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ORIGINAL PAPER

Characterization of the phenolic profile of commercial Montenegrin red and white wines

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Abstract

The phenolic characterization was conducted on 43 commercial red (Vranac, Kratošija, and Cabernet Sauvignon) and white (Krstač and Chardonnay) Montenegrin wines during the vintages of 2015 and 2016. Several phenolic groups were determined: total anthocyanins, low- and high-molecular-weight proanthocyanidins. Non-flavonoids, such as phenolic acids (seven hydroxycinnamic acid derivatives—tartaric esters and free forms, and hydroxybenzoic acid derivatives—gallic acid), and stilbenes (resveratrols *trans*/*cis* isomers and their 3-glucosides) were analyzed by HPLC–UV and HPLC–DAD, respectively. Red wines reported higher concentrations of phenolic compounds and antioxidant properties, these parameters being strongly correlated. Among red wines, Cabernet Sauvignon in 2015 reported the highest content of total phenols (2197 mg L^{-1}), low and high content of proanthocyanidins and antioxidant activity (20.0 mM L^{-1}), respectively, and the lowest content of stilbenes (1.15 mg L^{-1}). Vranac wines showed the highest content of anthocyanins (799–810 mg L^{-1}). Kratošija wines reported the highest content of hydroxycinnamic acids (92.0–97.7 mg L^{-1}) in both vintages. Among white wines, in the 2016 vintage, Krstač reported higher contents of total phenols and stilbenes (300 and 0.41 mg L^{-1}, respectively) when compared with Chardonnay wines (226 and 0.12 mg L^{-1}, respectively). The results showed that the variety influences the content and composition of hydroxycinnamic acid derivatives and stilbenes. The same was verified in vintages, where edaphoclimatic conditions probably influenced the results obtained. The wines studied were differentiated and discriminated. The factor variety was always present and sometimes it was difficult to classify wines according to the vintage, showing that the varietal factor prevails.

Keywords Vranac · Kratošija · Krstač · Phenolic groups · Hydroxycinnamic acids · Stilbenes

Introduction

Wines contain a number of phenolic molecules which are considered important parameters for the assessment of wine quality. Phenols contribute to the wine organoleptic/sensory characteristics such as color, astringency, bitterness and flavor, as well as to the chemical differentiation between red and white wines [1]. Phenolic compounds are ascribed with numerous positive effects to human health, especially due to their antioxidant properties [2, 3]. Several groups of phenolic compounds are responsible for these healthy properties: anthocyanins [4]; proanthocyanins [5, 6]; stilbenes [7]. Nevertheless, the complex mixture of these molecules and their synergetic action is also responsible for the biological properties of wines [8, 9].

Phenolic compounds in wines are mainly divided into non-flavonoid (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoid compounds (anthocyanins, flavan-3-ols and flavonols). The most abundant flavonoid groups of phenolic compounds are anthocyanins and flavan-3-ols in red wines, and flavonols, especially quercetin-3-glucoside in white wines. Among non-flavonoid phenols group, the hydroxycinnamic acids are the major group in

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white wines, responsible for the browning of wines as these components are easily oxidized [10].

Several factors influence the wines' phenolic composition. Nevertheless, the vineyard cultural and agronomical practices applied, allied to the environmental conditions during berry development such as soil, geographical location and weather conditions during the harvest period and above all, the varietal factors are the most important [2]. The technological process and winemaking techniques will also play an important role in the final phenolic composition of the obtained wines [11].

Since ancient times, Montenegro has had a long tradition in wine production, being considered the country of origin of the red varieties Vranac and Kratošija. These two varieties are very important in the wine production of the Western Balkan wine region. In the last 2 decades, the vineyard cultivation surface increased significantly in Montenegro and in other Western Balkan countries (Croatia, Bosnia and Herzegovina, Serbia, and N. Macedonia), resulting in an increase in wine production, and a higher presence of these wines in the European market and worldwide. In this sense, the chemical characterization of phenolic compounds and varietal discrimination of Montenegrin wines is an important issue, mainly for determining their regional authenticity. Wine origin should provide recognition and bring a touch of typicity to the wines obtained, the so-called "terroir". For the wine industry and the market sector, it is essential that wine authenticity can be recognized by the genetics (variety) and origin of the product. For national authorities, it is also important to control the authenticity of wines to monitor their label information [12].

The available literature devoted to the characterization of phenolic compounds in wines from the Western Balkan region is related to red wines (mostly wine from Vranac) [1, 10, 13–17].

Regarding the phenolic composition of Montenegrin wines, the data available in literature are mostly based on some global parameters for red wines performed by spectrophotometric methods. Kovač et al. [18] examined the influence of some oenological practices on the polyphenolic content of Vranac wine in Montenegro. Extractable phenolic compounds (total phenols, anthocyanins, low and high molecular procyanidins) in Montenegrin red wines were evaluated by Pajović et al. [19], whereas the analysis of polyphenols in Montenegrin Vranac wines was investigated by Pajović Šćepanović et al. [20]. The influence of the yield on the antioxidant capacity of some white and red wines from Montenegro was investigated by Košmerl et al. [21]. Preliminary studies of the phenolic composition and varietal discrimination of Montenegrin red wines, prepared under the same oenological practices have been performed applying the HPLC–DAD technique [22]. To the best of our knowledge, so far there is no available literature on the evaluation of phenolic groups or on the quantification of some individual non-flavonoid phenolic compounds in commercial red and white wines from different areas/localities and different vintages in Montenegro. Considering this, the objectives of the present work were: (1) to characterize phenolic groups and some individual phenols within the non-flavonoids phenol groups, and to determine the antioxidant activities of wines and the group affected by those properties; (2) to assess the influence of vintage on the commercial autochthonous wines Vranac, Kratošija, Krstač, as well as that of international Cabernet Sauvignon and Chardonnay, all 2015 and 2016 vintages from the Podgorički sub-region in Montenegro.

Materials and methods

Chemicals and reagents

The following chemicals and standards (all of HPLC grade) were used for the chromatographic analysis: methanol, formic acid, potassium dihydrogenphosphate, phosphoric acid (Merck, Darmstadt, Germany); distilled water, MilliQ water (Millipore Corporation, Billerica, MA, USA); standards: caffeic acid, ferulic acid, and *trans*-resveratrol (Sigma, Steinheim, Germany), caftaric acid (Dalton Chemical Laboratories Inc., Toronto, Canada) gallic acid, and *p*-coumaric acid (Roth, Karlsruhe, Germany).

For the spectrophotometric analyses and an estimation of the antioxidant activity, the following chemicals and standards were used: methanol, ethanol (Merck, Darmstadt, Germany), hydrochloric acid, sodium hydroxide, sodium bisulphite, L(+)-tartaric acid, potassium persulfate ($K_2S_2O_8$) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma, Steinheim, Germany), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (Fluka Germany). Ultra-pure water was of Milli Q grade (Millipore Corporation, Billerica, MA, USA). The reagents Folin–Ciocalteu and vanillin were from Merck Darmstadt, Germany.

Characterization of the assessed Montenegrin wine region

According to the recently finished zoning by EU regulations, the Montenegrin wine area is divided into four wine regions and fifteen sub-regions. The most important region is the Montenegrin Basin of Skadar Lake, where the Podgorica sub-region is included, covering 90% of the total vineyard area in Montenegro. According to the Geoviticulture Multicriteria Climatic Classification System [23], the weather in the Podgorica sub-region is classified as "very warm". Due to Huglin's heliothermal index, as well as to the sum

of growing degree days (GDD) for the period of the phenological cycle of the grapevines, the Podgorica sub-region is characterized as "Region V" (> 2220 GDD), which is a "warm region" according to the Winkler Index. The Podgorica vineyard district is favorable for the cultivation of vines and consequently, for the production of high-quality wines IPA (2017). The meteorological conditions for the experimental years assessed are summarized in Table 1.

Wine sampling

Forty-three commercial red and white wines from different *Vitis vinifera* L. autochthonous (Vranac, Kratošija and Krstač) and international reference varieties (Cabernet Sauvignon and Chardonnay) were collected during two consecutive vintages (2015 and 2016) directly from the commercial wineries located in different areas/locations of the Podgorica sub-region. Vranac and Kratošija are the most dominant red wines in Montenegro and in other Western Balkan countries, together with Cabernet Sauvignon wines. Krstač is only produced in Montenegro and it is the most abundant white wine in the country, followed by the popular Chardonnay. The criteria for the selection of the wineries were mainly their longest tradition and constant high quality of the wines produced. The technological process where the wines were produced varied among the wineries selected. Some wineries applied traditional processes (Lješkopolje), in others the process was semi-industrialized (Rogami and Piperi), while in others the process was fully industrial (Ćemovko Polje). The number of samples and the samples codes, variety, type of wine and appellation/area are listed in Table 2.

To be comparable, the wine samples from the two vintages were analyzed with the same age, that is, 1 year old in January (second year after vintage).

Spectrophotometric analyses

The spectrophotometric analyses were performed using a Varian Cary 100 spectrophotometer (Bio Tech, Maryland, USA), as described by Di Stefano and Guidoni [24] and Di Stefano et al. [25]. A preliminary clean-up of the phenols was performed with a classic Sep-Pak (0.35 g) C-18 columns supplied by Waters (Milford, MA, USA) to remove polar compounds such as sugars, organic acids, amino acids, and free SO_2, which might interfere with the assays.

Total phenols (TP)

The total phenols were assessed through the reduction of phosphotungstic–phosphomolybdic acids (Folin-Ciocalteu's reagent—FC) to blue pigments by phenols in an alkaline solution. Briefly, 1 mL of diluted wine (1/10 or 1/20 with 1 N of H_2SO_4) was mixed with 2 mL

Table 1 Meteorological data in the Podgorica sub-region during the two consecutive seasons studied (IHMS, 2017)

	Temperatures (°C)			Winkler Index	Huglin Index	Rainfall (mm)			Humidity		
	Annual average	Seasonal[a]	Monthly[b]			Annual precipitations	Seasonal[a]	Monthly[b]	Annual average	Seasonal[a]	Monthly[b]
2015	17.2	24.3	24.1	2620	3203	1176.0	243.0	43.6	57.0	47.3	50.0
2016	16.6	23.1	22.1	2397	2941	1993.7	675.7	84.4	62.0	55.2	58.0

[a]From April 1 to September 30

[b]Last month (30 days) before harvest

IHMS Institute for Hydrometeorology and Seismology of Montenegro

Table 2 Description of the wines studied

Wine code Number of samples	Variety	Type of wine	Appellation/location
V_1 ($n=3$)	Vranac	Red	Ćemovsko polje
V_2 ($n=3$)	Vranac	Red	Lješkopolje
V_3 ($n=3$)	Vranac	Red	Rogami
K_1 ($n=3$)	Kratošija	Red	Ćemovsko polje
K_2 ($n=3$)	Kratošija	Red	Lješkopolje
K_3 ($n=3$)	Kratošija	Red	Rogami
CS_1 ($n=3$)	Cabernet Sauvignon	Red	Ćemovsko polje
CS_2 ($n=3$)	Cabernet Sauvignon	Red	Piperi
CS_3 ($n=3$)	Cabernet Sauvignon	Red	Rogami
WK_1 ($n=3$)	Krstač	White	Ćemovsko polje
WK_2 ($n=2$)	Krstač	White	Lješkopolje
WK_3 ($n=2$)	Krstač	White	Rogami
WCh_1 ($n=3$)	Chardonnay	White	Ćemovsko polje
WCh_2 ($n=3$)	Chardonnay	White	Piperi
WCh_3 ($n=3$)	Chardonnay	White	Rogami

of methanol, 5 mL of distilled water, 1 mL of FC reagent, 4 mL of Na_2CO_2 solution (10%) and distilled water was added to a total volume of 20 mL. After 90 min, the absorbance was read at 700 nm against a blank prepared with distilled water in a 1 cm cuvette. When absorbance was between 0.3 and 0.6 AU (the linear range) the results were calculated as (+)-catechins equivalents = $186.5 \times A \times d$ in mL L^{-1}; where A is the absorbtion and d is sample dilution. The concentrations were determined by means of a calibration curve of gallic acid.

High-molecular-weight proanthocyanidins (HMP)

The HMP were evaluated by transformation into cyaniding [25]. The results were expressed against the corresponding blank as cyanidin chloride. The solution was prepared in two flasks (A and B) as follows: 9.5 mL of 95% ethyl alcohol, 2 mL of diluted wine (with 1 N H_2SO_4) with 3 mL of methyl alcohol and 12.5 mL of 0.3% FeSO4 in HCl. The flask A was placed to boil for 50 min ($T=102$ °C), while flask B was placed in ice for the same period. After that, both flasks were adjusted on the same temperature (19 °C) in the water bath and absorbance was read at 700 nm with blank—distilled water in 1 cm cuvette when absorption was between 0.3 and 0.6. The results were calculated as mg L^{-1} of cyanidin equivalents = $(A-B) \times 1162.5 \times d \times V$; where A is the absorbance of flask A content; B is the absorbance of flask B; d is the wine sample dilution; and V is the sample volume. The method provides a good estimation for the evaluation of HMP [26].

Low-molecular-weight proanthocyanidins: index of vanillin (LMP)

The catechins and proanthocyanidins reactive to vanillin were analyzed according to the optimized and controlled vanillin–HCl method of Broadhurst and Jones [27], following the conditions described by Di Stefano et al. [25] they were calculated as (+)-catechin (mg L^{-1}) by means of a calibration curve. A solution was prepared consisting of 2 mL of wines (diluted with 1 N H_2SO_4) and 5 mL of methanol. The mixture was then divided in two flasks (A and B). In flask A were added 6 mL of 4% vanillin in methanol and 3 mL of concentrated HCl. In flask B were added 6 mL of 4% vanillin in methanol and 3 mL of concentrated HCl. After 15 min of acclimatization of the samples at 15 °C, the absorbance was read at 700 nm using the content of flask B as blank. When the absorbance was between 0.2 and 0.4 AU (700 nm), the LMP was reported as (+)-catechin equivalents = $E \times d \times 0.4$ in mg L^{-1}; where E is the absorbance and d the sample dilution. This method provides a good estimation of catechins, free flavanols, and a low degree of proanthocyanidins.

Total anthocyanins (TA)

The total anthocyanins were determined on the basis of maximal absorbance in the visible range (536–542 nm) [25]. 5 mL of diluted wine (with 1 N H_2SO_4) were mixed with 3 mL of methanol and three drops of concentrated HCl, and 12 mL of a solution of ethanol/water/HCl (70:30:1). After 15 min the absorbance was read between 380 and 700 nm. The TA content was calculated as malvidin 3-glucoside chloride equivalents (mg L^{-1} of wine), being the

experimental value of the molar absorptivity of malvidin 3-glucoside chloride in ethanol/water/HCl (70:30:1), $\varepsilon = 30{,}100$ e.q. M^{-1} cm^{-1} at 542 nm [25].

HPLC analyses

HPLC–DAD analysis of phenolic acids

The HPLC separation and quantification of phenolic acids were performed according to the modified method of Vrhovšek et al. [28] on a Hewlett-Packard Series Instrument (Agilent Technologies, Santa Clara, CA) using a reversed-phase column POROSHELL 120 SB-C18 analytical column (150 × 2.1 mm, 2.7 µm) (Agilent Technologies, Santa Clara, CA), thermostated at 40 °C, with an auto injector (5 µL injection volume) and a diode array detector (DAD) set at 280 and 320 nm. 5 µL of 0.45 µm membrane-filtered samples were injected with a 0.25 mL min^{-1} flow rate. The solvents used were 0.5% formic acid pH 2.3 (solvent A) and methanol (solvent B), with the following gradient program: 3% B at 0 min, 6% B at 14 min, 7% B at 24 min, 13% B at 35 min and maintained until 47 min, 20% B at 57 min, 70% B at 60 min and maintained until 70 min, 3% B at 75 min, and post run times of 15 min. Determinations were carried out with an external standard method by comparing the retention times and ultraviolet (UV) spectra with commercial standards: caffeic acid, ferulic acid, caftaric acid, *p*-coumaric acid, gallic acid. The quantification of *trans*-coutaric acid, *cis*-coutaric acid, and fertaric acid was obtained by external calibration using caftaric acid.

HPLC analyses of stilbenes

The concentrations of four different resveratrols were analyzed according to a modified method of Eder et al. [29]. An HPLC system (type RRLC; Agilent Technologies, Santa Clara, CA) with an analytical column POROSHELL 120 SB-C18 (150 × 2.1 mm, 2.7 µm) (Agilent Technologies, Santa Clara, CA) was used. Using 0.5% formic acid (solvent A) and methanol (solvent B) as eluents, the following gradient was used: 3% B at 0 min, 4% B at 25 min, 20% B at 40 min, 30% B at 75 min and maintained until 80 min, 70% B at 85 min and maintained until 95 min, and 3% B at 98 min, with a post run time of 15 min. The injection volume was 5 µL of 0.45 µm membrane-filtered samples, with a flow rate of 0.25 mL min^{-1}. The UV detection was performed by measuring wavelengths of 280 and 320 nm. The identification and quantification of *trans*-resveratrol and *trans*-resveratrol glucoside (*trans*-piceid) was performed using pure standards. *Cis*-resveratrol and *cis*-resveratrol glucoside were obtained from the *trans*-isomers by UV irradiation (254 nm, 24 h) and were quantified using a molar *cis*/*trans* extinction ratio of 3.78/1.

Determination of the wines antioxidant activity

The antioxidant capacity of wines was estimated using the Trolox equivalent antioxidant capacity (TEAC) according to a modified spectrophotometric method [30], described by Pajović-Šćepanović et al. [22]. An ABTS solution was prepared by incubating 5 mL of 7 mM aqueous ABTS solution with 88 µL of potassium persulfate solution for 16 h in the dark. The obtained solution was further diluted in absolute ethanol until the initial absorbance value of 0.7 ± 0.05 at 734 nm was reached. The absorbance of 2 mL of ABTS solution at 734 nm (ABTS) was measured, 0.01 mL of adequately diluted wine was added. After exactly 6 min, the absorbance of the sample was measured. The results were expressed as mM L^{-1} of Trolox equivalent antioxidant capacity (TEAC).

Statistical analysis

To establish the significance of differences between the varietal wines, the origin of wines—sites/localities and their interaction for each studied parameter, a two-factorial analysis of variance (ANOVA) was applied. For those parameters and factors where significant differences were detected, an additional LSD test was applied to the significance level of $P < 0.05$.

The data obtained (19 variables, including phenolic compounds and ABTS antioxidant activity) were also used in a principal component analysis (PCA) and linear discriminant analysis (LDA) to verify if the wines could, respectively, differentiate and discriminate the wine samples according to the type of grape (white vs. red) and according to the variety, vintage, and location. These multivariate analyses were carried out according to the procedures described by Pajović-Šćepanović et al. [22]. All the statistical treatments were performed using the statistical package IBM SPSS Statistics 20 (IBM Corporation, New York, U.S.A.).

Results and discussion

Wine phenolic composition by spectrophotometrical methods

The total phenol content, low- and high-molecular weight proanthocyanidins (LMP and HMP, respectively), and the total anthocyanins in 1-year-old wines from Vranac, Kratošija, Cabernet Sauvignon, Krstač, and Chardonnay are reported in Table 3.

As expected, red wines contained a significantly higher amount of total phenol content, low- and high-molecular-weight proanthocyanidins than white wines. The total phenol content in the red wines varied between

Table 3 Content of polyphenols (mg L^{-1}) (TP—Total polyphenols, TA—total anthocyanins, LMP—low-molecular weight proanthocyanidins, HMP—high-molecular weight proanthocyanidins) and ABTS (mM L^{-1}) in the wines (Vranac—V, Kratošija—K, Cabernet Sauvignon—CS, Krstač—WK and Chardonnay—WCh) from the 2015 and 2016 vintages

	TP		TA		LMP		HMP		ABTS[a]	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
V_1	1834^{bc}	1780^{cd}	800^{a}	776^{b}	1082^{bc}	495^{c}	3088^{ab}	2137^{b}	17.5^{ab}	15.1^{c}
V_2	1623^{cd}	1551^{d}	815^{a}	738^{b}	818^{cd}	449^{c}	2102^{c}	1625^{c}	16.2^{ac}	15.0^{c}
V_3	2182^{ab}	2064^{ab}	814^{a}	883^{a}	1259^{ab}	1001^{b}	2714^{b}	2585^{ab}	20.1^{db}	18.2^{a}
Mean	1880^{B}	1798^{B}	810^{A}	799^{A}	1053^{A}	648^{B}	2635^{B}	2116^{B}	18.0^{B}	16.0^{B}
K_1	1470^{cd}	1239^{e}	253^{d}	220^{e}	995^{bc}	445^{ce}	1848^{c}	2012^{b}	15.3^{ac}	11.8^{d}
K_2	2063^{b}	1837^{bc}	308^{d}	484^{d}	1292^{ab}	819^{d}	2799^{b}	1880^{bc}	20.5^{db}	15.8^{bc}
K_3	1407^{d}	1200^{e}	407^{c}	296^{e}	451^{d}	297^{e}	1632^{d}	1177^{d}	13.8^{c}	10.8^{d}
Mean	1647^{C}	1425^{C}	323^{C}	333^{C}	913^{B}	520^{C}	2093^{C}	1690^{C}	17.0^{C}	13.0^{C}
CS_1	2133^{ab}	2227^{a}	457^{c}	584^{c}	1642^{a}	1183^{a}	3306^{ab}	2771^{a}	19.1^{b}	18.8^{a}
CS_2	1972^{b}	1724^{cd}	710^{b}	858^{a}	1112^{bc}	567^{c}	2939^{b}	2274^{b}	17.6^{ab}	15.8^{bc}
CS_3	2485^{a}	1867^{cb}	636^{b}	463^{d}	1587^{a}	1129^{ab}	3430^{a}	2454^{a}	22.8^{d}	16.8^{b}
Mean	2197^{A}	1939^{A}	601^{B}	602^{B}	1447^{A}	960^{A}	3225^{A}	2500^{A}	20.0^{A}	17.0^{A}
WK_1	389^{ab}	277^{ab}	–	–	51^{a}	26^{ab}	267	174	1.8^{abc}	1.4^{ab}
WK_2	427^{a}	301^{a}	–	–	47^{a}	37^{a}	282	179	2.1^{a}	1.5^{ab}
WK_3	408^{a}	322^{a}	–	–	52^{a}	31^{b}	296	177	2.0^{ab}	1.6^{a}
Mean	408^{A}	300^{A}	–	–	50^{A}	31^{A}	282	177	2.0^{A}	1.5
WCh_1	255^{c}	202^{c}	–	–	27^{b}	18^{d}	248	182	1.2^{c}	0.9^{b}
WCh_2	334^{bc}	229^{bc}	–	–	22^{b}	26^{c}	254	195	1.4^{bc}	1.0^{ab}
WCh_3	295^{c}	245^{bc}	–	–	25^{b}	25^{c}	269	188	1.2^{c}	1.1^{ab}
Mean	294^{B}	226^{B}	–	–	25^{B}	23^{B}	257	188	1.3^{B}	1.0
ANOVA										
Vintage	**		n.s.		**		**		**	

Different superscript small letters in the same row indicate significantly different mean values ($P<0.05$) for wines from different producers in the same vintage and for the red and white wines separately

Different superscript capital letters indicate significantly different mean values ($P<0.05$) for varietal wines and for the red and white wines separately

ANOVA was used to compare data between vintages (n.s. not significant and $^{**}P\leq0.05$)

[a]expressed as mM L^{-1} of Trolox equivalents

1407 and 2485 mg L^{-1} in the 2015 vintage, and from 1200 to 2227 mg L^{-1} in the 2016 vintage. These results are comparable to those reported for the Vranac and Kratošija wines produced in the Podgorički sub-region in the 2012 vintage, between 1265–2032 mg L^{-1} [21] and between 2000–2019 mg L^{-1} in Vranac wines during the seasons 2008–2010 [20]. Our results are also in accordance with the total phenol content for Macedonian (1394–3097 mg L^{-1}) [13] and Croatian red wines (1012–3264 mg L^{-1}) [14].

The content of total anthocyanins in the examined wines varied between 253–815 mg L^{-1} in 2015 and between 220–883 mg L^{-1} in 2016. The results are comparable to those reported for Montenegrin red wines from Vranac (600–870 mg L^{-1} in 2008 and 640–790 mg L^{-1} in 2009) produced with different fermentation methods in the Podgorički sub-region [31]. Our results are also in agreement with those of Košmerl et al. [21] regarding Montenegrin red wines from Vranac and Kratošija, as well as with Kallitraka et al. [32] regarding international Greek wines.

Regarding the contents of LMP, the variation was from 451 to 1642 mg L^{-1} in 2015 and from 297 to 1129 mg L^{-1} in 2016. The content of HMP was higher, ranging between 1632–3430 mg L^{-1} in 2015 and between 1177–2771 mg L^{-1} in 2016. Our results are within the range obtained in other studies that used the same techniques. Mattivi et al. [33] analyzed Italian red wines and reported a great variation in LMP, between 79–2431 mg L^{-1}, and in HMP, between 112–3550 mg L^{-1}. Baiano et al. [34] reported values between 953–2366 mg L^{-1} for LMP and 1581–3687 mg L^{-1} for HMP in Primitivo wine from the Puglia wine region in Italy.

The total phenol content was much lower in white wines than in red wines (Table 3). It ranged between 255–427 mg L^{-1} in 2015 and between 202–322 mg L^{-1} in 2016. The results obtained are in accordance with the total phenols content in white wines from Croatia (301–402 mg L^{-1}) [35], and a little higher than those of the varieties Krstač and Žižak (253.9 and 275 mg L^{-1}) from the Podgorički sub-region of Montenegro [21]

and those of Macedonian white wines from the varieties Tamjanika, Rizling, Smederevka, and Sauvignon Blanc (185–230 mg L^{-1}) [1]. The LMP content varied between 22–52 mg L^{-1} and 18–37 mg L^{-1} in 2015 and 2016, respectively. The HMP content ranged between 248–296 mg L^{-1} and 174–195 mg L^{-1} in 2015 and 2016, respectively.

The results obtained show that the levels of the different classes of phenolic compounds were dependent on grape variety (Table 3). In both vintages, Cabernet Sauvignon wines reported the highest content of the examined group of phenols, statistically different from the other two red wines, except for the content of anthocyanins. In previous studies, some authors reported a higher content of phenolic compounds in Cabernet Sauvignon wines when compared to other red wines [14, 19, 32]. The highest anthocyanins content obtained in this study was found in Vranac wines, which is in line with previous results for red wines in Montenegro [19, 21] and N. Macedonia [13]. The content of LMP and HMP in our study reported the same trend presented by Pajović et al. [19] for varietal wines in the Montenegrin region: Cabernet Sauvignon > Vranac > Kratošija.

Determination of individual phenolic non-flavonoid compounds in wines

For an accurate characterization of the phenolic composition of Montenegrin red and white wines, a detailed chromatographic analysis was performed using HPLC–DAD. Spectrophotometric methodologies for the evaluation of phenolic compounds are techniques which allow a fast screening of the phenolic content in wines [1]. Two classes of non-flavonoid phenolic compounds were investigated: Phenolic acids (hydroxycinnamic acid derivatives—tartaric esters and free forms and hydroxybenzoic acid derivatives—gallic acid) and stilbenes (resveratrols *trans*/*cis* isomers and their 3-glucosides).

Phenolic acids in red and white Montenegrin wines

As expected based on literature, the content of tartaric esters of HCA was generally higher than their free acids forms. In all the red and white wines, the concentration of HCA decreased in the following order: *trans*-caftaric acid > *trans*-coutaric acid > others (Table 4) [36–38].

Regarding red wines, the content of *trans*-caftaric acid ranged between 45.3–68.6 mg L^{-1} in 2015 and between 46.7–88.6 mg L^{-1} in 2016. The content of *trans*-coutaric acid varied from 8.5 to 20.5 mg L^{-1} and from 7.9 to 21.0 mg L^{-1} in 2015 and 2016, respectively. As it can be seen from the data presented in Table 4, statistically significant differences were observed for esters and free forms of HCA in the wines studied. The total content of HCA varied between 75.3–98.9 mg L^{-1} and 75.9–116 mg L^{-1} in the vintages from 2015 and 2016, respectively. The values for the total content of HCA are in accordance with the content reported for some Italian red wines [33] 20–120 mg L^{-1} and for some Austrian red wines 37.7–170.1 mg L^{-1} [39].

In the white wines, the contents of caftaric and *trans*-coutaric acid, and consequently the content of total HCA, were lower when compared to red wines (Table 4). The content of caftaric acid varied between 33.2–45.5 mg L^{-1} and 28.5–45.6 mg L^{-1}, whereas the content of *trans*-coutaric acid was 5.3–8.1 mg L^{-1} and 4.6–7.9 mg L^{-1} in 2015 and 2016, respectively. The content of total HCA in the white wines studied was 52.3–71.2 mg L^{-1} in 2015 and 47.8–65.4 mg L^{-1} in 2016. The results obtained for the total content of HCA in the white wines are comparable to those reported for Weiβburgunder and Chardonnay wines from Austria, which ranged between 26.5 and 65 mg L^{-1} [28]. However, our results were higher when compared to some Californian white wines, mostly Chardonnay (8.4–34.3 mg L^{-1}) [40].

Gallic acid was the only hydroxybenzoic acid derivative quantified in the wines (Table 5). In the red wines, the content of gallic acid varied between 27.1–66.1 mg L^{-1} in 2015 and 25.0–62.2 mg L^{-1} in 2016. These results are similar to some red wines in Greece (10.9–46.3 mg L^{-1}) [12] and to some Italian red wines (13.6–90.5 mg L^{-1}) [41]. The content of gallic acid in white wines varied between 0.9–2.4 mg L^{-1} and 1.4–1.9 mg L^{-1} in both vintages (2015 and 2016, respectively), and these results are in line with those obtained by Minussi et al. [42] when studying Italian white wines (0.6–3.5 mg L^{-1}).

The content of total HCA was higher in Kratošija wines, being statistically different from those found in Vranac and Cabernet Sauvignon (Table 4). The results obtained are comparable to those of Pajović-Šćepanović et al. [22], who studied red varietal wines produced from grapes from different locations but under the same oenological conditions. The percentage profile of hydroxycinnamic acids (caftaric/coutaric/fertaric acid) in the three analyzed red varietal wines is similar to those found in the other Montenegrin wine-growing regions [22]. The percentage profiles obtained in the varieties Vranac and Cabernet Sauvignon are also similar to those obtained in N. Macedonia [15]. There was evidence of varietal differences in the HCA profiles among the studied white wines Krstač and Chardonnay, mostly based on coutaric and fertaric forms. Those results confirmed previous studies where it was found that specific HCA profiles are responsible for the characterization of wines according to variety [33, 37].

The results obtained in the phenolic acids composition from the foreign varieties (Cabernet Sauvignon and Chardonnay) were compared between the wines obtained in Montenegro and in southern France. According to the studies of Laudrault et al. [43] and Teisedre and Laundrault [44], the

Table 4 Content of hydroxycinnamic acid derivatives (mg L^{-1}) in Montenegrin red and white wines from the 2015 and 2016 vintages (mean values ± SD)

	Caftaric acid		*cis*-Coutaric acid		*trans*-Coutaric acid		Fertaric acid		Caffeic acid		*p*-Coumaric acid		Ferulic acid		Total HCA	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
V_1	51.4^{c}	53.2^{d}	2.7^{c}	5.1^{b}	20.5^{a}	18.2^{b}	3.5^{c}	3.0^{cd}	6.4^{a}	4.6^{a}	4.5^{a}	3.7^{a}	3.0^{a}	0.9^{bc}	91.9^{ab}	88.7^{bc}
V_2	53.3^{bc}	46.7^{e}	1.7^{d}	3.2^{c}	17.2^{abd}	14.8^{d}	3.6^{c}	3.0^{cd}	3.9^{c}	4.5^{a}	3.4^{b}	2.6^{b}	2.4^{b}	1.0^{bc}	85.5^{ab}	75.9^{d}
V_3	49.3^{c}	57.8^{cd}	4.1^{a}	2.9^{d}	19.5^{ab}	21.0^{a}	3.7^{c}	3.3^{c}	3.6^{cd}	4.6^{a}	3.6^{b}	2.7^{b}	1.9^{b}	1.0^{bc}	85.8^{ab}	93.4^{b}
Mean	51.3^{B}	53.6^{C}	2.9^{B}	3.8^{AB}	19.1^{A}	18.0^{A}	3.6^{B}	3.1^{B}	4.6^{A}	4.6^{A}	3.8^{A}	3.0^{A}	2.4^{A}	0.9^{B}	87.7^{A}	86.0^{B}
K_1	68.6^{ab}	63.8^{b}	3.7^{abc}	6.5^{a}	11.1^{ef}	12.3^{e}	8.3^{a}	6.3^{b}	3.2^{d}	2.3^{cd}	2.0^{cd}	1.3^{de}	2.0^{b}	0.8^{c}	98.9^{a}	93.3^{b}
K_2	58.2^{abc}	64.0^{b}	3.2^{c}	1.6^{e}	8.6^{f}	7.9^{f}	8.0^{a}	6.2^{b}	3.9^{c}	2.6^{cd}	2.6^{c}	0.8^{e}	2.0^{b}	0.7^{c}	86.3^{ab}	83.8^{c}
K_3	69.4^{a}	88.6^{a}	1.7^{d}	1.8^{e}	8.5^{f}	13.5^{de}	6.8^{b}	7.5^{a}	2.8^{de}	1.8^{d}	0.9^{e}	0.8^{e}	0.7^{c}	2.0^{a}	90.7^{ab}	116.0^{a}
Mean	65.4^{A}	72.1^{A}	2.8^{B}	3.3^{B}	9.4^{C}	10.2^{C}	7.7^{A}	6.8^{A}	3.3^{C}	2.2^{C}	1.8^{C}	1.0^{C}	1.6^{B}	1.1^{AB}	92.0^{A}	97.7^{A}
CS_1	47.4^{c}	59.0^{c}	4.0^{ab}	3.7^{cd}	13.6^{de}	18.1^{b}	2.3^{d}	2.5^{d}	3.4^{cd}	3.0^{c}	3.5^{b}	2.0^{c}	1.2^{c}	0.9^{bc}	75.3^{b}	89.1^{bc}
CS_2	45.3^{c}	60.3^{c}	4.3^{a}	3.9^{c}	15.5^{bce}	15.3^{cd}	2.2^{d}	3.1^{cd}	5.1^{b}	2.2^{cd}	3.2^{b}	1.2^{de}	1.1^{c}	1.6^{ab}	76.1^{b}	87.7^{bc}
CS_3	52.1^{c}	55.5^{d}	3.1^{bc}	4.7^{b}	13.8^{de}	16.4^{c}	3.4^{c}	3.1^{cd}	2.9^{de}	2.5^{cd}	1.6^{d}	1.6^{cd}	1.9^{b}	1.3^{b}	78.9^{ab}	85.1^{c}
Mean	48.3^{B}	58.8^{B}	3.8^{A}	4.1^{A}	14.3^{B}	16.6^{B}	2.6^{C}	2.9^{B}	3.8^{B}	2.6^{B}	2.8^{B}	1.6^{B}	1.4^{C}	1.3^{A}	77.0^{B}	87.3^{B}
WK_1	38.6^{c}	28.5^{f}	4.5^{b}	4.5^{a}	7.7^{a}	5.0^{e}	6.3^{a}	4.9^{a}	1.9	1.9^{b}	2.0^{b}	1.8^{b}	1.4^{ab}	1.2^{a}	62.4^{bc}	47.8^{e}
WK_2	45.4^{a}	45.6^{a}	5.6^{a}	4.3^{a}	8.1^{a}	7.9^{a}	5.6^{b}	4.2^{ab}	2.5	1.0^{c}	2.3^{a}	1.0^{e}	1.6^{a}	0.9^{b}	71.2^{a}	65.0^{a}
WK_3	33.2^{e}	36.6^{d}	3.7^{c}	4.6^{a}	6.2^{bc}	6.3^{b}	3.3^{d}	4.7^{a}	2.5	1.7^{b}	1.6^{c}	1.4^{d}	1.7^{a}	1.1^{ab}	52.3^{e}	56.4^{c}
Mean	39.1^{B}	37.2^{B}	4.6^{A}	4.4^{A}	7.3^{A}	6.5^{A}	5.1	4.5^{A}	2.3	1.5^{B}	2.0^{A}	1.3^{B}	1.6^{A}	1.0	62.0^{A}	56.4^{B}
WCh_1	36.4^{d}	32.6^{e}	4.2^{b}	4.2^{a}	5.9^{c}	4.9^{c}	5.1^{bc}	3.6^{c}	1.8	1.9^{b}	1.2^{d}	1.5^{cd}	0.9^{c}	0.9^{b}	55.5^{d}	49.7^{d}
WCh_2	45.5^{a}	41.6^{b}	3.2^{d}	3.5^{b}	5.3^{c}	4.6^{d}	4.5^{c}	3.6^{c}	2.1	2.1^{b}	1.8^{c}	1.6^{bc}	1.0^{bc}	0.9^{b}	63.4^{b}	57.9^{b}
WCh_3	41.6_{b}	44.4^{c}	2.8^{e}	3.6^{b}	6.7^{b}	6.5^{b}	5.4^{b}	4.0^{bc}	2.5	3.1^{a}	1.9^{bc}	2.9^{a}	0.5^{d}	0.9^{b}	61.3^{c}	65.4^{a}
Mean	41.2^{A}	39.5^{A}	3.4^{B}	3.8^{B}	6.0^{B}	5.4^{B}	5.0	3.7^{B}	2.1	2.3^{A}	1.6^{B}	2.0^{A}	0.8^{B}	0.9	60.0^{B}	57.7^{A}
ANOVA																
Vintage	**		**		**		**		**		**		**		n.s.	

Different superscript small letters in the same row indicate significantly different mean values ($P < 0.05$) for wines from different producers in the same vintage and for the red and white wines separately

Different superscript capital letters indicate significantly different mean values ($P < 0.05$) for varietal wines and for the red and white wines separately

ANOVA was used to compare data between vintages (n.s. not significant and $^{**}P \leq 0.05$)

contents of caftaric, caffeic, and gallic acids were higher in wines obtained in France compared to our Montenegrin wines. Such difference could be attributed to the terroir and the edaphoclimatic conditions of the regions where the grapes were cultivated.

Stilbenes in red and white Montenegrin wines

Trans-resveratrol glucosides were the dominant forms of stilbenes for all the studied wines in both vintages, as confirmed by literature [11, 15, 36]. Regarding red wines, the *trans*-piceid content varied between 0.34–1.77 mg L^{-1} for the 2015 vintage and 0.80–2.58 mg L^{-1} in 2016. The second most abundant stilbenes were the *cis*-resveratrol glucosides, ranging between 0.24–1.25 mg L^{-1} and 0.67–2.09 mg L^{-1} in 2015 and 2016 red wines, respectively. The total stilbenes content varied from 1.00 to 3.48 mg L^{-1} in 2015 and from 2.40 to 5.70 mg L^{-1} in 2016. The results obtained for the 2015 vintage are in line with those presented for Vranac wines in the Podgorički sub-region for the period 2010–2012, 2.3–3.1 mg L^{-1} [44], and for some red wines from the 2015 vintage, 0.7–2.7 mg L^{-1} [22]. Our results for both vintages are also in line with those obtained by Kallitraka et al. [12] and Monagas et al. [11] in Greek and Spanish red wines, which reported contents of stilbenes ranging between 0.0–9.72 mg L^{-1} and 0.4–5.2 mg L^{-1}, respectively.

The distribution between the resveratrol forms in the studied white wines was similar to that observed in the red ones. *trans*-Resveratrol glucosides were the most abundant, with contents varying between 0.03–0.09 mg L^{-1} in 2015, and between 0.06–0.21 mg L^{-1} in 2016. The second most abundant resveratrol was *cis*-piceid for both varietal white wines in 2016, whereas in 2015 it reported the same level as *trans*-resveratrol. The content of total stilbenes ranged between 0.07–0.22 mg L^{-1} and 0.17–0.53 mg L^{-1} for the two consecutive vintages.

Table 5 Content of stilbenes and gallic acid (mg L^{-1}) in varietal Montenegrin red and white wines from the 2015 and 2016 vintages (mean values ± SD)

	Stilbenes										Hydroxybenzoic acid derivatives	
	trans-Resveratrol glucosides		*trans*-Resveratrol		*cis*-Resveratrol glucosides		*cis*-Resveratrol		Total stilbenes		Gallic acid	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
V_1	1.51^{b}	2.47^{a}	0.50^{ab}	0.55^{c}	0.86^{b}	2.09^{a}	0.15^{ab}	0.39^{ab}	3.02^{b}	5.50^{a}	44.8^{d}	45.9^{c}
V_2	1.77^{a}	2.58^{a}	0.41^{b}	0.38^{d}	1.25^{a}	1.22^{c}	0.06^{bcd}	$0.42a^{b}$	3.48^{a}	4.60^{c}	47.3^{c}	62.2^{a}
V_3	0.64^{e}	2.41^{a}	0.24^{c}	1.14^{a}	0.24^{e}	1.91^{a}	0.01^{d}	0.23^{c}	1.14^{de}	5.70^{a}	66.1^{a}	49.3^{b}
Mean	1.30^{A}	2.49^{A}	0.38^{B}	0.69^{A}	0.78^{A}	1.74^{A}	0.07^{A}	0.35^{B}	2.55^{A}	5.27^{A}	52.7^{A}	52.4^{A}
K_1	0.83^{d}	1.87^{c}	0.29^{c}	0.52^{c}	0.48^{c}	1.56^{b}	0.04^{cd}	0.34^{bc}	1.64^{d}	4.30^{d}	58.4^{b}	41.9^{d}
K_2	1.17^{c}	2.09^{b}	0.52^{a}	0.63^{c}	0.50^{c}	1.94^{a}	0.08^{bc}	0.25^{c}	2.27^{c}	4.90^{b}	40.1^{e}	39.4^{e}
K_3	1.20^{c}	1.75^{c}	0.54^{a}	0.55c	0.44^{c}	1.17^{c}	0.19^{a}	0.33^{bc}	2.37^{c}	3.80^{e}	37.3^{g}	36.2f
Mean	1.06^{B}	1.91^{B}	0.45^{A}	0.57^{B}	0.48^{B}	1.56^{B}	0.10^{B}	0.31^{B}	2.09^{B}	4.33^{B}	45.3^{B}	39.2^{B}
CS_1	0.38^{f}	1.08^{d}	0.52^{a}	0.40^{d}	0.32^{ed}	0.94^{d}	0.18^{a}	0.48^{a}	1.40^{d}	2.90^{f}	27.1^{h}	25.0^{h}
CS_2	0.34^{f}	0.80^{e}	0.26^{c}	0.57^{c}	0.38^{cd}	0.67^{e}	0.07^{c}	0.36^{abc}	1.05^{e}	2.40^{e}	27.9^{h}	39.6^{e}
CS_3	0.41^{f}	0.88^{d}	0.21^{c}	0.81^{b}	0.27^{e}	0.72^{de}	0.11^{b}	0.49^{a}	1.00^{e}	2.90^{f}	33.9^{f}	28.1^{g}
Mean	0.38^{C}	0.92^{C}	0.31^{C}	0.59^{B}	0.32^{C}	0.78^{C}	0.12^{B}	0.44^{A}	1.15^{C}	2.73^{C}	29.7^{C}	30.9^{B}
WK_1	0.06^{b}	0.11^{bc}	0.04^{b}	0.05^{ab}	0.05^{b}	0.09^{bc}	0.00^{c}	0.03^{c}	0.14^{b}	0.27^{cd}	2.3^{a}	1.9^{a}
WK_2	0.06^{b}	0.20^{a}	0.04^{b}	0.07^{a}	0.03^{c}	0.10^{b}	0.00^{c}	0.06^{b}	0.12^{b}	0.43^{b}	2.4^{a}	1.4^{b}
WK_3	0.09^{a}	0.21^{a}	0.06^{a}	0.06^{ab}	0.07^{a}	0.17^{a}	0.00^{c}	0.09^{a}	0.22^{a}	0.53^{a}	2.1^{b}	1.5^{b}
Mean	0.07^{A}	0.17^{A}	0.05^{A}	0.06	0.05^{A}	0.12^{A}	0.00^{B}	0.06^{A}	0.16^{A}	0.41^{A}	2.3^{A}	1.6
WCh_1	0.05^{b}	0.06^{d}	0.04^{b}	0.04^{b}	0.04^{bc}	0.05^{c}	0.01^{a}	0.02^{c}	0.14^{b}	0.17^{d}	0.9^{c}	1.9^{a}
WCh_2	0.06^{b}	0.09^{cd}	0.03^{b}	0.05^{ab}	0.04^{bcd}	0.06^{bc}	0.01^{ab}	0.02^{c}	0.14^{b}	0.22^{d}	1.3^{d}	1.4^{b}
WCh_3	0.03^{c}	0.13^{b}	0.02^{c}	0.07^{a}	0.02^{d}	0.08^{bc}	0.00^{b}	0.04^{c}	0.07^{c}	0.32^{c}	1.0^{c}	1.5^{b}
Mean	0.05^{B}	0.09^{B}	0.03^{B}	0.05	0.03^{B}	0.06^{B}	0.01^{A}	0.03^{B}	0.12^{B}	0.23^{B}	1.1^{B}	1.6
ANOVA												
Vintage	**		**		**		**		**		**	

Different superscript small letters in the same row indicate significantly different mean values ($P < 0.05$) for wines from different producers in the same vintage and for the red and white wines separately

Different superscript capital letters indicate significantly different mean values ($P < 0.05$) for varietal wines and for the red and white wines separately. ANOVA was used to compare data between vintages (n.s. not significant and ** $P \leq 0.05$)

Regarding red wines, the highest proportion of *trans* forms was found in all wines. A similar observation was made by Pajović-Šćepanović et al. [22] in Montenegrin wines from the same varieties prepared under the same oenological practices in the 2015 vintage. These wines contained 69.5%, 68.5%, and 65.1% *trans* forms (Vranac, Kratošija, and Cabernet Sauvignon, respectively), while for Vranac and Cabernet Sauvignon the results were 69.9% and 62.8%, respectively, for the 2006 vintage in N. Macedonia [15].

There was also a difference in the total concentration of stilbenes, which was significantly different among the red varietal wines studied. Vranac wines contained the highest content of total stilbenes, followed by Kratosija, while the lowest concentration was reported in Cabernet Sauvignon wines in both consecutive vintages (Table 5). The fact that wines from Cabernet Sauvignon reported a lower content of stilbenes in comparison to the other red wines was also verified in other wines from Montenegro, Greece, and Spain [11, 12, 22].

Regarding white wines, Krstač contained higher contents of stilbenes in both vintages and showed a higher variability among *trans* and *cis* forms compared to the wines from Chardonnay. The results presented in Table 5 indicate that the synthesis of stilbenes is affected by environmental stimuli, but genetic factors (grape variety) are also very important [17, 29, 45].

Antioxidant properties of the studied wines

The in vitro antioxidant activity of the studied wines was evaluated with the capacity to scavenge ABTS radicals, the results being reported in Table 3. The results showed that the antioxidant capacity of red wines was much higher when

compared to that of white wines, which is in agreement with previous studies [21, 35, 42].

On average, Cabernet Sauvignon wines showed the strongest activity, statistically different from the two other red wines Vranac and Kratošija. The antioxidant capacity (ABTS) of the studied red wines varied between 15.3–22.8 mmol L^{-1} of Trolox in 2015, and 10.8–18.8 mmol L^{-1} in 2016 (Table 3). These results are similar to those obtained by Pajović-Šćepanović et al. [22] (9.1–21.0 mmol L^{-1}), who studied red wines produced in the Podgoriči sub-region in the 2015 vintage. The results are also comparable to those from other studies where the same methodology was used. Šeruga et al. [14] and Baiano et al. [29] reported 7.9–24.2 mmol L^{-1} in Croatian wines and 9.98–23.35 mmol L^{-1} in Primitivo Italian wines.

We carried out the identification of the active phenolic classes and individual phenolic compounds that are responsible for the antioxidant properties of the red wines studied. The total phenol content was correlated with ABTS scavenging activity ($R^2 = 0.8906$ and $R^2 = 0.9254$, in 2015 and 2016, respectively). Both vintages under study reported low R^2, obtained by plotting the antioxidant capacity with the content of anthocyanins, low- and high-molecular proanthocyanidins. No correlations were found between the individual non-flavonoid phenolic compounds, the studied groups of phenolic acids (hydroxycinnamate acid and gallic acid), and stilbenes with antioxidant capacity. In literature, however, some groups of phenolic compounds such as hydroxycinnamate acid [38] and stilbenes [28], as well as gallic acid [42], can act more effectively comparatively to other phenolic groups and a direct linear correlation was proved between antioxidant capacity and the mentioned phenolic groups/compounds.

As expected, white wines in our study reported a considerable lower antioxidant capacity (Table 3). Krstač reported a higher antioxidant activity compared to Chardonnay. The average values of antioxidant activity for white wines varied between 1.2 and 2.1 mmol L^{-1} in 2015 and between 0.9 and 1.6 mmol L^{-1} in 2016. The total antioxidant capacity in white wines was also correlated with the concentrations of total phenols ($R^2 = 0.9057$ and $R^2 = 0.8987$ in 2015 and 2016, respectively), and not with the other groups of phenolic compounds studied, nor with individual non-flavonoid phenolic compounds.

Discrimination of grape varieties, vintage, and location influence in Montenegrin red and white wines

The variables studied and the results obtained in the phenolic profile and antioxidant activity of the Montenegrin red and white wines were applied in a PCA (Fig. 1). The results obtained from the PCA allowed a

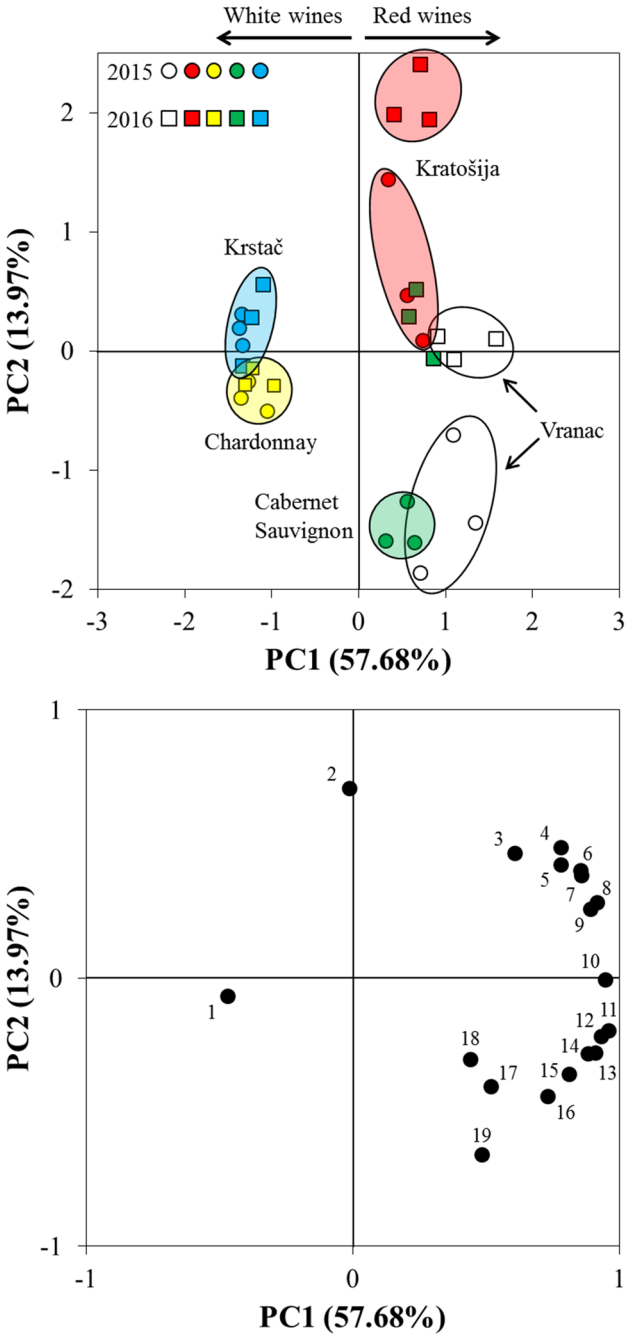

Fig. 1 Principal component analysis of the phenolic profile and antioxidant activity of Montenegrin red (Vranac, Kratošija, and Cabernet Sauvignon) and white (Krstač and Chardonnay) wines from 2015 and 2016 vintages. Principal components explain 71.65% of the total variance. 1—*cis*-coutaric acid; 2—fertaric acid; 3—*cis*-resveratrol; 4—caftaric acid; 5—*trans*-resveratrol; 6—total stilbenes; 7—*cis*-resveratrol glucosides; 8—*trans*-resveratrol; 9—total HCA; 10—gallic acid; 11—ABTS; 12—total phenols; 13—HMP; 14—*trans*-coutaric acid; 15—LMP; 16—caffeic acid; 17—total anthocyanins; 18—ferulic acid; 19—*p*-coumaric acid

clear differentiation between red and white wines. Red wines were represented in the entire positive region of PC1, while white wines were represented in the opposite region (Fig. 1). Interestingly, the PCA was able to separate the varieties and the year of vintage, but not the location. The differences within the same variety between vintages were greater in red wines, while in white wines the differences were smaller. In the case of red wines, the vintage has a clear differentiating impact on the phenolic profile, and consequently, on the antioxidant activity of red wines. The three varieties studied (Vranac, Kratošija, and Cabernet Sauvignon) were clearly separated according to the year of vintage (Fig. 1). In 2015, red wines contained higher content of TP, LMP, HMP, and antioxidant activity probably due to the higher temperatures and a lower rainfall registered during the growing season, mainly in the final period of harvest, providing better conditions for the accumulation of phenolic compounds. By contrast, the 2016 season was characterized by lower temperatures and higher humidity and rainfall (Table 1) which probably caused the incidence of fungi in the vineyards, leading to the synthesis of stilbenes in the grapes and consequently in the wines. This finding is in line with Raičević et al. [31] attributed higher levels of phenolic compounds (total phenols, total flavonoids and total flavan-3-ols) in wines from the Vranac variety in the Podgorički sub-region in 2008 in comparison to those from 2009 due to the changes in temperature and water availability (drought or precipitation). Higher levels of humidity are normally associated with the synthesis of stilbenes, while the temperature negatively influences the concentrations of resveratrols in grapes and wines [43]. This was also observed in our results since in the year of higher humidity, higher concentrations of the *cis* forms of resveratrols were found in the grapes and consequently in wines, in line with previous research [45, 46].

The same data were applied in an LDA to discriminate varieties and vintages. The obtained LDA is represented in Fig. 2. The discriminant model was able to correctly classify 100% of the original data according to their variety and vintage with only six variables (fertaric acid, *cis*-resveratrol, *trans*-resveratrol glucoside, gallic acid, ABTS antiradical activity, and ferulic acid). Nevertheless, this percentage decreased to 56.7% when a cross-validation was carried out. In all the varietal wines studied (except for Cabernet Sauvignon) using the cross-validation procedure, at least one sample from the vintage 2015 was classified as belonging to the vintage 2016. This fact proves that the season is important in the phenolic profile of wines, but they continue to preserve the typical varietal phenolic profile. Nevertheless, one wine from Vranac (vintage 2015) was classified as belonging to Cabernet Sauvignon (vintage 2015), possibly due to a similar phenolic profile, or a possible mixture of different wines.

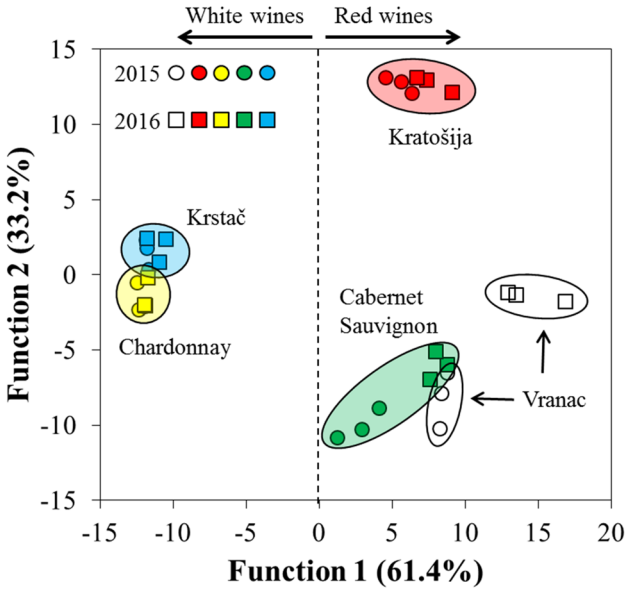

Fig. 2 Linear discriminant analysis of the varietal Montenegrin red (Vranac, Kratošija, and Cabernet Sauvignon) and white (Krstač and Chardonnay) wines from the 2015 and 2016 vintages, represented in a plane composed of the two main discriminant functions. The functions explain 94.6% of the total variance

Conclusion

The present study provides a contribution to the characterization of varietal Montenegrin red and white wines from different vintages. It was concluded that red wines are much richer in phenolic compounds compared to white wines. The antioxidant activity obtained was strongly correlated with the total phenol content of the wines.

Varietal and vintage effects were patent in the phenolic profile obtained. It was possible to differentiate wines according to variety and season; however, the wines were mainly discriminated by the variety and not so much by the vintage. Such results are probably related to the influence of climatic conditions during the vintages studied.

Regarding specific varieties, we concluded that Cabernet Sauvignon Montenegrin wines contained the highest contents of TP, LMP, HMP, and highest antioxidant activity. The same cultivar also reported the lowest content of stilbenes. Vranac wines showed the highest content of anthocyanins and stilbenes, whereas Kratošija wines had the highest content of hydroxycinnamic acids. Among white wines, Krstač reported higher total phenol content and total stilbenes, compared with Chardonnay wines.

The results obtained demonstrate that Montenegrin wines possess high levels of phenolic compounds and good antioxidant properties, supporting the potential of the sub-region to produce high-quality wines with important bioactive molecules.

This study contributes with information about the characteristics of commercial red and white wines from Montenegro. Such information will be helpful for Montenegrin national authorities to control wine authenticity, as well as to develop a case of study to apply for PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication) certifications, which is currently intended by the wine sector in Montenegro.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interests, and the article is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

References

1. Ivanova V, Stefova M, Chinnici F (2010) Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods. J Serb Chem Soc 75:45–49
2. Frankel EN, Waterhouse AL, Teissedre PL (1995) Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. J Agric Food Chem 43:890–894
3. Rodrigo R, Miranda A, Vergara L (2011) Modulation of endogenous antioxidant system by wine polyphenols in human disease. Clin Chim Acta 412:410–424
4. Vanzo A, Terdoslavich M, Brandoni A, Torres AM, Vrhovšek U, Passamonti S (2008) Uptake of grape anthocyanins into the rat kidney and the involvement of bilitranslocase (p NA). Mol Nutr Food Res 52:1106–1116
5. Gris EF, Mattivi F, Ferreira EA, Vrhovsek U, Pedrosa RC, Bordignon-Luiz MT (2011) Proanthocyanidin profile and antioxidant capacity of Brazilian *Vitis vinifera* red wines. Food Chem 44:2851–2860
6. Rigo A, Vianello F, Clementi G, Rossetto M, Scarpa M, Vrhovšek U, Mattivi F (2000) Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some Italian red wines. J Agric Food Chem 48:1996–2002
7. Vrhovšek U, Wendelin S, Eder R (1997) Quantitative bestimmung von hydroxyzimtsäuren und hydroxyzimtsäurederivaten (hydroxycinnamaten) in Weißweinen mittels HPLC. Mitt Klosterneuburg 47:164–172
8. Arnous A, Makris DP, Kafalas P (2001) Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. J Agric Food Chem 49:5736–5742
9. Mattivi F, Zulian C, Nicolini G, Valenti L (2002) Wine, biodiversity, technology, and antioxidants. Ann NY Acad Sci 957:37–56
10. Ivanova V, Vojnoski B, Stefova M (2011) Effect of the winemaking practices and aging on phenolic content of Smederevka and Chardonnay wines. Food Bioprocess Technol 4:1512–1518
11. Monagas M, Suarez R, Gómez-Cordovés C, Bartolomé B (2005) Simultaneous determination of nonanthocyanin phenolic compounds in red wines by HPLCDAD/ESI-MS. Amer J Enol Vitic 56:139–147
12. Kallitraka S, Mamalos A, Makris DP (2007) Differentiation of young red wines based on chemometrics of minor polyphenolic constituents. J Agric Food Chem 55:3233–3239
13. Ivanova-Petroleus V, Ricci A, Nedelkovski D, Dimovska V, Parpinello GP, Versari A (2015) Targeted analysis of bioactive phenolic compounds and antioxidant activity of Macedonian red wines. Food Chem 44:2851–2860
14. Šeruga M, Novak I, Jakobek L (2011) Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and spectrophotometric methods. Food Chem 124:1208–1216
15. Ivanova-Petropulos V, Ricci A, Nedelkovski D, Dimovska V, Parpinnelo GP, Versari A (2015) Targeted analysis of bioactive phenolic compounds and antioxidant activity of Macedonian red wines. Food Chem 171:414–420
16. Ivanova-Petropulos V, Durakova S, Ricci A, Parpinello GP, Versari A (2016) Extraction and evaluation of natural occurring bioactive compounds and change in antioxidant activity during red winemaking. J Food Sci Technol 53:2634–2643
17. Kostadinović S, Wilkens A, Stefova M, Ivanova V, Vojnoski B (2012) Stilbene levels and antioxidant activity of Vranec and Merlot wines from Macedonia: effect of variety and enological practices. Food Chem 135:3003–3009
18. Kovač V, Alonso E, Revilla E (1995) The effect of adding supplementary quantities of seeds during fermentation on the phenolic composition of wines. Amer J Enol Vitic 46:363–367
19. Pajovic R, Raicevic D, Popovic T, Sivilotti P, Lisjak K, Vanzo A (2014) Polyphenolic characterisation of Vranac, Kratosija and Cabernet Sauvignon (*Vitis vinifera* L. cv.) grapes and wines from different vineyard locations in Montenegro. S Afr J Enol Vitic 35:139–148
20. Pajović Šćepanovic R, Wendelin S, Eder R (2019) Assay of polyphenols in Montenegrin Vranac wines. Mitt Klost 69:65–75
21. Košmerl T, Bertalanič L, Maraš V, Kodžulović V, Šućur S, Abramovič H (2013) Impact of yield on total polyphenols, anthocyanins, reducing sugars and antioxidant potential in white and red wines produced from Montenegrin autochthonous grape varieties. Food Sci Technol 1:7–15
22. Pajović-Šćepanović R, Wendelin S, Forneck A, Eder R (2018) Phenolic composition and varietal discrimination of Montenegrin red wines (*Vitis vinifera* var. Vranac, Kratošija, and Cabernet Sauvignon). Eur Food Res Technol 244:2243–2254
23. Tonietto J, Carbonneau A (2004) A multicriteria climatic classification system for grape-growing regions worldwide. Agric For Meteorol 124:81–97
24. Di Stefano R, Guidoni S (1989) The analysis of total polyphenols in musts and wines. Vignevini 1(2):47–52
25. Di Stefano R, Cravero MC, Gentilini N (1989) Methods for the study of wine polyphenols. L'Enotecnico 5:83–89
26. Vrhovsek U, Mattivi F, Waterhouse AL (2001) Analysis of red wine phenolics: comparison of HPLC and spectrophotometric methods. Vitis 40:87–91
27. Broadhurst RB, Jones WT (1978) Analysis of condensed tannins using acidified vanillin. J Sci Food Agric 29:788–794

28. Vrhovšek U, Wendelin S, Eder R (1997) Effects of various vinification techniques on the concentration of *cis*- and *trans*-resveratrol and resveratrol glucoside isomers in wine. Am J Enol Vitic 48:214–219
29. Eder R, Wendelin S, Vrhovšek U (2001) Resveratrolgehalte von Trauben und Rotweinen in Abhängigkeit von Lesejahrgang und Lesetermin. Mitt Klosterneuburg 51:64–78
30. Rice-Evans CA, Miller JM, Paganga G (1996) Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Rad Biol Med 20:933–956
31. Raičević D, Božinović Z, Petkov M, Ivanova-Petropulos V, Kodžulović V, Mugoša M, Šućur S, Maraš V (2017) Polyphenolic content and sensory profile of Montenegrin Vranac wines produced with different oenological products and maceration. Maced J Chem Chem Eng 36:229–238
32. Kallitraka S, Tsoutsouras E, Tzourou E, Lanaridis P (2006) Principal phenolic compounds in Greek red wines. Food Chem 99:784–793
33. Mattivi F, Nicolini G (1997) Analysis of polyphenols and resveratrol in Italian wines. BioFactors 6:445–448
34. Baiano A, Terracone C, Gambacorta G, La Notte E (2009) Phenolic content and antioxidant activity of Primitivo wine: comparison among winemaking technologies. J Food Sci 74:C258–C267
35. Katalinić V, Milos M, Modun D, Musić I, Boban M (2004) Antioxidant effectiveness of selected wines in comparison with (+) catechin. Food Chem 86:593–600
36. Burns J, Gardner PT, O'Neil J, Crawford S, Morecroft L, McPhail DB, Crozier A (2000) Relationship among antioxidant activity, vasodilatation and phenolic content of wines. J Agric Food Chem 48:220–230
37. Vanzo A, Lavrenčić P, Ćuš F (2007) Vsebnost polifenolov v sorti Zelen. In: Ćuš F (ed) Proceeding Vinarski dan, Ljubljana, Conference Wine day, Ljubljana, pp 25–32
38. Vrhovšek U (1998) Extraction of hydroxycinnamoyltartaric acids from berries of different grape varieties. J Agric Food Chem 46:4203–4208
39. Zoechling WA, Reiter E, Eder R, Wendelin S, Liebner F, Jungbauer A (2009) The flavonoid kaempferol is responsible for the majority of estrogenic activity in red wine. Amer J Enol Vitic 60:223–232
40. De Beer D, Harbertson JF, Kilmartin PA, Roginsky V, Barsukova T, Adams DO, Waterhouse AL (2004) Phenolics: a comparison of diverse analytical methods. Am J Enol Vitic 55:389–400
41. Gambelli L, Santorini GP (2004) Polyphenols content in some Italian red wines of different geographical origins. J Food Comp Anal 17:613–618
42. Minussi RC, Rossi M, Bologna L, Cordi L, Rotilio D, Pastore GM (2003) Phenolic compounds and total antioxidant potential of commercial wines. Food Chem 82:409–416
43. Sun B, Spranger MI (2005) Review: quantitative extraction and analysis of grape and wine proanthocyanidin and stilbenes. Cienc Tec Vitivinic 20:59–89
44. Radovic B, Tesevic V, Kodzulovic V, Maras V (2015) Resveratrol concentration in Vranac wines. Vitis 54:169–171
45. Bavaresco L, Fregoni CMMB, Vezzulli S (2009) In: Roubelakis-Angelakis KA (ed) Grapevine molecular physiology & biotechnology. Springer, Dordrecht
46. Bavaresco L, Pezzutto S, Gatti M (2007) Mattivi F (2007) Role of the variety and some environmental factors on grape stilbenes. Vitis 46:57–61