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Varietal differentiation of grapes cv. 'Vranac', 'Kratošija' and 'Cabernet Sauvignon' from Montenegro according to their polyphenolic composition

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This paper presents the results of investigations into the polyphenolic profile of grapes from the red grape varieties 'Vranac', 'Kratošija' and 'Cabernet Sauvignon' grown in the Montenegrin wine region. Using an RP-HPLC method the composition of anthocyanins and hydroxycinnamic acids (HCA) in the fresh grapes was analysed. Results of the analysis of anthocyanins show that the Montenegrin grape varieties have the same order of the relative concentration of monoglucosid, non-acylated anthocyanins: Mv-3-gl > Pn-3-gl > Pt-3-gl > Dp-3-gl > Cy-3-gl, but with a different relative amount of anthocyanins in 'Vranac' and 'Kratošija'. The concentration of p-coumarylated derivates was higher than the concentration of acetylated ones in 'Vranac' and 'Kratošija'. In contrary, grapes of the variety 'Cabernet Sauvignon' have a relatively high amount of acylated derivates and more acetylated anthocyanins than p-coumarylated ones. Therefore the ratio between acetylated and coumarylated anthocyanins varied widely among the varieties: 'Vranac' 0.46, 'Kratošija' 0.15 and 'Cabernet Sauvignon' 2.07. The content of total anthocyanins varied significantly between the examined varieties: 'Vranac' (1360 mg/kg), 'Kratošija' (591 mg/kg) and 'Cabernet Sauvignon' (1116 mg/kg). The studied varieties were characterised by differences in the HCA profile (relative content of caftaric/coutaric/fertaric acid): 69/21/11 for 'Vranac', 75/11/14 for 'Kratošija' and 73/20/7 for 'Cabernet Sauvignon'. The values for trans-coutaric/trans-caftaric ratio varied significantly between the varieties and amounted to 0.236 for 'Vranac', 0.107 for 'Kratošija' and 0.213 for 'Cabernet Sauvignon'. The total HCA contents in the skin and pulp were different in the examined varieties. Principal component analysis showed that each studied variety had a unique profile compared to the content of anthocyanins and hydroxycinnamic acids. These properties are reliable criteria for varietal identification.
Keywords: polyphenolic profile, anthocyanins, hydroxycinnamic acids, 'Vranac', 'Kratošija', 'Cabernet Sauvignon'

Sortenmäßige Differenzierung von Trauben der Rebsorten 'Vranac', 'Kratošija' und 'Cabernet Sauvignon' aus Montenegro mittels ihrer Polyphenolzusammensetzung. *Dieser Beitrag stellt die Ergebnisse von Untersuchungen der Polyphenolprofile von Trauben der roten Rebsorten 'Vranac', 'Kratošija' und 'Cabernet Sauvignon' aus dem montenegrinischen Anbaugebiet dar. Mittels einer RP-HPLC-Methode wurde die Zusammensetzung der Anthocyane und Hydroxyzimtsäuren (HCA) in den frischen Trauben analysiert. Die Ergebnisse der Anthocyan-Analyse zeigen, dass die montenegrinischen Rebsorten die gleiche Ordnung der relativen Konzentrationen der monoglucosidischen, nicht-acylierten Anthocyane aufweisen: Mv-3-gl > Pn-3-gl > Pt-3-gl > Dp-3-gl > Cy-3-gl, jedoch mit einer unterschiedlichen relativen Menge an Anthocyanen in 'Vranac' und 'Kratošija'. In 'Vranac' und 'Kratošija' waren die Konzentrationen der p-coumarylierten Derivate höher als die der acetylierten. Im Gegensatz dazu haben Trauben der*

Sorte 'Cabernet Sauvignon' eine relativ hohe Menge an acylierten Derivaten und damit mehr acetylierte Anthocyane als p-coumarylierte. Daher variiert das Verhältnis zwischen acetylierten und coumarylierten Anthocyanen stark zwischen den Sorten: 'Vranac' 0,46, 'Kratošija' 0,15 und 'Cabernet Sauvignon' 2.07. Der Gehalt an gesamten Anthocyanen variiert signifikant zwischen den untersuchten Sorten: 'Vranac' (1360 mg/kg), 'Kratošija' (591 mg/kg) und 'Cabernet Sauvignon' (1116 mg/kg). Die untersuchten Sorten waren durch Unterschiede in ihren HCA-Profilen charakterisiert (relativer Gehalt von Caftarsäure/Coutarsäure/Fertarsäure): 69/21/11 für 'Vranac', 75/11/14 für 'Kratošija' und 73/20/7 für 'Cabernet Sauvignon'. Die Werte für das Verhältnis trans-Coutar-/trans-Caftarsäure variieren signifikant zwischen den Sorten und beliefen sich auf 0.236 für 'Vranac', 0.107 für 'Kratošija' und 0,213 für 'Cabernet Sauvignon'. Die Gesamtgehalte an HCA in der Beerenhaut und im Fruchtfleisch unterschieden sich in den untersuchten Sorten. Die Hauptkomponentenanalyse (PCA) zeigte, dass jede untersuchte Sorte ein einzigartiges Profil hinsichtlich der Anthocyangehalte und der Hydroxyzimtsäuren hat. Diese Eigenschaften sind zuverlässige Kriterien für die Sortenidentifizierung.
Schlagwörter: Polyphenolprofil, Anthocyane, Hydroxyzimtsäuren, 'Vranac', 'Kratošija', 'Cabernet Sauvignon'

Différenciation des raisins des cépages 'Vranac', 'Kratošija' et 'Cabernet Sauvignon' en provenance du Monténégro en fonction de leur composition polyphénolique. *Le présent article présente les résultats d'études des profils polyphénoliques de baies de raisin des cépages rouges 'Vranac', 'Kratošija' et 'Cabernet Sauvignon' en provenance de la région viticole monténégrine. La composition des anthocyanes et des acides hydroxycinnamiques (HCA) a été analysée dans les baies de raisin fraîches à l'aide d'une méthode RP-HPLC. Les résultats de l'analyse des anthocyanes montre que les cépages monténégrins présentent le même ordre de concentrations relatives d'anthocyanes monoglucosidiques non-acylés : Mv-3-gl > Pn-3-gl > Pt-3-gl > Dp-3-gl > Cy-3-gl, mais avec une quantité relative d'anthocyanes différente dans 'Vranac' et 'Kratošija'. Dans 'Vranac' et 'Kratošija', les concentrations des dérivés p-coumarylés ont été plus élevées que celles des dérivés acétylés. En revanche, les baies de raisin du cépage 'Cabernet Sauvignon' contiennent une quantité relativement élevée de dérivés acylés et donc plus d'anthocyanes acétylés que d'anthocyanes p-coumarylés. De ce fait, le rapport entre les anthocyanes acétylés et coumarylés varie fortement selon les cépages : 'Vranac' 0,46, 'Kratošija' 0,15 et 'Cabernet Sauvignon' 2.07. La teneur en anthocyanes totaux varie significativement entre les cépages étudiés : 'Vranac' (1360 mg/kg), 'Kratošija' (591 mg/kg) et 'Cabernet Sauvignon' (1116 mg/kg). Les cépages étudiés se caractérisaient par les différences dans leurs profils HCA (teneur relative en acide caftarique / acide coutarique / acide fertarique) : 69/21/11 pour 'Vranac', 75/11/14 pour 'Kratošija' et 73/20/7 pour 'Cabernet Sauvignon'. Les valeurs du rapport acide trans-coutarique / acide trans-caftarique variaient significativement entre les cépages et s'élevaient à 0.236 pour 'Vranac', à 0.107 pour 'Kratošija' et à 0,213 pour 'Cabernet Sauvignon'. Les teneurs totales en HCA dans la peau des baies et dans la pulpe différaient selon les cépages étudiés. L'analyse des composantes principales (PCA) a montré que chaque cépage étudié présente un profil unique en ce qui concerne les teneurs en anthocyanes et les acides hydroxycinnamiques. Ces caractéristiques sont des critères fiables pour l'identification des cépages.*
Mots clés : profil polyphénolique, anthocyanes, acides hydroxycinnamiques, 'Vranac', 'Kratošija', 'Cabernet Sauvignon'

Polyphenolic compounds play an important role in the quality of grapes and wines. They affect stability of the wine as well as its sensory characteristics and they are mainly responsible for colour, astringency and bitterness (Chira et al., 2009). These compounds also affect the wine structure and have a stabilizing influence as a basis for wine aging. Apart from the fact that they influence wine quality, they are considered to be important for human health, some of them being biologically active substances (Mattivi et al., 2002, Rodrigo et al., 2011).

Polyphenols are also important from the taxonomic point of view. It is a well-known fact that the pattern of some flavonoid classes, such as anthocyanins, is genetically controlled and that their distribution varies considerably among *Vitis Vinifera* varieties (Eder et al., 1994; Mazza, 1995; Mattivi et al., 2006). The anthocyanin profile is relatively stable for every variety, whereas the total concentration may vary between vintages, depending on climatic and agronomical factors (Mattivi et al., 2006). Therefore the composition of anthocyanins is considered to be one of the chemometric characteristcs allowing the differentiation of grape varieties (Eder et al., 1990; Mazza, 1995; Revilla et al., 2001) and wines (Eder et al., 1995; Revilla et al., 2001; Monagas et al., 2003). This also applies to

anthocyanin contents which is a varietal characteristic (Mattivi et al., 2002; Mattivi et al., 2006). In addition, the ratio between certain anthocyanin groups is considered to be a characteristic of a grape variety. The ratio between p-coumaroylates and acetylates has been assessed as a distinctive parameter for grape varieties and wines (González-Neves et al., 2007; Otteneder et al., 2004).
The HCA profile of wines depends to a great extent on the grape variety, but also on the vintage, the level of maturity and wine production technology (Pour-Nikfardjam, 2000). Despite these influencing factors, wines produced from different varieties have a specific HCA content, and, therefore, wine varieties can be classified on account of this (Mattivi and Nicolini, 1997). The trans-coutaric/trans-caftaric ratio, according to the authors, can also be used as a means of varietal differentiation of wines and grapes (De La Presa-Owens et al., 1995; Singleton et al., 1986). Preferably HCA profile analysis is used in combination with the results from the anthocyanin analysis (Calò et al., 1994).

Owing to its geographical origin and favourable climate, grape growing and winemaking have always been established in Montenegro and have traditionally been based on local varieties like 'Vranac' and 'Kratošija'. 'Kratošija' dominated until the emergence of phylloxera; however, its role was then taken over by 'Vranac', which features a lower heterogeneity and better skin coloration (Ulićević, 1966). Both of these varieties also grow in Macedonia, Bosnia and Herzegovina, Croatia and Serbia and are considered as local varieties of Western Balkan countries (Božinovic, 2005). Thanks to renewed investments in wine production, the vineyard areas in Montenegro are increasing, producing both local and international grape varieties. Among red varieties 'Vranac' is dominating, whereas 'Kratošija' is decreasing, and 'Cabernet Sauvignon' is increasing (Pajovic et al., 2011).

'Vranac' and 'Kratošija' are varieties which have been studied well, particularly with regard to their ampelographic characteristics, the mechanical structure of the cluster, the chemical composition of must (Ulićević, 1966; Pajović et al., 2011) and wine quality (Božinović, 2005; Pajović et al., 2002). Also some polyphenolic features of the varieties have been studied like the composition and contents of anthocyanins (Kovač, 1978; Pajović, 2011), the influence of the technological procedure on the polyphenolic content in wine (Kovač et al., 1995), as well as the polyphenolic potential of the grapes and wine varieties 'Vranac', 'Kratošija' and 'Cabernet Sauvignon' in the Montenegrin wine region (Pajović et al., 2012). The results of the cited studies point out the fact that the variety 'Vranac' has a more intense polyphenolic composition in comparison with the variety 'Kratošija', mostly on account of stronger colouration of the berry skin and the wine.
Although these grape varieties (especially 'Vranac') are very important for the wine industry in the Balkan region, there is, to our knowledge, no data available regarding its phenolic profile in the Montenegrin wine region. On the other hand, a significant number of studies are published on the anthocyanin composition of 'Cabernet Sauvignon', but there is no data on the phenolic profile of this international variety cultivated in Montenegro. The aim of this paper is to define the polyphenolic profile of the grape and wine varieties 'Vranac', 'Kratošija' and 'Cabernet Sauvignon', as well as to compare the findings obtained for the indigenous varieties ('Vranac' and 'Kratošija') with the results for the international variety 'Cabernet Sauvignon'.

Materials and Methods

Grape sampling

Grapes were sampled at the time of their technological ripeness in the year 2011. Grape samples of 'Vranac' (n = 6), 'Kratošija' (n = 6) and 'Cabernet Sauvignon' (n = 6) were collected from vineyards located in the Podgorica wine growing district. From each vineyard approximately 20 kg of grapes were representatively sampled. The vineyard locations were as follows: Šipčanik (L1), Rogami (L2), Kokoti (L3), Lješkoplje (L4), Nikolj Crkva (L5) and Kuči (L6) (Fig. 1). The majority of Montenegrin vineyards (almost 90 %) are located in the Podgorica district belonging to the Basin of Scadar Lake region (Fig. 1).

Physicochemical characteristics

100 berries were representatively taken from the 20 kg grape sample and weighed. Total soluble solids (TSS); titratable acidity and pH-value of the berry juice were determined according to OIV procedures (Oiv, 2011). These data are not presented in this paper.

Fig. 1: Vineyard locations of the sampled grapes

Sample preparation for HPLC

1000 berries, taken from the 20 kg of representatively sampled grapes from each location, were stored in the refrigerator at -80 °C (Irillab-800; Angelantoni, Perugia, Italy) for later phenolic analysis. Three months later, in January, samples were transferred to a refrigerator at -20 °C for a couple of days. Half an hour before preparation, the sample was placed into a refrigerator at 4 °C. Then the weight of 50 berries was measured. The pedicels were removed and the berries were manually separated in skins, seeds and pulp with a pistel. Skins were blotted on paper towels to remove any residual pulp, the weights of pulp, seeds and skin were determined with an analytical balance. Three samples of 50 berries were analyzed from each location.

Skin preparation for anthocyanin analysis was done according to the modified method of Eder et al. (1994). Five gram of skin were blended with 10 ml of methanol containing 0.01 % HCl for 10 minutes at 10.000 rpm (Homogenizer HD 4AP; Edmund Bühler, Tübingen, Germany). The extracts of skin were filtered with a 0.45 µm filter membrane (Multoclear 13; CS-Chromatographie Service, Langerwehe, Germany) before the HPLC analyses.

Skin preparation for hydroxycinnamic acids analyses was done with homogenization of 5 g of skin with 10 ml of 6 % (w/v) perchloric acid in water according to the modified method of Vrhovšek et al. (1997). Perchloric acid was added to precipitate proteins and inhibit polyphenoloxidase activities. The extraction was done by homogenization for 10 minutes at 10.000 rpm (Homogenizer HD 4AP; Edmund Bühler, Tübingen, Germany). The filtered extract of skin was passed through a 0.45 µm filter membrane (Multoclear 13; CS-Chromatographie Service, Langerwehe, Germany).

Must preparation for hydroxycinnamic acids analyses was done after addition of potassium metabisulfite (1 g) to inhibit polyphenol oxidase (PPO) and ascorbic acid (1 g) to reduce any quinone formed. The volume of unoxidized juice was measured to calculate the exact dilution, and a sample of it was taken to measure its HCA concentration. The filtered must was passed through a 0.45 µm membrane (Multoclear 13; CS-Chromatographie Service, Langerwehe, Germany) before HPLC analyses.

HPLC analyses of phenols

Analysis of anthocyanins was performed according to the modified method by Eder et al. (1990) with a HP system series II 1090 AminoQuant with DAD (Agilent, Santa Clara, USA). The separation was carried out using a LiChrospher 100, RP-18, (Merck, Darmstadt, Germany) steel cartridge (4 x 250 mm) filled with 5 µm particles, and furnished with a guard cartridge (4 × 4 mm), both thermostated at 40 °C. Two mobile phases were used: 5 nM phosphate buffer adjusted to pH-value 1.8 (solvent A) and methanol (solvent B). Both chemicals were from Merck (Darmstadt, Germany). Gradient elution was applied at a 0.8 ml/min flow rate according to the following program: linear gradient from 15 % B to 30 % B in 30 min, from 30 % B to 60 % B in 80 min, 60 % B for 90 min, from 60 % B to 15 % B in 92 min. The injection volume was 20 µl.

Delphinidin 3-glucoside (dp-3-gl), cyanidin 3-glucoside (cy-3-gl), petunidin 3-glucoside (pt-3-gl), peonidin 3-glucoside (pn-3-gl), malvidin 3-glucoside (mv-3-gl), and their relevant acetic acid (sum of acetylated) and p-coumaric acid esters (sum of p-coumarylated) were identified and quantified at 520 nm with an external calibration curve with standard substance malvidin 3-glucoside chloride (Extrasynthese, Genay, France).

The concentration of hydroxycinnamic acids was determined by HPLC according to the modified

method by Vrhovšek et al. (1997). A Rapid Resolution HPLC system (Agilent Technologies 1200, RRLC; Agilent, Santa Clara, USA) with an auto injector (5 µl injection volume) and a diode array detector set at 280 and 320 nm was used. The separation was carried out using a C18 reversed phase column (Zorbax SB-C18, 150 x 2.1 mm; Agilent, Santa Clara, USA) filled with 1.8 µm particles, thermostated at 40 °C. Two mobile phases were used: formic acid in water (0.5 %) adjusted to pH-value 2.3 (solvent A) and methanol (solvent B). Both chemicals are from Merck (Darmstadt, Germany). Gradient elution was applied at a 0.25 ml/min flow rate according to the following program: linear gradient from 4 % to 5 % B in 30 min, from 5 % B to 8 % B in 6 min, hold on 8 % for 4 min, from 8 to 12 % B for 20 min, from 12 % B to 21 % B in 10 min, from 21 % B to 70 % B in 5 min, hold on 70 % for 5 min, and back to the initial conditions of 4 % B in 7 min. Post-run time was 15 minutes.
Determinations were carried out with an external standard method by comparison of retention times and ultraviolet (UV) spectra with commercial standards: caffeic acid and ferulic acid (Sigma, Steinheim, Germany), caftaric acid (Dalton Chemical Laboratones Inc., Toronto, Canada) and p-coumaric acid (Roth, Karlsruhe, Germany).

Statistics

In order to establish the significance of differences between varieties, locations and their interaction for each studied parameter, a two factorial analysis of variance (ANOVA) was applied. For those parameters and factors where significant difference were detected, additionally an LSD test was applied to the significance level of $P < 0.05$. The relationships between cultivars grown on different locations were investigated by principal component analysis (PCA). Means for three replications were used to create a correlation matrix from which standardized principal component (PC) scores were extracted. Scatter plots of the first two PCs were created with Statistic/Graph. In order to determine which of the PCs accounted for the greatest amount of variation, the eigenvalues of the two PCs were compared for each trait. Data processing was performed using the statistical program Statistic (StatSoft, Inc., Tulsa, USA).

Results and Discussion

Anthocyanin profile of grapes

Table 1 summarizes the results of the anthocyanin profiles (percentages), i.e. the total concentration (in mg/kg) and ratio of certain groups of anthocyanins in the skin of fresh grapes for the varieties 'Vranac', 'Kratošija' and 'Cabernet Sauvignon'.
Among the anthocyanins detected in the berry skin mv-3-gl prevailed in all the grape varieties as it could have been expected on the basis of the data provided in literature (Eder et al., 1994; Revilla, 2001; Mattivi et al., 2006). Nevertheless, the relative amount of this anthocyanin statistically significantly varied in the varieties studied here. The highest content was found in 'Kratošija' (43.5 %), followed by 'Vranac' (40.8 %), whereas the variety 'Cabernet Sauvignon' contained the lowest values (38.6 %).
In the Montenegrin grape varieties 'Vranac' and 'Kratošija' the relative concentrations of monoglucosidic non-acylated anthocyanins were as follows: Mv-3-gl > Pn-3-gl > Pt-3-gl > Dp-3-gl > Cy-3-gl, but with a different relative amount of anthocyanins in 'Vranac' and 'Kratošija'. Non-acylated anthocyanins in 'Cabernet Sauvignon' grapes decreased in accordance with this pattern: Mv-3-gl > Dp-3-gl > Pt-3-gl > Pn-3-gl >Cy-3-gl (Table 1). The shown order and the relative amount of monoglucosidic anthocyanins in the variety 'Vranac' correspond with the findings presented by Bogićević (2009), who had studied this variety in the same locations. In addition, the anthocyanin profile found by Dimtrovska et al. (2011) is more in line with our values for 'Kratošija'. The order of monoglucosidic anthocyanins in 'Cabernet Sauvignon' determined here corresponds with that presented by Mattivi et al. (2006) and Romero-Cascales et al. (2005). The content of non-acylated anthocyanins for the studied varieties was higher than that of the acylated ones, which is in line with literature findings (Eder et al., 1994; Revilla et al., 2001; Revilla et al., 2009; Mattivi et al., 2006). Among the studied varieties, the highest relative amount of acylated derivates was found in 'Cabernet Sauvignon' and the lowest in 'Vranac'. Therefore, the ratio non-acylated/acylated anthocyanins differed statistically significantly between the studied varieties: 'Vranac' 2.26, 'Kratošija' 1.64 and the lowest in 'Cabernet Sauvignon' grapes 1.33.

Table 1: Anthocyanin profile (percentage), total concentration of anthocyanins in the fresh berry (mg/kg) and ratios for grape varieties 'Vranac', 'Kratosija' and 'Cabernet Sauvignon' (mean values ±SD)

	Non-acylated glucosides %					Acylated glucosides			Ratio	
Grape variety	*Dp-3-gl*	*Cy-3-gl*	*Pt-3-gl*	*Pn-3-gl*	*Mv-3-gl*	sum of acetylated	sum of p-coumarylated	total anthocyanins mg/kg	acetylated p-coumarylated glucosides	non-acylated acylated glucosides
Vranac										
L1	5.1±0.8 ^{cd}	2.8 ±0.48 ^{bc}	7.7±0.6 ^{c}	12.4±0.3 ^{a}	42.5±2.6 ^{a}	9.6± 0.1 ^{ab}	19.9±0.5 ^{bc}	1165±47 ^{bc}	0.48±0.01 ^{a}	2.38±0.06 ^{a}
L2	4.2±0.7 ^{d}	1.9±0.18 ^{d}	6.7±0.8 ^{d}	9.2±1.0 ^{c}	42.2±1.5 ^{a}	10.1±0.1 ^{a}	25.7±1.2 ^{a}	1091±134 ^{c}	0.39±0.01 ^{a}	1.80±0.10 ^{c}
L3	5.9±0.5 ^{bc}	2.8±0.14 ^{bc}	9.1±0.3 ^{b}	11.8±0.1 ^{a}	40.8±0.8 ^{a}	10.4±0.3 ^{a}	18.7±0.2 ^{c}	1367±58 ^{b}	0.55±0.01 ^{a}	2.43±0.03 ^{a}
L4	7.9±1.9 ^{a}	3.7±1.11 ^{a}	10.5±1.5 ^{a}	10.9±0.8 ^{b}	37.3±3.1 ^{b}	9.5±1.1 ^{ab}	20.1±3.1 ^{bc}	1335±176 ^{bc}	0.49±0.14 ^{a}	2.38±0.24 ^{a}
L5	7.1±0.1 ^{a}	3.03±0.11 ^{b}	9.9±0.2 ^{b}	9.7±0.4 ^{c}	40.9±0.3 ^{a}	8.8±0.4 ^{b}	20.6±0.1 ^{bc}	1873±385 ^{a}	0.43±0.02 ^{a}	2.39±0.04 ^{a}
L6	5.2± 0.8 ^{cd}	2.4±0.48 ^{cd}	7.6±0.5 ^{c}	11.6±1.25 ^{ab}	41.3±0.1 ^{a}	9.0±0.1 ^{b}	22.9±2.9 ^{b}	1326±184 ^{bc}	0.40±0.05 ^{a}	2.16±0.28 ^{b}
	5.9±1.5 ^{A}	*2.8±0.73* ^{A}	*8.6±1.51* ^{A}	*10.9±1.3* ^{A}	*40.8± 2.3* ^{B}	*9.6±0.7* ^{B}	*21.3±2.8* ^{B}	*1360±307* ^{A}	*0.46±0.08* ^{B}	*2.26±0.26* ^{A}
Kratošija										
L1	4.2±0.1 ^{a}	1.2±0.02 ^{b}	6.3±0.1 ^{ab}	5.9±0.4 ^{c}	44.6±0.3 ^{b}	4.7±0.2 ^{b}	33.0±0.6 ^{b}	714±123 ^{a}	0.14±0.01 ^{a}	1.65±0.06 ^{a}
L2	3.6±0.3 ^{a}	1.0±0.06 ^{b}	5.9 ±0.6 ^{b}	6.3±0.1 ^{c}	46.3±0.1 ^{a}	4.3±0.5 ^{b}	32.3±1.3 ^{b}	646±70 ^{ab}	0.14±0.02 ^{a}	1.73±0.07 ^{a}
L3	2.3±0.6 ^{b}	1.0±0.20 ^{b}	4.5±0.5 ^{c}	5.1±0.2 ^{c}	41.1±0.3 ^{c}	6.3±0.2 ^{a}	39.7±1.5 ^{a}	376±50 ^{c}	0.16± 0.00 ^{a}	1.19±0.10 ^{b}
L4	3.8±0.1 ^{a}	2.3±0.02 ^{a}	6.1±0.5 ^{b}	8.4±0.8 ^{b}	41.6±2.4 ^{c}	5.1±0.4 ^{ab}	32.7±1.8 ^{b}	451±90 ^{bc}	0.15±0.01 ^{a}	1.65±0.15 ^{a}
L5	4.6±1.1 ^{a}	2.6±0.46 ^{a}	7.2 ±0.8 ^{a}	9.8±0.1 ^{a}	40.0±0.1 ^{c}	4.8±0.3 ^{b}	30.9±2.6 ^{b}	794±62 ^{a}	0.16±0.02 ^{a}	1.79±0.20 ^{a}
L6	3.8±0.1 ^{a}	1.1±0.05 ^{b}	5.8±0.1 ^{b}	6.2±0.2 ^{c}	47.3±0.2 ^{a}	4.2±0.1 ^{b}	31.5±0.1 ^{b}	564±126 ^{abc}	0.13±0.01 ^{a}	1.80±0.01 ^{a}
	3.7±0.9 ^{B}	*1.5±0.69* ^{B}	*6.0±0.9* ^{B}	*7.0±1.7* ^{B}	*43.5±2.9* ^{A}	*4.9±0.8* ^{C}	*33.4±3.3* ^{A}	*591±168* ^{C}	*0.15±0.02* ^{B}	*1.64±0.23* ^{B}
Cabernet Sauvignon										
L1	11.5±0.3 ^{a}	2.1±0.19 ^{a}	8.5±0.3 ^{a}	7.4±0.1 ^{a}	36.8±0.2 ^{cd}	23.9±0.3 ^{d}	9.8±0.3 ^{e}	1510±145 ^{a}	2.43±0.04 ^{a}	1.96±0.05 ^{a}
L2	6.5±0.7 ^{c}	0.8±0.15 ^{b}	5.4±0.2 ^{b}	6.1±0.2 ^{b}	39.3±0.4 ^{b}	29.6±1.3 ^{c}	12.0±0.1 ^{de}	1034±8 ^{b}	2.46±0.10 ^{a}	1.40± 0.06c
L3	3.9±0.1 ^{d}	0.3±0.07 ^{bc}	3.5±0.1 ^{c}	3.3±0.2 ^{c}	35.7±0.2 ^{d}	37.5±0.3 ^{a}	15.8±0.3 ^{bc} c	750±99 ^{c}	2.37±0.07 ^{a}	0.87±0.01 ^{e}
L4	2.9±0.2 ^{d}	0.2±0.12 ^{c}	3.1±0.1 ^{c}	3.3±0.1 ^{c}	39.7±0.1 ^{b}	31.2±0.5 ^{b}	19.5±0.2 ^{a}	1068±46 ^{b}	1.60±0.04 ^{ab}	0.97±0.01 ^{de}
L5	8.5±0.1 ^{b}	1.7±0.06 ^{a}	7.6±0.4 ^{a}	6.1±0.1 ^{b}	37.9±0.3 ^{c}	20.8±1.4 ^{e}	17.3±0.9 ^{ab}	1527±353 ^{a}	1.21±0.14 ^{b}	1.62±0.04 ^{b}
L6	3.3±0.5 ^{d}	0.2±0.08 ^{c}	3.5±0.4 ^{c}	4.0±0.2 ^{c}	41.9±0.1 ^{a}	33.1±0.6 ^{b}	14.0±0.5 ^{cd}	808±33 ^{bc}	2.37±0.05 ^{a}	1.12±0.05 ^{d}
	6.1±3.2 ^{A}	*0.9±0.79* ^{C}	*5.3±2.2* ^{C}	*5.0±1.6* ^{C}	*38.6± 0.2*^{C}	*29.4±5.8* ^{A}	*14.8±3.4* ^{C}	*1116±343* ^{B}	*2.07± 0.7*^{A}	*1.33±0.39* ^{C}

Different superscript small letters in same row indicate significantly different means ($p < 0.05$) for locality.

Different bold superscript capital letters indicate significantly different means ($p < 0.05$) for grape varieties.

The content of acetylated and p-coumarylated anthocyanins varied significantly in the examined varieties. In the two autochtonous Montenegrin varieties, p-coumarylated derivates were more abundant than the acetylated ones, with varying relative amounts in 'Vranac' and 'Kratošija' (21.3 % and 33.4 %, respectively). Also, the values of acetylated anthocyanins varied in 'Vranac' and 'Kratošija' (9.6 % and 4.9 %, respectively). In 'Cabernet Sauvignon', among the acylated anthocyanins, acetylated glucosides prevailed, whereas p-coumarylated derivates accounted for 14.8 %. The Montenegrin varieties can be considered rich in coumarylated anthocyanins. The relative content of coumarylated anthocyanins found in 'Cabernet Sauvignon' grown in Montenegro was significantly higher than that reported by others authors (Revilla et al., 2001; García-Beneytez et al., 2002; González-Neves et al., 2007; Romero-Cascales et al., 2005). This observation provides evidence about the influence of the environmental factors on the anthocyanin profile and allows for a classification of grape varieties, according to the region of cultivation (regional markers).

In our study, the ratio between acetylated and coumarylated anthocyanin varied between the examined three varieties. The highest value of the acetylated/coumarylated anthocyanins ratio was measured in the variety 'Cabernet Sauvignon' with 2.07 which was statistically significantly different, whereas in the Montenegrin varieties the values were not significantly different with 0.46 and 0.15 ('Vranac' and 'Kratošija'). The values of this ratio in the grape varieties 'Vranac' and 'Cabernet Sauvignon', as measured in our research, matched with values (0.51 and 2.17) quoted by Dimitrovska et al. (2011).

The content of total anthocyanins significantly varied between examined varieties: 'Vranac' (1360 mg/kg), 'Kratošija' (591 mg/kg) and 'Cabernet Sauvignon' (1116 mg/kg). The values reported for the varieties 'Vranac' and 'Kratošija' are lower than those quoted by Pajović et al. (2011) for the same varieties, due to the different method of measuring them. However, the ratio of total anthocyanins among the varieties is pretty much the same in both studies.

ANOVA tests determined the statistically significant differences between the varieties for most of the tested parameters. While assessing the impact of the location, it was determined that the differences in the relative amount of non-acylated and acylated anthocyanins were statistically significant for the studied locations for all the three varieties. The greatest differences among the locations were with 'Cabernet Sauvignon',

whereas the fewest differences were observed for the variety 'Kratošija'.

Hydroxycinnamate profiles of grapes

In order to obtain a varietal characterization of the studied varieties, the ester forms of HCAs with tartaric acid (trans-caftaric acid, cis- and trans-coutaric acid, resp., and fertaric acid) and free forms (trans-caffeic, trans-p-coumaric and trans-ferulic acids) were examined. The HCA contents in the skin and pulp (in mg/kg) and the ratio of trans-coutaric acid/trans-caftaric acid for the varieties 'Vranac', 'Kratošija' and 'Cabernet Sauvignon' are presented in Table 2.
HCA were most present in the pulp of all the three varieties, whereas they were less present in the skin. In the pulp, there were only ester forms of HCAs, which prevailed in the skin as well, although the results from literature point out their rare occurrence in grapes (Mattivi and Nicolini, 1997; Vanzo, 2007).
In the pulp, the content of trans-caftaric acid was much higher than the content of coutaric acid for all the three varieties. The lowest values were obtained for 'Vranac', and the highest for 'Cabernet Sauvignon'. The results for the skin were reverse – the highest values for trans-caftaric acid were in 'Kratošija' and the lowest in 'Cabernet Sauvignon'. In the skin, the coutaric acid prevailed in 'Cabernet Sauvignon', whereas it was least present in 'Kratošija'. Such order among the varieties was found in the pulp as well. Trans-fertaric acid was more present in the pulp than in the skin for all the three varieties. The highest content of trans-fertaric acid was found in grapes of 'Kratošija' and the lowest in those of 'Cabernet Sauvignon', and the same results were found for the skin as well. The HCA values in the berries were in accordance with the findings cited in literature (Vrhovšek et al., 1998; Di Stefano and Maggiotitto, 1995), except for the content of trans-coutaric acid in 'Cabernet Sauvignon'. In the pulp, the content of trans-caftaric acid was higher than the content of coutaric acid. In the skin, there was more coutaric acid than in the pulp; the content of trans-fertaric was higher in the pulp than in the skin.
Statistically significantly the highest HCA content in the skin was found in 'Kratošija' (17.1 mg/kg), whereas its content was lower and equally distributed in 'Vranac' and 'Cabernet Sauvignon' (11.6 mg/kg and 10.8 mg/kg). The content of HCA in the pulp differed statistically significantly between the varieties: The highest HCA content was recorded in 'Cabernet Sauvignon' (37.5 mg/kg), whereas the values in 'Kratošija' were lower (34.9 mg/kg), and the lowest was found in 'Vranac' (21.2 mg/kg).

The total HCA content in the skin of all the tested varieties corresponded with the values described by Rodríguez-Montealegre et al. (2006), but they were lower than those cited by Di Stefano and Maggiotitto (1995). The HCA content in the pulp corresponds with the values found by De La Presa-Owens et al. (1995) and Singleton et al. (1986).
Figure 2, which presents the relative percentage of the tartaric esters of HCAs (skin and pulp), shows that caftaric acid was by far the prevailing HCA in the grapes, whereas coutaric acid was significantly less present and fertaric acid was least present. The studied varieties had varying HCA profiles (relative content of caftaric/coutaric/fertaric acid): the values for 'Vranac' were 69/21/11, for 'Kratošija' 75/11/14 and for 'Cabernet Sauvignon' 73/20/7. Montenegrin varieties can be considered rich in fertaric acid. The relative content of fertaric acid found in 'Cabernet Sauvignon' grown in Montenegro was significantly higher than that reported by Rodríguez-Montealegre et al. (2006) and Calò et al. (1994). The richness of the red grape varieties in fertaric acid grown in the Montenegrin wine region can be considered to be a consequence of regional influence.
We also determined the trans-coutaric/trans-caftaric ratio, which can be used for variety differentiation, according to De La Presa-Owens et al. (1995) and Singleton et al. (1986). The values for this ratio varied statistically significantly between the varieties and amounted to 0.236 for 'Vranac', 0.107 for 'Kratošija' and 0.213 for 'Cabernet Sauvignon'. The values of the trans-coutaric/trans-caftaric acid ratio for the variety 'Cabernet Sauvignon' in our research correspond well with the values in the skin of this variety reported by Rodríguez-Montealegre et al. (2006).
The varieties showed significant differences for the majority of the tested HCA contents in the berry, which was determined using ANOVA. Otherwise there were no significant differences between the growing locations with respect to trans-fertaric, caffeic and para-coumaric acid content in the skin, as well as with regard to the relative amount of caftaric and the relative amount of coutaric acid content. All the examined HCA contents showed a two-way interaction (at least, $P < 0.05$) between the variety and the location, except for the content of caffeic acid in the berry skin.

Table 2: Hydroxycinnamate composition of the skin and pulp and their ratio in varieties 'Vranac', 'Kratošija' and 'Cabernet Sauvingnon' (mean values ±SD)

	SKIN (mg/kg of fresh grapes)								*PULP* (mg/kg of fresh grapes)						*Ratio*
	trans - caftaric acid	*cis-coutaric acid*	*trans-coutaric acid*	*trans-fertaric acid*	*caffeic acid*	*para coumaric acid*	*ferulic Acid*	*total HCA in skin*	*trans-caftaric acid*	*cis-coutaric acid*	*trans-coutaric acid*	*trans-fertaric acid*	*total HCA in pulp*	*Total HCA in berry*	*t-coutaric /t-caftaric*
	Vranac														
L1	3.0±0.1^{b}	0.3±0.10^{b}	1.6±0.06^{b}	1.0±0.22^{b}	0.6±0.23	0.4±0.32^{ab}	0.0± c	6.8±0.3^{c}	7.1±0.6^{c}	0.5±0.09^{d}	1.0±0.05^{b}	1.6±0.32^{c}	10.2±0.3^{c}	17.0±0.1^{b}	*0.253±^{ab}*
L2	4.6±0.1^{ab}	0.7±0.01^{a}	2.6±0.4^{b}	0.9±0.22^{b}	0.2±0.42	0.4±0.33^{ab}	0.3±0.07^{bc}	9.7±0.2^{abc}	18.5±3.3^{ab}	0.9±0.05^{b}	2.8±0.61^{a}	2.0±0.22^{bc}	24.2±3.6^{ab}	33.9±3.8^{a}	*0.233±^{b}*
L3	3.7± 0.3^{ab}	0.5±0.04^{ab}	2.5±0.2^{b}	0.8±0.35^{b}	0.00±	0.5±0.13^{ab}	0.2±0.20^{c}	8.2±0.6^{bc}	21.5±0.3^{a}	0.9±0.02^{b}	2.8±0.07^{a}	2.0±0.38^{b}	27.2±0.2^{a}	35.4±0.8^{a}	*0.203±^{c}*
L4	7.7±0.2^{ab}	0.7±0.02^{a}	2.6±0.14^{b}	1.4±0.17^{a}	0.00±	0.6±0.21^{a}	0.9±0.06^{a}	13.8±0.5^{abc}	18.0±0.3^{ab}	0.8±0.02^{bc}	2.6±0.16^{a}	2.4±0.13^{a}	23.9±0.3^{ab}	37.7±0.8^{a}	*0.200±^{c}*
L5	7.5±0.2^{ab}	0.8±0.03^{a}	3.1±0.05^{b}	1.1±0.35^{ab}	0.8± 0.11	0.2±0.37^{b}	0.6±0.56^{bc}	14.1±0.9^{abc}	14.8±0.6^{b}	1.1±0.12^{a}	2.6±0.04^{a}	2.2±0.11^{ab}	20.7±0.7^{b}	34.8±1.4^{a}	*0.253±^{ab}*
L6	8.9±2.2^{a}	0.8±0.31^{a}	4.8±0.89^{a}	1.0±0.14^{b}	0.5±0.41	0.3±0.33^{ab}	0.7±0.02^{ab}	16.9±3.5^{a}	15.8±0.7^{b}	0.6±0.21^{cd}	1.9±0.35^{b}	1.9±0.10^{bc}	20.2±0.7^{b}	37.1±4.2^{a}	*0.270±^{a}*
	5.9±2.4^{B}	***0.6±0.21^{B}***	***2.9±1.06^{B}***	***1.0±0.28^{B}***	***0.4±0.37***	***0.4±0.28***	***0.5±0.37***	***11.6±3.9^{B}***	***16.0±4.8^{C}***	***0.8±0.21^{A}***	***2.3±0.71^{B}***	***2.0±0.33^{B}***	***21.1±5.7^{C}***	***32.7±7.6^{B}***	*0.236±^{A}*
	Kratošija														
L1	5.3±0.1^{d}	0.4± 0.11^{bc}	1.0±0.01^{d}	2.1±0.26^{bc}	0.3±44	0.1±0.12^{b}	0.1±0.20^{b}	9.3±0.8^{d}	28±.3.1^{b}	0.5±0.01^{b}	1.8±0.33^{bc}	3.7±0.33^{b}	34.8±3.1^{b}	44.1±4.0^{c}	*0.083±^{b}*
L2	4.3±0.1^{d}	0.2±0.01^{c}	0.7±0.05^{d}	2.1±0.11^{bc}	0.3±43	0.5±0.22^{a}	0.8±0.13^{a}	8.6±0.6^{d}	21.2±0.5^{c}	0.7±0.01^{a}	1.4±0.02^{c}	3.2±0.22^{c}	26.5±0.7^{c}	35.1±0.5^{c}	*0.080±^{b}*
L3	13.6±3.7^{b}	1.3±0.26^{a}	3.2±1.10^{b}	2.5±0.16^{a}	0.4±37	0.5±0.07^{a}	0.7±0.16^{a}	22.2±5.4^{b}	26.3±1.7^{b}	0.4±0.02^{b}	2.0±0.09^{b}	4.1±0.14^{b}	32.8±1.6^{b}	54.9±7.1^{b}	*0.127±^{a}*
L4	21.7±11.9^{a}	1.6±0.61^{a}	5.2±3.44^{a}	2.0±0.11^{c}	0.7±0.08	0.4±0.33^{ab}	0.3±0.28^{b}	31.9±15.5^{a}	35.4±0.8^{a}	0.5±0.04^{b}	2.6±0.18^{a}	3.8±0.09^{b}	42.3±1.0^{a}	74.2±16.4^{a}	*0.130±^{a}*
L5	10.9±3.5^{bc}	0.7±0.17^{b}	3.0±1.42^{bc}	2.3±0.03^{ab}	0.5±0.03	0.5±0.09^{a}	0.7±0.16^{a}	18.6±5.1^{bc}	39.4±8.8^{a}	0.5±0.04^{b}	2.6±0.35^{a}	3.8±0.29^{b}	46.2±8.9^{a}	64.8±14.0^{a}	*0.110±^{ab}*
L6	6.7±0.3^{cd}	0.3±0.02^{c}	1.3±0.13^{cd}	2.5±0.57^{a}	0.2±0.31	0.4±0.07^{ab}	0.5±0.12^{ab}	11.8±1.5^{cd}	19.6±1.3^{c}	0.7±0.34^{b}	1.7±0.04^{bc}	4.5±0.40^{a}	26.4±1.0^{c}	38.3±0.8^{c}	*0.110±^{ab}*
	10.4±7.6^{A}	***0.8±0.58^{B}***	***2.4±2.10^{C}***	***2.2±0.31^{A}***	***0.4±0.33***	***0.4±0.22***	***0.5±0.28***	***17.1±10.4^{A}***	***28.4±8.1^{B}***	***0.6±0.19^{B}***	***2.0±0.50^{C}***	***3.8±0.45^{A}***	***34.9±8.3^{B}***	***51.9±16^{A}***	*0.107±^{C}*
	Cabernet Sauvignon														
L1	7.4±1.4	1.1±0.02^{b}	5.1±0.79^{a}	0.6±0.05	0.5± 41	0.9±0.05^{a}	0.3±0.30	15.8±1.9^{a}	45.7±2.2^{a}	1.4±0.17^{a}	6.7± 0.77^{a}	1.4±0.11^{de}	55.1±2.8^{a}	71.0±0.9^{a}	*0.223±*
L2	4.2±1.0	1.6±0.17^{a}	3.4±0.88^{bc}	0.6±0.06	0.5±0.44	0.5±0.16^{ab}	0.6± 0.00	11.3±2.5^{ab}	35.5±0.1^{b}	0.7±0.02^{b}	5.0±0.20^{b}	1.7±0.02^{cd}	42.9±0.2^{b}	54.1±2.3^{b}	*0.210±*
L3	2.6±.2	0.4±0.04^{d}	1.3±0.06^{d}	0.7±0.07	0.2±0.38	0.5±0.05^{b}	0.4±0.38	6.1±0.6^{b}	20.0±0.9^{d}	0.4±0.09^{c}	3.7±0.22^{d}	1.2±0.03^{e}	25.2±1.0^{c}	31.3±1.1^{c}	*0.220±*
L4	4.7±0.2	1.1±0.07^{b}	4.2±0.05^{ab}	0.6±0.02	0.8±0.12	0.5±0.17^{ab}	0.3±0.30	12.4±0.2^{ab}	23.9±0.1^{cd}	0.6±0.20^{bc}	1.8±0.09^{e}	4.2±0.19^{a}	30.6±0.4^{d}	45.2±1.1^{b}	*0.213±*
5	4.4±0.2	0.9±0.15^{bc}	3.0±0.01^{bc}	0.6± 0.01	0.6±0.56	0.6±0.26^{ab}	0.6±0.05	10.6±0.7^{ab}	31.3±0.3^{b}	0.8±0.06^{b}	4.3±0.11^{c}	2.1±0.08^{b}	38.4±0.5^{c}	50.7±1.8^{b}	*0.203±*
L6	3.6±0.2	0.7±0.06^{cd}	2.2±0.02^{cd}	0.7±0.04	0.6±0.07	0.3±0.25^{b}	0.6±0.03	8.7±0.3^{b}	26.6±2.5^{c}	0.8±0.05^{b}	4.2±0.19^{c}	2.0±0.36^{bc}	32.7±3.3^{d}	43.7±3.4^{b}	*0.210±*
	4.5±7.6^{B}	***0.96±0.37^{A}***	***3.21±1.34^{A}***	***0.6±0.06^{C}***	***0.5±0.36***	***0.5±0.23***	***0.5±0.24***	***10.8±3.3^{B}***	***30.5±8.7^{A}***	***0.78±^{A}***	***4.29±0.32^{A}***	***2.07±1.0^{B}***	***37.5±10.1^{A}***	***49.3±12.5^{A}***	*0.213±^{B}*

Different superscript small letters in same row indicate significantly different means ($p < 0.05$) for locality.

Different bold superscript capital letters indicate significantly different means ($p < 0.05$) for grape varieties.

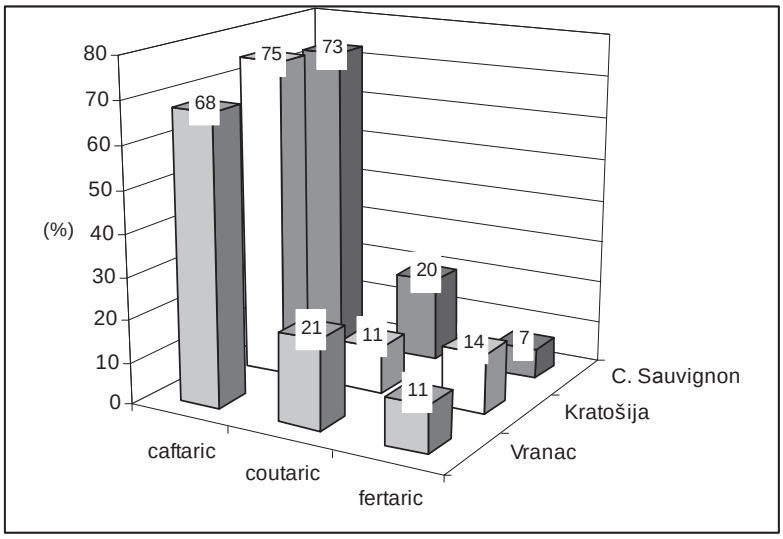

Fig. 2: Relative concentration of HCA for the studied varieties

PCA for grape varieties

The relationships between the three grape varieties grown at different locations were investigated by principal component analysis (PCA). The scatter plot was done with the distribution of components of anthocyanins and HCA in skin.

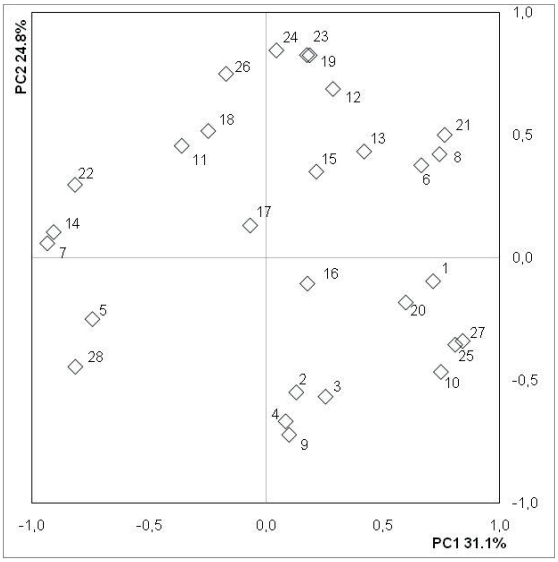

Fig. 3: The scatter plot of distribution of anthocyanin in skin (1 Dp-3-gl; 2 Cy-3-gl; 3 Pt-3-gl; 4 Pn-3-gl; 5 Mv-3-gl; 6 sum of acetates; 7 sum of p-coumarates; 8 acetyl/coumaryl ratio; 9 non- acylated/acylated ratio; 10 total anthocyanin) and HCA in skin (11 trans-caftaric acid; 12 cis-coutaric acid, 13 trans-coutaric acid; 14 trans-fertaric acid; 15 trans-caffeic acid; 16 para- coumaric acid without space 16 para-coumaric acid; 17 trans-ferulic acid; 18 total HCA), pulp (19 trans-caftaric acid; 20 cis-coutaric acid, 21 trans-coutaric acid; 22 trans-fertaric acid; 23 total HCA) and berry (24 total HCA; 25 t-coutaric/t-caftaric ratio; 26 relative amount (%) caftaric; 27 (%) coutaric; 28 (%) fertaric) content.

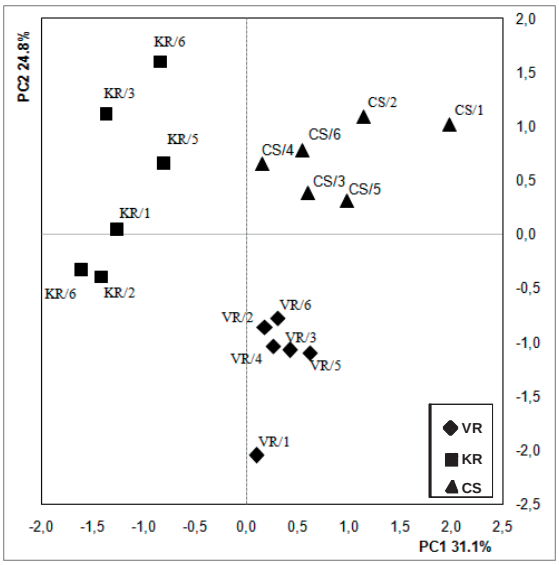

Fig. 4: Factor scores for the first two principle components for three grapevine varieties (VR – 'Vranac'; KR – 'Kratošija'; CS – 'Cabernet Sauvignon') grown on six locations (1-L1; 2-L2; 3-L3; 4-L4; 5 L5; 6-L6)

Results from the PCA indicated that the first two components show 55.9 % of the total variability observed. Correlation between the original variables and the first two principal components is shown in Figure 3. Variables with higher scores on PC1 (over 0.80 of absolute value) were sum of p-coumarylated anthocyanins content in skin (relative amount in %), trans-fertaric acid content in skin, trans-fertaric acid content in pulp, t-coutaric/t-caftaric ratio, relative amount of coutaric acid in berry and relative amount of fertaric acid in berry. The highest contribution of PC2 corresponded to the variables trans-caftaric acid content in pulp, total HCA content in pulp and total HCA content in berry. Starting from the negative to the positive values of PC1, the treatments indicated a general decrease in sum of p-coumarates content in skin, trans-fertaric acid content in skin, trans-fertaric acid content in pulp and relative amount of fertaric acid in berry and increase in t-coutaric/t-caftaric ratio and relative amount of coutaric acid in berry. Proceeding from negative to positive values of PC2 treatments showed increase in trans-caftaric acid content in pulp and total HCA content in pulp and in berry.

Figure 4 shows geometrical distances between varieties on different locations as determined on the basis of all studied characteristics. Based on the position on the scatter plot it can be observed that the grape varieties were separated in three distinct groups. This indicated that each variety had a unique profile compared to the content of anthocyanins and hydroxycinnamic acids. Also these properties can be used as reliable criteria for the varietal identification. It can also be noted that the grape variety 'Vranac' had the greatest stability of the content of these compounds. In the varieties 'Cabernet Sauvignon' and 'Kratošija' isolated groups were less homogeneous, depending on the location of growth.

Conclusion

Grapes of the varieties 'Vranac' and 'Kratošija' had a similar anthocyanin profile: the relative concentration of non-acylated anthocyanins followed the same order, p-coumarylated derivates were more abundant than

the acetylated ones, but the relative amount of each anthocyanin was different in the two varieties. On the other hand, 'Cabernet Sauvignon' grapes had a high relative amount of acylated derivates so that the acetylated anthocyanins were more abundant than p-coumarylated ones. In all the examined varieties cultivated in Montenegro, a high relative amount of p-coumarylated anthocyanins was found probably due to regional influences. The acetates/coumarates anthocyanin ratio varied considerably between the varieties. The content of total anthocyanins varied significantly between varieties, in the grapes 'Vranac' and 'Cabernet Sauvignon' is was almost twice as high as in 'Kratošija'. Out of the hydroxycinnamic acids analysed, caftaric acids prevailed, whereas coutaric acid showed much lower contents. Fertaric acids were least present in grapes of all the tested varieties, however, the varieties had a higher amount of fertaric acid than published before, probably due to regional influences. The varieties were characterised by differences in the HCA profile, total HCA content of the skin and the pulp and the trans-coutaric/trans-caftaric ratio.
Each grape variety had a unique profile when it comes to the composition of anthocyanins and hydroxycinnamic acids, which was confirmed using a multivariate method (principal component analysis). The method allowed for a clear classification of the examined grape varieties into three groups. A slight variation according to different growing locations was observed.

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