ASSAY OF POLYPHENOLS IN MONTENEGRIN VRANAC WINES

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The present paper results from a study on the polyphenols content of commercial wines from the Montenegrin variety 'Vranac', obtained from different localities, studied during three consecutive vintages, using spectrophotometric methodologies. During the three years under study, the commercial wines from the variety 'Vranac' reported a total polyphenols content varying from 1634 mg/l to 2722 mg/l. Total anthocyanins contents varied between 439 and 586 mg/l, while cathechins contents ranged from 230 to 307 mg/l. The vintage year caused variance in the data obtained. While only small variances were verified in the vintages of 2008 and 2009, considerable variations were observed in 2010. For instance, anthocyanins contents were lower and cathechins contents increased. Such variations could be ascribed to the edaphoclimatic conditions in each specific year. From the results obtained, it was possible to group the wines according to vintage year, and only one commercial wine remained separated from the other wines, due to its consistent composition during this three-year study. These results obtained from PCA, allow the conclusion that vintage years cause higher differentiation in the wines' composition than their cultivation site.
Keywords: total phenols content; total anthocyanins content; catechins; vintage year; locality

Untersuchung von Polyphenolen in montenegrinischen Vranac-Weinen. Die vorliegende Arbeit ist das Ergebnis einer Studie zum Polyphenolgehalt von kommerziellen Weinen der montenegrinischen Sorte 'Vranac' aus verschiedenen Regionen. Drei aufeinanderfolgende Jahrgänge wurden mittels spektrophotometrischer Methoden untersucht. In den drei untersuchten Jahren zeigten die handelsüblichen Weine der Sorte 'Vranac' einen Gesamtgehalt an Polyphenolen von 1634 mg/l bis 2722 mg/l. Der Gesamtgehalt an Anthocyaninen schwankte zwischen 439 und 586 mg/l, während der Gehalt an Catechinen zwischen 230 und 307 mg/l lag. Das Erntejahr war für die Varianzen bei den ermittelten Daten verantwortlich. Während in den Jahrgängen 2008 und 2009 nur geringe Varianzen nachgewiesen wurden, waren die im Jahr 2010 erheblich. So waren beispielsweise die Gehalte an Anthocyanen niedriger und die Gehalte an Catechinen höher. Solche Varianzen könnten auf die edaphoklimatischen Bedingungen des jeweiligen Jahres zurückzuführen sein. Aufgrund der erzielten Ergebnisse war es möglich, die Weine nach Jahrgang zu gruppieren, und aufgrund der konsistenten Zusammensetzung während dieser dreijährigen Studie blieb nur ein handelsüblicher Wein von den anderen Weinen getrennt. Diese Ergebnisse, die mittels PCA gefunden wurden, lassen den Schluss zu, dass Weinbaujahre eine höhere Differenzierung in der Zusammensetzung der Weine bewirken als ihr Anbauort.
Schlagwörter: Gesamtphenolgehalt, Gesamtanthocyangehalt, Catechine, Jahrgang, Lage

Polyphenols are one of the most common groups of secondary metabolites in plants, and an important parameter of grape and wine quality. They are responsible for the red color, bitterness and astringency (Chira et al., 2009) and affect the formation of the wine body, stabilization and basis for wine maturation. The main polyphenols of red wine are extracted from skin and seeds during maceration and alcoholic fermentation (Singleton and Esau, 1969). Their accumulation is influenced by a number of factors such as climate, soil, vintage, viticultural practices and yield (Downey et al., 2006). In scientific studies, great attention is paid to the importance of these components in the human diet because they can contribute to human health through different action mechanisms (Frankel et al., 1995; Rodrigo et al., 2011).
Thanks to a favorable geographical position and climate, grape growing and winemaking started to develop in Montenegro in ancient times. The wine region of Montenegro covers areas in the south, around the Lake Skadar basin, and the coastal area of the Adriatic Sea. The most important areas for grape growing and wine production are the Podgorica and Crmnica subregions, which belong to the Montenegrin region of the Lake Skadar basin. These wine subregions have favorable characteristics for growing red wine varieties. In the last decade, the viti-vinicultural sector in Montenegro and in Balkan countries has undergone a revival thanks to the expansion of the areas under vines. In 2007, only three wine producers were officially registered, whereas by the end of 2012, 55 wine producers were enrolled in the wine register in Montenegro. Wine production has developed in different localities, in particular in the areas of Podgorički and Crmnički subregions. Vranac wine still prevails among red wines (around 80 %) as well as in the total wine production in Montenegro (Pajović-Šćepanović et al., 2016).
During the three-year period of research (2008 to 2010), the mean growing temperatures were around 22 °C in Podgorički and around 21 °C in Crmnički. The sum of rainfall in the said growing period ranged from 552 to 796 mm in Podgorički and from 469 to 819 in Crmnički. A characteristic of these subregions are their high values for the heliothermic index (Huglin index), which ranged from 3450 to 3662 in Podgorički and from 2975 to 3116 in Crmnički during the research period (Pajović et al., 2013).
The majority of previous studies related to wine phenolic compounds in the Western Balkan region are related to red wines (mostly Vranac wine). The determination of polyphenol contents in Macedonian red and white wines has been performed by standardized spectrophotometric methods (Ivanova et al., 2010). Also for the Macedonian wine region, an assessment of how the phenolic composition of Vranac wine is affected by different vinification techniques was conducted (Ivanova et al., 2011), and target analyses of phenolic compounds and antioxidant properties of red wines were performed using HPLC-DAD analysis (Ivanova-Petropulos et al., 2015). Šeruga et al. (2011) determined the polyphenol content and antioxidant activity of some red wines in Croatia, using differential pulse voltammetry, HPLC and spectrophotometric methods.
Raičević et al. (2017) examined the influence of some oenological practices on the polyphenolic content of Vranac wine in Montenegro. Extractable phenol compounds (total phenols, anthocyanins, low and high molecular procyanidins) in red wines of Montenegro were evaluated by Pajović et al. (2014a), whereas the influence of yield on the antioxidant capacity of some white and red wines from Montenegro was investigated by Košmerl et al. (2013). Preliminary studies of the phenolic composition and varietal discrimination of Montenegrin red grapes, prepared under the same oenological practice, have been performed by applying the HPLC-DAD technique (Pajović-Šćepanović et al., 2018). The chemical composition of basic parameters for commercial red and white wines in Montenegro has been determined (Pajović et al., 2013; Pajović-Šćepanović et al., 2018).
As far as we know, there has been no study about the phenolic composition of commercial Vranac wine from Montenegro. Therefore, the purpose of this research is: first, to analyze and determine the polyphenolic content of Vranac wines from eight localities by means of spectrophotometric phenol analyses; secondly, to determine the influence of vintage years on the wine phenolic composition; and thirdly, to establish the phenolic uniformity of wines from different wine producers' locali-

ties. Based on the results obtained, this paper provides an overview of typization of the polyphenolic properties of commercial Vranac wines produced in Montenegro.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

The (+)-catechin standard was purchased from Fluka (Buchs, Switzerland); the vanillin (4-hydroxy-3-methoxybenzaldehyde) and Folin-Ciocalteu reagents were obtained from Merck (Darmstadt, Germany). The malvidin-3-O-glucoside standard was purchased from Extrasynthese (Genay, France). All the other reagents used were of analytical purity grade.

WINE SAMPLING

The wines were collected from eight wineries located in two sub-regions (Podgorički and Crmnički) belonging to the Wine Region of Lake Skadar. The chosen localities, subregion, and region were defined by the recently finished Zoning (2018) in Montenegro according to European Regulations. The locations were selected based on their importance for wine production in Montenegro: Lješkopolje (sample I), Ćemovsko polje (samples II and II), Rogami (sample V), Kokoti (sample VII), Beri (IX) from the Podgorički sub-region, and Limljani (sample IV) and Godinje (sample VIII) from the Crmnički subregion. The style of wine production from the chosen producers varies from traditional to industrial. Wine III is recognized as Vranac wine with a special technological process (addition of seeds at the beginning of alcoholic fermentation and maceration) which allows the enrichment of the wine with phenolic compounds.

The wines were collected from producers with a good reputation, directly from the winery. We took young wines (in January) from the vessel (tank or barrel), and three independent samples of each type of wine were collected in each of the years under study (2008 to 2010).

WINE ANALYSIS

Polyphenolic substances were analyzed by spectrophotometric methods with a Jenway 6405 (Keison Products, Chelmsford, U.K.) spectrophotometer. The wines were analyzed as young wines in January of the following year. All analyses were performed in triplicate. The chemical and sensorial characteristics of the wines and the edaphoclimatic conditions during the three crop seasons are presented in the paper by Pajović et al. (2013).

TOTAL PHENOLS CONTENT

The total phenols content was analyzed by the Folin-Ciocalteu method (Singleton et al., 1999), with gallic acid as standard, and the results were expressed as gallic acid equivalent (GAE). In brief, 1 ml of diluted wine (1/5 or 1/10 with distilled water) was mixed with 5 ml of Folin-Ciocalteu phenolic reagent, 20 ml of a Na_2CO_3 solution (20 %) was added, and distilled water was added to make up the total volume to 100 ml. After 60 min, the absorbance was read at 700 nm against a blank prepared with distilled water in a 1 cm cuvette. A calibration curve was constructed using a gallic acid standard solution (0 to 100 mg/l) using the same above-mentioned procedure ($R^2 = 0.9976$, $y = 0.0011x + 0.0063$).

TOTAL ANTHOCYANINS CONTENT

The total anthocyanins content was determined by spectrophotometry at 520 nm (Ribéreau-Gayon and Stonestreet, 1965). The method is based on the discoloration of anthocyanin with sodium hydrogen sulfite. The absorption reading is carried out at 520 nm in a 1 cm cuvette compared to distilled water as blank. By subtracting the absorbance of the sample without the added sodium hydrogen sulfite from the absorbance of the sample reacted with sodium hydrogen sulfite, a value was obtained and converted to mass concentration according to the calibration curve. The calibration curve was constructed with increasing concentrations (0 to 100 mg/l) of malvidin-3-glucoside ($y = 0.0367x + 0.0175$, $R_2 = 0.9989$). The results were expressed as milligrams of malvidin-3-O-glucoside equivalents per liter of wine (mg MAE/l).

CATECHINS CONTENT

The total cathechins content was determined by the vanillic method (Revilla et al., 1991) by use of (+)-catechin as a standard. Wine was diluted with water (1:10), and, after that, three sets of samples were prepared: (A) with wine, HCl, 96 % ethyl alcohol and vanillin; (B) control sample, the same as A, but with water instead of wine; and (C) sample, the same as A, but without vanillin. The absorption of the three samples was read after 30 min at $\lambda = 500$ nm. The blank used was distilled water. The difference in absorption (A – B – C) was interpolated in a linear regression equation using (+)-catechin as standard. The results are expressed in mg of (+)-catechins per liter (mg/l).

DETERMINATION OF COLOR INTENSITY AND HUE

The wine chromatic characteristics (color intensity and hue) were determined by the changed method of Glories (1984). Absorbance was measured at 420, 520 and 620 nm in a 1 cm cuvette (a 1 mm cuvette was used in the original method) in relation to double-distilled water. The value was multiplied by 10. Color intensity is presented as the sum of the absorbances at 420, 520 and 620 nm. The hue of the wine was defined as the A420/A520 ratio.

DATA ANALYSIS

In order to establish the significance of differences between the wines examined (origin of wine – sites/localities) the significance of differences between vintage years, and their interaction for each parameter studied, a two-factorial analysis of variance (ANOVA) was applied. The fulfilment of ANOVA requirements, namely the normal distribution of the residuals and the homogeneity of variance, were evaluated by means of the Shapiro-Wilk's test, and Levene's tests, respectively. For those parameters and factors, where significant differences were detected, an LSD test was additionally applied at a significance level of $p < 0.05$. An analysis of the experimental data was performed using the statistical package IBM SPSS Statistics 20 (IBM Corporation, New York, U.S.A.). The relationships between wines were investigated by principal component analysis (PCA). The phenolic composition of wines: total phenols, total anthocyanins, catechins, color intensity, and hue were used to create a correlation matrix. The data processing was performed using the statistical program Statgraphics Centurion XVI.I (StatSoft, Inc., Tulsa, USA).

RESULTS AND DISCUSSION

POLYPHENOLS COMPOSITION OF THE WINES EXAMINED

Due to the great importance of polyphenolic substances in wine, we assessed the content of total polyphenols, total anthocyanins and catechins by using a spectrophotometric technique (Table 1). This technique allows fast measurement and low reagent consumption for the estimation of polyphenols content, and the possibility of determining the level of wine quality (Ivanova et al., 2010).

Table 1: Content of total phenols, total anthocyanins, and catechins (mg/l) in Vranac wines from different localities/producers during three consecutive vintages (2008 to 2010) (mean values ± SD; n = 3)

	Total phenols			Total anthocyanins			Catechins		
	2008	2009	2010	2008	2009	2010	2008	2009	2010
I	1938 ± 179^{cd}	2079 ± 123^{c}	2154 ± 244^{c}	546 ± 43^{b}	483 ± 20^{de}	432 ± 24^{c}	240 ± 35^{bc}	210 ± 22^{d}	290 ± 24^{c}
II	2210 ± 116^{b}	2270 ± 153^{b}	2390 ± 108^{b}	529 ± 33^{b}	672 ± 19^{a}	413 ± 30^{b}	250 ± 22^{bc}	280 ± 19^{ab}	310 ± 30^{b}
III	2576 ± 95^{a}	2833 ± 138^{a}	2758 ± 153^{a}	522 ± 22^{bc}	624 ± 25^{b}	611 ± 24^{a}	270 ± 16^{ab}	300 ± 22^{a}	350 ± 24^{a}
IV	2176 ± 131^{b}	2151 ± 125^{bc}	2258 ± 187^{bc}	447 ± 28^{d}	499 ± 26^{dc}	418 ± 17^{cd}	230 ± 19^{c}	256 ± 22^{bc}	300 ± 17^{bc}
V	2236 ± 106^{b}	1751 ± 198^{ed}	1718 ± 189^{d}	690 ± 31^{a}	404 ± 17^{f}	521 ± 27^{b}	270 ± 23^{ab}	200 ± 26^{d}	250 ± 27^{d}
VI	2176 ± 124^{b}	2108 ± 99^{c}	2185 ± 123^{c}	480 ± 29^{cd}	445 ± 30^{ef}	409 ± 18^{cd}	250 ± 26^{bc}	240 ± 17^{c}	270 ± 19^{cd}
VII	1921 ± 150^{cd}	1896 ± 227^{d}	1667 ± 202^{d}	462 ± 28^{d}	471 ± 19^{de}	388 ± 26^{d}	240 ± 21^{bc}	200 ± 17^{d}	246 ± 26^{d}
VIII	1836 ± 153^{d}	1592 ± 163^{e}	1473 ± 29^{e}	504 ± 27^{bc}	421 ± 23^{f}	398 ± 20^{cd}	230 ± 29^{b}	199 ± 35^{d}	260 ± 20^{d}
IX	2006 ± 182^{c}	2041 ± 97^{cd}	1396 ± 51^{e}	462 ± 26^{d}	533 ± 40^{c}	321 ± 13^{e}	240 ± 29^{bc}	255 ± 22^{cb}	268 ± 13^{cd}
	$\mathbf{2119 \pm 337^{A}}$	$\mathbf{2080 \pm 418^{AB}}$	$\mathbf{2000 \pm 536^{B}}$	$\mathbf{516 \pm 76^{A}}$	$\mathbf{506 \pm 89^{A}}$	$\mathbf{446 \pm 86^{B}}$	$\mathbf{247 \pm 28^{B}}$	$\mathbf{238 \pm 42^{B}}$	$\mathbf{283 \pm 38^{A}}$

Different superscript lowercase letters in the same row indicate significantly different means ($p < 0.05$) for wines of the same vintage. Different bold superscript capital letters indicate significantly different means ($p < 0.05$) for wines between the vintages examined.

TOTAL POLYPHENOLS CONTENT IN THE WINES ANALYZED

The method used in this study was Folin-Ciocalteu's analysis, which is the most common method providing information on the total concentration of polyphenols. The results obtained are presented in Table 1 and show that the total phenols content in wines was statistically different between producers in each year under analysis: between 1836 and 2576 mg/l for the 2008 vintage, from 1592 to 2833 mg/l for the 2009 vintage, and from 1396 to 2758 mg/l for the 2010 vintage. Regarding the producers' localities examined and taking into account the average of the three vintages studied, the content varied between 1634 (VII) and 2722 mg/l (III). The majority of wine producers had a relatively uniform content of total phenols in the three vintage years investigated. The results presented are in compliance with the averages found for Vranac wines, in the Podgorički sub region: 1950 mg/l (Pajović et al., 2011) and 1906.5 mg/l (Košmerl et al., 2013).

These results are also in line with results for Vranac wines prepared with the addition of different oenological products, in the same vintage of 2008 (1950 to 3270 mg/l) and 2009 (2450 to 3280 mg/l), in the Podgorički subregion (Raičević et al., 2017). The results obtained are in the same range for total phenols content as those found for red wines from other Western Balkan countries: Macedonia (1394 to 3097 mg/l) (Ivanova-Petropulos et al., 2015) and Croatia (1012 to 3264 mg/l) (Šeruga et al., 2011). Regarding foreign wines, a similar range for total phenols was observed in Greek red wines (621to 3200.3 mg/l) (Kallithraka et al., 2006) as well as in wines from all over the world (1615 to 3791 mg/l) (Minussi et al., 2003).

ANTHOCYANINS CONTENT IN THE WINES ANALYZED

Anthocyanins are pigments responsible for the color of grapes which play a main role in the formation of polymeric pigments responsible for the color stability of red wines (Revilla et al., 2009). They are efficient antioxidants, preventing the development of diseases caused by oxidative stress in the organism (Rice-Evans et al., 1995; Vrhovšek, 1996). As shown in Table 1, the total anthocyanins content in the wines examined was 462 to 690 mg/l for the 2008 vintage, 421 to 671 mg/l for the 2009 season, and 321 to 611 mg/l for the 2010 vintage. The values for each of the vintage years examined varied significantly between wine producers. Regarding the average values for the three vintages studied, the highest content was found for the locality of Ćemovsko Polje (wines II and III – 571 and 586 mg/l, respectively), and the lowest was found in wine IX (439 mg/l) (Table 1).

The results obtained are in line with those of a previous study performed in the Podgorički subregion for Vranac wine. Pajović et al. (2012) reported values of 474 to 835 mg/l for red wines for the 2011 vintage; Raičević et al. (2017) found values for Montenegrin red Vranac wines of 600 to 870 mg/l for the 2008 vintage, and 640 to 790 mg/l for the 2009 vintage, produced with different fermentation methods. The results are also in compliance with results for Macedonian Vranac wines (395 and 1530 mg/l) (Ivanova-Petropulos et al., 2015).

CATECHINS CONTENT IN THE WINES ANALYZED

Flavanols are simple monomer compounds (catechins, epicatechins, etc.) and monomer compounds bound to high and low molecular weight proanthocyanidins. They are responsible for bitterness (low molecular weight proanthocyanidins) and astringency (high molecular weight proanthocyanidins) in wine (Lea, 1992). We analyzed the content of monomer and low molecular weight proanthocyanidins (dimers and trimers) using the vanillic method. The results are presented in Table 1 and show that the catechin content in wines was significantly different between producers in each year investigated: between 230 and 270 mg/l for the 2008 vintage, from 199 to 300 mg/l for the 2009 vintage, and from 246 to 350 mg/l for the 2010 vintage. Regarding producers' locality, the average content of the three vintages varied between 253 (V) and 307 mg/l (III).

The total catechin content in our research is comparable with that presented by Pajović et al. (2012) for Vranac wine from the Podgorički subregion (199 to 388 mg/l for the 2011 vintage) and with the results reported by Raičević et al. (2017) for the 2008 (247 to 374 mg/l) and 2009 vintages (314 to 390 mg/l). The results are also in line with those for red wines from other Western Balkan Countries: Slovenia (130 to 400 mg/l) as stated by Vrhovšek (1996), and Croatia for Plavac Mali wine (152.74 to 218.25 mg/l) (Osrečak et al., 2016).

Overall, the results presented for the spectrophotometric analysis of red Vranac wine from Montenegro suggest that the wines analyzed do not differ significantly from wines from other countries in terms of phenolic content. The greatest similarity was found with previous studies of the polyphenol content of wines from Montenegro, as well as from other Western Balkan countries where Vranac wine is most prevalent. Regarding the influence of wine producers' locality, the results showed that wines do not generally have uniformity regarding the compounds examined; only one wine (III) had a uniform total phenols and catechins content over the three vintages studied. Moreover, this wine had the highest phenols content among those examined, probably due to the special technology used for its production.

COLOR INTENSITY AND HUE OF THE WINES ANALYZED

Color intensity (IC) is the quantity of color in wine. It is determined as the sum of the absorbances at 420, 520 and 620 nm and depends on the wine and grape variety (Ribéreau-Gayon et al., 1983). Color hue is the development of color towards orange.

Table 2: Color intensity (IC) and hue (HC) of the wines examined during three consecutive vintages (mean values ± SD; n = 3).

Wine	Color intensity 2008	2009	2010	Hue 2008	2009	2010
I	3.58 ± 0.16^{b}	3.36 ± 0.12^{b}	2.76 ± 0.22^{bc}	0.31 ± 0.06	0.36 ± 0.05^{ab}	0.40 ± 0.09^{bc}
II	3.20 ± 0.25^{c}	4.37 ± 0.17^{a}	2.92 ± 0.21^{b}	0.29 ± 0.07	0.26 ± 0.09^{c}	0.34 ± 0.06^{c}
III	3.19 ± 0.18^{cd}	3.36 ± 0.24^{b}	3.48 ± 0.30^{a}	0.31 ± 0.09	0.27 ± 0.08^{c}	0.33 ± 0.06^{c}
IV	2.87 ± 0.22^{cd}	3.07 ± 0.19^{bc}	2.55 ± 0.21^{cd}	0.33 ± 0.06	0.30 ± 0.05^{bc}	0.39 ± 0.07^{bc}
V	4.45 ± 0.18^{a}	2.80 ± 0.26^{cd}	2.93 ± 0.27^{b}	0.30 ± 0.07	0.40 ± 0.09^{a}	0.32 ± 0.08^{c}
VI	3.14 ± 0.24^{cd}	2.81 ± 0.21^{cd}	2.60 ± 0.26^{bc}	0.37 ± 0.07	0.35 ± 0.06^{ab}	0.39 ± 0.04^{bc}
VII	2.85 ± 0.23^{cd}	3.08 ± 0.21^{bc}	2.57 ± 0.12^{cd}	0.34 ± 0.07	0.35 ± 0.05^{ab}	0.38 ± 0.08^{bc}
VIII	2.93 ± 0.28^{cd}	2.43 ± 0.13^{d}	2.41 ± 0.10^{d}	0.27 ± 0.06	0.28 ± 0.06^{ab}	0.46 ± 0.07^{ab}
IX	2.83 ± 0.26^{d}	3.20 ± 0.51^{b}	2.24 ± 0.15^{d}	0.30 ± 0.07	0.31 ± 0.06^{bc}	0.45 ± 0.07^{ab}
Mean	$\mathbf{3.23 \pm 0.54^{A}}$	$\mathbf{3.16 \pm 0.57^{A}}$	$\mathbf{2.72 \pm 0.40^{B}}$	$\mathbf{0.31 \pm 0.07^{B}}$	$\mathbf{0.33 \pm 0.08^{A}}$	$\mathbf{0.38 \pm 0.08^{A}}$

Different superscript lowercase letters in the same row indicate significantly different means ($p < 0.05$) for wines of the same vintage. Different bold superscript capital letters indicate significantly different means ($p < 0.05$) for wines between the vintages examined.

As shown in Table 2, the IC values of the wines examined were 2.83 to 4.45 for the 2008 vintage, 2.43 to 4.37 for the 2009 vintage, and 2.24 to 3.48 for the 2010 vintage. The values for each vintage year examined varied significantly between wine producers. Considering the three vintages studied, the highest average was found for the Ćemovsko Polje locality (wines II and III: 3.50 and 3.47, respectively), and the lowest was in wine VIII (2.59), with a similar distribution of total anthocyanins. Moreover, a good positive linear correlation between total anthocyanin contents and IC was observed (R^2 = 0.9403, R^2 = 0.7537, and R^2 = 0.946 for 2008, 2009 and 2010 vintages, respectively). The IC values presented in our paper are lower than those found for Vranac wines in the Podgorički subregion (Raičević et al., 2017). This fact may probably be ascribed to the lower anthocyanins contents in the wines investigated.

The HC values of the wines analyzed were significantly different between producers for the different vintages: 0.27 to 0.33 in 2008, 0.26 to 0.40 in 2009, and 0.36 to 0.46 in 2010. Regarding the producers' localities examined, the average of the three vintages varied between

0.30 (II and III) and 0.38 (VIII), which was inversely proportional to IC values. Corresponding correlation coefficients (R^2) were found between IC and HC: 0.7297, 0.5718 and 0.6909 in 2008, 2009 and 2010, respectively. The HC values obtained are close to those reported by Glories (1984) for young wines (0.20 to 0.30).

INFLUENCE OF VINTAGE YEAR ON THE PHENOLS CONTENT OF WINES

In light of the results of this study, it was observed that there were differences in the average content of the phenolic groups examined in the wines between the vintages studied. Wines from 2008 and 2009 had, on average, a higher total phenols content (2518 and 2447 mg/l, respectively), which was significantly different to the wines from the 2010 vintage (2353 mg/l). The average anthocyanins content was higher for the 2008 and 2009 vintages (416 and 406 mg/l, respectively), significantly different to the content observed in 2010 (346 mg/l) (Table 1). The IC values of the wines had the same distribution as the anthocyanins content, also showing higher values for the 2008 and 2009 vintages compared to wines from 2010. The HC was inversely proportional to the IC and was at its highest in 2010 (Table 2). Regarding the catechins content of the wines examined, the highest content was found for the 2010 vintage (283 mg/l), a significantly different one from those from the 2008 and 2009 vintages (247 and 238 mg/l, respectively). These differences, namely higher catechins contents and lower anthocyanins contents in the 2010 wines, can be explained by variations in weather conditions between the growing seasons, especially in rainfall and its distribution. The 2010 vintage had a seasonal rainfall of 498 and 483 mm, and a rainfall during the harvest month of 102 and 212 mm in the Podgorički and Crmnički subregions, respectively. On the other hand, the 2008 and 2009 vintages were characterized by a reduced seasonal rainfall (401 and 412.5 mm for Podgorički and 393 and 410 mm for Crmnički, respectively) and rainfall in the harvest month (75.6 and 65.3 mm for Podgorički and 63 and 103 mm for Crmnički, respectively), compared to 2010.

These findings about the phenols groups for different climatic conditions partly match the previous finding that higher temperatures and humidity at the end of grapes maturation are advantageous for producing higher concentrations of total catechins and low molecular weight oligomers, while such effect was shown to be the opposite of that observed for anthocyanins in grape samples and consequently in wine (Mateus et al., 1999).

PRINCIPAL COMPONENT ANALYSIS

PCA was applied in order to obtain further information on what affects the different phenolic composition of the wines examined, if it is the vintage year or the wine producers' locality. A correlation matrix was created based on the phenolic composition of wine in terms of total phenols, total anthocyanins and cathechins contents, IC, and HC. The PCA results indicated that the first principal component (PC1) accounts for 62.94 % of the variability, and the second principal component (PC2) accounts for 24.41 % of the variability, i.e. together, PC1 and PC2 account for 87.35 % of the total variance. The correlation between the original variables and the first two principal components is shown in Figure 1. The projection for wines showed that the grouping of samples was mainly observed according to vintage, with a clear separation of the 2010 wines from 2008 and 2009 ones. Thus, wines from 2010 were located in the positive region of PC2, while wines from 2008 and 2009 were located in the negative region of PC2. Regarding the producers' localities examined, we could not find a grouping of wines, except for wine III from Ćemovsko Polje, which was located in the positive region of PC1 due to its high total phenols and catechins content. The other producers did not show any grouping for the three-year period (Fig. 1). The wine groupings presented in the PCA plots confirm the conclusion drawn in the previous section about grouping the wines according to the vintage year, and separating only one wine on the basis of uniformity and high total phenols content.

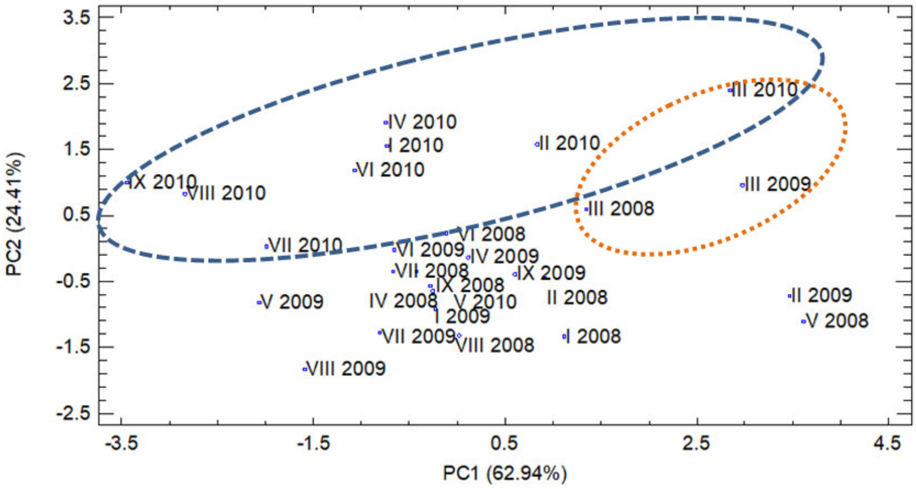

Fig. 1: Scatter plot for the distribution of phenolic compounds in the wines studied during three consecutive vintages; the two principal components explain 87.35 % of the total variability.

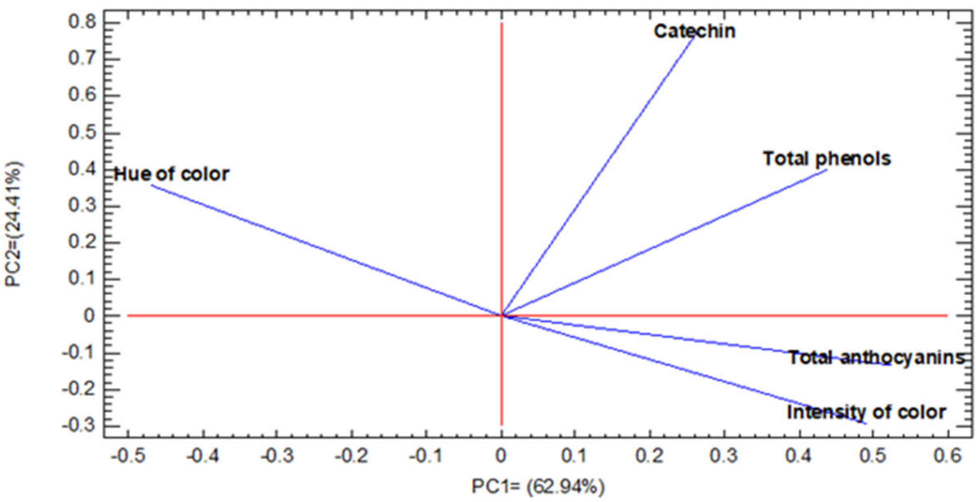

Fig. 2: Factor score for the two principal components of wines

The present work allowed concluding that for a given variety, the phenolic composition of the wine obtained is primarily affected by the vintage year and intrinsically related to edaphoclimatic conditions and only secondly by the growing region and locality. The combination of these two variables is responsible for the genuineness and the typical profile of the various wines. The information retrieved from this work is not only important to producers and the industry, but also to consumers. Montenegrin Vranac red wines possess a considerable amount of important bioactive components influenced by the location and vintage year conditions. Therefore, this is the first step towards understanding how producers and industrials can obtain and predict wines of great quality, while also contributing to providing guidance to consumers on the best wine-growing areas. The results point out that the wine-growing area of Ćemovsko Polje produces wines with uniform phenolic profiles regardless of the vintage year and of edaphoclimatic conditions. This information is also vital in order to obtain wines with a consistent quality profile, thus improving consumers' loyalty to a certain product. If such observation is maintained for several vintage years, the wines produced in that area must be considered for special certification, such as certificates of origin. Furthermore, the specific characteristics of a given variety (Vranac), allied to the cultivation in a specific delimited area with particular edaphoclimatic conditions and oenological practices should be a case of study to apply for a Protected Designation of Origin (PDO) or a Protected Geographical Indication (PGI). Such certification could have an impact on the wine industry in Montenegro and boost the important industrial and agricultural sectors.

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