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Maceration duration and grape variety: key factors in phenolic compound enrichment of Montenegrin red wine

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ABSTRACT

The present study focuses on the effect of increasing maceration times (0, 2, 4, 6, and 10 days) on the extraction of phenolic compounds into two Montenegrin wines made with the autochthonous varieties Vranac and Kratošija. Significant differences were found in the total acidity, pH and alcohol content of these two varietal wines, which showed different responses to the maceration time. The total content of phenolic compounds (phenols, tannins and anthocyanins) increased significantly with longer maceration time, which is consistent with the existing literature. Specifically, the longest maceration time (10 days) consistently resulted in the highest levels of all parameters analysed. In addition, the anthocyanin profile determined by HPLC showed the interaction between maceration time and variety on the concentrations of the individual anthocyanins. Compared to Kratošija, Vranac wines consistently showed higher anthocyanin contents. In addition, the structural analysis of the tannins revealed that the mean degree of polymerisation (mDP), the percentage of prodelphinidins (%PD) and the percentage of galloylation (%Gall) changed with increasing maceration time. Significant correlations were found between the antioxidant activity values and the analytical parameters, including the structural characteristics of the tannins. This study provides important information on the interaction between maceration time and various varietal characteristics that influence the phenolic composition of Montenegrin wines and contributes to a deeper understanding of winemaking practices and wine quality.

KEYWORDS: Vranac, Kratošija, low molecular weight proanthocyanidins, high molecular weight proanthocyanidins, antioxidant activity, maceration time, anthocyanins, tannin structural characteristics.

INTRODUCTION

Phenolic compounds are important constituents of red wine composition, contributing to its style and quality. Proanthocyanidins and anthocyanins account for a major part of red wine polyphenols due to their role in red wines: anthocyanins are responsible for the red colour and proanthocyanidins are associated with colour stability and wine preservation during ageing (Ribéreau-Gayon *et al*., 2006). The structure of proanthocyanidins varies depending on the constitutive sub-units, mean degree of polymerisation (mDP) and linkage position (Lorrain *et al*., 2011), and the structure influences the sensorial properties of the wine; for example, low molecular weight proanthocyanidins are bitter, and astringency increases with proanthocyanidin chain length (Chira *et al*., 2011; Peleg, 1999), higher mDP and higher percentages of galloylation (%Gall) (Lisjak *et al*., 2020; Vidal *et al*., 2003). Additionally, flavonoids are important for human health due to their antioxidant properties (Cimino *et al*., 2007; Gris *et al*., 2011; Rigo *et al*., 2000; Rodrigo *et al*., 2011).

The level of anthocyanins and proanthocyanidins in grapes is determined by several factors, such as grape variety and climatic and pedological conditions (Mattivi *et al*., 2009). During the skin maceration period of winemaking, phenolic compounds are transferred from the grapes to the must. The wine's phenolic composition and content depend on the original grape potential and the extraction and winemaking techniques employed. Winemaking technology (e.g., skin maceration time, temperature, the intensity of pressing, yeast selection, SO_2 addition low-temperature prefermentative techniques, modification of the skin juice ratio, and the addition of maceration enzymes, among others) can influence the phenolic content of finished wine (Busse-Valverde *et al*., 2012; Ivanova *et al*., 2011).

The changes that occur in phenolic compounds have been investigated in several studies. Most of the authors have found that anthocyanins are extracted from the skins and reach maximum values in the earliest stages of fermentation, but extraction of proanthocyanidins from the seeds needs longer maceration time (Gomez-Plaza *et al*., 2001; Ivanova-Petropulos *et al*., 2016; Kovac *et al*., 1992; Vrhovšek *et al*., 2002). Some authors have also reported a correlation between antioxidant capacity, phenolic composition and maceration time (Kocabey *et al*., 2016; Plavša *et al*., 2012). Due to the importance of proanthocyanidins and anthocyanins in red wine quality, it is important to understand how winemaking practices influence each varietal wine's final qualitative and quantitative composition.

Grape and wine production in Montenegro is traditionally based on two autochthonous red grape varieties, Vranac and Kratošija. Kratošija was dominant until the phylloxera outbreak, after which Vranac became the preferred grape due to its better skin colouration and lower heterogeneity of its population (Ulićević, 1966). Vranac became an important regional red variety in Western Balkan countries (Macedonia, Serbia, Bosna and Hercegovina, and Croatia), whereas Kratošija decreased in importance (Pajović-Šćepanovic *et al*., 2019a). A study performed from 2015 to 2019 showing a genetic relationship between Kratošija and Vranac also confirmed the previous findings of Calò *et al.* (2008) that Kratošija has the same genetic profile as Primitivo from Puglia, Italy and Zinfandel from California, USA (Maraš *et al*., 2020). Due to the good reputation of Zinfandel and Primitivo, Montenegrin winemakers recognised the importance of Kratošija grape and started preparing its varietal wines a few years ago.

Due to the importance of both varieties, Montenegro researchers performed many parallel studies of Vranac and Kratošija grapes and wines. The spectrophotometric evaluation of extractable phenolic compounds (total phenol, total anthocyanins, LMPs and high molecular weight proanthocyanidins - HMPs) in Montenegrin red grapes and wines showed that their content was similar in Vranac and Kratošija for analysed groups, except anthocyanins which were almost twice as low in Kratošija grapes and wines. These two varieties were considered to have medium to long ageing potential (Pajovic *et al*., 2016). Additionally, analyses of the skin and seeds of two Montenegrin red varieties showed that the grapes in Kratošija had a higher content of total flavan-3-ols. By contrast, Vranac seeds contained a relatively higher content of epicatechin-3-O-gallate (Pajović-Šćepanović *et al*., 2019a). A study of the anthocyanin's composition performed by HPLC–DAD showed that both varieties have the same anthocyanin profile, order of anthocyanins mono-glycosides, but different percentages. The total content of anthocyanins was much lower in Kratošija, and its wine contained a higher content of malvidin-3-O-coumarylglucoside than the Vranac wines (Pajović-Šćepanović *et al*., 2018). The influence of yield on the antioxidant capacity of Montenegrin wines was investigated by Košmerl *et al.* (2013). The authors reported that a higher content of total polyphenols, anthocyanins and reducing sugars, as well as a stronger reducing power and DPPH scavenging ability, were found in Vranac wines compared to Kratošija ones.

Although the phenolic and anthocyanin profiles and contents of Vranac and Kratošija wines have been studied in several previous investigations (Košmerl *et al*., 2013; Pajovic *et al*., 2016; Pajović-Šćepanović *et al*., 2018; Pajović-Šćepanović *et al*., 2019a), to the best of our knowledge, there is no available literature on the evolution of both tannins (including their structural characteristics) and anthocyanins during the skin maceration of these Montenegrin autochthonous wines. Therefore, the purpose of this study was to fill this gap by exploring the influence of various maceration times (0, 2, 4, 6, and 10 days) on the chemical composition of Vranac and Kratošija wines produced under standardised winemaking protocols in the Podgorički sub-region of Montenegro. By determining various analytical parameters, the present research sought to elucidate how different maceration durations impact the composition and characteristics of tannins and anthocyanins in these autochthonous wines.

MATERIALS AND METHODS

1. Chemicals and reagents

Methanol, ethanol, hydrochloric acid, sodium hydroxide, sodium bisulphite, and L(+)-tartaric acid were purchased from Sigma Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, 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)

2. Grape sampling

The Montenegrin wine area is divided into four wine regions and fifteen subregions. The Montenegrin Basin of Skadar Lake is the most important region, and it includes the Podgorica subregion, which covers 90% of the total vineyard area in Montenegro.

Grapes from cv. Vranac and Kratošija (*Vitis vinifera* L.) were sampled in 2021 from experimental vineyards of the Biotechnical Faculty, Lješkopolje (L4 42°24′01.42”N, 19°12′34.94”E, 31 m.a.s.l.) in Podgorica sub-region. The vineyards were planted in 2005 with rows oriented north–south. Winkler index was 2609.5, seasonal rainfall from April to September (376.1 mm), and irrigation occurred during the vegetation phase.

The grapes were manually harvested at full technological maturity and carefully transported to the winery in 20 L boxes. The total soluble solids (TSS), titratable acidity, and pH of the grape juice were measured following International Organization of Vine and Wine procedures (OIV, 2023). The physicochemical composition of the must is presented in Table 1.

3. Vinification

Wines were prepared in the winery in the Biotechnical Faculty at the University of Montenegro. They were produced in duplicate in 50 L tanks, each containing 35 kg of grapes.

The grapes were destemmed and crushed. Addition of sodium metabisulphite (10 g SO_2/100 kg grapes) took place in crushed grapes. Vinification was conducted with inoculation using rehydrated yeast (LALVIN ICV D254, Lallemand) prepared using 10 g of dry yeast per 100 kg of grapes). Five different maceration times were assayed for each grape variety: 0, 2, 4, 6, and 10 days. All the vinifications were conducted at 20-25 °C. The cap was punched down twice daily throughout the fermentation pomace contact period, and the temperature was recorded. The first decantation of free-run wine (different times for each combination) was performed with a vertical screw press (basket press). The second decantation of wine from the sediment was conducted one month after the first decantation with the addition of SO_2. The wines were stored under typical cellar conditions (in the dark, temperature of 12 °C to 16 °C, 70% humidity) until analysis.

The samples of wines were marked, based on maceration time, as follows: Vranac (V0, V2, V4, V6, and V10) and Kratošija (K0, K2, K4, K6, and K10). Four months after the fermentation, spectrophotometric and HPLC analyses of polyphenols in the wines were conducted.

4. Chemical analyses of wine

Fourier-transform infrared (FTIR) spectroscopy analyses were performed to evaluate the chemical composition of wines (alcohol, total acidity and pH). FT-IR spectroscopy was performed according to Resolution OIV/OENO 390/2010 (2010) using a FOSS- WineScan machine (FT 120 Reference Manual, Fa. Foss, Hamburg, Germany).

5. Spectrophotometric analyses

A Varian Cary 100 spectrophotometer (Bio Tech, Maryland, USA) was used for the spectrophotometric analyses following the methodology of Di Stefano and Guidoni (1989) and Di Stefano *et al.* (1989). Using a classic Sep-Pak (0.35 g) C-18 column provided by Waters (Milford, MA, USA), a preliminary clean-up of the phenols was carried out to exclude polar compounds that could potentially interfere with the assays, such as sugars, organic acids, amino acids and free SO_2.

6. Total phenols (TP)

The total phenols content was determined by reducing phosphotungstic-phosphomolybdic acids (Folin-Ciocalteu's reagent—FC) to blue pigments using phenols in an alkaline solution (Di Stefano & Guidoni, 1989). In brief, 1 mL of diluted wine (1/10 or 1/20 with 1 N of H_2SO_4) was mixed with 2 mL of methanol, 5 mL of distilled water, 1 mL of FC, and 4 mL of a 10% solution of Na_2CO_2; distilled water was then added to make up a total volume of 20 mL. After 90 min, absorbance was measured at 765 nm by comparing with a blank comprising 1 cm cuvette filled with distilled water. The results were computed as (+)-catechins equivalents = 186.5 × A × d in mL/L when absorbance was within the linear range of between 0.3 and 0.6 AU. Here, A represents absorption and d represents sample dilution.

TABLE 1. Physicochemical parameters of two varieties at harvest.

Variety	TSS Brix	Total acidity (g/L of tartaric acid)	pH
Vranac	23.4b	4.50	3.94
Kratošija	26.1a	5.25	3.91

a,b Different letters in the same column show significant differences according to Tukey test ($P < 0.05$).

(+)-catechin was used as a standard to create a calibration curve for determining the concentrations (Arnous *et al.*, 2001).

The wine total phenol index (TPI) was also measured. After diluting the wine with water in the flask, the absorbance at 280 nm was measured in a quartz cuvette, and the value was computed using the dilution factor (Ribéreau-Gayon *et al.*, 2006).

7. High molecular weight proanthocyanidins (HMP)

By converting them into cyanidins, the HMP were assessed (Di Stefano *et al.*, 1989). Cyanidin chloride was used to express the results in relation to the blank. Two flasks designated A and B, were used to prepare the solution, which consisted of 9.5 mL of 95% ethyl alcohol, 2 mL of diluted wine (containing 1 N H_2SO_4), 3 mL of methyl alcohol, and 12.5 mL of 0.3% SO_4 in HCl. Flask A was boiled for 50 min (at 102 °C), and flask B was kept in ice for the same amount of time. Then, both flasks were maintained at 19 °C until the absorbance, measured at 700 nm using distilled water in a 1 cm cuvette as a blank, was between 0.0 and 0.6. The formula for calculating the results was mg/L of cyanidin equivalents = $(A - B) \times 1162.5 \times d \times V$, where A stands for flask A content absorbance, B for flask B absorbance, d for wine sample dilution, and V for sample volume. This methodology provides a reliable approximation of HMP content (Vrhovsek *et al.*, 2001).

8. Low molecular weight proanthocyanidins: vanillin index (LMP)

Using the optimised and controlled vanillin–HCl method of Broadhurst and Jones (1978) and the conditions outlined by Di Stefano *et al.* (1989), the catechins and proanthocyanidins reactive to vanillin were analysed. The results were calculated as (+)-catechin (mg/L) using a calibration curve. The solution was prepared with 5 mL of methanol and 2 mL of wine (diluted with 1 N H_2SO_4). After that, the mixture was split between two flasks (A and B). Six millilitres of 4% vanillin in methanol and 3 mL of concentrated HCl were added to flask A. Six millilitres of 4% vanillin in methanol mL and 3 mL of concentrated HCl were added to flask B. Following a 15-min acclimatisation period at 15 °C, the absorbance was measured at 700 nm, utilising flask B's contents as a reference. The LMP content was expressed as (+)-catechin equivalents = $E \times d \times 0.4$ in mg/L when the absorbance was between 0.2 and 0.4 AU (700 nm); here, E stands for absorbance and d for sample dilution.

Catechins, free flavanols, and a low degree of proanthocyanidins can all be well-estimated using this method.

9. Total anthocyanins (TA)

The maximum absorbance in the visible range (536-542 nm) was used to calculate the total anthocyanins (Di Stefano *et al.*, 1989). Twelve millilitres of an ethanol/water/HCl (70:30:1) solution was combined with 3 mL of methanol, three drops of concentrated HCl, and 5 mL of diluted wine (with 1 N H_2SO_4). The absorbance was measured between 380 and 700 nm after 15 minutes. According to Di Stefano *et al.* (1989), the TA content was determined using the experimental value of the molar absorptivity of malvidin 3-glucoside chloride in ethanol/water/HCl (70:30:1), with ε = 30,100 eq./M/cm at 542 nm.

10. Colour intensity (CI) and hue of wines (HC)

Total absorbance at 420, 520, and 620 nm was used to calculate colour intensity (Ribéreau-Gayon *et al.*, 2006). The ratio of A420 to A520 determines the wine's hue. The samples were measured directly through a 1 mm optical path at 420, 520, and 620 nm, and the results were multiplied by 10.

11. Total tannin analysis

Total tannin content was determined by employing the precipitation method with methylcellulose (Mercurio and Smith, 2008).

12. Antioxidant activity

Antioxidant activity was determined using the DPPH stable radical method (Brand-Williams *et al.*, 1995). The results are expressed as Trolox equivalents (TE mg/L).

13. Tannin structural characteristics

The mean polymerisation degree of tannins (mDP), the percentage of galloylation (%Gall) and the percentage of prodelphinidin content (%PD) were determined according to the method described by Chira *et al.* (2012). In more detail, tannins were isolated using a C18 (Lichrolut C18, 5 g octadecyl bonded end-capped silica, 25 mL vol) SPE cartridge (Merck®, Darmstadt, Germany).

The cartridge was activated by sequentially adding 25 mL methanol, 25 mL distilled water and the wine sample (10 mL). It was then washed with 50 mL distilled water and left to dry for 15 min. Elution of tannins was accomplished with 50 mL methanol. The eluate was evaporated and lyophilised to obtain a dry powder, which was then dissolved in methanol (to reach a final content of 5 g/L). Acid-catalysed depolymerisation took place in the presence of phloroglucinol (50 g/L phloroglucinol, 10 g/L ascorbic acid, 0.1N HCl, in methanol) for 20 min at 50 °C. The reaction was stopped by adding 1 mL of aqueous sodium acetate (40 mM). The reaction products [(+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC) and (-)-epicatechin gallate (ECG), as well as their phloroglucinol adducts], were analysed by LC/MS on a Shimadzu 2010A (Shimatzu® Corporation) coupled to a single quadrupole mass spectrometer equipped with an electrospray ion source (Kyraleou *et al.*, 2017). All analyses were performed in triplicate.

14. Individual anthocyanin analyses

HPLC was used to determine the monomeric anthocyanins according to the method described in Kyraleou *et al.* (2015). The equipment used was a Jasco AS-1555 Intelligent Sampler, a Jasco PU 2089 Plus Quaternary Gradient Pump, a Jasco MD-910 diode array detector, and a Jasco

TABLE 2. Chemical and phenol composition of two Montenegrin wines produced with different maceration durations (0, 2, 4, 6, and 10 days).

	Chemical compounds				Phenolic compounds						
	Vranac										
	Alcohol (vol %)	Total acidity (g/L)	Volatile acidity (g/L)	pH	TP (mg/L (+)catchin)	TA (mg/L)	TPI	LMP (mg/L (+)catchin)	HMP (mg/L cyanidin chloride)	CI	HC
0	13.8 ± 0.2ab	4.5 ± 0.1d	0.45 ± 0.06d	3.78 ± 0.01ab	215.6 ± 14.4c	94.1 ± 5.8b	13.9 ± 0.7e	161.1 ± 3.5e	146.7 ± 0.2c	0.73 ± 0.02	0.63 ± 0.01a
2	14.2 ± 0.1a	4.0 ± 0.3e	0.56 ± 0.01c	3.85 ± 0.01a	416.7 ± 31.7c	344.2 ± 1.6b	23.9 ± 1.2d	470.4 ± 9.8d	521.9 ± 188.4b	1.23 ± 0.17	0.57 ± 0.03b
4	13.8 ± 0.2ab	5.4 ± 0.2b	0.93 ± 0.02a	3.75 ± 0.07bc	799.7 ± 182.0b	774.6 ± 221.3a	32.5 ± 1.8c	958.4 ± 3.9c	721.4 ± 30.3b	1.59 ± 0.23	0.51 ± 0.02c
6	13.1 ± 0.5c	5.1 ± 0.2c	0.56 ± 0.10c	3.65 ± 0.03d	1015.1 ± 225.5a	762.9 ± 5.3a	37.1 ± 1.2b	1089.7 ± 14.3b	1219.2 ± 129.4a	1.65 ± 0.08	0.50 ± 0.02c
10	13.5 ± 0.2bc	5.7 ± 0.1a	0.84 ± 0.01b	3.70 ± 0.01cd	1152.2 ± 10.4a	911.8 ± 15.8a	45.2 ± 0.8a	1293.5 ± 60.8a	1020.6 ± 17.5a	1.75 ± 0.07	0.50 ± 0.02c
	13.7 ± 0.4B	4.9 ± 0.6	0.67 ± 0.19	3.75 ± 0.06	719.3 ± 382.7	577.5 ± 329.3A	30.6 ± 11.2	794 ± 423.3	725.9 ± 398.9	1.47 ± 0.43A	0.57 ± 0.05
	KratoŠija										
0	15.1 ± 0.2a	5.0 ± 0.1cd	0.59 ± 0.01c	3.84 ± 0.10a	178.4 ± 8.6d	30.6 ± 2.2c	11.1 ± 0.5d	122.6 ± 2.1c	148.8 ± 2.3d	0.52 ± 0.03b	0.60 ± 0.03a
2	15.3 ± 0.1a	4.8 ± 0.4d	0.61 ± 0.03c	3.82 ± 0.12a	425.8 ± 21.3c	130.2 ± 2.9bc	21.3 ± 0.4c	248.7 ± 2.0c	300.6 ± 3.6cd	0.82 ± 0.0b	0.59 ± 0.01b
4	14.9 ± 0.2a	5.7 ± 0.1b	0.79 ± 0.02a	3.62 ± 0.03b	724.3 ± 3.1b	228.5 ± 0.3b	29.3 ± 0.3b	1037.9 ± 104.4b	440.3 ± 57.0c	1.16 ± 0.06a	0.54 ± 0.01d
6	15.0 ± 0.6a	6.2 ± 0.3a	0.70 ± 0.01b	3.60 ± 0.10b	1067.4 ± 103.8a	332.3 ± 63.6a	37.9 ± 1.3a	1178.2 ± 120.9a	842.5 ± 32.2b	1.19 ± 0.1a	0.52 ± 0.02d
10	14.0 ± 0.2b	5.3 ± 0.1bc	0.82 ± 0.08a	3.68 ± 0.07ab	1219.9 ± 130.0a	299.3 ± 76.4a	38.7 ± 1.3a	1243.0 ± 20.8a	1273.0 ± 188.8a	1.23 ± 0.12a	0.51 ± 0.03d
	14.8 ± 0.5A	5.4 ± 0.6	0.70 ± 0.10	3.71 ± 0.12	723.2 ± 406.0	204.2 ± 120.9B	27.6 ± 10.8	767.1 ± 500.7	601.1 ± 428.6	1.05 ± 0.35B	0.55 ± 0.05

a-e Different superscript small letters in the same row indicate significantly different mean values ($P < 0.05$) for varietal wines.
A,B Different superscript capital letters indicate significantly different mean values ($P < 0.05$) for varietal wines on average.

LC-Net II/ADC. The column was a Restek Pinnacle II C18 (250 × 4.6 mm, 5 µm). Eluent A was 10**%** aqueous formic acid solution, eluent B was methanol, and the flow rate was 1 mL/min.

15. Statistical analysis

A two-factorial analysis of variance (ANOVA) was applied to determine the significance of the differences in each studied parameter between the varietal wines and maceration duration, and their interaction. For those parameters and factors where significant differences were detected, an additional LSD test was applied with a significance level of $P < 0.05$. Normal distribution of the data and variance homogeneity were assumed.

The data obtained (17 variables, including chemical and phenolic compounds, elements of the structure of proanthocyanidins and ABTS antioxidant activity) were also used in a principal component analysis (PCA) to verify if the wines could respectively differentiate and discriminate the wine samples according to the variety and maceration duration. All the statistical treatments were performed using the statistical package IBM SPSS Statistics 20 (IBM Corporation, New York, USA).

RESULTS AND DISCUSSION

1. Chemical composition of wines

The chemical compositions of Vranac and Kratošija wines prepared with varying maceration durations (0, 2, 4, 6, and 10 days) are presented in Table 2. Significantly different alcohol contents were observed among the examined wines ($P < 0.05$). More specifically, in Vranac wines, the alcohol content was lower in samples subjected to 6 and 10 days of maceration, while in Kratošija wine, this difference was only observed in samples subjected to 10 days of maceration compared to those with 0, 2, and 4 days (Table 2). The average alcohol content in this study for Vranac and Kratošija wines (13.7 vol % and 14.8 vol %, respectively) was consistent with previously reported values for wines of the same varieties produced within the same wine subregion (14.0 vol % and 14.6 vol %, respectively), based on a seven-year average (Pajovic-Šćepanović *et al.*, 2016).

Total acidity content also exhibited significant differences ($P < 0.05$) among wines subjected to different maceration durations, with the highest levels observed in Vranac wine after 10 days and Kratošija wine after 6 days. This finding aligns with results reported by Kocabey *et al.* (2016), demonstrating an increasing trend in total acid content with prolonged maceration. The average total acidity content in the present study was 4.9 g/L for Vranac wine and 5.4 g/L for Kratošija wine. The values for Vranac wine were lower compared to those reported in the literature (between 5.53 g/L and 6.72 g/L) (Radonjić, 2020). The values for Kratošija wine are consistent with previously reported values (Košmerl *et al.*, 2013; Pajović *et al.*, 2013) of 5.7 g/L and 5.1 g/L, respectively. Furthermore, pH values displayed significant differences ($P < 0.05$) among Vranac wines for all maceration durations, with a trend of increasing pH values over time. By contrast, Kratošija wines showed lower discrepancies in pH values with different maceration durations. This observation may be attributed to a relatively strong correlation between total acid content and wine pH values ($R^2 = 0.658$). Interestingly, the pH values in this study were higher than those reported in previous research (Pajovic *et al.*, 2016; Pajović-Šćepanović *et al.*, 2016), particularly when compared to commercial wines. This difference in total acid content and pH values could be explained by the common practice of not adjusting acidity during wine production in Montenegro (Eder *et al.*, 2023). Moreover, ANOVA results revealed a significant difference in alcohol content between the two examined varietal wines ($P < 0.05$).

2. Phenolic content

The phenolic composition of Vranac and Kratošija wines subjected to varying maceration durations (0, 2, 4, 6, and 10 days) is summarised in Table 2. Total phenolic content in the wines exhibited an increasing trend with longer maceration durations, with the highest values observed in wines macerated for 10 days, irrespective of varietal type (Vranac or Kratošija). More specifically, there was no significant variation ($P > 0.05$) between wines prepared with 6 and 10 days of skin maceration (Table 2). The total phenolic index demonstrated a strong correlation with total phenolic content ($R^2 = 0.9477$) and exhibited significant differences ($P < 0.05$) among the different maceration durations for both investigated varietal wines. Consistent with findings in the literature (Gómez-Plaza *et al.*, 2001; Ivanova *et al.*, 2011; Kovač *et al.*, 1992; Pajović *et al.*, 2012), the observed increase in total phenolic content with longer maceration durations was anticipated.

In this study, the total phenol content ranged from 1000 to 1200 mg/L for both varietal wines with maceration durations of 6 and 10 days (Table 2). These findings are consistent with results from previous studies concerning experimental wines. For instance, Pajovic *et al.* (2016) reported values of around 1200 mg/L for Vranac wine and above 1000 mg/L for Kratošija wine from the 2011 and 2012 vintages macerated for 6 days. Similarly, Lisjak *et al.* (2020) found total phenol content to be above 1100 mg/L for Cabernet Sauvignon wine prepared after 7 and 9 days of maceration. Notably, a higher total phenol content, such as 2000 mg/L, is characteristic of commercial Vranac wines in Montenegro (Eder *et al.*, 2023).

The extraction of anthocyanins in Vranac wines was rapid, with high levels reached even after 2 and 4 days of maceration, consistent with previous literature for this wine variety (Ivanova-Petropulos *et al.*, 2016; Kovač *et al.*, 1992; Pajović *et al.*, 2012). The highest total anthocyanin value was attained after 10 days of maceration, with no significant differences observed compared to wines produced with 6 days of skin contact. By contrast, Kratošija wines reached maximum anthocyanin content after 6 days, followed by a slight decrease with longer maceration, although no significant differences were observed among wines

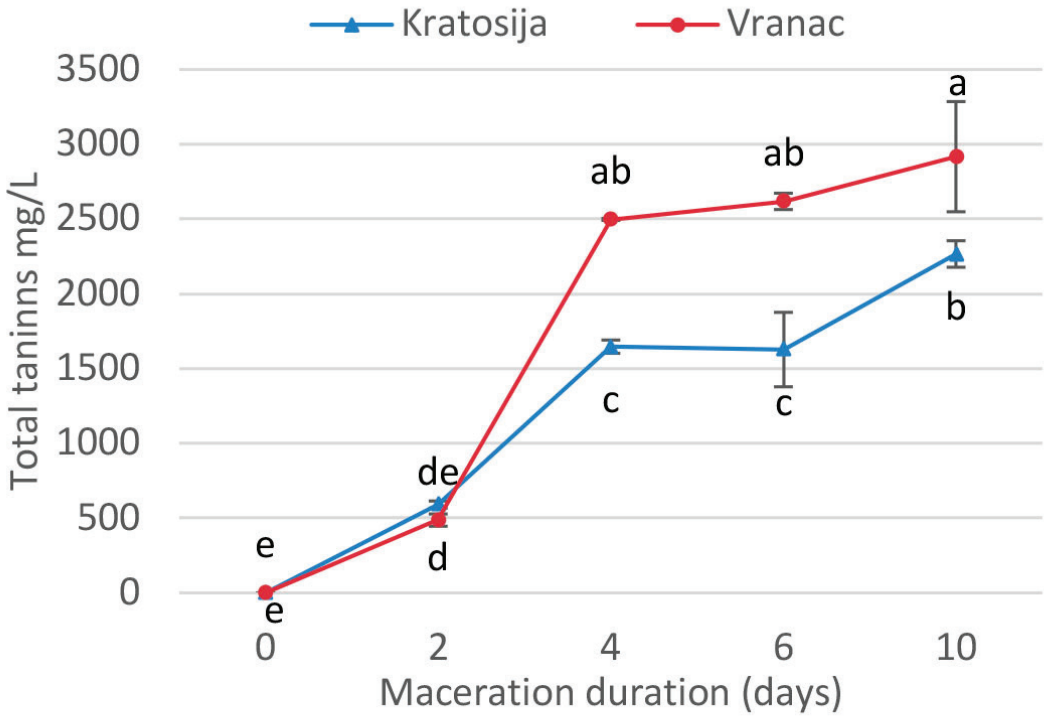

FIGURE 1. Concentration of tannins (mg/L) in Vranac and Kratošija wines with different maceration durations (0, 2, 4, 6, and 10 days).

a-e Different letters indicate significantly different mean values ($P < 0.05$) among examined wines. Error bars represent standard deviation.

macerated for 4, 6, and 10 days (Table 2). These results for Vranac wines are consistent with previous studies by Gómez-Plaza *et al.* (2001), Lisjak *et al.* (2020), and Plavša *et al.* (2012), in which maximum anthocyanin values were found in wines after 10 and 11 days of maceration. However, previous research on Vranac wines showed decreased total anthocyanin content after 7 days of maceration, with no statistical difference observed with longer periods (Ivanova *et al.*, 2011; Kovač *et al.*, 1992; Pajović *et al.*, 2012). The anthocyanin content for Vranac wines after 4 and 6 days of maceration was approximately 750 mg/L, which is consistent with relevant values for Vranac wines in 2011 and 2012 (637 and 834 mg/L, respectively) (Pajovic *et al.*, 2016). These results are also comparable with those presented by Eder *et al.* (2023) for the same varietal wine from the 2014 and 2015 vintages (787 mg/L and 745 mg/L, respectively). By contrast, Kratošija wines exhibited lower anthocyanin contents (Pajović-Šćepanović *et al.*, 2018) of around 300 mg/L, which is consistent with previous findings (317 mg/L and 429 mg/L, respectively) for the same varietal wine (Pajovic *et al.*, 2016). This value for Kratošija wines also aligns with values for Primitivo wines (ranging from 254 mg/L to 348 mg/L) produced using different winemaking technologies (Baiano *et al.*, 2009).

The colour intensity of wines increased with maceration time, reaching its highest value in wines macerated for 10 days, and with no significant differences observed among wines macerated for 4, 6, and 10 days for both varietal wines (Table 2). This trend is due to the similar anthocyanin content in these young wines. Moreover, colour intensity was positively correlated with anthocyanin content ($R^2 = 0.8664$), which is consistent with the findings by Gómez-Plaza *et al.* (2001). However, some researchers have reported decreased colour intensity with longer maceration durations due to the conversion of anthocyanin into non-pigment forms (Busse-Valverde *et al.*, 2012; Ivanova *et al.*, 2011; Kovač *et al.*, 1992). The values for these examined wines ranged from 0.52 to 1.47, which is within the range of 0.3 to 1.8 cited by Ribéreau-Gayon *et al.* (2006).

The hue values of the wines decreased with duration of maceration in both varietal wines, with no significant differences observed between wines macerated for 4, 6, and 10 days. The values ranged from 0.50 to 0.63, which is within the range for young wines (Ribéreau-Gayon *et al*., 2006).

The LMP content increased with maceration time, with the highest content found in wines macerated for 10 days for both varietal wines (Table 2). While all maceration durations showed significant differences in Vranac wine, Kratošija wine exhibited similar values for 6 and 10 days of maceration. The estimated LMP content in wines macerated for 6 and 10 days ranged between 1000 mg/L and 1200 mg/L. These values were higher than values reported for Vranac (266–518 mg/L) and Kratošija (355–556 mg/L) from the 2011 and 2012 vintages (Pajovic *et al.*, 2016), as well as for Vranac (982 mg/L) as a

TABLE 3. Degree of polymerisation (mDP), percentage of prodelphinidin (%PD), and percentage of galloylation (%Gall), in Vranac and Kratošija wines with different maceration durations (2, 4, 6, and 10 days).

	VRANAC			KRATOŠIJA		
Days	mDP	%PD	%Gall	mDP	%PD	%Gall
2	2.71 ± 0.38c	6.51 ± 2.8b	1.81 ± 0.01c	3.1 ± 0.23b	7.81 ± 0.45c	1.32 ± 0.08c
4	3.92 ± 0.18b	6.49 ± 0.39b	2.20 ± 0.01bc	3.3 ± 0.31b	8.23 ± 0.37c	1.31 ± 0.02c
6	4.01 ± 0.02b	7.85 ± 0.17b	2.90 ± 0.26b	3.54 ± 0.08ab	14.85 ± 0.96b	2.53 ± 0.41b
10	4.81 ± 0.10a	13.8 ± 0.35a	5.43 ± 0.60a	3.98 ± 0.32a	19.26 ± 0.08a	4.79 ± 0.01a
Mean	3.86	8.66	3.09	3.48	12.54	2.49

a-d Different superscript small letters in the same row indicate significantly different mean values ($P < 0.05$) for varietal wines. A,B Different superscript capital letters indicate significantly different mean values ($P < 0.05$) for varietal wines on average.

three-year average from 2014–2016 (Eder *et al.*, 2023). This discrepancy may be attributed to the slow polymerisation reactions of proanthocyanidins (Table 3).

Two methods were employed to determine the tannin content in wines: HMPs and total tannins (Table 2). As expected, the results obtained by both methods were highly correlated ($R^2 = 0.744$). The HMPs method measures the total amount of proanthocyanidins consisting of at least five units (Vrhovšek *et al.*, 2001), while the method for tannin determination estimates highly polymerised tannin units. The HMP values increased with maceration time, reaching their highest value after 6 days for Vranac wines and 10 days for Kratošija wines. Significant differences were found ($P < 0.05$) for all duration times for both wines. The HMP content in Vranac wines (1219 mg/L and 1021 mg/L for 6 and 10 days of maceration, respectively) was slightly lower compared to data reported for the same varietal wine from Montenegro and the 2011 and 2012 vintages (1214 mg/L and 1455 mg/L, respectively) (Pajovic *et al.*, 2016), and much lower than commercial Vranac wines (2330 mg/L) (Eder *et al.*, 2023). The HMP contents of Kratošija wine were 843 mg/L and 1273 mg/L after 6 and 10 days of maceration, respectively, in agreement with values of 1024 mg/L reported by Pajovic *et al.* (2016).

The results of total tannin content are presented in Figure 1 and are consistent with findings by Busse-Valverde *et al.* (2012), who also noted a similar increase with maceration time. Significant differences were observed for each maceration duration, aligning with findings reported by AEB Groupe (2022). The maximum tannin content for both varieties was attained after 10 days of maceration (2.9 g/L for Vranac and 2.2 g/L for Kratošija wines), a trend supported by literature where tannin concentrations typically range between 1 and 4 g/L in red wines, including those from the Vranac variety (Kovač *et al.*, 1992; Quaglieri, 2018; Raičević *et al.*, 2017).

Several authors have reported an increase in flavan-3-ol content (catechin and HMPs) with prolonged skin fermentation, which is attributed to ethanol enhancing the extraction of skin and seed flavanols (Casassa *et al.*, 2012; Gómez-Plaza *et al.*, 2001; Ivanova-Petropulos *et al.*, 2016; Kovač *et al.*, 1992; Lisjak *et al.*, 2020; Vrhovšek *et al.*, 2002).

A two-way ANOVA was conducted to assess the influence of maceration time and variety on the various phenolic parameters determined in this study. All examined parameters (total phenols, total phenol index, total acidity, LMPs, HMPs, and total tannins) exhibited the lowest content at day 0 and the highest after 10 days of maceration. Significant differences ($P < 0.05$) were observed between the values obtained after 0, 2, 4, and 6 maceration days compared to the corresponding values between 6 and 10 maceration days (no significant differences) (Table 2).

Regarding the influence of variety on the content of examined parameters, the ANOVA revealed significant differences ($P < 0.05$) in total anthocyanins, colour intensity, and total tannins among the two varietal wines (Figure 1). However, the remaining analytical parameters examined did not exhibit significant differences. These results are consistent with previous studies in which the two autochthonous Montenegrin varieties, Vranac and Kratošija, showed the highest differences in anthocyanin content of all other examined phenolic groups (Košmerl *et al.* 2013, Pajovic *et al.,* 2016).

3. Structural composition of tannins

The structural compositions of proanthocyanidins, including mDP, %PD, and %Gall, for Vranac and Kratošija wines subjected to varying maceration durations are detailed in Table 3. This study is the first to investigate the tannin structural characteristics of both these wines. The mDP values exhibited an increasing trend with prolonged maceration duration in both Vranac and Kratošija wines, reaching maximum content after 10 days. Significant differences ($P < 0.05$) in mDP values were observed among different maceration durations for Vranac wines, while Kratošija wines exhibited no significant differences until day 6. This

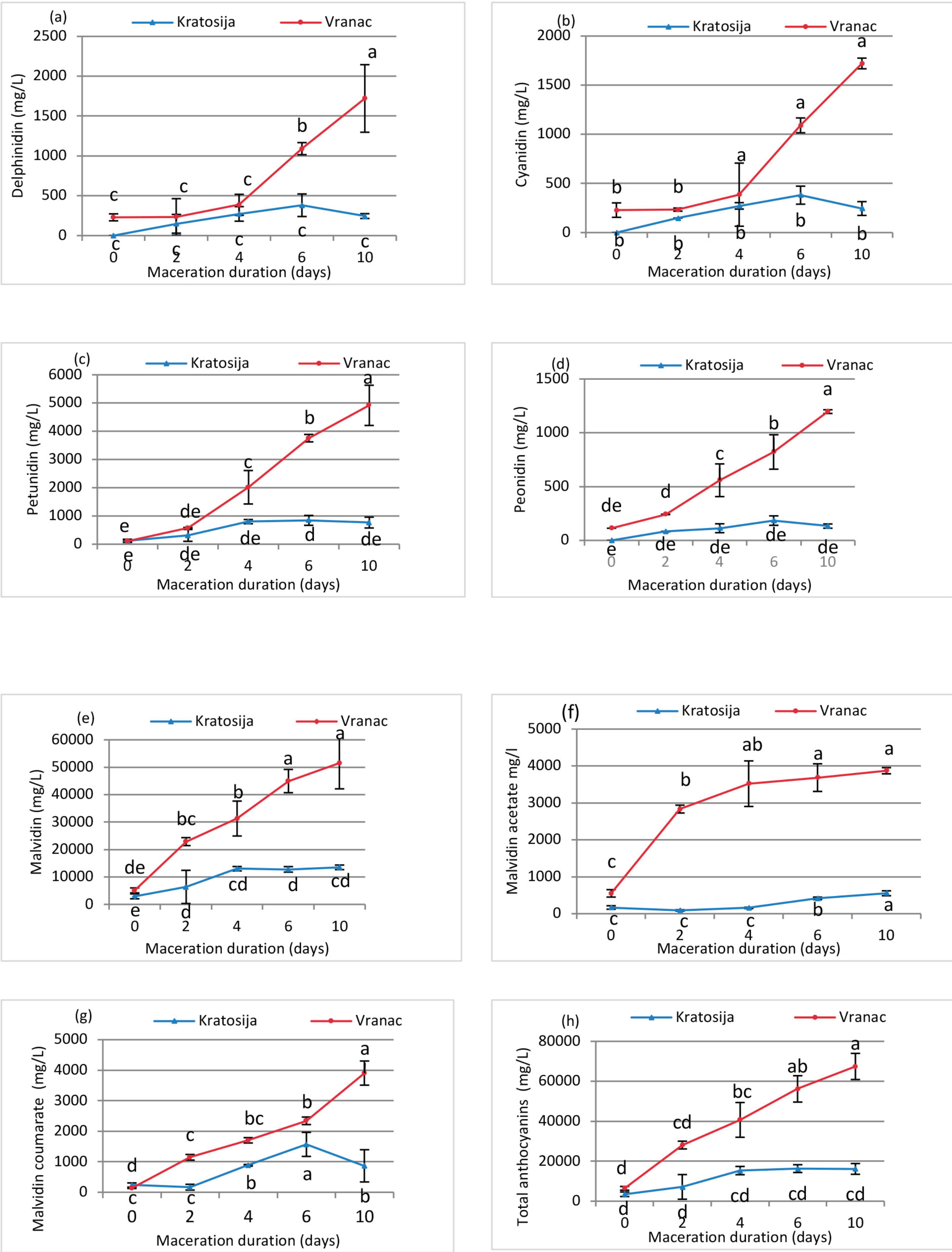

FIGURE 2. Concentration of monomeric anthocyanins: (a) delphinidin, (b) cyanidin, (c) petunidin, (d) peonidin, (e) malvidin, (f) malvidin acetate and (g) malvidin coumarate in Vranac and Kratošija wines with different maceration durations (0, 2, 4, 6, and 10 days).

a-e Different letters indicate significantly different mean values (*P* < 0.05) among examined wines. Error bars represent standard deviation.

trend is consistent with previous studies, which reported maximum mDP values after 10 and 11 days of maceration for Cabernet Sauvignon wines (Busse-Valverde *et al.*, 2012; Lisjak *et al.*, 2020).

Vranac wines displayed higher mDP values (4.81) than Kratošija wines (3.98), although both fell within the reported range in the literature (2.0-17.0) (Casassa, 2017). The mDP value obtained for Vranac wine (4.81) aligns well with the respective value reported for Monastell wines (4.93) (Busse-Valverde *et al.*, 2012). However, they are lower than the values reported by Monagas *et al.* (2003) (ranging from 6.9 to 13.0), Fernandez *et al.* (2007) (ranging from 7.4 to 13.6), and Hanlin *et al.* (2009) (with a value of 21.0). These disparities likely arise from the inherent influence of grape variety. Both Vranac and Kratošija are unique indigenous Montenegrin varieties known for their lower flavan-3-ol content compared to Cabernet Sauvignon (Pajović *et al.*, 2014; Pajović-Šćepanović *et al.*, 2019a). This variability in flavan-3-ol content among grape varieties could account for the observed differences in mDP values between Vranac and Kratošija wines.

The extraction of prodelphinidins exhibited an increasing trend with the duration of maceration in both examined varietal wines, reaching its peak after 10 days, with significant differences observed among the various maceration lengths (Table 3). However, contrasting observations have been reported in previous research, with a rapid increase in prodelphinidins being noted during the initial stages of maceration, followed by a subsequent decline after 5 days of extraction (González-Manzano *et al.*, 2006; Lisjak *et al.*, 2020).

The %PD values obtained for Vranac and Kratošija after 10 days of maceration (13.8 and 19.26, respectively) closely align with the range of 17.8–18.4 found by Chira *et al.* (2012) in Cabernet Sauvignon wines and with the values reported by Lisjak *et al.* (2020) (18.8 %PD) for a wine macerated for 7 days.

The %Gall content also exhibited an increase with increasing maceration time. The maximum value for both wines was obtained after 10 days of maceration, with a significant difference being observed ($P < 0.05$) compared to the values obtained for other durations. This finding is in accordance with the findings of other authors who reported that the extraction of galloylated units requires longer maceration durations (Busse-Valverde *et al.*, 2012; González-Manzano *et al.*, 2006; Lisjak *et al.*, 2020).

The maximum %Gall values for Vranac and Kratošija wines after 10 days of maceration were 5.43 and 4.79, respectively. These values fall within the range of respective values reported for wines macerated for 11 (23.1) (Lisjak *et al.*, 2020) and 10 days (1.48–3.58) (Busse-Valverde *et al.*, 2012). Although the seeds of both Vranac and Kratošija grapes are rich in gallic acid, the lower proportion of seeds compared to the flesh might have resulted in lower %Gall values in these wines (Pajović-Šćepanovic *et al.*, 2019b).

All tannin structural parameters exhibited an increasing trend with longer maceration periods. ANOVA analyses revealed that the highest values of all analytical parameters were achieved after 10 days of maceration, significantly differing from values obtained for other durations. However, it is plausible that even longer maceration periods beyond 10 days could yield different parameter trends (Busse-Valverde *et al.*, 2012; González-Manzano *et al.*, 2006; Lisjak *et al.*, 2020).

Furthermore, variety did not exert any influence on the analysed structural characteristics of tannins, which is consistent with the findings related to proanthocyanidin content presented in Table 2 (TP, LMPs, HMPs, total tannins), with no significant differences being observed between the two wines.

4. Anthocyanin composition

We also examined the influence of maceration time on the concentration of individual anthocyanins. Figure 2 illustrates the concentrations of individual anthocyanins detected in the wines. Our study focused on determining the concentrations of five non-acylated glucosides (delphinidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-glucoside, peonidin 3-glucoside, and malvidin 3-glucoside) and two acylated derivatives (malvidin-3-glucoside acetylglucoside and malvidin-3-O-coumarylglucoside).

The concentration of all five mono-glucoside anthocyanins increased during maceration. In Vranac wines, the highest concentration was observed after 10 days of maceration, with no statistical differences ($P > 0.05$) compared to wines macerated for 6 days. Conversely, Kratošija wines exhibited the maximum content of all anthocyanin monomers after 6 days of maceration, with no significant differences observed compared to the wines macerated for 4 or 10 days (Figures 2a, 2b, 2c, 2d, and 2e). These findings align with previous research by Gómez-Plaza *et al.* (2001), which also reported a 10-day maceration period as optimal for achieving maximum anthocyanin content in Monastrell wine. However, contrasting results were reported by Ivanova *et al.* (2011), who found that the maximum anthocyanin values for Vranac wine were reached on the sixth day, followed by a slight decrease after that, which is more consistent with the observations of this study for Kratošija wines. Consistent with previous literature (Kallitraka *et al.*, 2005; Monagas *et al.*, 2003; Revilla *et al.*, 2001), malvidin 3-glucoside emerged as the dominant anthocyanin, exhibiting a higher increase during maceration in Vranac wines than in Kratošija wines.

Other major anthocyanins investigated in this study (also noted by Revilla *et al.* (2001) were two acylated derivatives of malvidin: specifically, malvidin-3-acetylglucoside and malvidin-3-O-coumarylglucoside. The results obtained in this study demonstrate that the concentration of malvidin-3-acetylglucoside increased during maceration in both wines. In Vranac wines, the concentration of malvidin-3- coumarylglucoside increased over contact time, reaching its peak at 10 days. Similarly, Kratošija wines exhibited the highest concentrations at 6 days of fermentation

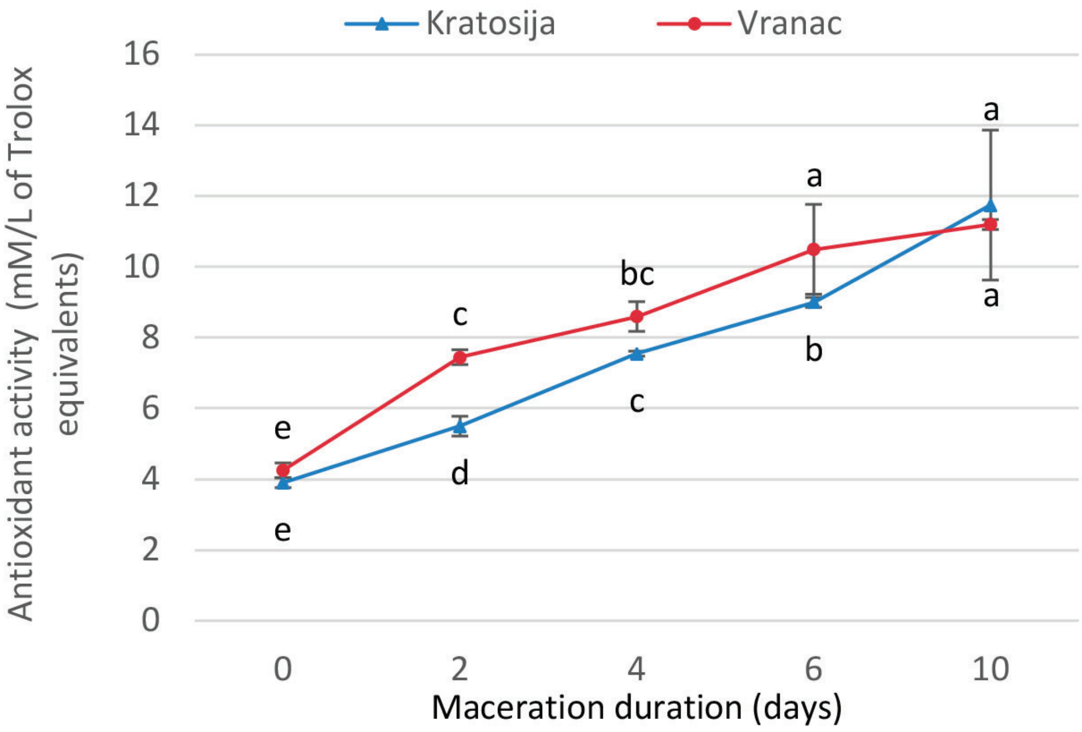

FIGURE 3. Antioxidant capacity (mM/L of Trolox equivalents) in Vranac and Kratošija wines with different maceration durations (0, 2, 4, 6, and 10 days).

a-e Different letters indicate significantly different mean values ($P < 0.05$) among examined wines. Error bars represent standard deviation.

(Figure 2g). Significant differences in the concentration of this compound were observed between both contact time and the two wine varieties (Figure 2g). In Vranac wines, the concentration of malvidin-3-acetylglucoside increased over contact time, reaching its peak at 6 days, while in Kratošija wines the highest concentrations was observed at 10 days of fermentation (Figure 2g).

Despite clear differences in concentration, all examined individual anthocyanins were equally extracted during fermentation, with maceration time having no impact on the retention of individual anthocyanins, which is consistent with findings reported by Revilla *et al.* (2001). Additionally, minor variations were observed in Vranac wine, such as the percentage of delphinidin 3-glucoside in wines with no maceration and the percentages of petunidin 3-glucoside and peonidin 3-glucoside after 0 and 2 days of maceration.

The values obtained by HPLC analysis were lower than those determined spectrophotometrically (Table 2) due to the different method employed. However, the overall trend in anthocyanin concentration across different wine samples was similar. Moreover, a highly significant correlation was found between the results for total anthocyanins (Table 2) and malvidin 3-glucoside content ($R^2 = 0.9313$), as well as between total anthocyanins analysed by HPLC (Figure 2e) ($R^2 = 0.9391$).

Regarding the influence of grape variety, ANOVA tests revealed significant differences ($P < 0.05$) for all examined anthocyanin compounds, including non-acylated glucosides. Vranac wines exhibited a higher concentration of both individual and total anthocyanin contents compared to Kratošija wines, consistent with previous research in the Montenegrin wine region (Pajović-Šćepanović *et al.*, 2018). However, Kratošija wines contained a higher content of malvidin-3-O-coumarylglucoside compared to the non-esterified forms. These findings align with previous research, where Vranac and Kratošija wines showed total anthocyanin contents of 800 and 300 mg/L, respectively (determined spectrophotometrically), and 394 and 131 mg/L, respectively (analysed by HPLC) (Pajović-Šćepanović *et al.*, 2018, Pajović-Šćepanović *et al.*, 2019b). The higher content of malvidin-3-O-coumarylglucoside in Kratošija wines is a characteristic trait of this variety, consistent with previous findings regarding Montenegrin grapes and wines (Pajović *et al.*, 2014; Pajović-Šćepanović *et al.*, 2018). Wines with a genetic profile similar to Kratošija, such as Primitivo and Crljenjak Kaštelanski, also exhibit higher content of malvidin-3-O-coumarylglucoside compared to other anthocyanin forms (Mattivi *et al.*, 2006; Mekinić *et al.*, 2019).

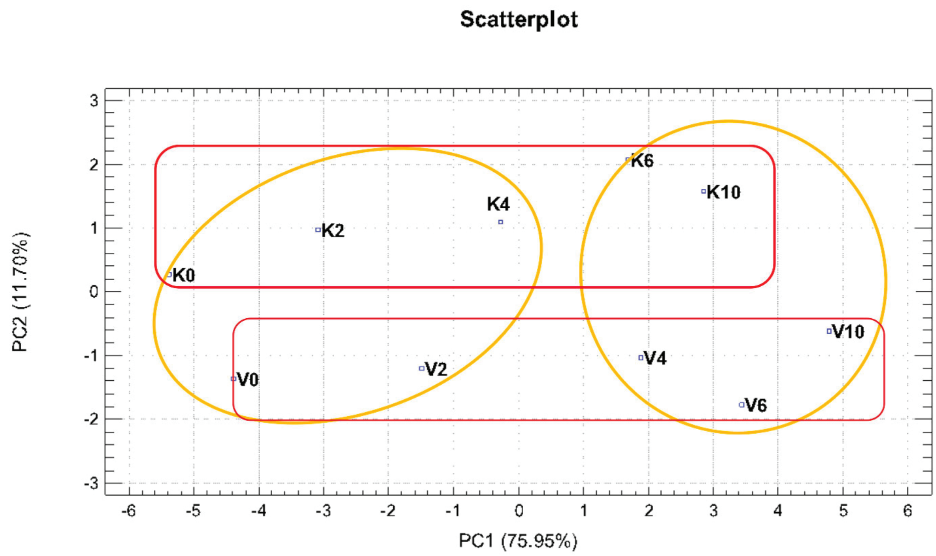

FIGURE 4. Principle component scatterplot for two principal components for Vranac (V) and Kratošija (K) red wines with different maceration durations (0, 2, 4, 6, and 10 days).

5. Antioxidant activity

The antioxidant properties of Montenegrin wines derived from various maceration durations were investigated here for their capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, as depicted in Figure 3. The results reveal a significant variation in antioxidant activity across different maceration durations. Specifically, wines without maceration exhibited the lowest scavenging activity values, whereas those subjected to a 10-day maceration period demonstrated the highest antioxidative potential. More specifically, Vranac wines subjected to up to 6 days of maceration did not exhibit significant differences ($P > 0.05$) in antioxidant activity. Conversely, Kratošija wines exhibited distinct antioxidant activities across varying maceration durations (Figure 3). These observations align with prior investigations suggesting augmentation of antioxidant activity during maceration, as documented in studies by Kocabey *et al.* (2016) and Mekinić *et al.* (2019). The enhancement in antioxidant activity underlines the dynamic relationship between maceration duration and the resultant phenolic composition, elucidating its implications for the overall quality and health-promoting attributes of Montenegrin wines.

Varietal diversity did not yield significant differences ($P > 0.05$) in the antioxidant characteristics of the wines, as determined by analysis of variance (ANOVA). The recorded antioxidant values ranged from 3.9 to 11.75 mmol/L Trolox across the studied varietals. Specifically, the values obtained for wines subjected to 6 and 10 days of maceration fell

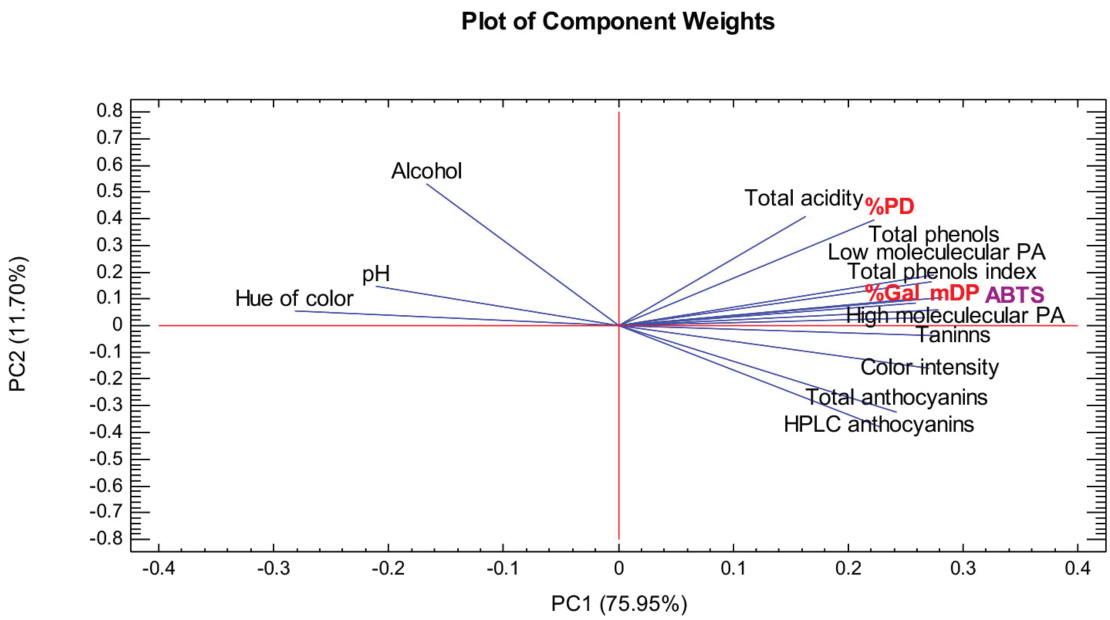

FIGURE 5. Plot of component weight for two principal components of the wines studied.

within the range reported by previous investigations utilising the same methodology for assessing antioxidant properties, with values ranging from 7.8 to 24.2 mmol/L Trolox (Baiano *et al.*, 2009; Pajović-Šćepanović *et al.*, 2019b; Šeruga *et al.*, 2011).

Significant correlations were obtained between the antioxidant activity values and several analytical parameters of the wines determined spectrophotometrically. Specifically, significant correlations were observed between TP and TPI results and antioxidant activity values (R^2 = 0.705 and 0.909, respectively). This finding is consistent with previous research (Frankel *et al.*, 1995; Mattivi *et al.*, 2002; Kallithraka *et al*., 2009), reinforcing the relationship between these phenolic constituents and the antioxidative potential of wines. Furthermore, a robust correlation was identified between proanthocyanidin components (HMPs, LMPs, and total tannins) and antioxidant activity, with R^2 values of 0.884, 0.852, and 0.743, respectively. Additionally, structural attributes of proanthocyanidins, such as mDP, %PD, and %Gall, exhibited significant correlations with antioxidant activity values with R^2 values of 0.693, 0.824, and 0.855, respectively. These correlations underscore the pivotal role of proanthocyanidins in determining the antioxidant capacity of wines. The results are in accordance with those obtained by earlier studies indicating that the antioxidant potential of red wines is predominantly influenced by their flavan-3-ol and proanthocyanidin content (Cimino *et al.*, 2007; Eder *et al.*, 2023; Gris *et al.*, 2011; Rigo *et al.*, 2000). Notably, Cimino *et al.* (2007) and Rigo *et al.* (2000) reported substantial correlations between proanthocyanidins, total phenols, and scavenging capacity values, utilising similar spectrophotometric methodologies employed in this investigation. Indeed, the phenolic compounds exert their antioxidant effects through several mechanisms, primarily attributable to their concentration and the inherent free radical scavenging properties conferred by the hydroxyl groups within their chemical structures (Paganda *et al.*, 1999).

6. Principal component analyses

Principal component analysis (PCA) was employed to gain deeper insights into the factors influencing the differentiation among the studied wines, specifically examining the relative contributions of variety and maceration duration. A correlation matrix was constructed based on 17 wine compounds and physicochemical characteristics to facilitate this analysis. These included phenolic compounds quantified via spectrophotometry, the structural components of proanthocyanidins, total anthocyanin content determined by HPLC, and antioxidant activity.

The results of PCA revealed that the first (PC1) and second principal components (PC2) collectively accounted for 87.20% of the total variance, indicating that a substantial proportion of the overall variability can be attributed to these components. The correlation between the original variables and the first two principal components is illustrated in Figure 4. Upon projection of the wines onto the PCA plot, it became apparent that the grouping of wine samples primarily aligned with varietal distinctions, despite the absence of statistically significant differences in their composition. Vranac wines were clustered within the negative region of PC2, primarily attributed to their elevated levels of total anthocyanins (as determined spectrophotometrically and by HPLC), the resulting higher colour intensity, and their higher tannin content. Conversely, Kratošija wines exhibited grouping in the positive region of PC2, characterised by higher alcohol content, elevated pH levels, and colour hue (Figures 4 and 5). All other examined matrix components exhibited similar ranges, with no significant differences observed between the varietal wines (as indicated in Tables 2 and 3 and Figures 1, 2h, and 3).

Regarding the influence of maceration duration, the wines exhibited distinct groupings based on the content of examined matrix components. Specifically, wines subjected to shorter maceration durations (0 and 2 days) for both varieties and 4 days for Kratošija wines were clustered within the negative part of PC1. This clustering was primarily attributed to the comparatively lower content of the analysed compounds in these wines. Conversely, wines produced with longer maceration durations (6 and 10 days for both varieties and 4 days for Vranac) were grouped in the positive part of PC1, indicative of higher levels of the examined matrix components (Figure 4). The observed grouping patterns were predominantly driven by variations in the content of phenolic compounds, as evidenced by data presented in Tables 1 and 2 and Figures 1 and 3.

These findings demonstrate the significant impact of both the varietal distinction and the maceration duration on the phenolic composition and overall chemical profile of the wines, as elucidated by their distinct groupings in the PCA analysis (Figure 4).

CONCLUSION

This study explored the differences in chemical composition of two Montenegrin wines, Vranac and Kratošija, produced with different maceration durations. These two varieties are important in Western Balkan countries, with the Kratošija variety being spread worldwide under different synonyms.

The chemical composition analysis revealed significant varietal differences in alcohol content, total acidity, pH values, and phenolic compounds. Vranac wines generally exhibited higher total acidity and lower pH values than Kratošija wines. Additionally, phenolic content increased with maceration time, with the highest values being observed after 10 days of maceration. These findings are consistent with previous literature and highlight the impact of maceration duration on wine phenolic composition.

The structural composition analysis of proanthocyanidins showed increased mDP, %PD, and %Gall with longer maceration durations. Vranac wines displayed higher mDP and %PD than Kratošija wines, indicating varietal-specific differences in proanthocyanidin composition.

The anthocyanin composition analysis revealed variations in individual anthocyanin concentrations with maceration time. Both Vranac and Kratošija wines showed increased anthocyanin contents with longer maceration time. However, Vranac wines exhibited higher total anthocyanin concentrations compared to Kratošija wines. Additionally, in Vranac wines, the maximum concentration of anthocyanins was reached after 10 days, while in Kratošija, it was achieved within 6 days.

Principal component analysis revealed that grape variety and maceration time significantly influenced the composition of the examined compounds. While significant differences were only observed for a few parameters (e.g., anthocyanins, colour intensity, alcohol content and total tannins), the wines from different varieties were distinctly separated on the scatterplot.

This study highlights the relationship between varietal characteristics, maceration duration, and wine phenolic composition. Understanding these factors is essential for winemakers to optimise wine quality and production techniques according to specific grape varieties. Further research could explore additional parameters influencing wine phenolic composition, such as fermentation temperature and grape maturity, to provide comprehensive insights into wine production processes.

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