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Polyphenols, anthocyanins, and *trans*-resveratrol in red wines from the Hungarian Villány region

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Abstract

Epidemiological data indicate that moderate red wine consumption leads to less coronary heart disease and shows anti-carcinogenic effects. Especially, red wine phenolics have been linked to these effects. Sixty-seven red wines from the Hungarian Villány region, of vintages from 1996 to 2003 were analysed for their polyphenolic and anthocyanin composition by means of RP-HPLC/UV–Vis. Varieties with generally high concentrations of polyphenols are Kékfrankos, Merlot and Zweigelt, while anthocyanin content was highest in Shiraz, Oportó, Kékfrankos and Zweigelt. In agreement with other authors, our results show that *trans*-resveratrol content is mainly dependent on variety and vintage year. Principal component analysis (PCA) revealed that flavan-3-ols and delphinidin- and petunidin-3-glucosides are mainly responsible for the separation of wines according to polyphenolic composition. We could not discriminate between varieties or wineries based on polyphenol content, but could for vintage years.

Keywords: Polyphenol; Anthocyanin; HPLC; Principal component analysis; Classification

1. Introduction

Polyphenols are an important group of secondary plant compounds. They not only play an important role in the plant itself as protective agents against fungus attack and UV irradiation (Matern & Grimmig, 1993), but also – according to several epidemiological studies – have a positive effects on human health: they decrease the incidence of coronary heart disease, reduce platelet aggregation, and provide antioxidative and anti-carcinogenic protection (de Lange et al., 2003; Gaziano et al., 1993; Kuulasmaa et al., 2000; Renaud & de Lorgeril, 1992; Stoclet et al., 2004; Tjonneland, Gronbaeck, Stripp, & Overvad, 1999). From a winemaking perspective, they serve as antioxidants, colouring components (especially anthocyanins in red wine), and contribute to the mouthfeel and bitterness of wine (Boulton, 2001; Pour Nikfardjam, 2002; Vidal et al., 2004).

Many studies have determined the polyphenolic composition of wines from various winemaking regions of the world. But these studies mostly focus only single polyphenols, such as *trans*-resveratrol, or on subgroups of the polyphenols, such as flavonoids or hydroxycinnamic acids (Clare, Skurry, & Shalliker, 2004; Kerem, Bravdo, Shoseyov, & Tugendhaft, 2004; Pour Nikfardjam, Rechner, Patz, & Dietrich, 1999; Pour Nikfardjam, László, & Dietrich, in press; Ritter, Götz, & Dietrich, 1994; Soleas, Tomlinson, Diamandis, & Goldberg, 1997; Vitrac, Monti, Vercauteren, Deffieux, & Mérillon, 2002; Vrhovsek, Wendelin, & Eder, 1997). Recently,

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more detailed studies have been published (del Alamo Sanza, Nevares Domínguez, Cárcel Cárcel, & Navas Gracia, 2004; González-Neves et al., 2004; Makris, Psarra, Kallithraka, & Kefalas, 2003; Netzel et al., 2003; Pour Nikfardjam, 2002; Pour Nikfardjam, László, & Dietrich, 2003; Rodríguez-Delgado, González-Hernández, Conde-González, & Pérez-Trujillo, 2002). Despite many technical and analytical improvements, a comprehensive analysis of the polyphenol content of wines, especially in Hungary, is lacking.

The polyphenolic fingerprint can be a very useful tool for the classification of wines. de la Presa-Owens, Lamuela-Raventós, Buxaderas, and de la Torre-Boronat (1995) showed that principal component analysis (PCA) of the polyphenolic fraction can separate wine according to variety. In more recent studies, polyphenolic fingerprints have not only been used to identify the geographic origin of red and white wines, but even their winery origin and the winemaking technology used (Isabel Spranger et al., 2004; Peña-Neira, Hernandez, Garcia-Vallejo, Estrella, & Suarez, 2000; Rodríguez-Delgado et al., 2002; Tinttunen & Lehtonen, 2001). Other methods, such as Fourier-transform-infrared (FTIR) techniques, have also been successfully used for the discrimination of red wine cultivars (Edelmann, Diewok, Schuster, & Lendl, 2001).

The aim of our project was to characterise Hungarian red wines according to their polyphenol and anthocyanin contents and to provide more data on the current polyphenol status of Hungarian red wine from the southernmost Hungarian wine region Villány. Furthermore, we wanted to find whether the polyphenolic fingerprint could be used as a means of classifying wines according to variety and origin.

2. Materials and methods

2.1. Wines

Sixty-seven red wines from two wineries (Bock Pincészet and Polgár Pincészet; both located in Villány/Hungary) from the vintage years 1996–2003 and of various grape varieties were analysed. Several cuvees were also analysed. Their varietal composition was as follows: Cuvee: Cabernet Sauvignon, Cabernet franc, Merlot, Kékfrankos, Kékoportó; Royal Cuvee: Cabernet Sauvignon, Pinot noir; Rubin Cuvee: Kékoportó, Kékfrankos, Merlot.

2.2. Chemicals

All reagents used were of analytical grade unless otherwise stated. HPLC water was from Szkarabeusz (Pécs, Hungary), and acetonitrile (gradient grade) and phosphoric acid from Merck (Darmstadt, Germany). All polyphenol standards were from Extrasynthese (Genay, France).

2.3. Polyphenol analysis

Polyphenolic analysis, by means of HPLC, was performed according to Bonerz (2003). Briefly, a Perkin–Elmer (Wellesley, USA) 200 Series HPLC system, consisting of degasser, autosampler, pump, column oven and PDA detector, was used. Injection volume was 20 μl. A ChromSep (LiChrospher) RP-18 end-capped 250 × 4.6 mm, 5 μm (Varian, Budapest, Hungary), column was used for separation and kept at 30 °C. Chromatograms were recorded at 280 and 520 nm. The gradient consisted of two eluents: (A) water/phosphoric acid (99.5/0.5; v/v); (B) acetonitrile/water/phosphoric acid (50/49.5/0.5; v/v/v). Flow rate was 1.0 ml min^{-1}. Separation of components was achieved as follows: the concentration of A was kept constant for 2 min, then the concentration of B was increased over 5 min to 20%, then further increased to 40% over 18 min, followed by a hold of 6 min. B was increased to 80% over 4 min and then to 100% over 5 min, followed by a hold of 2 min. Equilibrium time to original conditions was 15 min.

Some polyphenols were calculated as their respective free acids: caftaric acid, coutaric acid, fertaric acid, and GRP (grape reaction product, 2-*S*-glutathionyl caftaric acid). All anthocyanins were quantified as malvidin-3-glucoside.

2.4. trans-Resveratrol analysis

The HPLC system for *trans*-resveratrol analysis consisted of a Gynkotek (Germering, Germany) M 480 GT pump, a Rheodyne 8125 (20 μl loop) injector and a Gynkotek M 340 S UV diode array detector. A 250 × 4.6 mm column, packed with 6 μm particle size C_{18} material (Szabó, Ohmacht, Huck, Stögl, & Bonn, 2005) was used for the separations. A Chromeleon® (Softron GmbH, Germering, Germany) data management software system was used for the control of the equipment and for data evaluation. Quantification was carried out using the peak areas method. A multi-step gradient method was applied, using methanol–water–acetic acid (10/90/1; v/v/v) mixture as solvent A and methanol–water–acetic acid (90/10/1; v/v/v) mixture as solvent B at a flow rate of 1.5 ml/min. The gradient profile was: 0.0–18.0 min, from 0% to 40% B; 18.0–25.0 min, from 40% to 100% B; 25.0–27.0 min, 100% B. Chromatographic separations were monitored at 306 nm (Márk, Pour Nikfardjam, Avar, Figler, & Ohmacht, in press).

2.5. Statistics

Statistical analysis was carried out using Excel® (Microsoft Corp., Redmond, USA), SPSS® (SPPS

Corp., Chicago, USA), and S-PLUS® (MathSoft Inc., Cambridge, USA).

3. Results and discussion

3.1. Polyphenol content

Mean polyphenol content were highest in Kékfrankos, Merlot, and Zweigelt, while mean anthocyanin contents were highest in Shiraz, Oportó, Kékfrankos and Zweigelt (Tables 1–3, Fig. 1). These higher values are probably due to the higher proportion of younger wines in these groups, where less oxidative destruction of polyphenols and anthocyanins associated with ageing would have occurred. Monomeric anthocyanins are particularly susceptible to various degradation reactions, such as copigmentation (Boulton, 2001). This degradation is likely responsible for the logarithmic decrease in anthocyanin content observed in this study (Fig. 2).

With polyphenols, this exponential trend was not observed, rather it assumed a more or less linear shape (Fig. 3). In the 1998 vintage, a very high wine polyphenol content was found. 1998 was an exceptionally good year for red wine production in southern Hungary. During the most important months for polyphenol biosynthesis and accumulation (August–October) the weather was very dry and hot except for a very few days of heavy rainfall in 1998. In August there was a very hot period with daily highs of 35–36 °C, while there was no rainfall at all except on 21st, 22nd and 24th with a total of 102 mm. September and October were also very dry, e.g., in October, no rainfall at all was recorded except for the 18th, 25th, and 29th (data taken from our meteorological weather station: Lufft HP-100, Fellbach, Germany). Thus, the increased water stress during the ripening period may have led to higher polyphenol production (Schultz, 2000; Spayd, Tarara, Mee, & Ferguson, 2002).

In 2001, polyphenol content was exceptionally low (Fig. 3). Here also weather conditions were a decisive parameter. Compared to the past 50 year (1950–2000), mean temperature increased by 0.68 °C, while sunshine was reduced by 107 h. Rainfall was increased by 134 mm over the whole vegetation period. Especially in September, a lot more rainfall than normal (+167 mm), lower temperature (−2.1 °C), and less sunshine (−78 h) were recorded, leading to an abnormally high proportion of grey-rotted berries, which in turn caused higher oxidative degradation of polyphenols and anthocyanins through the polyphenol oxidases of the fungus.

3.2. trans-Resveratrol content

In agreement with the results of Frankel, Waterhouse, and Teissedre (1995); Goldberg et al. (1995), and Pour Nikfardjam et al. (1999), *trans*-resveratrol content was mainly dependent on variety and vintage year (Figs. 4 and 5). The highest mean resveratrol concentrations were found in Kékfrankos (2.8 mg/l), Merlot (3.9 mg/l), Pinot noir (3.2 mg/l), Zweigelt (2.6 mg/l) and Rubin Cuvee (3.0 mg/l), with the latter being made up of Kékoportó, Kékfrankos, and Merlot. The overall mean resveratrol concentration was 2.3 mg/l. These results support the data published by Királyné Véghely, Kerényi, and Tyihák (1996); Király-Véghely, Tyihák, Albert, Németh, and Kátay (1998), and Kállay and Török (1998). Király-Véghely et al. (1998) found a mean concentrations of 2.6 mg/l for *trans*-resveratrol in *Vitis vinifera* L. wines.

2002 was clearly the vintage year with the highest *trans*-resveratrol concentration followed by 2001. Mean *trans*-resveratrol concentration was 4.9 mg/l in 2002, about 114% more than the average (2.3 mg/l), and 2.4 mg/l in 2001. According to vine-growing records, 2002 was an exceptionally good year with regard to health status of grapes; we speculate that no extensive destruction of *trans*-resveratrol through the stilbene-oxidase provided by *Botrytis cinerea* could occur.

3.3. Canonical discriminant analysis

Canonical discriminant analysis on normalised data sets of the vintage years 1999–2003 was performed on the following analytes: gallic, caftaric, caffeic, and *p*-coumaric acids, tyrosol, catechin, procyanidin B_2, epicatechin, rutin, *trans*-resveratrol, quercetin, and delphinidin-, petunidin-, peonidin- and malvidin-3-glucosides. Vintage had a significant influence on polyphenolic composition of the wines and the wines could be grouped according to vintage year (Fig. 6). However, good separation could not be achieved based on variety or winery (results not shown). These results are thus in opposition to the findings of Peña-Neira et al. (2000) and Tinttunen and Lehtonen (2001), in which wines from different geographic origins and wineries were discriminable using polyphenolic profiles.

3.4. Principal component analysis

Principal component analysis (PCA) showed that the discrimination between wines is mainly due to the positive correlation between catechin, epicatechin, procyanidin B_2 and tyrosol, and between petunidin- and delphinidin-3-glucosides, respectively, while there is no correlation between the two polyphenol groups (Fig. 7). This leads to the conclusion that mainly flavan-3-ols account for the distinctive difference between vintages. These compounds were especially high in Pinot noir and Zweigelt (tyrosol), Pinot noir, Kékoportó, and Merlot (catechin), Cabernet franc and Shiraz (procyanidin B_2), and Merlot and Kékfrankos, and Cabernet

Table 1
Polyphenol contents (mg/l) of various red wines from the Hungarian Villány region

Variety	Gallic acid	Tyrosol	Caftaric acid	Catechin	GRP	Procyanidin B2	Caffeic acid	Epicatechin	*p*-Coumaric acid	Fertaric acid	Rutin	Ferulic acid	*trans*-Resveratrol	Quercetin
Cabernet franc ($n = 5$)														
Mean	45.3	46.5	42.1	62.1	3.5	83.2	14.6	92.0	9.2	2.5	9.5	0.0	0.8	3.7
Std. dev.	33.2	47.5	11.2	29.1	4.9	53.6	14.3	76.5	6.7	4.3	5.5	0.0	0.5	5.2
Cabernet Sauvignon ($n = 10$)														
Mean	57.8	89.1	53.5	81.8	1.1	43.7	23.1	102.8	6.6	3.4	13.1	0.0	2.8	5.6
Std. dev.	30.5	67.6	19.5	47.3	3.6	44.8	8.6	64.9	7.6	10.3	8.5	0.0	2.4	4.3
Cabernet Sau/fr ($n = 6$)														
Mean	70.9	54.9	54.8	69.0	0.0	51.8	20.0	113	9.2	0.9	17.2	0.0	1.2	2.4
Std. dev.	22.6	33.9	13.0	29.9	0.0	33.2	14.5	40.2	7.2	2.3	11.7	0.0	0.8	3.1
Cuvee ($n = 4$)														
Mean	60.3	72.3	53.3	73.8	0.0	25.4	27.1	110	10.5	1.3	20.2	0.0	1.9	3.5
Std. dev.	9.5	34.3	6.1	21.3	0.0	41.1	8.5	27.6	6.9	2.6	9.6	0.0	0.4	3.8
Kadarka ($n = 2$)														
Mean	57.9	56.0	62.8	77.0	3.1	3.0	5.9	81.9	0.0	3.3	12.1	0.0	0.9	8.7
Std. dev.	6.3	74.8	8.8	37.7	4.4	4.3	8.4	11.7	0.0	4.7	17.2	0.0	1.1	2.5
Kékfrankos ($n = 6$)														
Mean	46.0	82.9	87.0	71.5	0.0	44.2	37.0	126	9.5	0.0	13.6	0.0	2.8	11.3
Std. dev.	10.1	63.2	26.7	33.0	0.0	37.0	21.8	104	5.3	0.0	10.4	0.0	1.7	5.0
Merlot ($n = 10$)														
Mean	65.9	81.2	51.6	89.1	2.5	47.5	18.5	126	10.2	3.0	16.9	2.6	3.9	11.2
Std. dev.	23.7	51.9	19.8	43.0	8.0	43.6	11.1	59.1	10.3	8.5	17.2	7.4	4.0	11.9

Table 2
Polyphenol contents (mg/l) of various red wines from the Hungarian Villány region

Variety	Gallic acid	Tyrosol	Caftaric acid	Catechin	GRP	Procyanidin B2	Caffeic acid	Epicatechin	*p*-Coumaric acid	Fertaric acid	Rutin	Ferulic acid	*trans*-Resveratrol	Quercetin
Oportó ($n = 4$)														
Mean	29.7	63.5	52.4	98.6	0.0	56.7	37.4	75.6	3.7	8.1	19.4	0.0	1.2	9.8
Std. dev.	11.5	71.5	33.4	51.1	0.0	91.2	12.8	83.3	7.5	16.2	14.5	0.0	0.7	8.0
Pinot noir ($n = 4$)														
Mean	45.2	117	55.5	103	0.0	33.9	28.9	64.6	8.9	8.3	9.7	0.0	3.2	7.5
Std. dev.	15.3	65.5	25.5	46.4	0.0	24.2	16.4	68.5	8.7	12.2	8.1	0.0	0.5	2.0
Portugieser ($n = 3$)														
Mean	43.0	67.2	50.2	77.2	4.7	51.7	25.7	87.6	0.4	7.1	14.7	0.7	1.4	5.8
Std. dev.	19.8	75.3	18.3	57.4	8.1	60.5	18.9	71.9	0.7	12.3	10.3	1.2	0.9	3.8
Royal Cuvee ($n = 4$)														
Mean	79.2	82.9	54.6	75.9	0.0	67.5	23.3	98.2	5.8	0.0	13.6	0.0	1.9	5.8
Std. dev.	11.4	53.2	20.8	28.4	0.0	33.1	9.8	43.4	4.4	0.0	16.2	0.0	0.6	3.3
Rubin Cuvee ($n = 3$)														
Mean	53.5	89.3	66.1	79.8	0.0	61.0	30.8	95.9	4.3	11.5	13.3	1.3	3.1	12.2
Std. dev.	2.1	18.2	2.1	32.8	0.0	22.7	19.8	32.6	5.0	10.1	3.1	2.3	2.8	2.6
Shiraz ($n = 2$)														
Mean	50.0	84.3	40.4	68.2	0.0	65.6	24.4	99.7	8.3	0.0	15.5	2.0	1.1	13.4
Std. dev.	8.6	14.3	2.7	5.4	0.0	12.1	2.3	35.6	3.8	0.0	0.4	2.8	0.2	1.8
Zweigelt ($n = 4$)														
Mean	58.3	86.7	77.5	73.4	3.4	60.2	30.6	111	8.0	7.9	6.8	1.9	2.7	6.0
Std. dev.	11.4	77.7	7.5	50.5	6.8	47.1	14.3	51.4	8.2	9.3	5.4	2.3	1.7	6.3
Total ($n = 67$)														
Mean	56.1	77.6	57.3	79.4	1.3	50.1	24.6	103	7.5	3.8	14.2	0.7	2.3	7.4
N	67.0	67.0	67.0	67.0	67.0	67.0	67.0	67.0	67.0	67.0	67.0	67.0	67.0	67.0
Std. dev.	22.5	54.3	21.1	37.7	4.3	43.7	14.4	60.4	7.2	8.3	11.0	3.0	2.2	6.7

Table 3
Anthocyanin contents (mg/l) of various red wines from the Hungarian Villány region (calculated as malvidin-3-glucoside)

Variety	Delphinidin-3-glucoside	Petunidin-3-glucoside	Peonidin-3-glucoside	Malvidin-3-glucoside
Cabernet franc ($n = 5$)				
Mean	63.4	73.0	39.3	656
Std. dev.	72.8	96.5	44.4	696
Cabernet Sauvignon ($n = 10$)				
Mean	92.8	74.2	43.5	565
Std. dev.	61.2	41.2	24.2	375
Cabernet Sau/fr ($n = 6$)				
Mean	24.3	18.8	11.4	129
Std. dev.	26.2	18.6	10.2	129
Cuvee ($n = 4$)				
Mean	69.3	56.9	32.1	394
Std. dev.	25.6	11.5	8.5	76.9
Kadarka ($n = 2$)				
Mean	48.4	46.2	41.0	415
Std. dev.	53.7	56.7	49.8	534
Kékfrankos ($n = 6$)				
Mean	64.4	72.4	82.3	797
Std. dev.	49.4	64.6	65.6	671
Merlot ($n = 10$)				
Mean	53.7	49.6	38.2	276
Std. dev.	55.3	46.9	37.7	261
Oportó ($n = 4$)				
Mean	63.3	103	64.3	1411
Std. dev.	44.3	63.7	42.3	880
Pinot noir ($n = 4$)				
Mean	43.0	39.9	48.1	316
Std. dev.	22.8	24.6	35.6	156
Portugieser ($n = 3$)				
Mean	56.8	57.5	43.7	560
Std. dev.	55.6	51.3	46.4	500
Royal Cuvee ($n = 4$)				
Mean	15.2	14.0	11.7	138
Std. dev.	11.4	15.8	11.3	164
Rubin Cuvee ($n = 3$)				
Mean	45.3	45.6	34.3	352
Std. dev.	38.1	27.2	22.4	166
Shiraz ($n = 2$)				
Mean	152	220	128	1810
Std. dev.	43.4	109.3	42.7	923
Zweigelt ($n = 4$)				
Mean	71.5	88.0	61.7	754
Std. dev.	74.6	84.8	61.8	867
Total ($n = 67$)				
Mean	60.7	62.7	45.0	545
N	67.0	67.0	67.0	67.0
Std. dev.	53.0	60.4	42.0	581

Sauvignon/franc cuvee (epicatechin). These varieties are well known for their rich tannin structure and extensive mouthfeel and certainly flavan-3-ols play an important role in this oral sensation (Vidal et al., 2004). Beside these compounds, caftaric and gallic acid also showed high concentrations in the wines (Table 1).

With regard to anthocyanins, delphinidin- and petunidin-3-glucoside had significant influences on the separation between vintages (Fig. 7). These anthocyanins are known to be more prone to oxidative degradation due to their higher number of hydroxyl groups compared to malvidin-3-glucoside (Montreau, Lattes, & Margulis,

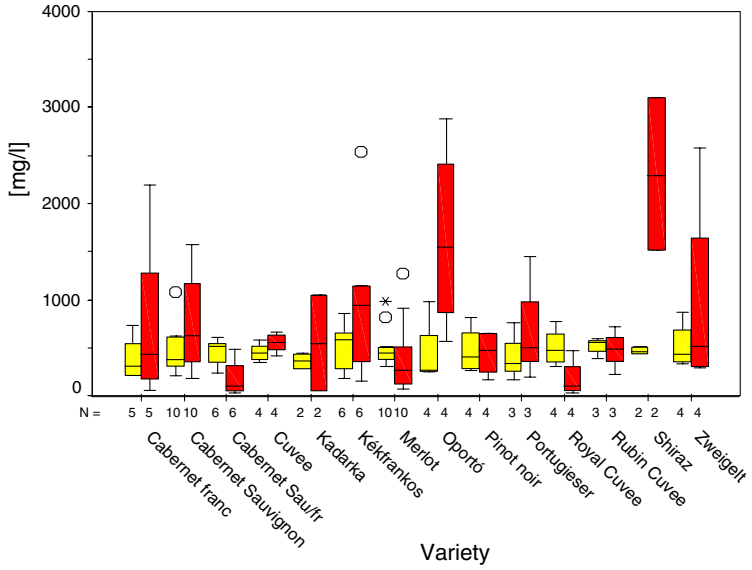

Fig. 1. Mean polyphenol (yellow) and anthocyanin (red) contents of Hungarian red wines from the Villány region ($n = 67$); outliers shown as circles, extremes as stars.

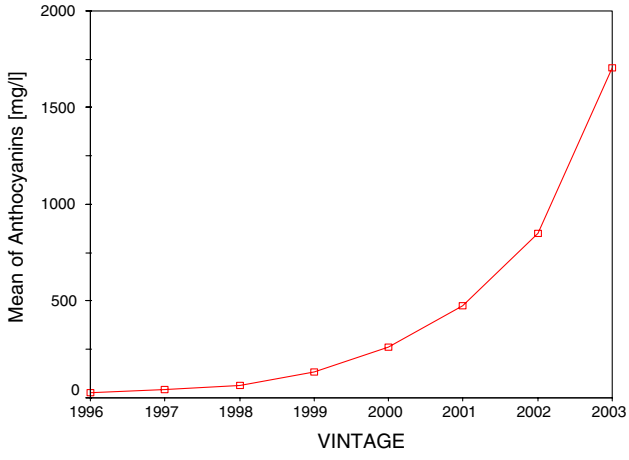

Fig. 2. Mean anthocyanin contents of wines from the Hungarian Villány region from different vintages ($n = 67$).

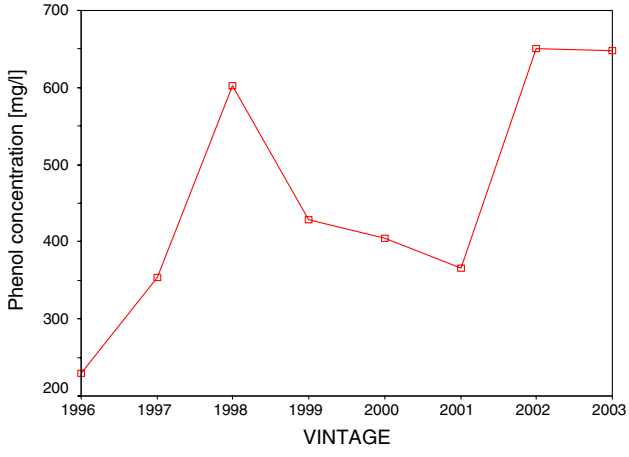

Fig. 3. Mean polyphenol contents of red wines from the Hungarian Villány region ($n = 67$).

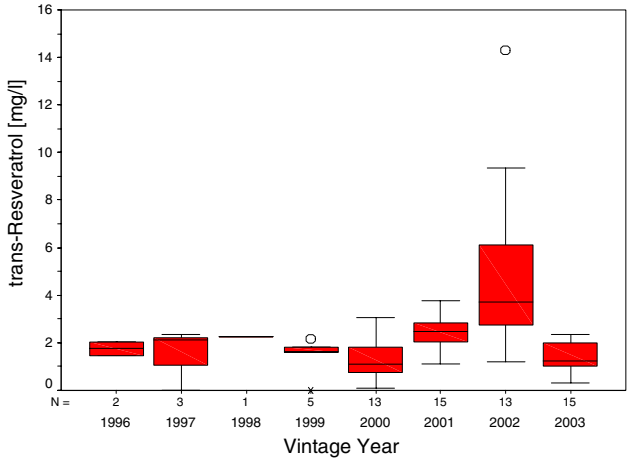

Fig. 4. *trans*-Resveratrol contents of Hungarian red wines from the Hungarian Villány region ($n = 67$); outliers shown as circles, extremes as stars.

1970) and also rapidly undergo reactions with procyanidins, acetaldehyde and hydroxycinnamic acids forming new chemical compounds (Bloomfield, Heatherbell, & Pour Nikfardjam, 2003; Dallas, Ricardo-da-Silva, & Laureano, 1996; Darias-Martin, Carrillo, Diaz, & Boulton, 2001; Darias-Martin et al., 2002). Their rate of oxidative degradation or involvement in copigmentation reactions, thus significantly influences the classification of red wines according to their polyphenolic profiles. Furthermore, this also explains why wines older than the 1999 vintage could not be classified, as monomeric anthocyanins had almost completely degraded and thus could not be used for statistical classification (results not shown).

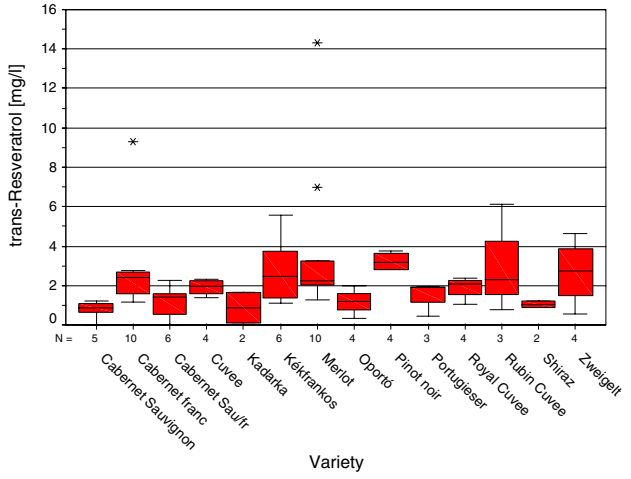

Fig. 5. *trans*-Resveratrol contents of Hungarian red wines from different varieties and cuvees from the Hungarian Villány region ($n = 67$); outliers shown as circles, extremes as stars.

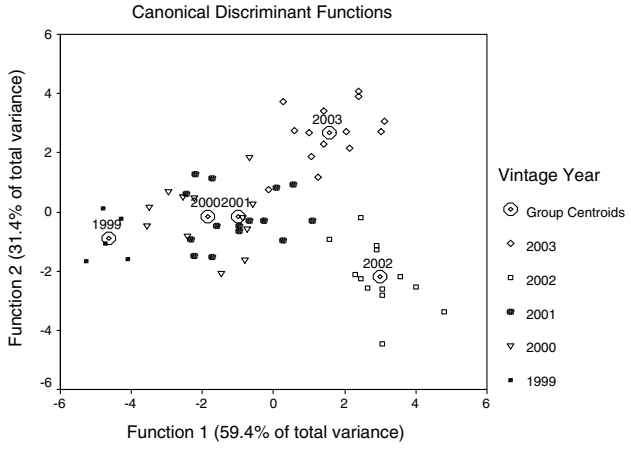

Fig. 6. Canonical discriminant analysis of polyphenolic composition of Hungarian red wines from the Villány region according to vintage year ($n = 61$).

4. Conclusions

Our results provide the largest database available to-date on polyphenol, anthocyanin, and *trans*-resveratrol content of Hungarian red wines from the Villány region. Varieties with generally high concentrations of polyphenols are Kékfrankos, Merlot and Zweigelt, while anthocyanin content were highest in Shiraz, Oportó, Kékfrankos, and Zweigelt. In accordance with other authors, our results show that *trans*-resveratrol content is mainly dependent on variety and vintage year. Wine-making technology has also been identified as an important influencing factor (Clare et al., 2004). PCA revealed that flavan-3-ols and delphinidin- and petunidin-3-glucosides are the main components responsible for the separation of wines based on polyphenolic composition. We could not distinguish between varieties or

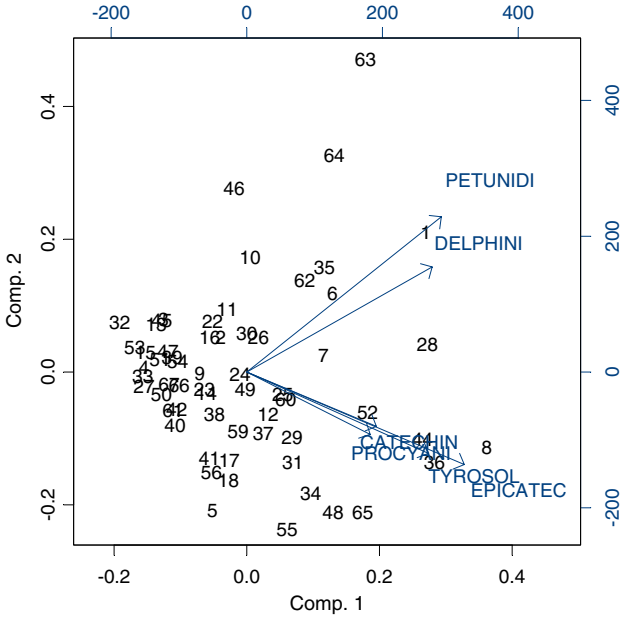

Fig. 7. Principal component analysis of Hungarian red wines from the Villány region (vintage 1999–2003) over 15 polyphenols ($n = 61$; only the 6 most important components are shown).

origin based on polyphenol content, but could for vintage years. Because of various degradation processes occurring during ageing, such as oxidation and copigmentation, it is important to keep in mind that polyphenols and anthocyanins are subject to a vast variety of chemical changes, which affect their analytical determinability and thus have direct consequence for a desired classification of varietal wines according to their polyphenolic and anthocyanin profile.

5. Variety synonyms

Kékoportó (Oportó): Portugieser, Portugais Bleu; *Kékfrankos*: Blaufränkisch, Lemberger.

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