 0.05$) and between caftaric acid and *p*-coumaric acid ethylesters (Pearson coefficient = 0.76, $P < 0.05$).

From the results found, it is evident that the amount of *p*-coumaric acid ethylester depends on the changes of several other hydroxycinnamates.

Multivariate statistical analysis

Principal component analysis (PCA) is a multivariate method analysing quantitatively the dependent variables. The aim is to reach the new orthogonal variables called principal components. Principal component analysis was performed with all variable values from all non-flavonoid phenolic compounds. The two first components represent 46.8% of total variance (CV1 = 26.1%, CV2 = 20.7%). On the basis of PCA method, the variables for canonical discriminant analysis were selected (Table 5). The following non-flavonoid phenolic compounds were thus eliminated from further assessment: vanillic acid, syringic acid, and *cis*-piceid. These compounds turned out to be nonsignificant for the wine differentiation on the basis of geographical origin using PCA method.

In order to increase the discrimination value obtained by PCA method, the geographical origin of wines was verified by canonical discriminant analysis (CDA). This method includes only those variables which proved a high significance for the differentiation of the geographical origin using PCA method.

Canonical discriminant 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 in which the principal components summarise total variation (Moreno-Rojas *et al*. 2010). Canonical discriminant analysis is often used for analysis of wines on

Table 5. Factor coordinates of the variables for PC1 and PC2 based on the covariance matrix

	PC 1	PC 2
Galic acid	0.6674	–0.1866
Protocatechuic acid	–0.1063	–0.6900
p-Hydroxybenzoic acid	–0.1609	–0.6149
Vanillic acid	0.2727	0.1239
Syringic acid	–0.0489	–0.4491
Caffeic acid	0.5839	–0.4536
Caftaric acid	–0.6027	–0.2898
p-Coumaric acid	0.5384	–0.7453
p-Coutaric acid	–0.6229	–0.2256
Ferulic acid	0.1080	–0.5771
Fertaric acid	–0.6259	–0.2545
Ethyl caffeate	0.8028	–0.0473
p-Coumaric acid ethylester	0.8897	–0.1336
Ferulic acid ethylester	0.6627	–0.4017
trans-Resveratrol	–0.2453	–0.5407
cis-Resveratrol	–0.0741	–0.7026
trans-Piceid	–0.5122	–0.4362
cis-Piceid	–0.3949	–0.4190

Table 6. Standardised canonical discriminant functions for geographical origin-based differentiation

	CV 1	CV 2
Galic acid	0.6086	–0.5760
Protocatechuic acid	0.0693	**1.1403**
p-Hydroxybenzoic acid	0.8161	**–1.3633**
Caffeic acid	0.4218	0.1112
Caftaric acid	**–1.0945**	**–1.0289**
p-Coumaric acid	0.4222	0.2207
p- Coutaric acid	**2.2303**	0.3371
Ferulic acid	–0.0854	0.3121
Fertaric acid	0.3728	0.8699
Ethyl caffeate	0.6105	0.2894
p-Coumaric acid ethylester	–0.3221	0.9467
Ferulic acid ethylester	0.0898	–0.4942
trans-Resveratrol	**–2.7239**	0.1738
cis-Resveratrol	**1.1981**	–0.4287
trans-Piceid	0.9881	–0.3247

the basis of their geographical origins, e.g. red wines from South Africa (Minnar & Booyse 2004), wines from South Italy (Galgano *et al.* 2008) or Italian red wines (Galgano *et al.* 2011).

Canonical discriminant analysis (CDA) was therefore used for the determination of chemical markers which are also the most important for the wine differentiation on the basis of their geographical origin in this study.

The resulted explained variance represents 57.5% for CV1 and 24.9% for CV2 which is 82.4% of total variation. The differences between the regions were significant (Wilk's lambda = 0.0171 at the value of P = 0.00). The Wilks' lambda value near zero indicates a good discrimination function of the individual CV (Table 6).

It can be seen from the result achieved that the CV1 most considerably correlates with *p*-coutaric acid and *cis*-resveratrol in the positive way, and with *trans*-resveratrol and caftaric acid in the negative way. The CV2 is positively connected with protocatechuic acid, and negatively with *p*-hydroxybenzoic acid and caftaric acid.

Trans-resveratrol was lso found as chemotaxonomical marker of Croatian wines on the basis of its geographical origin (Rastija *et al.* 2009). Protocatechuic acid as a parameter for white wines differentiating based on their geographic origins was also confirmed in the study of Greek wines (Kallithraka *et al.* 2001). Similar parameter as in this study (protocatechuic acid) proved to be a suitable discriminant parameter of the geographical origin for the evaluation of Italian and Spanish wines (Andreu-Navarro *et al.* 2011). Furthermore, gallic acid is stated as a discriminant parameter of the geographical origin, e.g. for Greek wines (Kallithraka *et al.* 2001), Spanish wines (Pena-Neira *et al.* 2000), and Italian and Spanish wines (Andreu-Navarro *et al.* 2011). The importance of gallic acid as a discriminant parameter was not confirmed in this study.

A good differentiation can be seen of wines according to the individual regions (Figure 1). CV1 differentiates well the regions e (Litoměřice) and b (Slovácko) from the region d (Znojmo) and a (Mikulov). CV2 differentiates then the regions e (Litoměřice) from the regions a (Mikulov), and c (Velké Pavlovice).

The use of canonical discriminant analysis was successful in to classifying correctly 95.4% of all

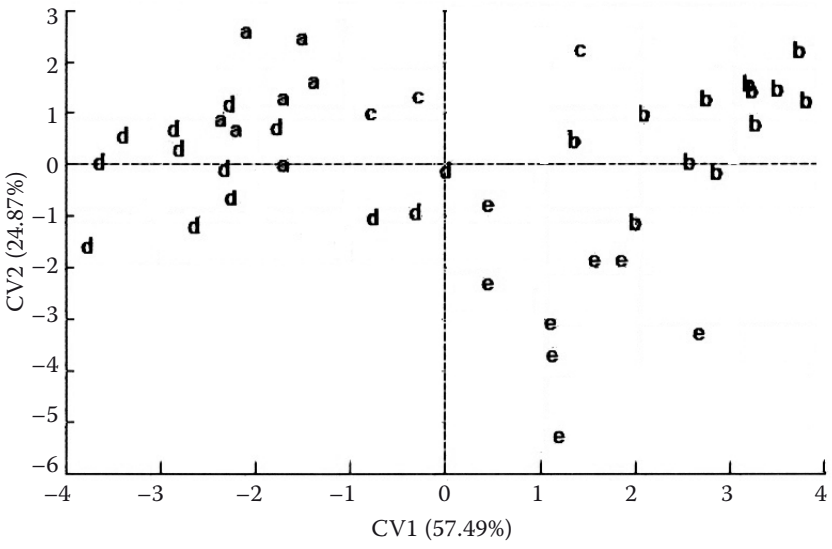

Figure 1. Results of canonical variate analysis for first (CV1) and second (CV2) variate analysis of wines from different regions

a – Mikulov, b – Slovácko, c – Velké Pavlovice, d – Znojmo, e – Litoměřice

the wines studied. In 4 regions, it the individual wines were also successfully classified according to their geographical origin with 100% accuracy. From the whole collection of 43 wines of cv. Riesling, only 2 wines from the region Znojmo were not correctly classified.

CONCLUSIONS

The results of this study show that it is possible to classify white wines according to their geographical origin on the basis of the non-flavonoid phenolic compounds profile. The ANOVA and discriminant analysis showed a clear impact of terroir on the non-flavonoid phenolic compounds profile in Riesling wines. Among the main discriminant parameters which allowed classifying white wines in this study belong protocatechuic acid, *p*-hydroxybenzoic acid, caftaric acid, *p*-coutaric acid, *trans*-resveratrol, and *cis*-resveratrol. The results also proved that, from the group of non-flavonoid phenolics, the compounds belonging above all to stilbenes seem to be more significant discriminating parameter of the geographical origin.

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