

Varietal Differentiation of White Wines on the Basis of Phenolic Compounds profile

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Abstract

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The authenticity of grapevine varieties is a very important topic in the Czech Republic, where varietal wines is very important for wine drinkers. The wines from 7 grapevine varieties were investigated. Sixteen phenolic compounds belonging among hydroxybenzoic and hydroxycinnamic acids, stilbenes, and flavan-3-ols were analysed by HPLC method. The aim of this study was to find markers of varietal origin of wines among the phenolic compounds studied. The analytical parameters obtained were evaluated for this purpose by CVA (canonical varietal analysis) method. It proved to be successful in detecting the following grapevine variety authenticity markers: hydroxycinnamic acids (i.e. *p*-coumaric acid and caffeic acid), hydroxybenzoic acids (protocatechuic acid and syringic acid), and flavan-3-ols ((-)-epicatechin and (+)-catechin).

Keywords: authenticity; grapevine variety; HPLC; chemometric analysis; *Vitis vinifera* L.

From the economic point of view, the grapevine is the most important fruit species in the world. The genus *Vitis* L. includes more than 50 species. Of them, the most widespread and cultivated is the European grapevine, *Vitis vinifera* L. (PAVLOUŠEK *et al.* 2011). It is mainly grown at latitudes from 50°N to 30°N and 40°S to 30°S, that approach the 10°C and 20°C isotherms (MULLINS *et al.* 1992). Within the framework of the *Vitis vinifera* L. species is a great biodiversity and it is estimated that more than 9600 varieties are cultivated worldwide (GALET 2000). The most important fungal diseases and grapevine pests were introduced to the European vineyards towards the end of the 19th century (PAVLOUŠEK 2010). This fact then became an impetus that started the process of breeding and selection of new, interspecific grapevine varieties with increased resistance to fungal diseases as a tool of biological fight against these pathogens.

The characteristics of a wine are mainly determined by the grape variety used in its production, and it is therefore important to establish a pattern

of common characteristics that would allow to identify the wines produced by different grape varieties and at same time scale create differentiation criteria (ALEIXANDRE *et al.* 2002).

In the Czech Republic, wine is classified on the basis of the grapevine variety. This means that, from the viewpoint of customers, the varietal origin of the consumed wine represents a very important parameter of quality. Wine is a complex beverage with a very varied chemical composition. The quality of the produced grapes and wine is dependent on the contents of primary and secondary metabolites.

A “primary metabolite” is directly involved in the growth, reproduction, and development of grapevine while a “secondary metabolite” is not directly involved in those processes, but usually has an important ecological function like the defence against pests and diseases. Many of these primary and secondary metabolites from plants are renowned for their beneficial effects on human health (ALI *et al.* 2010).

Phenolic compounds represent a very important group of secondary metabolites occurring in grapes and wine. The evaluation of the profile of phenolic compounds seems to be a very suitable method of defining the authenticity of the individual varieties.

Biosynthesis of these compounds is strictly controlled by genes controlling the production of the individual enzymes involved in the relevant biosynthetic pathways. The phenolic profile of a given variety reflects to a great extent its genetic potential (MAKRIS *et al.* 2006). Already in the past, the profile of phenolic compounds was used to differentiate red and white wines from South Africa (DE VILLIERS *et al.* 2005), Villányi varietal red wines in Hungary (POUR NIKFARDJAM *et al.* 2006), varietal red wines in Greece (MAKRIS *et al.* 2006), different varietal red wines in Europe and the United States (VÁCLAVÍK *et al.* 2011), and also Argentinian red wines in the Mendoza province (FANZONE *et al.* 2012).

The high performance liquid chromatography (HPLC) belongs to the methods that are popular and most frequently used for the analyses of phenolic compounds contained in grapes and wine (POUR NIKFARDJAM *et al.* 2006; RADOVANOVIČ *et al.* 2010; VÁCLAVÍK *et al.* 2011; FANZONE *et al.* 2012).

The application of sophisticated statistical procedures (the so-called chemometrics) enables to evaluate the analytical data and use the results as a suitable tool enabling the classification of wines on the basis of their varietal origins (ARVANITOYANNIS *et al.* 1999). Statistical and in particular multivariate methods offer the possibility of a fast and efficient extraction of the information originating from large sets of data (DE VILLIERS *et al.* 2005).

The main aim of this study was the discrimination of authentic wines on the basis of grapevine varieties. The wines originated from grapes harvested in the identical stage of ripeness and processed using the identical method of vinification. The aim was chemometric classification of varietal wines on the basis of phenolic profiles.

MATERIAL AND METHODS

Evaluated were the wines made of white grapevine varieties of either European (E) or interspecific (I) origin, namely: Aurelius (E), Chardonnay (E), Müller Thurgau (E), Moravian Muscat (E), Hiber-

nal (I), Malverina (I), and Merzling (I). The wines originated from two localities, i.e. Perná (the wine-growing subregion Mikulov) and Sádek (the wine-growing subregion Znojmo). The characteristics of the Perná site: seasonal average temperature 9°C, seasonal precipitation rate 552 mm, sum of active temperature 2900°C, geological bedrock is limestone, and the soil is sandy-loam with a higher content of lime. The characteristics of the Sádek site: seasonal average temperature 8°C, seasonal precipitation rate 480 mm, and sum of active temperature 2700°C. Geological bedrock is granite and orthorul. The soil is loam-sandy.

The vintage period comprised the years 2005 and 2006. Laboratory analyses were performed in the laboratory of the Department of Viticulture and Enology in Lednice (Mendel University in Brno, Czech Republic).

Vinification technology. All kinds of white wine were made using an identical technology of vinification. Fermentation and ageing of wine took place in glass ballons with the capacity of 50 l. The harvested grapes were destemmed, macerated for a short period of time (2 h at the temperature of 15°C), and pressed to the must yield of 60%. The obtained must was clarified with bentonite Seporit (Ersbloeh Geisenheim AG, Geisenheim, Germany) used in the dose of 100 g/hl. The yeast nutrition preparation Enovit (AEB Group, Brescia, Italy) and pure yeast culture were added into the clear must in doses of 10 g/hl, respectively. After the end of fermentation, the young wine was racked and saturated with sulphur dioxide to the content of 30 mg/l free SO₂. Thereafter, the young wine was bottled (without any further clarification and/or filtration) for analyses.

Determination of phenolic compounds. In all wine samples, the following phenolic compounds were determined: 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. The concentrations of the individual phenolic compounds were determined using an unpublished method with direct injection of the sample as described below. The samples of wine were centrifuged at 3000g for 6 min and diluted in the ratio 1:1 with 100mM HClO₄.

The chromatographic system Shimadzu LC-10A consisted of two pumps LC-10ADvp, a column thermostat with manual injection valve, a DAD detector SPD-M10Avp (all from 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 μ m, 3 \times 150 mm; Grace, Derrfield, USA) equipped with a guard column (3 \times 7.5 mm *i.d.*) filled with the same sorbent. The temperature of separation was 60°C. The mobile phases were as follows: A = 15mM HClO₄ and B = 15mM HClO₄, 10% MeOH, 50% acetonitrile. The flow rate of 0.6mM/min was used. The gradient programme is described in Table 1.

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

The detection wavelength was 200 nm for (+)-catechin and (–)-epicatechin, 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.

Chemicals. 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 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 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 – 15mM HClO₄; B – 1mM HClO₄, 10% MeOH, 50% acetonitrile

The stock standard solution was prepared by accurately weighing about 10 mg of each phenol in a 25 ml volumetric flask. The standard was dissolved in 10 ml of acetonitrile and made up to the mark 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 a 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).

Statistical analysis. The obtained data were processed in relation to the wine-growing regions and expressed by mean values and standard deviations. Then the multivariate chemometric methods were applied, followed by canonical variate analysis (CVA) as a supervised learning technique for the differentiation of the wines into groups on the basis of the grapevine variety and finding markers which show a significant discrimination value. All statistical analyses were performed with the use of a statistical program UNISTAT Version 5.0 (Unistat, Brno, Czech Republic).

RESULTS AND DISCUSSION

In grapes, gallic, and protocatechuic acids belong to the most important hydroxybenzoic acids occurring in grapes. The contents of hydroxybenzoic acids in wine are strongly dependent on the grapevine variety (RENTZSCH *et al.* 2009).

Table 2 shows the mean values and standard deviations of hydroxybenzoic acid in wines from different grapevine varieties. In wines made from grapevine varieties under this study, the highest content of hydroxybenzoic acids was that of protocatechuic acid in the variety Aurelius (1.66 mg/l) and the second one was found out in variety Malverina (1.56 mg/l). Gallic acid was another most frequent hydroxybenzoic acids and its content ranged from 0.81 to 1.17 mg/l in varieties Malverina and Moravian Muscat, respectively. Our values, however, were markedly lower than those found out in South African wines (2.0–4.7 mg/l) (DE VILLIERS *et al.* 2005). Comparable contents of gallic acids were estimated in Brazilian Riesling wine (BALLUS *et al.* 2012). The obtained results demonstrated that gallic acid and protocatechuic

Table 2. Mean values and standard deviations of hydroxybenzoic acids in varietal white wines from Czech Republic.

| Acid (mg/l) | Variety | | | | | | |
|--------------------------|-------------|-------------|-------------|-------------|-------------|-----------------|----------------|
| | Aurelius | Chardonnay | Hibernal | Malverina | Merzling | Moravian Muscat | Müller Thurgau |
| Gallic | 0.87 ± 0.21 | 0.91 ± 0.32 | 0.88 ± 0.10 | 0.81 ± 0.53 | 0.73 ± 0.16 | 1.17 ± 0.39 | 1.07 ± 0.55 |
| Protocatechuic | 1.66 ± 0.64 | 1.22 ± 0.59 | 1.09 ± 0.38 | 1.56 ± 0.13 | 1.28 ± 0.56 | 1.44 ± 1.14 | 1.36 ± 0.92 |
| <i>p</i> -Hydroxybenzoic | 0.91 ± 0.19 | 0.53 ± 0.16 | 0.63 ± 0.33 | 0.59 ± 0.21 | 0.90 ± 0.67 | 0.73 ± 0.33 | 0.63 ± 0.50 |
| Syringic | 0.77 ± 0.50 | 0.75 ± 0.44 | 0.51 ± 0.07 | 0.96 ± 0.31 | 0.48 ± 0.17 | 0.45 ± 0.13 | 0.68 ± 0.35 |

acid were the most important hydroxybenzoic acids occurring in the evaluated wines made of white grapevine varieties.

The group of hydroxycinnamic acids represented another important group of phenolic compounds occurring in white wine. They usually originate from hydroxycinnamic tartaric esters that also occur in grapes (CHEYNIER *et al.* 2010). Out of them, caftaric acid was the most important and amounted to as much as 50% of the total content of hydroxycinnamic acids in wine (RENTZSCH *et al.* 2009).

Table 3 shows the mean values and standard deviations of hydroxycinnamic acid in wines from different grapevine varieties. The highest content of caftaric acid was found out in Malverina and Hibernal, 39.73 and 32.49 mg/l, respectively. These interspecific varieties showed a high content of this hydroxybenzoic acid. Markedly lower contents of caftaric acid were found out in South African varietal wines (8.8–14.3 mg/l) (DE VILLIERS *et al.* 2005) while, on the contrary, very high content of this acid was found out in the variety Riesling, which originated from the Canadian province Ontario (NAGEL *et al.* 1979).

Of hydroxycinnamic acids, *p*-coumaric acid is another important compound and its content in

varietal wines was higher than that of caffeic acid. Its contents were also very different between the individual grapevine varieties and ranged from 4.85 mg/l in Merzling to 8.66 mg/l in Hibernal.

The ratio of caftaric and *p*-coumaric acids in grapes is controlled genetically and for that reason it can be used when identifying and determining the origin of grapevine varieties (RITTER *et al.* 1994). In the individual varieties, the ratios of caftaric and *p*-coumaric acids were diverse and this observation was corroborated also by POUR NIKFARDJAM *et al.* (2007) who published values ranging from 0.5 to 10.6. In this study, the observed differences were significantly greater (17.99–49.66) for varieties Moravian Muscat and Malverina, respectively, but the chemometric statistical analysis did not demonstrate that the ratio between caftaric and *p*-coumaric acids could be a marker of varietal authenticity. *p*-Coumaric acid is another important hydroxycinnamic acid; in the analysed samples of white wine, its content ranged from 0.70 mg/l to 1.26 mg/l (in varieties Merzling and Moravian Muscat, respectively).

Stilbenes are also an important group of phenolic compounds. They are naturally present in many plant families, e.g. *Pinaceae*, *Myrtaceae*, *Fagaceae*,

Table 3. Mean values and standard deviations of hydroxycinnamic acids and ratio of caftaric acid/*p*-coumaric acid in varietal white wines from Czech Republic

| Acid (mg/l) | Variety | | | | | | |
|---|--------------|--------------|---------------|---------------|---------------|-----------------|----------------|
| | Aurelius | Chardonnay | Hibernal | Malverina | Merzling | Moravian Muscat | Müller Thurgau |
| Caffeic | 2.03 ± 1.13 | 1.61 ± 0.42 | 1.38 ± 0.15 | 2.67 ± 1.39 | 1.10 ± 0.37 | 1.89 ± 0.54 | 1.96 ± 0.86 |
| Caftaric | 20.46 ± 5.00 | 30.55 ± 8.81 | 32.49 ± 22.66 | 39.73 ± 19.25 | 17.86 ± 15.81 | 22.67 ± 16.89 | 30.24 ± 19.18 |
| <i>p</i> -Coumaric | 1.09 ± 0.92 | 0.98 ± 0.56 | 1.09 ± 0.49 | 0.80 ± 0.30 | 0.70 ± 0.39 | 1.26 ± 0.96 | 0.71 ± 0.74 |
| <i>p</i> -Coutaric | 6.54 ± 3.87 | 7.87 ± 3.92 | 8.66 ± 4.10 | 7.15 ± 5.16 | 4.85 ± 3.37 | 8.32 ± 6.68 | 6.66 ± 4.17 |
| Ferulic | 0.50 ± 0.21 | 0.52 ± 0.16 | 0.62 ± 0.21 | 1.30 ± 0.81 | 0.59 ± 0.16 | 0.50 ± 0.21 | 0.46 ± 0.16 |
| Fertaric | 3.15 ± 0.64 | 3.01 ± 0.23 | 5.92 ± 1.80 | 6.05 ± 1.37 | 2.87 ± 0.55 | 2.01 ± 0.78 | 3.20 ± 1.09 |
| Ratio of caftaric acid/ <i>p</i> -coumaric acid | 18.77 ± 3.45 | 31.17 ± 4.62 | 29.81 ± 4.06 | 49.66 ± 6.67 | 25.51 ± 4.21 | 17.99 ± 3.56 | 42.59 ± 7.78 |

Table 4. Mean values and standard deviations of stilbenes in varietal white wines from Czech Republic

| Chemical parameters (mg/l) | Variety | | | | | | |
|-------------------------------|-----------|------------|-----------|-----------|-----------|--------------------|------------------|
| | Aurelius | Chardonnay | Hibernal | Malverina | Merzling | Moravian Muscat | Müller hurgau |
| <i>Trans</i> -resveratrol | 0.75±0.46 | 0.66±0.39 | 0.46±0.04 | 1.23±0.75 | 0.28±0.11 | 0.62±0.31 | 0.66±0.59 |
| <i>Cis</i> -resveratrol | 0.90±0.32 | 0.62±0.26 | 0.57±0.24 | 1.79±0.73 | 0.36±0.12 | 0.54±0.18 | 0.67±0.30 |
| <i>Trans</i> -piceid | 0.32±0.32 | 0.27±0.19 | 0.29±0.22 | 0.50±0.43 | 0.15±0.14 | 0.22±0.18 | 0.30±0.42 |
| <i>Cis</i> -piceid | 0.96±0.46 | 0.68±0.40 | 0.85±0.36 | 1.95±1.77 | 0.61±0.37 | 0.82±0.45 | 0.70±0.47 |

Liliacea, *Moraceae*, *Papilionaceae*, and *Vitaceae*. They are synthesised by several species within the family of *Vitaceae* including *Vitis vinifera*. Stilbenes are compounds possessing antifungal activity which enables the plants to fight off a pathogen attack (BAVARESCO & FREGONI 2001). Resveratrol exists in two isomer forms (*cis*- and *trans*-). 3-*O*- β -D-Glucosides of *cis*- and *trans*-resveratrol are called piceids (RENTZSCH *et al.* 2009). *Trans*-resveratrol, *trans*- and *cis*-piceids were identified in grapes (BAVARESCO *et al.* 2007). *Cis*-resveratrol is not present in grapes, but only in wine (WATERHOUSE 2002). *Trans*-resveratrol is the most important compound belonging to this group of polyphenols. Table 4 shows the mean values and standard deviations of stilbenes in wines from different grapevine varieties. The highest content of *trans*-resveratrol was found out in variety Malverina (1.23 mg/l) and it can be said that this was a relatively high level as far as white wines are concerned because, according to RODRIGUEZ-DELGADO (2002), its usual level in white wine is 0.3 mg/l. POUR NIKFARDJAM *et al.* (1999) mentioned that the concentration of *trans*-resveratrol in white German wines ranged from 0 to 0.7 mg/l, and that the mean value was 0.3 mg/l. The levels of *trans*-resveratrol in Czech Riesling wines were also lower and ranged from 0.04 to 0.82 mg/l (KUMŠTA *et al.* 2012).

The major flavan-3-ol monomers found in grapes and wine include (+)-catechin, (–)-epicatechin, and (–)-epicatechin-3-*O*-gallate. Flavan-3-ols belong to the class of flavonoid phenolic compounds. Table 5 shows the mean values and standard deviations of

flavan-3-ols in wines from different grapevine varieties. The highest and the lowest contents of (+)-catechin were found out in varieties Moravian Muscat and Malverina (13.35 and 7.45 mg/l, respectively). A comparable content of this compound was found out in South African Chardonnay, 7.6 mg/l (DE VILLIERS *et al.* 2005), and in white wines from Greece (ANASTASIADI *et al.* 2009). In Brazilian Riesling, the content of this phenolic compound was lower, 3.28 mg/l (BALLUS *et al.* 2012). The highest and the lowest contents of (–)-epicatechin was detected in wines made of varieties Moravian Muscat and Malverina (8.79 mg/l and 4.50 mg/l, respectively). Similar levels were found out also in the varieties Chardonnay (5.80 mg/l) and Sauvignon Blanc (5.90 mg/l) from South Africa (DE VILLIERS *et al.* 2005).

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 in which the principal components summarise the total variation (MORENO-ROJAS *et al.* 2010). Canonical variate analysis is used for the analysis of wines on the basis of their varietal origin (DE VILLIERS *et al.* 2005).

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

The resulting explained variance represented 61.67% for CV 1 and 22.56% for CV 2, which is

Table 5. Mean values and standard deviations flavan-3-ols in varietal white wines from Czech Republic

| Chemical parameters (mg/l) | Variety | | | | | | |
|-------------------------------|-------------|--------------|-------------|-------------|-------------|--------------------|-------------------|
| | Aurelius | Chardonnay | Hibernal | Malverina | Merzling | Moravian Muscat | Müller Thurgau |
| (+)-Catechin | 9.10 ± 1.29 | 10.65 ± 4.56 | 8.63 ± 2.43 | 7.45 ± 3.44 | 8.17 ± 1.19 | 13.35 ± 5.14 | 9.70 ± 4.79 |
| (–)-Epicatechin | 5.31 ± 0.54 | 5.84 ± 2.48 | 5.39 ± 2.39 | 4.50 ± 1.57 | 4.77 ± 1.49 | 8.79 ± 5.39 | 5.32 ± 2.23 |

Table 6. Eigenvalues, percent variance and standard canonical discriminant functions for varietal origin-based differentiation

| Parameter | Discriminant function | | | |
|--|-----------------------|--------|--------|--------|
| | 1 | 2 | 3 | 4 |
| Eigenvalue | 60.517 | 22.141 | 8.425 | 5.870 |
| Variance (%) | 61.67 | 22.56 | 8.61 | 5.98 |
| Cannonical correlation | 0.9918 | 0.9782 | 0.9456 | 0.9244 |
| Wilk' λ | 0.0000 | 0.0003 | 0.0062 | 0.0589 |
| <i>P</i> value | 0.0000 | 0.0014 | 0.0777 | 0.4504 |
| Gallic acid | 2.851 | 0.483 | -0.001 | 1.244 |
| Protocatechuic acid | 2.030 | 8.648 | -4.837 | -2.010 |
| <i>p</i> -Hydroxybenzoic acid | -2.545 | -2.890 | 0.543 | 0.972 |
| Syringic acid | 4.775 | -0.253 | 1.318 | -1.561 |
| Caffeic acid | 1.125 | -4.194 | 2.341 | 1.817 |
| Caftaric acid | -11.666 | 5.478 | -3.890 | -0.657 |
| <i>p</i> -Coumaric acid | -0.810 | 1.222 | 0.367 | -2.505 |
| Caftaric acid/ <i>p</i> -coumaric acid | 4.311 | 0.063 | -0.183 | -1.440 |
| <i>p</i> -Coutaric acid | 9.445 | -9.259 | 6.243 | 3.138 |
| Ferulic acid | -1.217 | -1.219 | -1.494 | -3.271 |
| Fertaric acid | -2.930 | 1.268 | 1.274 | -0.790 |
| <i>Trans</i> -resveratrol | 2.427 | -0.852 | 2.341 | -2.172 |
| <i>Cis</i> -resveratrol | 2.123 | 0.859 | -0.258 | 0.740 |
| <i>Trans</i> -piceid | 1.046 | 0.466 | 0.952 | -2.168 |
| <i>Cis</i> -piceid | 2.446 | 0.697 | -0.948 | 2.091 |
| (+)-catechin | -3.351 | 2.341 | -3.710 | 2.314 |
| (-)-epicatechin | -0.866 | -5.165 | 3.590 | -1.954 |

84.23% of total variation. The differences between both regions were significant (Wilk's lambda = 0.0000 at the value of $P = 0.0000$). The Wilks' lambda value near zero indicates a good discrimination function of the individual CVs (Table 6). It can be seen from the results achieved that the canonical variate 1 most considerably correlates with *p*-coutaric acid and syringic acid in the positive way and with caftaric acid and (+)-catechin in the negative way. The CV 2 is positively related with protocatechuic acid and caftaric acid, and negatively with *p*-coutaric acid and (-)-epicatechin.

A graphic presentation of the studied wines defined by the first two canonical variates (CVs) is shown in Figure 1, in which a good differentiation can be seen of wines according to the grapevine variety. The canonical variate 1 differentiates well the varieties Merzling (b), Hibernal (c) from the varieties Aurelius (a), Malverina (e), Müller Thur-

gau (f), and Moravian Muscat (g). Canonical variate 2 then differentiates the varieties Malverina (e) and Müller Thurgau (f) from Chardonnay (d) and Moravian Muscat (g). With the use of canonical variate analysis, it was possible to classify correctly 100.00% of all varietal wines under this study.

In this study (both in the case of CV 1 and CV 2), the compounds belonging to the class of hydroxycinnamic acids (i.e. *p*-coutaric acid and caftaric acid) showed to be the most important markers of varietal discrimination, and also some hydroxybenzoic acids (protocatechuic acid, syringic acid) and flavan-3-ols ((-)-epicatechin and (+)-catechin) proved to be important.

Similar markers of varietal origin were detected also in studies dealing with the evaluation of wines originating from other wine-producing countries of the world. In South African white wines, the following phenolic compounds were identified as

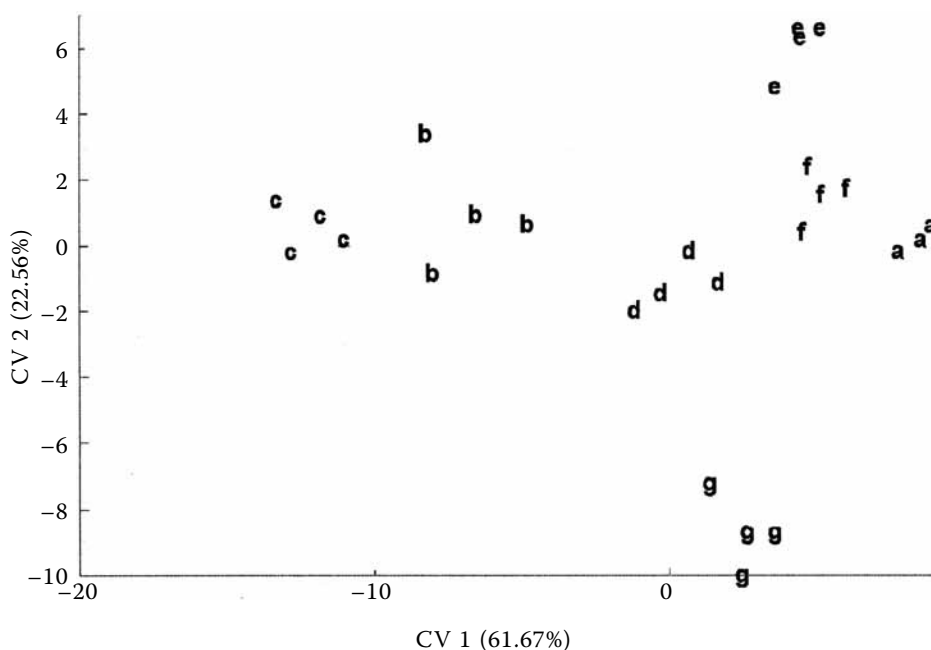


Figure 1. Results of canonical variate analysis for first (CV1) and second (CV2) canonical variate of wines from different varieties

a – Aurelius, b – Merzling, c – Hibernal, d – Chardonnay, e – Malverina, f – Müller Thurgau, g – Moravian Muscat

responsible for the discrimination of individual varieties: caftaric acid, *p*-coumaric acid, (+)-catechin, (–)-epicatechin, ferulic acid, and caffeic acid (DE VILLIERS *et al.* 2005). In young red wines from Greece, MAKRIS *et al.* (2006) successfully used caftaric acid, *p*-coumaric acid, and (–)-epicatechin for the discrimination of individual varieties. In another classification of Greek varietal wines, ANASTASIADI *et al.* (2009) used (+)-catechin, (–)-epicatechin, *p*-coumaric acid, and syringic acid. In Germany, white wines originating from the region Rhenish Palatinate (Pfalz) were classified above all by means of (+)-catechin, (–)-epicatechin (ALI *et al.* 2011).

CONCLUSIONS

This study proved that the profiles of phenolic compounds can not be used for the discrimination of interspecific varieties from those of *Vitis vinifera* L. In spite of this, however, it can be concluded that phenolic compounds can be successfully used for the discrimination of wines made from different grapevine varieties. As the most important and efficient markers, it is possible to recommend hydroxycinnamic acids and flavan-3-ols. Of hydroxycinnamic acids, important discriminant parameters are *p*-coumaric acid and caftaric acid. A very high discriminating value has been shown also by (+)-catechin and (–)-epicatechin. This conclusion corresponds also with the results of other studies mentioned in the text.

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