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Characterisation of Vranec, Cabernet Sauvignon and Merlot wines based on their chromatic and anthocyanin profiles

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Abstract: Wines of three different grape varieties, Vranec, Cabernet Sauvignon and Merlot, were characterised in terms of their anthocyanin and chromatic profiles, total polyphenols and antioxidant potential. The total, monomeric, polymeric and co-pigmented anthocyanins were determined by spectrophotometry and the individual anthocyanin compounds were quantified using HPLC–DAD. The chromatic profile was evaluated according to colour density, hue, % red, % blue, % yellow and brilliance (*dA*). The established data were submitted to analysis of variance and principle component analysis in order to evaluate their potential for differentiation of wines according to variety and vintage. The Vranec wines showed distinctive characteristics, with the highest content of anthocyanins and values of colour intensity, % red and *dA*, compared to the other two studied varieties. The content of petunidin-3-glucoside, peonindin-3-glucoside and anthocyanin acetates were established as possible markers for the differentiation of Vranec wines from Cabernet Sauvignon and Merlot wines. However, none of the assayed parameters could be used for the differentiation of Cabernet Sauvignon from Merlot wines. It was observed that wine age limits the successful classification of the wines by variety according to anthocyanins. The chromatic parameters allowed young (aged up to 1 year) to be distinguished from old Vranec wines.

Keywords: wine; anthocyanins; Vranec; colour; differentiation.

INTRODUCTION

Wine is a complex product composed of various compounds of different nature. One of the most important components of wines that influence their quality parameters are polyphenols. Polyphenols contribute to the organoleptic characteristics, such as colour, astringency and bitterness and also exert antimicrobial and antioxidant properties.[1] Anthocyanins are polyphenols of the flavo-

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noid family that play a key role in the colour characteristics and colour stability of wines.[2] Anthocyanins are transferred into the wine from grape skins during the process of vinification. The free monomeric anthocyanins are responsible for the bluish-red colour of young wines, however, during maturation and ageing, the colour of wines transforms to brick-red as a result of the development of polymeric pigments. The extent of red colour in wines depends on the type and concentration of anthocyanins, pH, level of free SO_2 and the degree of polymerisation and co-pigmentation.[3]

Knowledge of the chemical composition of wine and its relation to the grape variety is of great importance in oenology. Several groups of compounds have been proposed for authentication purposes of wines and anthocyanins are among them. The amount of anthocyanins in wines is affected by several factors, such as grape variety, soil, climate, the winemaking techniques, *etc*. However, there are indications that the anthocyanin pattern or fingerprint of grapes is relatively constant and independent of the environmental conditions. As a result, anthocyanins are considered as markers of variety and are used for the differentiation and classification of grapes and wines.[4,5]

There are many publications in which the anthocyanin composition of wines produced from the international grape varieties Cabernet Sauvignon and Merlot is studied,[6–10] but, to the best of our knowledge, there has been almost no detailed research on the anthocyanin profile of wines obtained from these grape varieties cultivated in R. Macedonia. There are also little data on the composition of Vranec wines,[11,12] which is an indigenous Balkan variety.

The aim of this research was to study and compare the colour characteristics, anthocyanin composition, content of total phenolics and antioxidative potential of red wines obtained from three grape varieties, Vranec, Cabernet Sauvignon and Merlot, and produced in four consecutive vintages (2005–2008). Furthermore, based on the considerable number of data obtained from the analysed chemical parameters, the possibility for the classification of the wines according to age and variety was evaluated.

EXPERIMENTAL

Wine samples. A set of 32 monovarietal red wines of vintage ranging from 2005 to 2008, produced and bottled in different local wineries were taken directly from the producers. Most of the samples were of Vranec, an autochthonous Balkan variety (17), then Merlot (8) and Cabernet Sauvignon (7). All wines were collected during the first three months of 2009 and were immediately analysed for their total phenolic content, individual anthocyanins, colour components and antioxidant capacity.

Chemicals. For the chromatographic determinations, acetonitrile (gradient grade, HPLC) and potassium dihydrogen phosphate (*p.a.*) were obtained from Merck (Darmstadt, Germany). Malvidin-3-glucoside purchased from Extrasynthese (Genay Cedex, France) was used for anthocyanin quantification. 2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and gallic acid were from Sigma–Aldrich (St. Louis, MO, USA). Nitric acid, 60 % (*puriss*), used for the determination

of the elements was also purchased from Sigma–Aldrich (St. Louis, MO, USA). Deionised water was obtained from an ultra pure water system TKA Lab micro (Niederelbert, Germany).

Total phenolic content. The total phenols in the wines were determined spectrophotometrically (Cary 50 SCAN, Varian, Inc., USA) at 765 nm, according to the method of Slinkard and Singleton[13] using Folin–Ciocalteu reagent and gallic acid as a standard substance. The results were calculated as the mean of three measurements and are expressed as gallic acid equivalents (*GAE*, mg L^{-1}).

Determination of the colour components. The colour components, colour intensity (*CI*), hue (*H*), brilliance (*dA*), percent red (%*R*), percent yellow (%*Y*) and percent blue (%*B*) were determined by direct measurement of the absorbance of the wines at 420, 520 and 620 nm in 2 mm cuvette after filtering of the samples. *CI* was calculated as the sum of the absorbances at 420, 520 and 620 nm and *H* as the ratio between the absorbance at 420 nm and the absorbance at 520 nm. The other colour variables, *dA*, %*R*, %*Y* and %*B* were calculated according to Glories.[14]

Determination of co-pigmented, monomeric, polymeric and total anthocyanins. The content of monomeric, co-pigmented, polymeric and total anthocyanins was determined according to the method based on the colorimetric effects that wines exert after addition of SO_2 and acetaldehyde.[15] The spectrophotometric measurements were performed in a 2 mm cuvette at 520 nm. The obtained readings were corrected for a 10 mm optical path and used for the calculation of the co-pigmented, monomeric, polymeric and total anthocyanins expressed as absorption units (a.u.) and percents.

Antioxidant activity. The antioxidant capacity of the wines was determined as their antiradical DPPH• activity ($I_{50\%}$). The stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) was used in the procedure described by Brand–Williams.[16] The effect of distinct wine aliquots on DPPH• inhibition (*I*%) was calculated using Eq. (1):

$$I\% = 100\frac{A_0 - A_i}{A_0} \quad (1)$$

where A_0 is the absorbance of the blank and A_i is the absorbance of the sample.

According to the calculated values, a graph was constructed relating the DPPH• inhibition (%) and the wine quantity (mL). From the graph, the quantity of wine (mL) that caused 50 % inhibition of the DPPH radical was determined and expressed as $I_{50\%}$.

Determination of anthocyanins by HPLC. The anthocyanins were determined using a Shimadzu Prominence liquid chromatography system comprising a quaternary pump LC-|-20AT, a photodiode detector SPD-M20A, a column oven CTO-20AC, an autosampler SIL--20AC, an on-line degasser DGU-20A5 and a bus communications module CBM-20A. The system was controlled by Class VP 7.3 software. Separation of the compounds was achieved using a Purospher STAR RP-18e column (250 mm×4 mm I.D, 5 μm particle size) supplied by Merck, Darmstadt, Germany, protected with a guard column of the same packing material (4 mm×4 mm). The chromatographic conditions were as described in a previous study.[17] The anthocyanins were identified by the retention time, elution order and the spectral characteristics of the compounds. The amounts of the anthocyanins identified in the samples are expressed as malvidine-3-glucoside equivalents.

Statistical analysis. The means and standard deviations were calculated using Microsoft Excel® 2003. For comparison of the means, one-way ANOVA and Tukey's post-hoc test were applied at the 95 % significance level ($p < 0.05$). In addition, multivariate analysis including Principal Component Analysis (PCA) was performed using SPSS® 18.0 software.

RESULTS AND DISCUSSION

Chemical profile of the wines

The mean values of the colour components, content of anthocyanins and total phenols, and the antioxidant capacity of the wines (2005–2008 vintages) of Vranec, Cabernet Sauvignon and Merlot varieties are summarized in Table I, while Table II presents the mean values of the analyzed parameters of wines grouped by vintage.

TABLE I. Colour components, content of anthocyanins and total phenols and antioxidant capacity of the red wines grouped by grape variety, Vranec, Cabernet Sauvignon and Merlot; the results are expressed as means ± *SD*; different letters in the superscript in a same row indicate statistically significant means ($p < 0.05$)

Wine characteristic	Vranec (n=17)	Cabernet Sauvignon (n=7)	Merlot (n=8)
Colour intensity (*CI*)	15.4±4.3[a]	10.6±1.8[b]	9.15±2.3[b]
Hue (*H*)	0.66±0.12[a]	0.77±0.11[a]	0.76±0.12[a]
%*R*	54.1±4.0[a]	50.7±3.0[a]	51.1±3.0[a]
%*B*	10.8±0.8[a]	10.7±1.1[a]	10.4±1.6[a]
%*Y*	35.1±3.8[a]	38.6±3.3[a]	38.5±4.0[a]
dA / %	57.2±6.8[a]	51.1±5.7[a]	51.8±5.8[a]
Monomeric anthocyanins, a.u.	1.84±1.01[a]	1.12±0.55[a]	1.2±0.7[a]
Co-pigmented anthocyanins, a.u.	1.12±0.68[a]	0.71±0.39[a]	0.83±0.42[a]
Polymeric anthocyanins, a.u.	5.6±1.6[a]	3.9±0.7[b]	3.2±0.8[b]
Total anthocyanins, a.u.	8.6±2.6[a]	5.7±1.6[b]	5.1±1.4[b]
Monomeric anthocyanins, %	20.5±6.55[a]	19.04±5.05[a]	21.37±7.60[a]
Co-pigmented anthocyanins, %	12.92±7.25[a]	11.78± 3.95[a]	16.43±7.71[a]
Polymeric anthocyanins, %	66.58±9.33[a]	69.18±5.64[a]	62.20±9.50[a]
Total phenols, GAE, mg L^{-1}	2383±596[a]	2323±279[a]	2023±188[a]
Antiradical DPPH$^{\bullet}$ activity $I_{50\%}$, µL wine	21.2±6.3[a]	20.6±5.0[a]	23.0±2.8[a]

Differences in average values of the studied parameters were observed amongst the wines of different variety (Table I). The Vranec variety wines had the highest colour intensity compared to the Cabernet Sauvignon and Merlot wines. This is due to the higher content of total and polymeric anthocyanins observed in Vranec wines, since anthocyanins are related and contribute to the colour intensity of wines.[3] Vranec wines have also shown the highest values for percent red (%*R*), brilliance (*dA*) and monomeric anthocyanins. However, although they were the wines richest in anthocyanins, their content of total phenols was not markedly higher compared to the Cabernet Sauvignon and Merlot wines. The lowest average phenolic content (2023 mg L^{-1} GAE) was measured in the Merlot wines, but this value did not differ significantly from the other two studied wine varieties. ANOVA and the Tukey post hoc test used on the obtained data for the wine characteristics revealed statistically significant differences among the wines only for three parameters: colour intensity and contents of polymeric and total

anthocyanins. These parameters allow differentiation of Vranec wines from the Cabernet Sauvignon and Merlot varieties, but not between the Merlot and Cabernet Sauvignon wines. Regarding the antioxidant capacity, Vranec, Cabernet Sauvignon and Merlot wines exhibited very similar values of DPPH• activity ($I_{50\%} \approx 20$), which did not change with ageing of the wines (Table II).

TABLE II. Colour components, content of anthocyanins and total phenols and antioxidant capacity of the red wines of Vranec, Cabernet Sauvignon and Merlot variety grouped by vintage, 2005–2008; the results are expressed as means ± *SD*; different letters in the superscript in a same row indicate statistically significant means ($p < 0.05$)

Wine characteristic	2005 (n=3)	2006 (n=6)	2007 (n=6)	2008 (n=17)
Colour intensity (*CI*)	14.4±2.5^a	9.7±2.2^a	13.1±4.8^a	13.4±4.9^a
Hue (*H*)	0.80±0.10^a	0.79±0.12^a	0.74±0.09^a	0.65±0.12^a
%*R*	49.5±2.7^a	50.6±3.4^a	51.1±2.5^a	54.4±3.8^a
%*B*	10.8±0.2^a	9.8±0.7^a	11.1±0.8^a	10.8±1.3^a
%*Y*	39.6±3.0^a	39.6±3.4^a	37.8±2.8^a	34.8±3.9^a
dA / %	48.8±5.6^a	50.8±6.5^a	51.9±4.7^a	57.7±6.4^a
Monomeric anthocyanins, AU	1.0±0.2^a	0.87±0.19^a	1.4±0.7^a	1.9±1.0^a
Co-pigmented anthocyanins AU	0.53±0.3^a	0.42±0.18^a	1.1±0.6^a	1.2±0.6^a
Polymeric anthocyanins AU	5.6±1.1^a	3.6±1.1^a	4.9±2.2^a	4.8±1.7^a
Total anthocyanins AU	7.2±1.0^a	4.9±1.0^a	7.3±3.0^a	7.8±2.8^a
Monomeric anthocyanins, %	14.4±1.4^a	18.1±3.7^a	18.3±4.7^a	23.0±7.1^a
Co-pigmented anthocyanins, %	7.9±5.8^a	9.4±5.3^a	14.8±6.9^a	15.5±6.7^a
Polymeric anthocyanins, %	77.7±4.4^a	72.5±6.0^{ab}	66.9±7.6^{ab}	61.4±7.4^b
Total phenols, GAE, mg L^{-1}	2105±86^a	1982±211^a	2469±682^a	2370±482^a
Antiradical DPPH activity $I_{50\%}$, μL wine	20.1±2.2 ^{a}	27.4±6.2^a	20.3±5.1^a	20.0±4.3^a

Additionally, the Pearson's coefficients of correlation were calculated from the data of the analysed characteristics of the wine samples. It was found that the total anthocyanins showed highly positive correlations with the colour intensity (r = 0.967), %*R* (r = 0.743) and brilliance, *dA* (r = 0.740), and negative correlations with hue (r = –0.780) and %*Y* (r = –0.811).

Comparing the average values of wine characteristics grouped by vintage (Table II), it was observed that the %*R* and *dA* were higher in younger wines than in older wines, while older wines were characterized by higher %*Y* and hue values. The younger wines were also richer in monomeric and co-pigmented anthocyanins (Table II). In young red wines, a co-pigmentation phenomenon occurs that involves molecular association between anthocyanins and other, usually, non-coloured organic molecules. The colour exhibited by these co-pigmented complexes could be 30–50 % higher than that of the anthocyanin free forms.[18] On the other hand, during storage and ageing of wines, the co-pigmented complexes break up and polymerization reactions and condensation of anthocyanins with other wine constituents occur. As a result, the concentration of

the monomeric anthocyanins decreases in favour of the polymeric anthocyanins. The newly formed polymeric pigments are compounds with different spectral characteristics that affect the chromatic properties of the wines, changing the hue to higher values.[9,19] In addition, the proportion of yellow colour of aged wines was simultaneously enhanced with increasing degree of polymerization.[20] The data obtained in this study for the analysed colour parameters are in agreement with the results reported by Cliff *et al.*,[21] who compared 4 different wine varieties over 7 vintages and found higher contents of monomeric, co-pigmented and total anthocyanins in the younger wines, regardless of the variety.

The applied ANOVA and Tukey's post-hoc test showed that among the wines of different vintages, statistically significant difference existed only in the content of polymeric anthocyanins. Actually, clear differentiation was possible only between wines produced in 2005 and 2008 or between mature wines with considerable content of polymeric anthocyanins and younger wines in which the polymerization processes were still in an early phase. The wines of 2006 were neither significantly different from the wines of 2007, nor from the wines produced in 2005 and 2008. Concerning the content of total phenols and the antioxidant capacity, statistically significant differences were not evidenced among the wines of different vintages. This indicates that the antioxidant activity of the wines was not related to the age of the wine, but rather with the concentration of phenolic compounds present in the wine. It is known that the antioxidant capacity and the content of phenolics strongly correlate and that wines containing higher levels of phenolic compounds are usually associated with stronger antioxidant activity.[22–24]

In the wine samples, 14 anthocyanin compounds were unambiguously detected and quantified and the average values of individual anthocyanins grouped according to variety are presented in Table III. In order to facilitate the comparison of anthocyanin profiles of the wines, the anthocyanin concentrations were normalized, *i.e.*, the compounds were expressed as percent of the total. The anthocyanin profiles of the analysed wine samples were submitted to statistical tests for wine differentiation.

It was observed that the profiles of the monomeric anthocyanins of the wines were distinctive and related to the grape variety. Malvidine-3-glucoside was the predominant compound in the wines of all grape varieties, which is in agreement with literature data.[4,10] Malvidine acetyl and coumaroyl derivatives were also found in abundance compared to the other anthocyanins. The proportion of malvidine-3-glucoside in the Cabernet Sauvignon wines (36.5 %) was lower compared to the Vranec (44.1 %) and Merlot (40.3 %) wines. On account of this, the proportion of malvidine acetate was substantially higher in the Cabernet Sauvignon (14.3 %) than in Vranec (7.9 %) wines, but almost the same as in the Merlot (14.4 %) wines. The relative content of malvidine-3-glucoside that was

found in the analysed wine samples was slightly lower compared to the Cabernet Sauvignon wines studied by Burns *et al.*[6] and Arozarena *et al.*[19]

TABLE III. Antocyanin composition of Vranec, Cabernet Sauvignon and Merlot wines produced in 2006–2008; De-3-gl (delphinidin-3-*O*-glucoside), Cy-3-gl (cyanidin-3-*O*-glucoside), Pt-3-gl (petunidin-3-*O*-glucoside), Pn-3-gl (peonidin-3-*O*-glucoside), Mv-3-gl (malvidin-3-*O*--glucoside), De-ac (delphinidin-3-*O*-acetylglucoside), Cy-ac (cyanidin-3-*O*-acetylglucoside), Pt-ac (petunidins-3-*O*-acetylglucoside), Pn-ac (peonidin-3-*O*-acetylglucoside), De-coum (delphinidin-3-*O*-*p*-coumarylglucoside), Mv-ac (malvidin-3-*O*-acetylglucoside), Pt-coum (petunidin-3-*O*-*p*-coumarylglucoside), Pn-coum (peonidin-3-*O*-*p*-coumarylglucoside), Mv-coum (malvidin-3-*O*-*p*-coumarylglucoside); the results are expressed as means ± *SD*; different letters in superscript in the same row indicate statistically significant means ($p < 0.05$)

Antho-cyanin	Vranec		Cabernet Sauvignon		Merlot	
	mg L^{-1}	%	mg L^{-1}	%	mg L^{-1}	%
De-3-gl	7.01±3.72	5.93±1.01^a	3.69±0.54	5.92±1.00^a	6.37±2.67	6.01±1.45^a
Cy-3-gl	3.12±1.01	2.63±1.45^a	1.92±0.18	3.09±0.96^a	2.40±0.35	2.27±1.44^a
Pt-3-gl	10.09±6.22	8.53±0.79^a	3.89±0.61	6.24±1.29^b	7.12±3.98	6.73±0.69^b
Pn-3-gl	9.91±6.11	8.38±0.70^a	3.13±0.80	5.02±1.81^b	6.08±3.08	5.74±1.24^b
Mv-3-gl	52.11±35.57	44.07±9.43^a	22.74±7.40	36.50±4.43^a	42.72±31.64	40.34±7.58^a
De-ac	2.81±0.53	2.37±1.87^a	2.31±0.15	3.71±0.86^a	2.97±0.78	2.80±1.35^a
Cy-ac	2.39±0.35	2.02±0.46^a	1.95±0.13	3.13±0.68^a	2.23±0.36	2.11±1.48^a
Pt-ac	2.61±0.77	2.21±1.39^a	2.20±0.31	3.53±0.47^a	2.93±1.04	2.77±0.88^a
Pn-ac	3.43±1.25	2.90±1.18^a	2.17±0.27	3.48±0.54^a	3.30±1.51	3.12±0.79^a
Mv-ac	9.31±5.35	7.88±1.79^a	8.90±5.28	14.29±5.68^a	15.20±12.22	14.35±4.41^a
De-coum	2.43±0.67	2.06±1.53^a	1.87±0.07	3.00±0.77^a	2.22±0.38	2.10±1.17^a
Pt-coum	2.63±0.79	2.22±1.45^a	1.84±0.05	2.95±0.92^a	2.31±0.44	2.18±1.17^a
Pn-coum	3.12±1.19	2.63±1.31^a	1.93±0.12	3.10±0.83^a	2.69±0.72	2.54±1.08^a
Mv-coum	7.30±3.98	6.17±1.08^a	3.77±0.64	6.04±0.60^a	7.36±4.01	6.95±0.88^a
Glucosides	82.23±52.62	69.54±13.38^a	35.37±9.53	56.77±9.49^a	64.69±41.72	61.08±12.40^a
Acetates	14.73±2.90	17.38±4.90^b	8.63±0.85	28.14±2.56^a	11.44±3.70	25.15±4.50^a
Couma-rates	15.47±6.63	13.08±5.37^a	9.40±0.89	15.09±3.12^a	14.58±5.55	13.76±4.30^a
Total	112±62	100±24	53±11	100±15	91±51	100±21

The proportion of malvidine-3-glucoside in the Vranec wines varied in the range 22.4–48.8 %, depending on the wine age. Older wines contained significantly lower levels of this anthocyanin, which indicated its degradation or transformation during the processes of wines ageing. One assumption is that malvidine-3-glucoside in the presence of fermenting yeasts is involved in addition reactions with pyruvate molecules forming vitisin A, a compound that is not detected in grapes but exists in abundance in young wines and decreases with their maturation.[25,26] Mv-3-glucoside also reacts with caffeic acid building pinotin A, the concentration of which increases with ageing and reaches a maximum in about 5-year-old wines.[27]

The highest content of total anthocyanins expressed as malvidin-3-glucoside equivalents was found in the Vranec wines, with an average value of 112.4 mg L^{-1}. Very large variations in the concentrations of total anthocyanins among samples of this variety were observed as a result of the different age of the wines. In general, younger wines were richer in monomeric anthocyanins, detectable by liquid chromatography, than older ones. For Cabernet Sauvignon wines, the average concentration of total monomeric anthocyanins was 53.4 mg L^{-1}, a value lower in comparison to the data reported by González-Neves *et al.*[7,28] A possible explanation for this discrepancy is the domination of older wine samples in the set of Cabernet Sauvignon wines analysed in the present study and the fact that the mentioned literature values referred to wines analysed immediately after completion of the alcoholic fermentation or wines of maximum 1-year old. In the Merlot wines, the content of total monomeric anthocyanins varied from 70 mg L^{-1} for mature wines (3 years) to 185 mg L^{-1} for young wines (1 year).

Statistical analysis of the anthocyanin components with ANOVA ($p < 0.05$) and the Tukey's post hoc test showed possible differentiation of Vranec from Merlot and Cabernet Sauvignon wines according to the content of petunidin-3--glucoside and peonidin-3-glucoside, but not between Cabernet Sauvignon and Merlot wines (Table III). Between wines of Vranec and the other two varieties, Cabernet Sauvignon and Merlot, statistically significant differences were also verified in the proportion of anthocyanin acetates, which agrees with the results published by Arozarena *et al.*[29] According to these authors, differentiation of several varieties of Spanish wines was possible based on their anthocyanin fingerprint, with malvidine-3-glucoside being the most discriminating compound. On the other hand, Kallithraka *et al.*[30] did not obtain consistent anthocyanin profiles even for monovarietal wines and thus considered the anthocyanin composition inappropriate for establishing wine authenticity. According to their study, significant changes in anthocyanins distribution occurs during vinification and particularly during maturation of wines as a consequence of considerable transformations of the monomeric anthocyanins to co-pigmented and polymeric forms.

Anthocyanin ratios in the wines

Genetic factors are determinant for every grape cultivar to have its own specific anthocyanin pattern.[4,31,32] It is assumed that the anthocyanin pattern is constant and independent of the environmental conditions, so it was proposed that it could be used for discrimination of the grape varieties. Moreover, the established ratios between some anthocyanins were considered as parameters upon which the identity of the grape could be verified[7] and the same principle could be used in certifying the authenticity of single variety wines.[33]

Based on the measured concentrations of individual anthocyanins in the studied wines, several anthocyanin ratios were calculated and are presented in Table

IV. These ratios were statistically analysed to identify variations that could be used for differentiation of the wine varieties. Analysis of variance showed that the ratios ΣGluc/ΣAcet, Pt3gl/De3gl, De3gl/Pn3gl and Mv3gl/Mvac in Vranec wines differed significantly from those of Cabernet Sauvignon and Merlot wines, but they did not differ between Merlot and Cabernet Sauvignon wines. These findings indicate that Vranec wines are characterized by a specific anthocyanin profile that distinguishes them from other international varieties of wines, such as Cabernet Sauvignon and Merlot.

TABLE IV. Ratios of anthocyanin compounds detected in Vranec, Cabernet Sauvignon and Merlot wines; De – delphinidins, Pt – petunidins, Pn – peonidins, Mv – malvidins, Coum – coumaroyl derivatives, Acet – acetyl derivatives and Gluc – monoglucosides; different letters in superscript in the same row indicate statistically significant means ($p < 0.05$)

Anthocyanin ratios	Vranec	Cabernet Sauvignon	Merlot
ΣMv/ΣPn	3.98±1.0	4.90±1.78	4.89±2.06
ΣDe/ΣPn	0.86±0.25	1.10±0.11	0.99±0.11
ΣPt/ΣPn	0.78±0.30	0.43±0.40	0.45±0.38
(ΣMv+ΣPt+ΣDe)/ΣPn	5.84±0.90	7.06±1.97	6.89±2.06
ΣGluc/ΣAcet	3.91±1.01^{a}	2.11±0.48^{b}	2.37±0.17^{b}
ΣGluc/ΣCoum	4.84±1.59	3.90±0.78	4.11±1.27
ΣCoum/ΣAcet	0.86±0.24	0.55±0.14	0.61±0.14
Mv3gl/ΣGLuc	0.60±0.09	0.63±0.08	0.62±0.09
Pt3gl/De3gl	1.37±0.18^{a}	1.06±0.10^{b}	1.09±0.18^{b}
De3gl/Pn3gl	0.76±0.12^{b}	1.21±0.24^{a}	1.07±0.16^{a}
Pt3gl/Pn3gl	1.02±0.06^{b}	1.26±0.16^{a}	1.15±0.13^{ab}
Mv3gl/Pn3gl	4.86±1.18	7.49±2.79	6.40±2.47
Mv3gl/Mvac	5.29±1.40^{a}	3.08±1.29^{b}	3.10±0.59^{b}
Mv3gl/Mvcoum	6.44±2.07	5.90±1.19	5.34±1.40
Mvac/Mvcoum	1.24±0.45	2.25±1.14	1.80±0.68

Differentiation of the wines

In order to further study the possibilities for classification and differentiation of wines, Principle Component Analysis (PCA) was performed. PCA is a multivariate method that produces new set of variables obtained as a result of the linear combination of the original descriptors. The new variables or principal components are calculated so as to retain most of the information present in the original data set, but reduced in the least number of variables.[34]

PCA was applied to the data of individual anthocyanins determined in the studied wines. Of the fourteen variables, three components with eigenvalues greater than 1 were obtained, accounting for 87.5 % of the variation. The first component explained 54.1 %, the second 23.1 %, and the third component explained 12.2 % of the total variation. After the third component, the percentage of explained variation drastically decreased, so it could be concluded that the most relevant information is obtainable from the first three components. The

component matrix showing the distribution of variables in the three components is presented in Table V. The predominant variables grouped into the first component include: peonidin coumarate, cyanidin-3-glucoside, delphinidin-coumarate, delphinidin acetate, malvidin-3-glucoside and petunidin acetate. The second component contained peonidin-3-glucoside and petunidin-3-glucoside. Cyanidin acetate dominated in the third component.

TABLE V. Component matrix (coefficients) of the three principal components used for grouping of Vranec, Cabernet Sauvignon and Merlo by anthocyanins

Variables	Component		
	1	2	3
pncoum	0.988	0.027	–0.016
cy3gl	0.980	0.086	0.055
decoum	0.976	–0.128	–0.046
deac	0.962	–0.198	–0.038
mv3gl	–0.950	0.300	–0.023
ptac	0.917	–0.359	–0.053
ptcoum	0.809	–0.115	–0.250
de3gl	0.715	0.272	0.253
mvcoum	0.630	0.110	–0.524
pt3gl	0.233	0.949	0.147
pn3gl	0.216	0.938	0.176
mvac	–0.482	–0.807	–0.289
cyac	0.027	–0.483	0.773
pnac	0.352	–0.434	0.539

The score plot of the first two components is shown in Fig. 1. The principle component analysis confirmed that wines of the local Vranec variety could be easily distinguished from Merlo and C. Sauvignon, while the latter two were harder to separate. The first group, located in the positive part of the principal component 2 (PC 2) and the negative part of the principal component 1 (PC 1) consisted of samples of Vranec wines. The second group, in the central part, with negative values for PC 2, covered the Merlot wines, while a third group was located in the negative part of PC 2 and contained the Cabernet Sauvignon wines. Difficulties achieving ideal varietal classification of Cabernet Sauvignon and Merlot wines according to anthocyanins were also reported by other authors.[24] On the other hand, González-Neves *et al.*[7] obtained satisfactory differentiation of Cabernet Sauvignon from Merlot and Tannat wines, but not between the latter two varieties.

Deviation from the correct grouping was observed in two samples of Vranec wine and one sample of the Cabernet Sauvignon variety. Since these three wines were produced in the 2006 vintage unlike the rest that were of recent date, it was supposed that the observed deviations were due to the age of the samples. This finding tends to support the results published by De Villiers *et al.*,[2] who did not

achieve a successful classification of South African wines of a large range of vintages by their anthocyanin profile. These data may support the assumption that the variety characteristics are obscured as a result of changes in the anthocyanin pattern during ageing and the age of wines becomes a limiting factor in their classifications by anthocyanins.

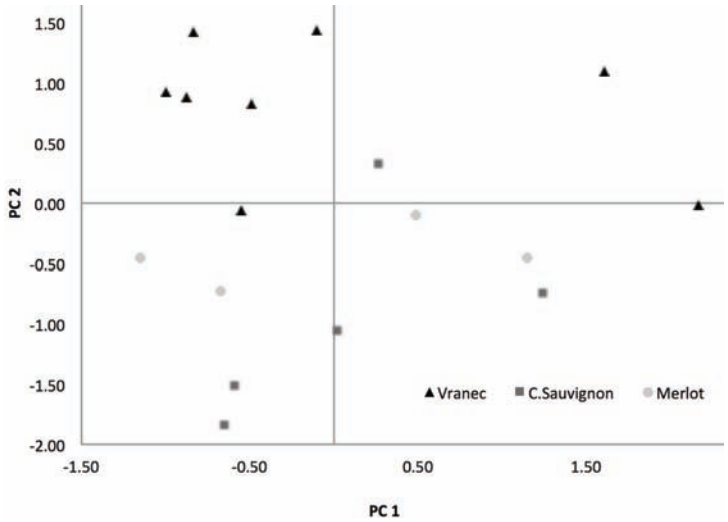

Fig. 1. Score plot of the principal components PC 1 and PC 2 of the wines according to variety.

Since statistical analyses (ANOVA) of the wine colour characteristics and type of anthocyanins obtained by spectrophotometric measurements (Table I) showed that colour intensity and content of total and polymeric anthocyanins could be used as indicators for differentiation of Vranec wines from other wine varieties, an attempt was made to group the wines of the Vranec variety according to age using multivariate methods based on spectrophotometric data.

The component matrix of the variables used for the classification of Vranec wines is presented in Table VI. Of the total number of variables, three main components were extracted that together described 94.5 % of the variation. Most of the variables, such as %*Y*, hue, total anthocyanins, monomeric anthocyanins, brightness, %*R*, colour intensity and polymeric anthocyanins were located in the first component. Percent blue (%*B*) dominated in the second component, while the co-pigmented anthocyanins were descriptors of the third component.

The best segmentation of wine samples according to age was obtained by the loading plot of the first (PC1) and third (PC3) component, as shown in Fig. 2. Relatively clear differentiation of older wines (produced in vintages 2005–2007) was observed in the lower left part of the plane, along the negative parts of PC1 and PC3. In the upper right part of the plane, mostly in the positive part of PC3,

were grouped wines produced in the 2008 harvest (aged 1 year at the time of analysis). Hue and %*Y* emerged as descriptors for the old wines, while young wines correlated with monomeric and total anthocyanins, %*R* and brightness (*dA*).

TABLE VI. Component matrix of the three principal components used for grouping Vranec wines according to age by chromatic parameters; AC – anthocyanins

Variables	Component		
	1	2	3
%*Y*	–0.957	0.220	–0.083
Hue	–0.935	0.294	–0.085
Total AC	0.890	0.435	–0.019
Monomeric AC	0.927	–0.036	–0.049
dA	0.909	–0.386	0.073
%*R*	0.886	–0.422	0.098
Colour intensity	0.849	0.359	–0.347
Polymeric AC	0.701	0.575	–0.363
Co-pigmented AC	0.355	0.362	0.832
%*B*	0.022	0.898	0.169
Monomeric AC	0.927	–0.036	–0.049

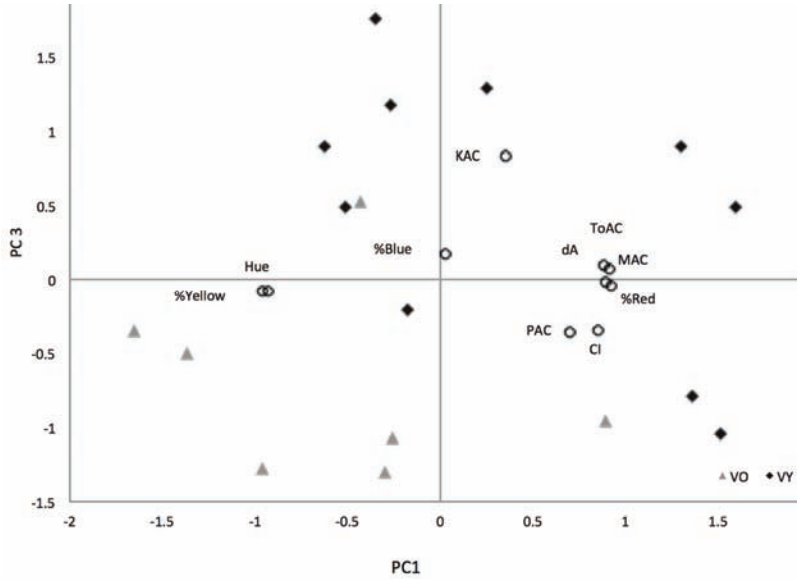

Fig. 2. Distribution of Vranec wines according to age - Score plot and superimposed loading plot of PC 1 and PC 3 using the chromatic characteristics determined by spectrophotometry; VO – old wines (2005, 2006 and 2007) and VY – young wines (2008).

CONCLUSIONS

The results of this study represent a certain contribution to a database of the chemical properties of red wines obtained from the most common grape varieties

grown in Macedonia. The wines of the autochthonous grape variety Vranec were distinguished from Cabernet Sauvignon and Merlot wines by their specific chromatic and anthocyanin profiles, particularly highest colour intensity, %*R*, %*dA*, monomeric, co-pigmented and total anthocyanins. Concerning the individual anthocyanins, Vranec wines contained higher percentage of petunidin-3-glucoside and peonidin-3-glucoside and lower percentage of acetates. The content of total phenols among the investigated wine varieties did not vary significantly, although it was slightly higher in Vranec. The antioxidative potential was similar for all the wine varieties and independent of the wine age. The results obtained using principal component analysis showed that differentiation of the Vranec wines from Cabernet Sauvignon and Merlot varieties could be feasible according individual anthocyanins. Chromatic properties allowed the differentiation of Vranec wines into two groups, young (aged to 1 year) and old. However, further studies are necessary to compile data of a greater number of wines corresponding to several vintages to confirm these observations.

ИЗВОД

КАРАКТЕРИСТИКЕ ВИНА ВРАНЕЦ, КАБЕРНЕ СОВИЊОН И МЕРЛО У ПОГЛЕДУ БОЈЕ И САДРЖАЈА АНТОЦИЈАНА

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Три врсте вина, Вранец, Каберне Совињон и Мерло, су испитане у погледу садржаја антоцијана, карактеристика боје, количине укупних полифенола и антиоксидативног потенцијала. Спектрофотометријски су одређивани укупни, мономерни, полимерни и копигментисани антоцијани, а поједина једињења су квантификована методом HPLC–DAD. Хроматни профил је одређен према интензитету боје, као % црвене, плаве и жуте боје и % сјаја (*dA*). Добијени подаци су анализирани у циљу процене њихове примене у разликовању вина према врсти и години. Вина Вранец су имала најкарактеристичнија својства, са највећим садржајем антоцијана и највећим интензитетом црвене боје и сјаја у односу на друге две врсте вина. Као могући маркери у диференцијацији вина Вранец од вина Каберне Совињон и Мерло могли би послужити петунидин-3-глукозид, пеонидин-3-глукозид и ацетати антоцијана. Са друге стране, ни један од анализираних параметара није могао разликовати вина Каберне Совињон и Мерло. Одређивање врсте вина према садржају антоцијана ипак има своја ограничења, повезана са старошћу вина. Параметри боје могу да послуже у разликовању младих (до 1 године старости) од старих вина само у случају врсте Вранец.

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