Study of the effects of climatic conditions on the phenolic content and antioxidant activity of Austrian and Montenegrin red wines

Reinhard Eder[1], Radmila Pajović-Šćepanović[2], Danijela Raičević[2], Tatjana Popović[2], Karin Korntheuer[1], Silvia Wendelin[1], Astrid Forneck3 and Christian Philipp[1]

[1]Federal College and Research Institute for Viticulture and Pomology, Klosterneuburg, Austria

[2]Biotechnical Faculty, University of Podgorica, Montenegro

[3]University of Natural Resources and Life Sciences, Vienna; Department of Crop Sciences, Institute for Viticulture and Pomology, Tulln, Austria

*corresponding authors: reinhard.eder@weinobst.at and radmilap@ucg.ac.me

Abstract

We evaluated the chemical composition and antioxidant activity of 30 red wines from Austria (cv. Cabernet-Sauvignon, Blaufraenkisch and Zweigelt) and 20 wines from Montenegro (cv. Cabernet-Sauvignon and Vranac) from three consecutive vintages: 2014, 2015 and 2016. We determined spectrophotometrically the contents of total phenol (TP), total anthocyanin (TA) and low- and high-molecular-weight proanthocyanidins (LMP and HMP respectively). We identified and quantified 18 phenolic compounds (hydroxycinnamic acids, hydroxybenzoic acids, stilbenes and flavan-3-ols) by high-performance liquid chromatography. In addition, we used Fourier-transform infrared spectroscopy for chemical analyses of the main wine parameters. Austrian autochthonous wines exhibited a similar chemical composition (alcohol 12.4 vol%, pH 3.38). Blaufraenkisch wines showed higher TP, HMP and LMP than Zweigelt wines; however, TA content was similar. Blaufraenkisch wines also showed higher phenolic acid, flavan-3-ol and stilbene contents than Zweigelt wines. Montenegrin autochthonous Vranac wines showed a typical chemical composition (alcohol 13.0 vol%, pH 3.42), and medium to high levels of phenols: TP, HMP, LMP, TA, HCA and flavan-3-ols. On the other hand, they showed a moderate stilbene content. Cabernet-Sauvignon wines from Austria and

Montenegro exhibited some similarities in phenolic composition: TP, HMP, LMP and TA. There were notable variations in the phenolic acid and flavan-3-ol contents, especially the stilbene content, which was much higher in the Austrian wines than in the Montenegrin wines. These findings evidence an important impact of climatic conditions on these compounds. The antioxidant activity in all investigated wines was high and correlated strongly with TP, total phenol index, HMP and LMP in wines. The vintage influenced the chemical composition and content of all examined phenolic groups, except flavan-3-ols. Wines from the vintage with the best maturity (2015) contained the highest content of spectrophotometrically determined phenolic compounds and lowest level of phenolic acids and stilbenes. Principle component analysis showed that wines were mainly discriminated by variety and origin but not by vintage.

Blaufraenkisch, Zweigelt, Vranac, Cabernet-Sauvignon, phenol composition

Introduction

Wine has long been appreciated for its high contents of phenolic compounds, which are important for wine quality. Phenolic compounds, especially anthocyanins, flavanols, catechin and other flavonoids, are responsible for the sensory characteristics of wine, particularly colour, astringency, bitterness and flavour (Cheynier *et al.*, 2006; Liszt *et al.*, 2015; Sterneder *et al.*, 2021). In addition, phenolic compounds have various positive effects on human health, especially due to their antioxidant properties (Frankel *et al.*, 1995; Rodrigo *et al.*, 2011). The antioxidative value of wines is mainly attributed to their total phenolic content (Minussi *et al.*, 2003), as well as to the specific chemical structure of some phenols, such as flavan-3-ols and proanthocyanidins (Gris *et al.*, 2011; Rigo *et al.*, 2000), anthocyanins (Vanzo *et al.*, 2008) and stilbenes (Vrhovšek *et al.*, 1997a). The profile and ratio of some phenolic compounds can be used to differentiate grape and wine varieties (Eder *et al.*, 1994; Mattivi and Nicolini, 1997; Jaitz *et al.*, 2010; Monagas *et al.*, 2005; Pajović-Šćepanović *et al.*, 2019a).

There are two categories of phenolic compounds: flavonoids, which are the most abundant in red wine, consist of anthocyanins, flavan-3-ols and flavonols, while non-flavonoids comprise hydroxybenzoic and hydroxycinnamic acids and stilbenes (Eder, 2019). Furthermore, condensed anthocyanins can be detected in aged wines (Eder *et al.*, 2014).

The concentration and composition of phenolic compounds in grapes and red wine differ depending on the grape variety (Jaitz *et al.*, 2010; Kallithraka *et al.*, 2005; Pajović-Šćepanović *et al.*, 2018). Additionally, the phenolic composition of wines is affected by the quality and yield of the grapes, which are influenced by growing conditions (Costa *et al.*, 2015). Wine characteristics are also affected by the interaction between regional climate and geological and soil conditions, wine region, weather conditions during the harvest period, vintage (Gris *et al.*, 2011) and vineyard management (Košmerl *et al.*, 2013). The chemical composition of each wine variety produced in a specific terroir reflects the locality (Costa *et al.*, 2015).

Winemaking techniques are also important parameters that influence the final phenolic composition of the obtained wines (Monagas *et al.*, 2005; Scutaraşu *et al.*, 2020). Thus, it is usually possible to determine a specific type of wine from the wine origin.

For the wine industry, it is important to determine the authenticity of its products in order to be able to comply with public regulations; thus, applicable wine analyses based on wine components (e.g., phenolic compounds) are needed (Popîrdă *et al.*, 2021). Here, we focus on the wine-producing European countries Austria and Montenegro, which are located in different climatic zones. Austria is particularly known for its white wines, but it is also important in the production of red wines, with autochthonous varieties, such as Blaufraenkisch and Zweigelt. Austrian vineyards are located in the European Union (EU) wine zone B. According to the Winkler Index, Montenegro, a Mediterranean country, belongs to wine zones CII and CIII; however, it is not an EU member state. Montenegro has a dominant production of red wines, mostly the autochthonous Vranac variety (Pajović-Šćepanović *et al.*, 2019b).

The phenolic composition of Austrian red wines has been investigated for many years. Results have been published concerning anthocyanins (Eder, 2014), phenolic acids and flavan-3-ols (Jaitz, 2010; Vrhovšek *et al.*, 1997b) and stilbenes (Eder *et al.*, 2001). These results have led to the establishment of routine protocols for analysing Austrian wines. However, a more detailed study of specific phenolic groups in wines has not yet been conducted. There has been recent interest in evaluating phenolic compounds in grapes (Košmarel *et al.*, 2013; Pajovic *et al.*, 2014a; Pajović *et al.*, 2014b; Pajović-Šćepanović *et al.*, 2019a) and in wines (Pajović-Šćepanović *et al.*, 2018; Pajović-Šćepanović *et al.*, 2019b). In recent decades, international French grape varieties (e.g., Cabernet-Sauvignon) have been cultivated in Austria and Montenegro. In this study, Cabernet-Sauvignon is used as a common and international standard variety in both countries, an approach that enables a good comparison of the data (Costa *et al.*, 2015).

Comparable data regarding the phenolic composition of Austrian and Montenegrin red wines are scarce in the literature. Thus, to increase the knowledge of the phenolic content of Austrian and Montenegrin red wines, we evaluated the chemical and phenolic composition of three autochthonous red wines (Blaufraenkisch and Zweigelt from Austria and Vranac from Montenegro) from three consecutive vintages (2014, 2015 and 2016). For comparative reasons, we evaluated Cabernet-Sauvignon wines from both countries to investigate the influence of the climatic region and specific terroir on the expression of phenolic substances. We aimed to increase the knowledge of (a) the phenol composition of typical Austrian and Montenegrin red wines with distinct geographic origins, (b) the influence of different climatic conditions, which existed during the three vintages (2014-2016) and (c) the adaptability of Cabernet-Sauvignon to the growing conditions in Austria and Montenegro. Our overall goal was to improve the methodological resolution of terroir-based wine analytical protocols.

Materials and methods

1. 1. Chemicals and reagents

The following chemicals and standards (all high-performance liquid chromatography [HPLC] grade) were used for the chromatographic analysis: methanol, formic acid, potassium dihydrogen phosphate, phosphoric acid (all from Merck, Darmstadt, Germany); distilled water, MilliQ water (Millipore Corporation, Billerica, MA, USA); and phenol standards, including caffeic acid, ferulic acid, trans-resveratrol (all from Sigma, Steinheim, Germany), caftaric acid (Dalton Chemical Laboratories Inc., Toronto, Canada) gallic acid and *p*-coumaric acid (all from Roth, Karlsruhe, Germany). For the spectrophotometric analyses and estimation of the antioxidant activity, the following chemicals and standards were used: methanol, ethanol (both from Merck), hydrochloric acid, sodium hydroxide, sodium bisulphite, L(+)-tartaric acid, potassium persulfate (K2S2O8), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (all from Sigma), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (Fluka, Germany) and Milli Q grade (Millipore Corporation). The Folin-Ciocalteu reagent and vanillin were from Merck.

2. 2. Austrian and Montenegrin wine samples

The analysed wine varieties, the number of samples, the country of origin and the wine-growing region for the examined vintages (2014-2016) are given in Table 1. Fifty commercial red wines were collected during three consecutive vintages (2014-2016) directly from the commercial wineries: 30 Austrian wines consisting of Blaufraenkisch, Zweigelt and Cabernet-Sauvignon from the Burgenland and Lower Austria wine regions, and 20 Montenegrin wines consisting of Vranac and Cabernet-Sauvignon mostly from the Skadar Lake wine region, a Podgorica sub-region, which covers 90 % of the wine-growing region in Montenegro (Table 1).

The criterion for the selection of the commercial wineries was that they mainly have a long history of consistently producing high-quality wines. To ensure comparability, the wine samples from the three vintages were analysed at the same age; i.e., 1 year old.

Table 1. Wine origin and variety, the number of samples and wine-growing regions for three consecutive vintages (2014-2016).

	Variety	Number	Wine growing region	
Austria			Burgenland	Lower Austria
	Cabernet-Sauvignon	10	5	5
	Blaufraenkisch	10	9	1

	Zweigelt	10	4	6
Montenegro			Skadar Lake wine region	Coastal wine region
	Cabernet-Sauvignon	10	9	1
	Vranac	10	9	1

3. 3. Characterisation of the assessed Austrian and Montenegrin wine regions

Table 2 provided the climatic conditions (the Huglin Index, total precipitation, seasonal and vegetal precipitation and cool climate indices) for the Austrian and Montenegrin wine regions (localities) where most of the wines are produced.

Table 2. Climate condition of the examined vintages.

	Austria (Klosterneuburg, Agneshof)	**Montenegro** (Podgorica sub-region)
Huglin Index	**2014:**1902 **2015**: 2200 **2016:** 2076	**2014:** 3109 **2015**: 3203 **2016:** 2941
Total precipitation	**2014:** 853 mm **2015:** 582 mm **2016:** 836 mm	**2014**: 2117.7 mm **2015:** 1176.0 mm **2016:** 1993.7 mm
Precipitation (April-September)	**2014**: 664 mm **2015**: 279 mm **2016:** 483 mm	**2014:** 796 mm **2015:** 243 mm **2016**: 675 mm
Cool climate indices	**2014:** 12.0 °C **2015:** 11.3 °C **2016**: 12.2 °C	**2014:** 16.4 °C **2015:** 18.7 °C **2016:** 17.0 °C

Based on the Huglin Heliothermal Index, the Klosterneuburg, Agneshof region in Austria is classified as temperate (H-1) and temperate warm (H+1), and the Podgorica sub-region in Montenegro is very warm (H+3). There are also differences in night cool index/cool climate

indices: the Klosterneuburg, Agneshof region in Austria has very cool nights (CI+2) and the Podgorica sub-region in Montenegro has temperate nights (CI-1). This classification is based on the Geoviticulture Multicriteria Climatic Classification System (Tonietto and Carbonneau, 2004).

Total precipitation was much higher in Montenegro than in Austria, but seasonal precipitation was similar in both countries.

The values for the climatic conditions of the Podgorica sub-region are as expected, because it has a subtropical climate and belongs to the CIII zones according to the Winkler Index (Pajović-Šćepanović *et al.*, 2019b). The data for the Klosterneuburg, Agneshof locality come from the Federal Research Institute for Enology weather station.

Despite the marked differences in climatic parameters between these two wine regions, there is evidence of a similar trend for the examined vintages in both countries. Specifically, the 2015 vintage showed the highest Huglin Index values and lowest level of total and seasonal precipitation compared with the 2014 and 2016 vintages (Table 2).

4. 4. Wine analysis

4.1. 4.1. Fourier-transform infrared (FTIR) spectroscopy analysis

FT-IR spectroscopy was performed according to OIV/OENO Resolution 390/2010 using a FOSS- WineScan machine (FT 120 Reference Manual, Fa. Foss, Hamburg, Germany).

4.2. 4.2. Spectrophotometric analyses of phenolic groups

Spectrophotometric analyses were performed as described by Di Stefano and Guidoni (1989) and Di Stefano *et al.* (1989) using a Varian Cary 100 spectrophotometer (Bio Tech, Gaithersburg, MD, USA). A classical Sep-Pak (0.35 g) C-18 column from Waters (Milford, MA, USA) was used for a preliminary clean-out to remove any polar compounds, such as sugars, organic acids, amino acids and free sulphur dioxide (SO_2), which could have interfered with the assays.

4.3. 4.3. Total phenols (TP)

TP were analysed using colourimetric determination by means of a photometer with the addition of the Folin-Ciocalteu reagent, which reduces phenolic substances to monohydroxyphenols by reacting with tungstophosphoric acid and molybdophosphoric acid. A realistic estimation of TP was obtained after the preliminary cleaning of the samples to remove compounds that interfere with the assay (Di Stefano and Guidoni, 1989). One millilitre of wine was diluted wine with 1 N H_2SO4 and then mixed with 2 ml of methanol, 5 ml of distilled water, 1 ml of Folin-Ciocalteu reagent and 4 ml of 10 % Na_2CO_2. Distilled water was added to a total volume of 20 ml in the flask. After 90 min, the absorbance was read at 700 nm against a blank prepared with distilled water in a 1 cm glass cuvette. When absorbance was between 0.3 and 0.6 AU (the linear range), TP was calculated as ml (+)-catechin equivalents per l of wine with the equation $186.5 \times A \times d$, where A is the absorbance and d is sample dilution. The concentrations were determined by means of a calibration curve generated with gallic acid.

4.4. 4.4. Total phenol index (TPI)

The TPI was also determined in the wines. Absorbance at 280 nm was measured in a quartz cuvette (after dilution wine with water in the flask) and the value was calculated according to the dilution factor.

4.5. 4.5. High-molecular-weight proanthocyanidins (HMP)

HMP were determined by applying the method of Di Stefano *et al.* (1989) and were evaluated by conversion to cyanidin. The following solution was prepared in two flasks (A and B): 9.5 ml of 95 % ethanol, 2 ml of diluted wine (with 1 N H_2SO_4), 3 ml of methanol and 12.5 ml of 0.3 % $FeSO_4$ in HCl. Flask A was boiled for 50 min at 102 °C in a water bath with a glass Graham condenser, while flask B was placed on ice for the same amount of time. Both flasks were adjusted to the same temperature (19 °C) in a water bath and then the absorbance was read at 700 nm with a blank (distilled water) in a 1 cm glass cuvette (target absorbance between 0.3 and 0.6). The absorbance of the corresponding blank (cyanidin chloride) was subtracted from each measurement. HMP are expressed as mg cyanidin equivalents per l of wine based on the equation $(A - B) \times 1162.5 \times d \times V$, where A is the absorbance of flask A, B the absorbance of flask B, d the wine sample dilution and V the sample volume.

4.6. 4.6. Low-molecular-weight proanthocyanidins (LMP)

Catechins and proanthocyanidins reactive with vanillin were analysed by the optimised and controlled vanillin HCl method of Broadhurst and Jones (1978) under conditions described by Di Stefano *et al.* (1989) and calculated as (+)-catechin (mg L^{-1}) using a calibration curve. A solution was prepared with 2 ml of wine (diluted with 1 N H_2SO_4) and 5 ml of methanol. The mixture was divided into two flasks (A and B). Both flasks contained 6 ml of 4 % vanillin in methanol and 3 ml of concentrated HCl. After adjusting the flask temperatures to 15 °C for 15 min in a water bath, the absorbance was read at 700 nm in the glass cuvette using the content of flask B as the blank. When the absorbance was between 0.2 and 0.4 AU (700 nm), LMP was calculated as mg (+)-catechin equivalents per l^- of wine using the equation $E \times d \times 0.4$, where E is the absorbance and d the sample dilution. This method provides a good estimate of catechins, free flavanols and a small amount of proanthocyanidins.

4.7. 4.7. Total anthocyanins (TA)

TA were determined based on the measurement of maximum absorbance at 536-542 nm (Di Stefano *et al.*, 1989). Five millilitres of wine (diluted with 1 N H_2SO_4) was mixed with 3 ml of methanol, 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 in glass cuvette. TA was calculated as mg malvidin-3-glucoside chloride equivalent per l^- of wine, which corresponds to the experimental value of the molar absorbance of malvidin-3-glucoside chloride in ethanol/water/HCl (70:30:1), $\varepsilon = 30100$ at 542 nm (Di Stefano et al., 1989).

4.8. 4.8. HPLC analyses of phenolic acids and flavan-3-ols

The phenolic profile of the wines was determined by HPLC according to the modified method of Vrhovšek (1997b). The HPLC system (Agilent Technologies, Santa Clara, CA, USA) included an autoinjector (5 µl injection volume) and a diode array detector (DAD) at 280 and 320 nm. Separation was performed using a POROSHELL 120 SB-C18 analytical column (150 × 2.1 mm, 2.7 µm) (Agilent Technologies) maintained at 40 °C. Five microlitres of filtered sample (0.45-µm membrane) was injected at a flow rate of 0.25 ml/min. Two mobile phases were used: 0.5 % formic acid pH 2.3 (solvent A) and methanol (solvent B) with the following gradient programme: 3 % B at 0 min, 6 % B at 14 min, 7 % B at 24 min, 13 % B at 35 min and held until 47 min, 20 % B at 57 min, 70 % B at 60 min and held until 70 min, and 3 % B at 75 min, with a 15-min lag time. Phenolic acids were identified using an external standard method by comparing retention times and UV spectra with the following commercial standards: caffeic acid, ferulic acid, caftaric acid, *p*-coumaric acid, gallic acid, tyrosol, (+)-catechin, (-)-epicatechin, procyanidin B1 and procyanidin B2. Trans-coutaric acid, cis-coutaric acid and fertaric acid were quantified by external calibration with caftaric acid.

4.9. 4.9. HPLC analyses of resveratrol

The concentrations of four forms of resveratrol were analysed according to a modified method by Eder *et al.* (2001). An HPLC system (RRLC type; Agilent Technologies) with a POROSHELL 120 SB-C18 analytical column (150 × 2.1 mm, 2.7 µm) (Agilent Technologies) was used. Using 0.5 % formic acid (solvent A) and methanol (solvent B) as the eluent, the following gradient was employed: 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 lag time of 15 min. Five microlitres of filtered sample (0.45-µm membrane) was injected at a flow rate of 0.25 ml/min. UV detection was performed by measuring the absorbance at 280 and 320 nm. Trans-resveratrol and trans-resveratrol glucoside (trans-piceid) were identified and quantified using pure standards. Cis-resveratrol and cis-resveratrol glucoside were extracted from trans-isomers by UV irradiation (254 nm, 24 h) and quantified with a cis/trans extinction molar ratio of 3.78/1.

4.10. 4.10. Determination of the antioxidant activity of the wines

The antioxidant capacity of the wines was estimated using Trolox-equivalent antioxidant capacity (TEAC) according to a modified spectrophotometric method (Rice-Evans *et al.*, 1996) described by Pajović-Šćepanović *et al.* (2018). ABTS solution was prepared by incubating 5 ml of a 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 at 734 nm was 0.7 ± 0.05. The absorbance of 2 ml of ABTS solution at 734 nm was measured, and 0.01 ml of appropriately diluted wine was added. After exactly 6 min, the absorbance of the sample was measured in a glass cuvette. The results as expressed as mol Trolox/l.

4.11. 4.11. Statistical analysis

We assumed that the collected data followed a normal distribution and showed homoscedasticity. We used two-way analysis of variance (ANOVA) to assess the significance

of the factors wine variety and origin and their interaction between these factors for each studied parameter. When the ANOVA revealed a significant difference, we applied a post hoc least significant difference test. We considered $P < 0.05$ to be statistically significant.

We also used the data (19 variables, including phenolic compounds and ABTS antioxidant activity) for principal component analysis (PCA) and linear discriminant analysis (LDA) to determine whether they could differentiate the wine samples according to the type of grape (white vs red), variety and vintage. We performed these multivariate analyses according to the procedures described by Pajović-Šćepanović *et al*. (2018). We used SPSS Statistics 20 (IBM Corp., Armonk, NY, USA) for all statistical analyses.

Results and discussion

To obtain new and relevant data on the phenolic composition of autochthonous wines from Austrian and Montenegro, we combined spectrophotometric analysis (four phenolic groups) and chromatographic methods (HPLC-UV and HPLC-DAD) to identify individual phenolic compounds.

1. 1. Chemical composition of wines

We first analysed the influence of vintage and region on the chemical composition of the wine samples (Table 3). The 2014 vintage wines had the lowest alcohol content and the highest total acidity, whereas the 2015 vintage wines had the highest alcohol content and lowest total acidity. The other tested parameters also showed significant differences between the three vintages. These differences can be explained by the different meteorological conditions for each vintage (Table 2).

Two-way ANOVA revealed that the chemical composition of the wines was influenced by both variety and vintage ($p < 0.05$). The influence of the variety was best reflected in the alcohol content, total acidity and pH. Considering the average of the three vintages, Austrian Cabernet-Sauvignon had the highest alcohol content, followed by Montenegrin Cabernet-Sauvignon and Vranac. The wines were considered to be dry, based on the fructose and residual sugar concentrations.

Acidity was evaluated based on tartaric and lactic acids, total acidity and pH. Total acidity was 5.1-6.2 g/L, with small but significant differences between the varieties. Austrian Cabernet-Sauvignon had the highest total acidity, followed by Montenegrin Cabernet-Sauvignon. Autochthonous Austrian Zweigelt and Blaufraenkisch had the lowest total acidity. The pH showed the same pattern as total acidity, with lower values in autochthonous Austrian varieties and higher values in Cabernet-Sauvignon, which can be explained by the buffering effects of minerals. Volatile acidity was in the low-medium permitted range, with all values below 1.0 g/L and the highest values in Cabernet-Sauvignon. The SO_2 values were very low in Montenegrin wines (free SO_2 between 7 and 11 mg/L, total SO_2 between 18 and 32 mg/L) and moderate-low in Austrian wines (free SO_2 between 10 and 40 mg/L, total SO_2 between 22 and 76 mg/L), and all were within the legal framework of the EU wine law (Table 3).

The data for the examined Austrian wines showed a higher alcohol content and a lower acidity compared with earlier investigations (Eder *et al.*, 1992; Eder *et al.*, 2004), which can be explained by changes in winemaking style. However, the same parameters are in line with the results of Zoechling *et al.* (2009), indicating that these changes are recent. All the presented parameters for the chemical components of the Montenegrin wines agree with previous results (Pajović-Šćepanović *et al.*, 2016), except the SO_2 content, which was very low in the wines of the present study.

Table 3. Basic wine parameters (average of 10 locations) of Austrian and Montenegrin red wines from three vintages (2014-2016).

Country		Vintage	Rel. density 20/20	Ethanol vol %	Fructose g l^{-1}	Residual sugar g l^{-1}	Acidity g l^{-1}
Austria	CS	2014	0.9949	13.2^{ef}	0.2^{ij}	0.5^{f}	6.2^{a}
		2015	0.9950	14.3^{b}	1.5^{b}	1.9^{b}	5.1^{g}
		2016	0.9953	14.7^{a}	1.9^{a}	2.3^{a}	5.3^{efg}
		Mean	**0.9951^{A}**	**14.0^{A}**	**1.2^{A}**	**1.5^{A}**	**5.6^{A}**
	BLF	2014	0.9941	11.9^{i}	0.3^{ghi}	0.5^{f}	5.8^{b}
		2015	0.9935	13.1^{f}	0.5^{e}	1.4^{c}	5.4^{cde}
		2016	0.9938	12.7^{g}	0.6^{cd}	0.7^{f}	5.5^{c}
		Mean	**0.9938^{B}**	**12.6^{C}**	**0.4^{C}**	**0.8^{C}**	**5.5^{A}**
	ZW	2014	0.9937	11.2^{j}	0.1^{j}	0.1^{j}	5.5^{cd}
		2015	0.9924	13.6^{c}	0.5^{de}	0.3^{hi}	5.3^{ef}
		2016	0.9928	12.5^{h}	0.3^{fg}	0.3^{hi}	5.3^{fg}
		Mean	**0.9930^{C}**	**12.4^{D}**	**0.3^{E}**	**0.2^{D}**	**5.3^{C}**
Montenegro	CS	2014	0.9950	12.5^{gh}	0.4^{f}	0.5^{f}	5.5^{c}
		2015	0.9944	13.5^{cd}	0.7^{c}	1.1^{d}	5.5^{cd}
		2016	0.9942	13.4^{de}	0.5^{de}	1.2^{d}	5.3^{fg}
		Mean	**0.9949^{A}**	**13.1^{B}**	**0.5^{B}**	**0.9^{B}**	**5.4^{B}**
	VR	2014	0.9958	12.0^{i}	0.2^{hi}	0.4^{fg}	5.4^{def}
		2015	0.9938	14.1^{b}	0.7^{c}	1.3^{c}	5.3^{fg}

	2016	0.9942	13.2^{ef}	0.3^{fgi}	0.9^{e}	5.3^{ef}
	Mean	**0.9947^{A}**	**13.0^{B}**	**0.3^{D}**	**0.8^{C}**	**5.3^{C}**
Influence of vintage	2014	**B**	**C**	**C**	**C**	**A**
	2015	**A**	**A**	**A**	**A**	**B**
	2016	**B**	**B**	**B**	**B**	**B**

Different superscript lowercase letters in the same row indicate significantly different means ($P < 0.05$) for varieties from different vintages.
Different superscript uppercase letters indicate significantly different means ($P < 0.05$) for varieties based on the 3-year average.
Different uppercase letters indicate significantly different means ($P < 0.05$) between the examined vintages.
Abbreviations: BLF = Blaufraenkisch; CS = Cabernet-Sauvignon; VR = Vranac; ZW = Zweigelt.

2. 2. Spectrophotometric analysis of phenolic content

The results (average of 10 wines) of the four phenolic compound parameters (TP, the TPI, HMP and LMP) and TA are reported in Table 4. We found differences in mean TP content between the vintages ($p < 0.05$). All the examined phenolic parameters showed the lowest values in 2014. Except for TA, the highest phenolic parameter values occurred in 2015. TA was highest in 2016. TP and the TPI were not significantly different between 2015 and 2016.

Table 4. Contents of spectrophotometrically-determined phenolic groups and antioxidant activity (ABTS radical scavenging) in Austrian and Montenegrin red wines from three vintages (2014-2016).

Country	Variety	Vintage	TP	TPI	TA	HMP	LMP	Antioxidant activity (ABTS radical scavenging)
Austria	**Cabernet-Sauvignon (n = 10)**	2014	2545 ± 526^{b}	71.5 ± 12.8^{c}	594 ± 138^{e}	2941 ± 569^{d}	1311 ± 407^{d}	22.5 ± 5.3^{c}
		2015	2995 ± 664^{a}	75.5 ± 9.0^{b}	628 ± 122^{cd}	3274 ± 1022^{a}	1391 ± 330^{c}	24.5 ± 6.5^{b}
		2016	3017 ± 735^{a}	88.7 ± 24^{a}	608 ± 145de	3349 ± 1175^{a}	1453 ± 596^{b}	26.6 ± 6.6^{a}

		Mean	**2853 ± 665^{A}**	**78.6 ± 18^{A}**	**607 ± 137^{B}**	**3261 ± 1002^{A}**	**1384 ± 445^{A}**	**25.0 ± 6.2^{A}**
	Blaufraenkisch (n = 10)	2014	1631 ± 255^{e}	49.1 ± 8.0^{h}	412 ± 145^{i}	1754 ± 428^{h}	686 ± 200^{i}	12.9 ± 3.1^{g}
		2015	1795 ± 328^{e}	51.1 ± 7.1^{h}	483 ± 130^{gh}	1877 ± 711^{g}	831 ± 174^{h}	15.0 ± 3.1^{f}
		2016	1762 ± 475^{g}	52.9 ± 7.1^{gh}	461 ± 108^{gh}	1872 ± 1086^{g}	817 ± 511^{h}	15.7 ± 4.8^{f}
		Mean	**1740 ± 325^{E}**	**51.0 ± 9.3^{E}**	**452.2 ± 123^{D}**	**1834 ± 762^{D}**	**778 ± 326^{D}**	**14.5 ± 3.6^{D}**
	Zweigelt (n = 10)	2014	1354 ± 267^{h}	38.7 ± 6.0^{i}	472 ± 102^{gh}	1496 ± 484^{i}	494 ± 185^{j}	11.2 ± 1.9^{h}
		2015	1379 ± 321^{h}	42.5 ± 6.5^{j}	492 ± 126^{g}	1543 ± 372^{i}	518 ± 309^{j}	12.9 ± 2.1^{g}
		2016	1389 ± 177^{h}	41.8 ± 6.6^{j}	538 ± 160^{f}	1424 ± 259^{j}	515 ± 210^{j}	9.9 ± 2.2^{h}
		Mean	**1374 ± 278^{D}**	**41.0 ± 6.6^{D}**	**501 ± 131^{C}**	**1486 ± 373^{E}**	**508 ± 233^{E}**	**11.3 ± 2.4^{E}**
Montenegro	**Cabernet-Sauvignon (n = 10)**	2014	2301 ± 416^{c}	55.3 ± 10.3^{f}	623 ± 110^{cde}	2571 ± 635^{e}	1072 ± 244^{f}	16.4 ± 2.2^{e}
		2015	2316 ± 536^{c}	60.5 ± 14.6^{f}	633 ± 87^{c}	3211 ± 689^{b}	1548 ± 797^{a}	19.1 ± 4.2^{d}
		2016	2387 ± 950^{c}	54.6 ± 19.5^{fg}	749 ± 232^{b}	3010 ± 1055^{c}	1253 ± 654^{e}	18.0 ± 5.1^{d}
		Mean	**2335 ± 652^{B}**	**56.8 ± 15.1^{C}**	**668 ± 163^{A}**	**2930 ± 824^{B}**	**1303 ± 623^{B}**	**17.8 ± 4.8^{B}**
	Vranac (n = 10)	2014	1975 ± 134^{e}	51.6 ± 3.2^{h}	454 ± 100^{h}	1932 ± 383^{g}	881 ± 186^{g}	15.0 ± 0.9^{f}
		2015	2068 ± 321^{d}	65.8 ± 7.5^{d}	787 ± 108^{a}	2906 ± 367^{d}	1249 ± 332^{e}	18.0 ± 2.8^{d}
		2016	2017 ± 245de	59.5 ± 8.2^{f}	745 ± 117^{b}	2151 ± 415^{e}	816 ± 159^{h}	15.9 ± 2.5^{e}
		Mean	**2020 ± 244^{C}**	**58.9 ± 9.2^{B}**	**662 ± 183^{A}**	**2330 ± 561^{C}**	**982 ± 299^{C}**	**16.3 ± 2.7^{C}**
Influence of vintage		2014	**B**	**B**	**C**	**C**	**C**	**C**

	2015	**A**	**A**	**B**	**A**	**A**	**A**
(450 samples)							
	2016	**A**	**A**	**A**	**B**	**B**	**B**

The values represent the mean ± standard deviation (mg/L except for antioxidant activity, which is expressed as mmol Trolox/l).

Different superscript lowercase letters in the same row indicate significantly different means ($P < 0.05$) for varieties from different vintages.

Different superscript uppercase letters indicate significantly different means ($P < 0.05$) for varieties based on the 3-year average.

Different uppercase letters indicate significantly different means ($P < 0.05$) between the examined vintages.

Based on the two-way ANOVA, all the examined phenolic compound parameters were significantly different between the varieties ($p < 0.05$).

Considering the average of the three vintages, Austrian Cabernet-Sauvignon had the highest TP (2853 mg/L), followed by Montenegrin Cabernet-Sauvignon (2335 mg/L) and Vranac (2020 mg/L). Austrian autochthonous Blaufraenkisch and Zweigelt had considerably lower TP contents, specifically 1740 and 1374 mg/L respectively.

The TPI corresponded well with the TP content in the examined wines. Austrian Cabernet-Sauvignon showed the highest value (78.6 mg/L), followed by Montenegrin Vranac (58.9 mg/L) and Cabernet-Sauvignon (56.8 mg/L). Austrian Blaufraenkisch and Zweigelt had the lowest TPI (Table 4).

The TA content was similar in Montenegrin Cabernet-Sauvignon and Vranac (668 and 662 mg/L respectively) and Austrian Cabernet-Sauvignon (607 mg/L). Austrian Blaufraenkisch and Zweigelt had lower values (452 and 501 mg/L$^-$ respectively). These values (given as an average of three years), including the minimum and maximum values (430-1140 mg/L), are in good agreement with data reported by Aleixandre-Tudo *et al.* (2017).

Consistent with findings reported by Mattivi *et al.* (2002), HMP and LMP were the most prevalent phenolic compounds. Cabernet-Sauvignon from Austria (3261 mg/L for HMP and 1384 mg/L for LMP) and Montenegro (2930 mg/L for HMP and 1303 mg/L for LMP) had the highest contents and showed small but significant differences. Due to the observed values of these flavan-3-ol-based proanthocyanidins the examined samples can be classified as medium-to-long aged wines (Mattivi *et al.*, 2002). HMP and LMP of the other wines were lower: Vranac (2330 and 982 mg/L respectively), Blaufraenkisch (1834 and 778 mg/L respectively) and Zweigelt (1486 and 508 mg/L respectively); the differences between the varieties were significant.

The results for the Blaufraenkisch and Zweigelt wines are consistent with the study by Zoechling *et al.* (2009). Furthermore, the HMP and LMP values for Blaufraenkisch wines are consistent with Vrhovšek *et al.* (2002). The results for the Montenegrin wines are comparable with the results of previous studies, in which the authors used the same methods for for the

whole group of examined compounds (Pajovic *et al.*, 2014a; Pajović-Šćepanović *et al.*, 2019b) and for total phenols (Košmarel *et al.*, 2013; Šćepanović *et al.*, 2019).

It is interesting that the Cabernet-Sauvignon wines (especially the ones from Austria) showed the highest content of all examined phenolic compounds. Cabernet-Sauvignon is well known for producing wines with a high tannin content (Muñoz *et al.*, 2021). Therefore, this variety often expresses the highest polyphenolic content when comparing different red wine varieties (De Beer *et al.*, 2004; Kallithraka *et al.*, 2006; Pajovic *et al.*, 2014a).

3. 3. Phenolic acids

We investigated two classes of non-flavonoid phenolic acids: phenolic acids (hydroxycinnamic acid [HCA] derivatives: tartaric esters and free forms) and hydroxybenzoic acid derivatives (gallic acid and the alcohol-related substance tyrosol). Table 5 shows the results for the HCA content (averages of 10 wines). The most abundant HCA are the conjugated forms with tartaric acid (caftaric acid, trans-and cis-coutaric acid and fertaric acid), consistent with results published by Vrhovšek (1998).

We performed a two-way ANOVA to determine how variety and vintage influence the content of each HCA and the total HCA content in the wines. Regarding the variety, Austrian Cabernet-Sauvignon (total HCA: 165.4 mg/L) Blaufraenkisch (total HCA: 161.8 mg/L) exhibited the highest total HCA content in all the examined vintages ($P < 0.05$). These values and the values for Zweigelt (total HCA: 85.0 mg/L), presented as 3-year averages, are in good agreement with data reported for some Austrian red wines (37-170.0 mg/L) by Zoechling *et al.* (2009).

Table 5. Contents of hydroxycinnamic acid derivatives in Austrian and Montenegrin red wines from three vintages (2014-2016).

	Variety	Vintage	Caftaric acid	cis-Coutaric acid	trans-Coutaric acid	Fertaric acid	Caffeic acid
Austria	**CS**	2014	126.0 ± 55.5^{a}	5.9 ± 1.3^{c}	30.9 ± 12.6^{a}	4.2 ± 0.6^{a}	3.6 ± 0.8^{c}
		2015	123.3 ± 55.3^{a}	4.3 ± 1.0^{c}	29.1 ± 13.1^{a}	4.0 ± 0.7^{ab}	4.2 ± 0.6^{c}
		2016	107.6 ± 63.8^{a}	8.7 ± 2.6^{a}	28.2 ± 17.1^{a}	3.2 ± 1.1^{cd}	3.2 ± 1.2^{c}
		Mean	**119.0 ± 58.8^{A}**	**6.3 ± 2.5^{A}**	**29.4 ± 14.3^{A}**	**3.8 ± 0.9^{A}**	**3.7 ± 0.7^{B}**
	BLF	2014	127.0 ± 33.6^{a}	3.6 ± 0.8^{cd}	21.3 ± 5.3^{b}	4.0 ± 0.7^{a}	18.9 ± 15.2^{b}
		2015	76.9 ± 40.1^{b}	1.9 ± 0.9^{cde}	13.1 ± 7.5^{d}	3.3 ± 1.0^{c}	24.1 ± 16.0^{b}
		2016	117.0 ± 54.8^{ab}	4.5 ± 2.0^{c}	17.7 ± 8.9^{bcd}	3.5 ± 1.1^{bc}	31.8 ± 16.5^{a}
		Mean	**107.0 ± 48.4^{B}**	**3.4 ± 1.7^{c}**	**17.2 ± 8.0^{bc}**	**3.7 ± 1.0^{A}**	**24.9 ± 16.7^{A}**
	ZW	2014	71.8 ± 14.6^{b}	2.6 ± 0.4^{ef}	14.1 ± 3.2^{cd}	2.2 ± 0.3^{e}	20.8 ± 7.6^{b}
		2015	27.9 ± 28.8^{d}	0.9 ± 0.5^{i}	5.5 ± 5.6^{e}	1.6 ± 0.6^{f}	20.5 ± 5.2^{b}
		2016	35.6 ± 26.6^{cd}	1.9 ± 0.7^{f}	6.0 ± 4.0^{e}	1.2 ± 0.5^{f}	25.2 ± 12.3^{ab}
		Mean	**45.1 ± 30.1^{D}**	**1.8 ± 0.9^{D}**	**8.5 ± 5.9^{D}**	**1.6 ± 0.6^{D}**	**22.1 ± 9.0^{A}**
Montenegro	**CS**	2014	53.7 ± 10.5^{bcd}	3.6 ± 0.6^{cde}	13.7 ± 2.6^{cd}	2.6 ± 0.7^{de}	2.5 ± 0.5^{c}

		2015	57.6 ± 8.4^{bcd}	4.0 ± 0.4^{c}	15.0 ± 2.5^{bcd}	3.2 ± 0.7^{cd}	2.8 ± 0.8^{c}
		2016	62.0 ± 7.0^{bc}	4.0 ± 0.5^{c}	16.3 ± 2.5^{bcd}	3.0 ± 0.5^{cd}	2.5 ± 0.6^{c}
		Mean	**57.8 ± 9.3^{C}**	**3.8 ± 0.5^{B}**	**15.0 ± 2.6^{C}**	**2.9 ± 0.6^{C}**	**2.6 ± 0.6^{B}**
	VR	2014	62.3 ± 8.9^{bc}	3.7 ± 1.0^{cd}	18.4 ± 2.3^{bcd}	3.5 ± 0.5^{bc}	2.8 ± 0.6^{c}
		2015	64.6 ± 12.8^{bc}	2.9 ± 1.1^{de}	20.5 ± 4.5^{bcd}	3.5 ± 0.5^{bc}	4.3 ± 1.7^{c}
		2016	61.6 ± 8.2^{bc}	4.3 ± 0.5^{c}	18.5 ± 3.5^{bcd}	3.0 ± 0.3^{cd}	3.7 ± 1.8^{c}
		Mean	**62.8 ± 10.2^{C}**	**3.6 ± 1.6^{BC}**	**19.1 ± 3.6^{B}**	**3.3 ± 0.5^{B}**	**3.6 ± 1.6^{B}**
Influence of vintage		2014	**A**	**B**	**A**	**A**	**B**
		2015	**B**	**C**	**B**	**B**	**AB**
		2016	**AB**	**A**	**AB**	**B**	**A**

The values represent the mean ± standard deviation (mg l^{-1}).
Different superscript lowercase letters in the same row indicate significantly different means ($P < 0.05$) for varieties from different vintages.
Different superscript uppercase letters indicate significantly different means ($P < 0.05$) for varieties based on the 3-year average.
Different uppercase letters indicate significantly different means ($P < 0.05$) between the examined vintages.
Abbreviations: BLF = Blaufraenkisch; CS = Cabernet-Sauvignon; VR = Vranac; ZW = Zweigelt

Due to the notable differences in the absolute HCA contents between the varieties, our subsequent discussion refers to percentages of HCA (Mattivi and Nicolini, 1997). Austrian autochthonous Blaufraenkisch and Zweigelt contained the highest relative percentage of caftaric acid (81.1 % and 78.1 % respectively), and a relatively high percentage of caffeic acid. Montenegrin Cabernet-Sauvignon and Vranac contained a lower total HCA content (85.0 and 96.8 mg/L respectively), a higher percentage of fertaric acid and total HCA, which is in line with previous finding for Montenegrin red wines (Pajović- Šćepanović *et al.*, 2018, Pajović- Šćepanović *et al.*, 2019b).

Cabernet-Sauvignon from both countries showed a similar HCA profile: the relative caftaric acid, coutaric acid and fertaric acid contents were 72.9 %, 23.7 % and 3.7 % for Austria, and 71.0 %, 24.0 % and 4.9 % for Montenegro. The trans-coutaric acid/trans-caftaric acid ratio, which is also described as a varietal characteristic (Singleton *et al.*, 1986), was 0.248 for Austrian Cabernet-Sauvignon and 0.251 for Montenegrin Cabernet-Sauvignon, confirming their varietal authenticity. The differences in the absolute amount of HCA compounds between Austrian and Montenegrin Cabernet-Sauvignon can be explained by different climatic conditions in the wine-growing regions and winemaking practices.

We noted the influence of vintage based on the differences in tartaric esters of HCA, with the highest total content in 2014, whereas the highest free HCA content was in 2016 ($P < 0.05$). However, we only noted these differences in Austrian wines; there were no significant differences in Montenegrin wines.

HCA derivatives showed the highest values in 2014 and the lowest values in 2015 ($P < 0.05$) (Table 6). The gallic acid content (given as a 3-year average) ranged from 26.9 to 58.4 mg/L, indicating that gallic acid was the most prevalent HCA derivative in the examined varieties. This finding is in good agreement with findings of Chira *et al.* (2009). The gallic acid content was 37.2 and 26.9 mg/L in Austrian autochthonous Blaufraenkisch and Zweigelt wines respectively, which is in good agreement with values obtained for the same varieties by Zoechling *et al.* (2009). The gallic acid content in Vranac wines (55.5 mg/L) is consistent with a previous study (Pajović-Šćepanović *et al.*, 2019b).

Cabernet-Sauvignon from Austria and Montenegro showed different gallic acid contents: 58.4 and 42.4 mg/L respectively. Both values are consistent with Cabernet-Sauvignon produced in Brazil, Argentina and Chile (37.92-62.71 mg/L; Granato *et al.*, 2011). The differences in gallic acid among the examined varieties could also be caused by specific winemaking technologies, including the addition of oenological hydrolysable tannins (Ivanova-Petropulos *et al.*, 2015).

The tyrosol contents were very similar in all wines (around 30 mg/L) and showed little difference between regions and varieties.

4. 4. Stilbenes

Four stilbenes were determined in the present study: cis- and trans-resveratrol and the two corresponding glucoside forms (cis- and trans-piceid) (Table 6). The trans forms of stilbenes were dominant in most of the analysed wines, representing 34 %-66.8 % of total stilbenes - a result we expected based on the literature (Burns *et al.*, 2000; Ivanova-Petropulos *et al.*, 2015; Monagas *et al.*, 2005; Pajović-Šćepanović *et al.*, 2018).

Table 6. Contents of stilbenes and hydroxybenzoic acids in Austrian and Montenegrin red wines from three vintages (2014-2016).

Country	Variety	Vintage	Stilbenes					Hydroxybenzoic acid derivatives	
			trans-Resveratrol glucosides	trans-Resveratrol	cis-Resveratrol glucosides	cis-Resveratrol	Total stilbenes	Gallic acid	Tyrosol
Austria	CS	2014	5.5 ± 2.3^{a}	4.0 ± 1.3^{a}	3.1 ± 1.2^{cd}	1.3 ± 0.5^{cd}	14.0 ± 4.5^{bc}	68.4 ± 17.8^{a}	30.9 ± 9.6^{cdef}
		2015	3.0 ± 1.2^{cd}	3.4 ± 1.5^{abc}	3.1 ± 1.4^{cd}	1.8 ± 1.0^{bc}	11.3 ± 3.3^{cd}	61.4 ± 12.9^{ab}	22.6 ± 6.9^{fg}

		2016	4.1 ± 1.9^{abc}	2.6 ± 1.6^{c}	6.1 ± 2.6^{b}	2.3 ± 2.2^{b}	15.0 ± 6.3^{ab}	45.4 ± 13.5^{bcd}	39.8 ± 12.3^{ab}
		Mean	**4.2 ± 2.1^{A}**	**3.3 ± 1.6^{A}**	**4.1 ± 2.3^{B}**	**1.8 ± 1.4^{B}**	**13.5 ± 5.0^{B}**	**58.4 ± 17.6^{A}**	**31.1 ± 12.0^{A}**
		2014	5.2 ± 2.7^{ab}	3.8 ± 1.4^{ab}	3.4 ± 1.5^{c}	2.1 ± 0.9^{bc}	14.6 ± 5.5^{bc}	37.7 ± 13.3^{de}	31.0 ± 8.4^{cde}
		2015	2.8 ± 1.9^{cd}	3.2 ± 1.1^{abc}	3.3 ± 1.4^{cd}	2.1 ± 1.1^{bc}	11.5 ± 4.5^{cd}	37.6 ± 10.2^{de}	31.1 ± 5.5^{cde}
	BLF	2016	5.5 ± 3.3^{a}	3.0 ± 1.5^{bc}	7.7 ± 2.9^{a}	3.3 ± 2.0^{a}	19.5 ± 7.3^{a}	36.3 ± 20.9^{de}	38.3 ± 10.1^{abc}
		Mean	**4.5 ± 2.9^{A}**	**3.3 ± 1.4^{A}**	**4.8 ± 2.9^{A}**	**2.5 ± 1.5^{A}**	**15.2 ± 6.7^{A}**	**37.2 ± 15.3^{B}**	**33.5 ± 8.8^{A}**
		2014	2.1 ± 1.2^{de}	2.9 ± 1.2^{bc}	2.2 ± 1.3^{cdef}	1.3 ± 0.7^{cd}	8.5 ± 3.8^{de}	25.2 ± 8.3^{e}	31.7 ± 10.7^{bcde}
		2015	0.5 ± 0.3^{i}	1.1 ± 0.4^{de}	1.5 ± 0.4^{cde}	0.8 ± 0.4^{de}	3.9 ± 0.9^{fgh}	23.6 ± 9.2^{e}	34.2 ± 6.5^{abcd}
	ZW	2016	1.1 ± 1.7^{ei}	1.3 ± 1.0^{d}	2.8 ± 1.8^{cde}	1.9 ± 1.8^{bc}	7.1 ± 5.0^{efg}	31.9 ± 14.0^{de}	33.9 ± 10.4^{abcd}
		Mean	**1.1 ± 1.0^{C}**	**1.7 ± 1.2^{B}**	**2.1 ± 1.1^{C}**	**1.3 ± 1.2^{C}**	**6.4 ± 3.4^{C}**	**26.9 ± 11.3^{C}**	**33.3 ± 9.3^{A}**
Montenegro	**CS**	2014	0.6 ± 0.7^{i}	0.5 ± 0.3^{de}	0.6 ± 0.5^{g}	0.4 ± 0.3^{e}	2.0 ± 1.6^{h}	42.8 ± 11.5^{cd}	27.3 ± 8.4^{defg}
		2015	0.3 ± 0.2^{i}	0.2 ± 0.1^{e}	0.3 ± 0.2^{g}	0.1 ± 0.1^{e}	0.8 ± 0.5^{h}	38.8 ± 23.3^{cde}	34.9 ± 14.9^{abcd}

	2016	0.9 ± 0.8^{ei}	0.8 ± 0.9^{de}	1.1 ± 1.2^{fg}	0.6 ± 0.9^{de}	3.5 ± 3.1^{gh}	45.6 ± 27.7^{bcd}	41.2 ± 14.6^{a}
	Mean	**0.7 ± 0.9^{C}**	**0.5 ± 0.6^{C}**	**0.6 ± 0.7^{D}**	**0.4 ± 0.6^{D}**	**2.1 ± 2.3^{D}**	**42.4 ± 21.8^{B}**	**34.5 ± 14.1^{A}**
VR	2014	2.9 ± 0.8^{cd}	1.4 ± 0.9^{d}	2.1 ± 0.7^{def}	0.6 ± 0.4^{de}	7.0 ± 1.9^{ef}	63.9 ± 25.3^{a}	20.1 ± 5.4^{g}
	2015	1.6 ± 0.7^{dei}	0.5 ± 0.3^{de}	1.1 ± 0.6^{fg}	0.1 ± 0.0^{de}	3.3 ± 1.2^{gh}	46.3 ± 17.8^{bcd}	23.6 ± 6.9^{efg}
	2016	3.8 ± 1.7^{bc}	0.8 ± 0.4^{de}	3.2 ± 1.5^{cd}	0.3 ± 0.2^{e}	8.2 ± 3.4^{de}	56.2 ± 43.3^{abc}	29.4 ± 9.0^{def}
	Mean	**2.8 ± 1.5^{B}**	**0.9 ± 0.7^{C}**	**2.1 ± 1.3^{C}**	**0.3 ± 0.3^{D}**	**6.2 ± 3.1^{C}**	**55.5 ± 31.3^{A}**	**24.4 ± 8.1^{B}**
Influence of vintage	2014	**A**	**A**	**B**	**B**	**B**	**A**	**A**
	2015	**B**	**B**	**C**	**B**	**C**	**B**	**B**
	2016	**A**	**B**	**A**	**A**	**A**	**AB**	**AB**

The values represent the mean ± standard deviation (mg l^{-1}).

Different superscript lowercase letters in the same row indicate significantly different means ($P < 0.05$) for varieties from different vintages.

Different superscript uppercase letters indicate significantly different means ($P < 0.05$) for varieties based on the 3-year average.

Different uppercase letters indicate significantly different means ($P < 0.05$) between the examined vintages.

Abbreviations: BLF = Blaufraenkisch; CS = Cabernet-Sauvignon; VR = Vranac; ZW = Zweigelt

It is known that the biosynthesis of stilbenes is affected by microbiological and/or abiotic stress, and that grape variety, growing region (altitude), weather during ripening, intensity of fungal attack and cultural techniques have a strong impact on the content of stilbenes (Bavaresco *et al.*, 2003; Bavaresco *et al.*, 2007; Eder *et al.*, 2001). Based on the two-way ANOVA, variety and vintage had a significant effect ($P < 0.05$) on the content of stilbenes.

Blaufraenkisch showed the highest total stilbene content in each vintage (15.2 mg/L on average), followed by Austrian Cabernet-Sauvignon (13.5 mg/L). Vranac showed a similar total stilbene content (6.4 and 6.2 mg/L respectively). Finally, Montenegrin Cabernet-Sauvignon had the lowest content (2.1 mg/L).

The data for Austrian autochthonous Blaufraenkisch and Zweigelt wines correspond well with data presented by Eder *et al.* (2001): in their 6-year study, Blaufraenkisch had the highest content of trans-stilbene (7.6 mg/L on average), but for some vintages (1998 and 1995) there were much higher values (14.1 and 13.4 mg/L respectively). The value for Zweigelt wines (5.5 mg/L) in the present study align with the values published in that study, as well as with data presented by Zoechling *et al.* (2009) for the 2004 and 2005 vintages (2.02 and 3.17 mg/L respectively) of Zweigelt and Blaufraenkisch (10.27 mg/L).

The mean total content of trans-forms of resveratrol for Vranac wines was 3.7 mg/L, which is in agreement with the values (3.85 and 3.1 mg/L respectively) obtained by Raičević *et al.* (2020) for the trans-forms of resveratrol in the 2008 and 2009 vintages of Vranac wines in the Podgorica sub-region. In addition, Radović *et al.* (2015) reported mean total stilbene contents of 2.31-3.11 mg/L for the 2010-2012 vintages of the same wine variety, which aligns well with the values obtained in this study (Vranac: 6.2 mg/L and Cabernet-Sauvignon: 2.1 mg/L) in the same wine region. Our results are very similar to those of Pajović *et al.* (2018), who reported values of 2.55 and 1.15 mg/L for the 2015 vintage of Vranac and Cabernet-Sauvignon wines respectively, which were produced under the same conditions with grapes from different locations in Montenegro. The values for stilbenes in wines from Montenegro are in line with those from some Mediterranean countries, such as Spain (Monagas *et al.*, 2005) and Greece (Kallithraka *et al.*, 2006).

Montenegrin Cabernet-Sauvignon had much lower stilbene concentrations compared with Austrian Cabernet-Sauvignon. This difference can be explained by the influence of the wine-growing region and climatic conditions, especially humidity. Our data correspond very well with a previous study (Pajović-Šćepanović *et al.*, 2018). The results of our study confirm the results of Goldberg *et al.* (1995) and Goldberg *et al.* (1996), who showed that Cabernet-Sauvignon from Mediterranean countries (Italy, Spain and Greece) and new world wine regions (California, Australia and South Africa) had lower stilbene contents compared with wines from Burgundy, Switzerland and Bordeaux.

The statistical analysis of the results showed a significant influence of vintage on stilbene content ($P < 0.05$). All the examined varieties showed the lowest stilbene content for the 2015 vintage and the highest value for the 2016 vintage. This can be explained by the meteorological conditions as presented by Pajović-Šćepanović *et al.* (2019b) and shown in Table 1: 2015 had a higher temperature and lower humidity compared with 2016. A high temperature during the ripening period is negatively linked to the quantity of resveratrol in grapes and wines (Bavaresco *et al.*, 2007)

5. 5. Flavan-3-ols

The flavanol monomers (catechin and epicatechin) and procyanidin dimers (B_1 and B_2) were identified and quantified (Table 7). We found that neither variety nor vintage had a significant influence on the content of flavan-3-ols in the examined wines. Nevertheless, based on the

content of flavan-3-ols, we were able to divide the four varieties into two groups. On the other hand, we noted significant differences in the content of flavan-3-ols (except for procyanidin B_2) between the vintages. We did not expect this result based on the literature (Chira *et al.*, 2009; Gris *et al.*, 2011). In a 10-year study (Mattivi *et al.*, 2002), the authors found the composition of flavan-3-ols to vary significantly depending on grape variety and vintage, and to also be influenced by environmental conditions.

Table 7. Contents of flavan-3-ols in Austrian and Montenegrin red wines from three vintages (2014-2016).

Country	Variety	Vintage	Catechin	Epicatechin	Procyanidin B1	Procyanidin B2	Total flavan-3-ols
Austria	CS	2014	84.5 ± 25.2^{a}	42.0 ± 17.9^{abc}	69.4 ± 19.7^{a}	47.6 ± 17.2^{a}	243.5 ± 78.0^{a}
		2015	69.4 ± 14.2^{ab}	43.8 ± 12.4^{ab}	44.1 ± 16.0^{cdef}	39.1 ± 10.8^{abc}	196.4 ± 44.5^{abcde}
		2016	87.1 ± 31.9^{a}	47.4 ± 20.4^{a}	54.9 ± 15.3^{abc}	40.5 ± 9.6^{ab}	229.9 ± 74.1^{ab}
		Mean	**80.3 ± 25.8^{A}**	**44.4 ± 17.2^{A}**	**56.1** ± 19.8^{A}	**42.4 ± 13.4^{A}**	**223.3 ± 69.3^{A}**
	BLF	2014	63.7 ± 18.7^{bc}	42.1 ± 19.1^{abc}	56.8 ± 9.0^{abc}	37.8 ± 13.8^{abcd}	200.4 ± 57.9^{abcd}
		2015	69.8 ± 22.7^{ab}	49.5 ± 22.8^{a}	52.5 ± 8.3^{bcd}	45.7 ± 18.3^{a}	217.5 ± 69.9^{ab}
		2016	58.4 ± 37.7^{bcd}	44.9 ± 39.8^{ab}	59.6 ± 22.1^{ab}	48.7 ± 32.0^{a}	211.6 ± 128.6^{abc}
		Mean	**64.0 ± 27.7^{B}**	**45.5 ± 28.6^{A}**	**56.3 ± 17.7^{A}**	**44.1 ± 22.9^{A}**	**209.8 ± 90.1^{A}**
	ZW	2014	41.3 ± 16.1^{def}	30.4 ± 18.9^{cdef}	28.4 ± 11.0^{gh}	24.2 ± 15.3^{de}	124.3 ± 53.4^{f}
		2015	38.2 ± 16.0^{ef}	38.4 ± 26.1^{abcd}	25.9 ± 12.4^{h}	34.4 ± 24.5^{abcde}	136.9 ± 78.2^{ef}
		2016	43.8 ± 39.5^{cdef}	36.9 ± 24.6^{abcd}	34.7 ± 14.6^{efgh}	35.6 ± 17.7^{abcde}	151.0 ± 87.5^{cdef}
		Mean	**41.1 ± 26.1^{C}**	**35.2 ± 23.4^{B}**	**29.7 ± 13.2^{C}**	**31.4 ± 20.0^{C}**	**137.4 ± 74.4^{B}**
Montenegro	**CS**	2014	44.9 ± 13.2^{cdef}	16.9 ± 10.3^{f}	42.1 ± 11.3^{cdefg}	21.5 ± 9.9^{e}	125.4 ± 42.3^{f}
		2015	47.3 ± 23.6^{cdef}	21.8 ± 12.5^{ef}	43.0 ± 26.0^{cdefg}	22.3 ± 17.6^{e}	134.3 ± 78.7^{f}

	2016	50.2 ± 21.4^{bcdef}	19.7 ± 11.7^{c}	46.6 ± 26.7^{bcde}	29.8 ± 14.5^{bcde}	146.3 ± 69.2^{def}
	Mean	**47.5 ± 19.8^{C}**	**19.4 ± 11.6^{C}**	**43.9 ± 21.5^{B}**	**24.5 ± 14.7^{C}**	**135.3 ± 65.1^{B}**
VR	2014	52.9 ± 8.8^{bcde}	26.8 ± 7.0^{def}	49.8 ± 12.9^{bcd}	30.2 ± 8.7^{bcde}	159.8 ± 36.1^{bcdef}
	2015	47.8 ± 10.9^{cdef}	19.5 ± 8.4^{def}	38.5 ± 18.0^{defgh}	25.3 ± 12.6^{cde}	128.2 ± 47.1^{f}
	2016	32.3 ± 18.4^{f}	14.1 ± 10.4^{c}	31.5 ± 17.7^{fgh}	23.2 ± 12.6^{de}	101.1 ± 62.6^{f}
	Mean	**43.1 ± 15.7^{C}**	**20.1 ± 10.1^{C}**	**39.9 ± 17.9^{B}**	**26.2 ± 13.3^{C}**	**129.7 ± 54.8^{B}**
Influence of vintage	2014					**A**
	2015					**B**
	2016					**A**

The values represent the mean ± standard deviation (mg l^{-1}).
Different superscript lowercase letters in the same row indicate significantly different means ($P < 0.05$) for varieties from different vintages.
Different superscript uppercase letters indicate significantly different means ($P < 0.05$) for varieties based on the 3-year average.
Different uppercase letters indicate significantly different means ($P < 0.05$) between the examined vintages.
Abbreviations: BLF = Blaufraenkisch; CS = Cabernet-Sauvignon; VR = Vranac; ZW = Zweigelt

The total flavan-3-ol content was highest in Austrian Cabernet-Sauvignon (average: 223.3 mg/L) and Blaufraenkisch (average: 209 mg/L). There were lower contents in Zweigelt (average: 137.4 mg/L), Vranac (average: 129.7 mg/L) and Montenegrin Cabernet-Sauvignon (average: 135.3 mg/L), with no significant differences between the varieties (Table 7). We found that the flavan-3-ol content was the highest among all the phenolic compounds analysed in the Austrian and Montenegrin wines. Thus, our data confirm the statement by Kennedy and Waterhouse (2000) that flavan-3-ols are the most abundant class of phenols in wines.

Austrian Cabernet-Sauvignon and Blaufraenkisch showed higher flavan-3-ol monomer concentrations than procyanidin dimer concentrations, with more (+)-catechin than

epicatechin and more procyanidin B_1 than procyanidin B_2. These differences were smaller for Zweigelt, which showed a similar level of both monomers and both dimers. Zoechling *et al.* (2009) reported moderate flavan-3-ol concentrations in Austrian Zweigelt and Blaufraenkisch. Comparing the two Austrian autochthonous varieties, it is remarkable that Blaufraenkisch contains considerably more (+)-catechin and epicatechin (64.0 and 45.5 mg/L respectively) than Zweigelt (41.0 and 35.2 mg/L respectively), which is reflected by Blaufraenkisch having a more bitter and harsher taste than Zweigelt wines. The same tendency can be observed for the bitter-tasting procyanidins, which certainly contribute to the mentioned sensorial difference between the two varieties.

Wines produced in Montenegro had similar contents of flavan-3-ols and the percentage distribution between the monomers and dimers was also balanced. In terms of monomers, the concentration of (+)-catechin was higher than epicatechin, and procyanidin B_1 was the main dimer. A previous study of Montenegrin wines showed lower contents of flavanol-3-ols due to the traditional production technology, but a similar distribution of monomers and dimers (Pajović-Šćepanović *et al.*, 2018). This composition of flavan-3-ols is linked to their composition in grapes, in which (+)-catechin has been found to be the main monomer in skin and procyanidin B_1 the main dimer in the seed (Pajović-Šćepanović *et al.*, 2019a).

Cabernet-Sauvignon produced in both regions showed considerable differences in the total flavan-3-ol and individual flavan-3-ol composition. The differences in absolute values can be explained by the different wine regions (climate and soil, etc.) as well as the wine processing technology. However, independently of the production area, (+)-catechin was the most abundant monomer and procyanidin B_1 was the dominant dimer.

6. 6. Antioxidant properties of the studied wines

We evaluated the antioxidant activity of the Austrian and Montenegrin red wines based on the capacity to scavenge ABTS radicals. The results of the antioxidative activity are shown in Table 2; the range is 11.3-25.0 mmol Trolox/l. The two-way ANOVA revealed that both variety and vintage significantly influenced the antioxidant properties of the examined wines.

Regarding the influence of variety, all examined wines showed significant differences ($P < 0.05$). Blaufraenkisch and Zweigelt showed 14.5 and 11.3 mmol Trolox/l, while the value for Vranac wines was 16.3 mmol Trolox/l. Austrian Cabernet-Sauvignon showed the highest value (average: 25.0 mmol Trolox/l), followed by Montenegrin Cabernet-Sauvignon (average: 17.8 mmol Trolox/l). According to Fernández-Pachón *et al.* (2004), the examined wines have high antioxidant activity, because they have values above 11 mmol Trolox/l. The results are comparable to studies that used the same methodology to estimate antioxidant activity. For example, Pajović-Šćepanović *et al.* (2019b) cited antioxidant capacity between 15.3 and 22.8 mmol Trolox/l, and Baiano *et al.* (2009) reported values of 7.9-24.2 mmol Trolox/l.

The 2015 vintage had the highest antioxidant activity and the 2014 vintage had the lowest ($P < 0.05$). These results correspond with the concentrations of the phenolic groups determined spectrophotometrically.

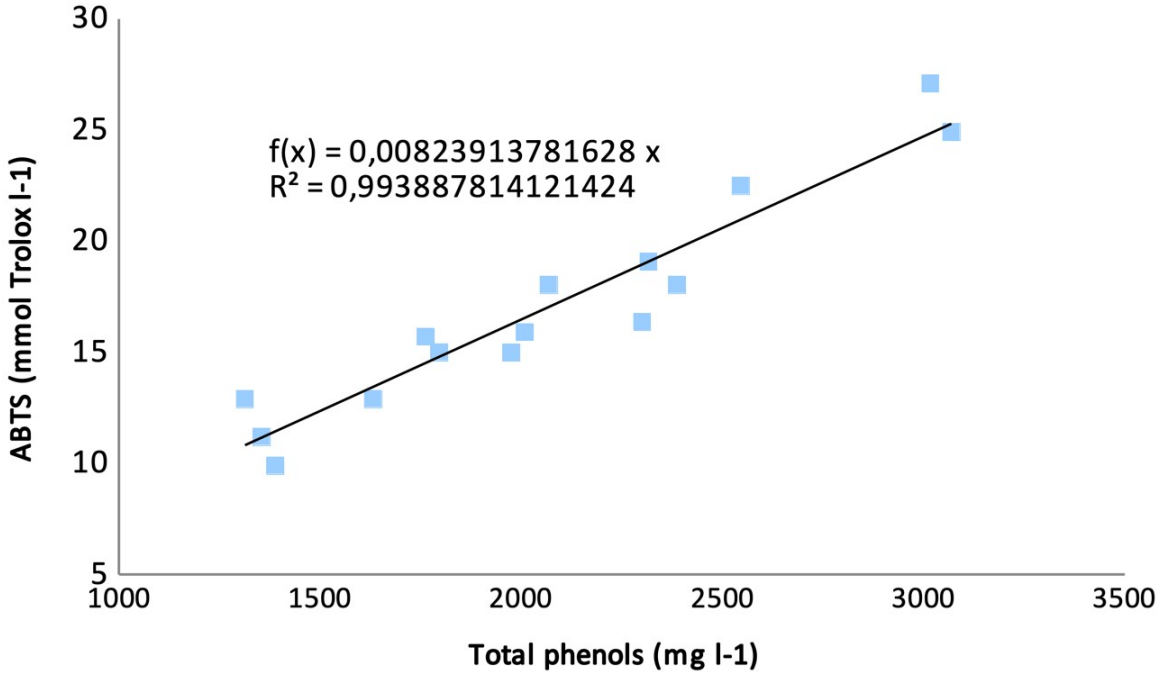

Figure 1. Correlation between total phenols and antioxidant activity of the examined Austrian and Montenegrin red wines.

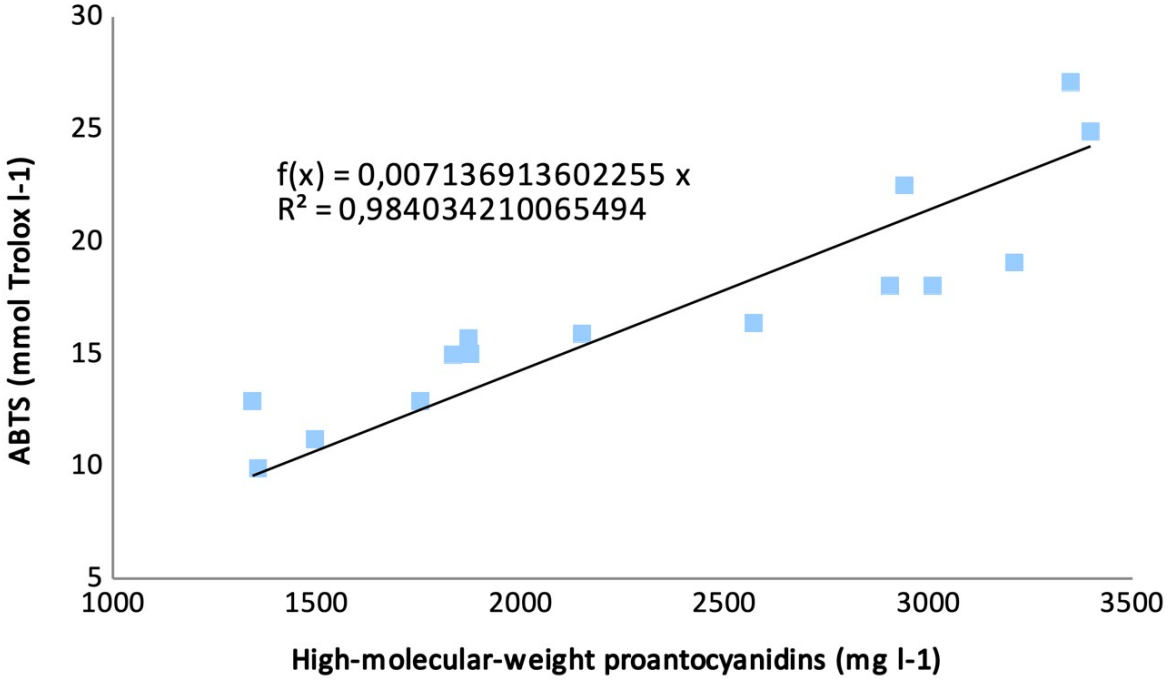

Figure 2. Correlation between high-molecular-weight proanthocyanidins and antioxidant activity of the examined Austrian and Montenegrin red wines.

7. 7. Correlation between antioxidant capacity and phenolic substances

We performed a correlation analysis to determine which phenolic groups or individual phenolic compounds have similar behaviour to the ABTS radical scavenging capacity, and therefore would have an effect on the antioxidant properties of the analysed red wines. We

noted a high correlation between TP and the TPI and ABTS scavenging capacity (r^2 = 0.914 [Figure 1] and r^2 = 0.9321 respectively). This finding is consistent with the literature (Frankel *et al.*, 1995; Mattivi *et al.*, 2002). We also found a strong correlation between HMP and LMP and ABTS scavenging capacity (r^2 = 0.775 [Figure 2] and r^2 = 0.6041 respectively). Previous studies have shown that the antioxidant potential of red wines is mainly related to their flavan-3-ol and proanthocyanidin contents (Gris *et al.*, 2011; Rigo *et al.*, 2000). In a study using similar spectrophotometric methods, Rigo *et al.* (2000) reported a high correlation between LMP and TP and scavenging capacity. Although spectrophotometrically determined proanthocyanidins have antioxidant activity, we found no significant correlation between individual flavan-3-ols or other phenolic compounds and ABTS radical scavenging capacity.

8. 8. Differentiation of wines based on the phenolic compound composition

We performed PCA to explore the ability of the phenolic compound composition to differentiate the examined wines based on wine variety, vintage or region. The PCA provided good discrimination of wines according to variety and region, but there was no significant separation by vintage.

We evaluated the influence of wine regions based on the differences of phenolic composition of Austrian and Montenegrin Cabernet-Sauvignon. These varieties showed similar chemical and phenolic compositions based on spectrophotometric measurements and a similar HCA profile; therefore, they were located close together on the PCA scatterplot. However, Austrian Cabernet-Sauvignon contained much more phenolic acids and flavan-3-ols compared with Montenegrin Cabernet-Sauvignon. The biggest difference was in the stilbene content, which was much lower in Montenegrin Cabernet-Sauvignon due to the much warmer climate in Montenegro.

The projection of the first two principal component axes (PC1 and PC2, explaining 76.37 % of the total variability) enabled good separation according to wine variety and region (country of origin). Thus, the Austrian autochthonous wines are separated from the other wines; they are located in the positive region of PC2. Austrian Cabernet-Sauvignon is located in the positive region of PC1 (Figure 3) together with Blaufraenkisch. Factor 1 separates these wines from the others due to the higher content of almost all examined phenolic groups (e.g., stilbenes, most HCA and flavan-3-ols; Figure 4). Zweigelt wines are located in the negative region of PC1 and the positive region of PC2, based on the lower content of almost all compounds, except caffeic acid and *p*-coumaric acid.

The Montenegrin wines (Vranac and Cabernet-Sauvignon) are located in the negative region of both PC1 and PC2 (Figure 3). These wines are characterised by high TA and ferulic acid contents and a high trans-coutaric acid/trans-caftaric acid ratio. The proximity of these wines (especially Cabernet-Sauvignon) to the Austrian Cabernet-Sauvignon (negative region of PC2) comes from the high TP, TPI, HMP and LMP contents (Figure 4). The PCA proved that the examined wines can be separated according to wine variety and region. Although there were significant differences for most of the examined phenolic compounds between the vintages, PCA did not show separation according to vintage. This result, however, is very similar to results in the literature (Gris *et al.*, 2011).

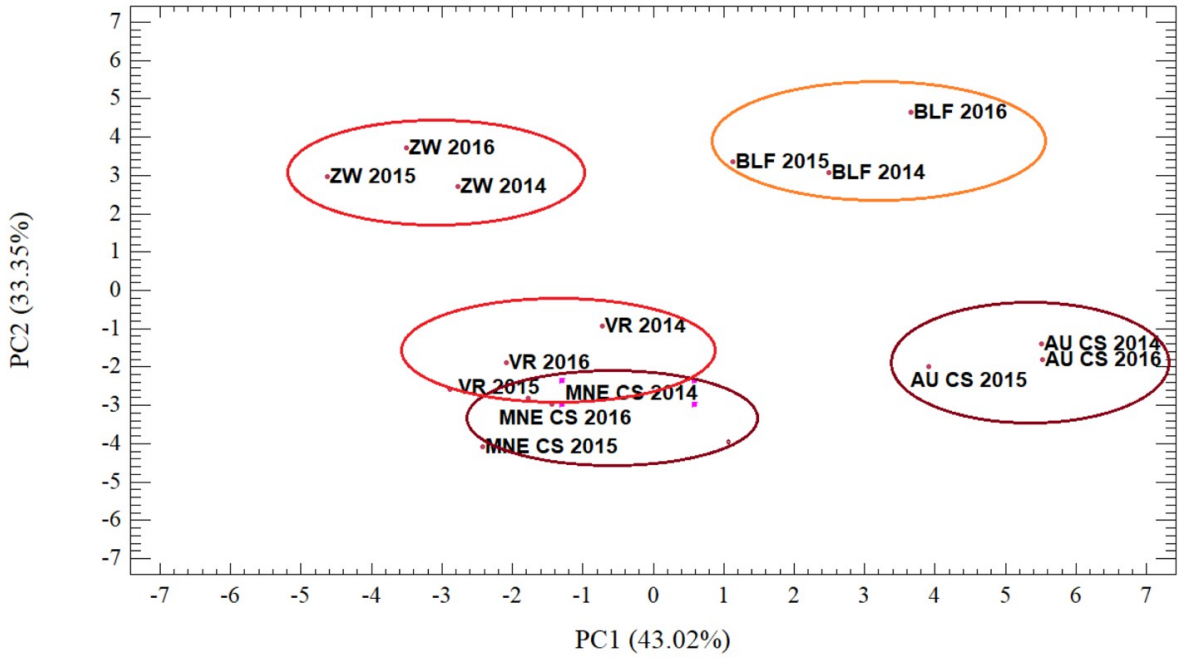

Figure 3. Principal component analysis scatterplot of principal components 1 and 2 (PC1 and PC2, respectively) for the analysed red wines from Austria (AU CS = Cabernet-Sauvignon; BLF = Blaufraenkisch; ZW = Zweigelt) and Montenegro (MNE CS = Cabernet-Sauvignon; VR = Vranac).

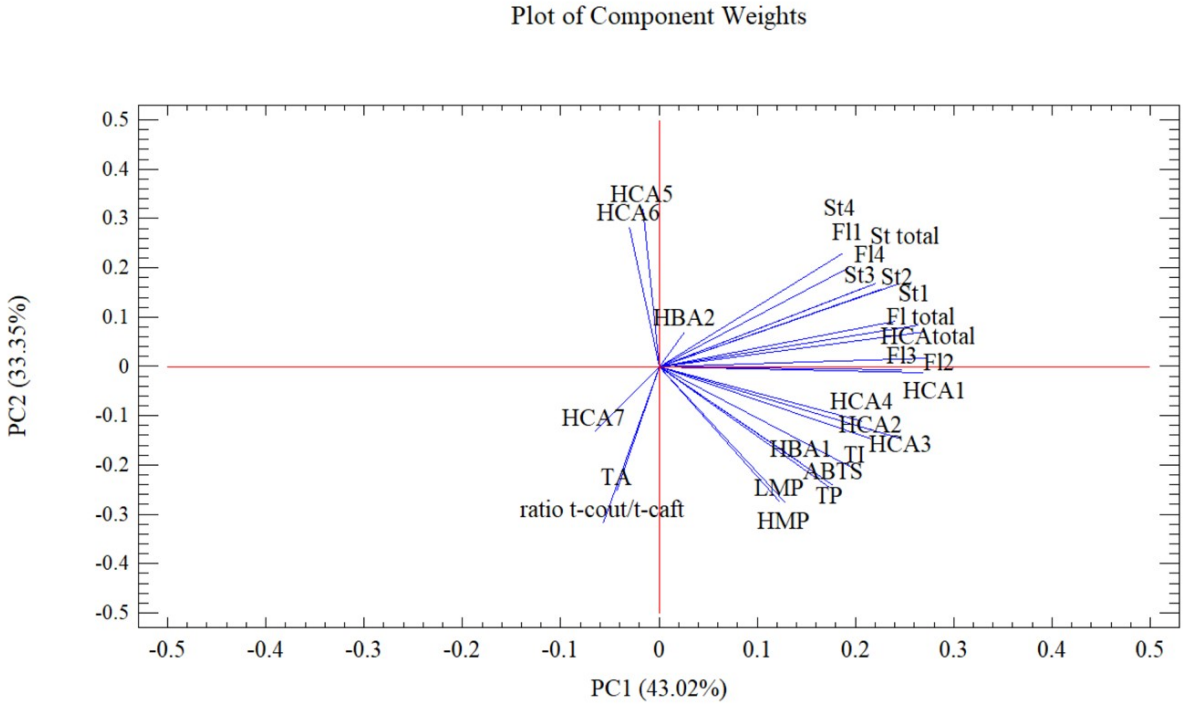

Figure 4. Principal component analysis scatterplot of component weights: hydroxycinnamic acid (HCA) derivatives: 1 – caftaric acid, 2 – cis-coutaric acid, 3 – trans-coutaric acid, 4 – fertaric acid, 5 – caffeic acid, 6 – *p*-coumaric acid, 7 – ferulic acid, HCA total, ratio – trans-coutaric acid/trans-caftaric acid ratio; hydroxybenzoic acid (HBA) derivatives: 1 – gallic acid, 2 – tyrosol; stilbenes (St): 1 – trans-resveratrol glucosides, 2 – trans-resveratrol, 3 – cis-resveratrol glucosides, 4 – cis-resveratrol, St total; flavan-3-ols (Fl): 1 – catechin; 2 – epicatechin; 3 – procyanidin B1; 4 – procyanidin B2, Fl total; polyphenols measured spectrophotometrically: TA – total phenols content, TI – total phenol index, TA – total anthocyanins, HMP – high-molecular-weight proanthocyanidins, LMP – low-molecular-weight proanthocyanidins, and ABTS.

Conclusion

We have presented the detailed chemical and phenolic composition and antioxidant activity of red wines from Austria (Cabernet-Sauvignon, Blaufraenkisch and Zweigelt) and Montenegro (Cabernet-Sauvignon and Vranac) of three vintages (2014, 2015 and 2016). We used HPLC-DAD to quantify individual phenolic acids, stilbenes and flavan-3-ols, and spectrophotometric methods to evaluate phenolic groups. Our results reveal a variation in phenolic composition depending on wine variety and vintage and influenced by the wine region. Austrian autochthones wines had similar chemical compositions, but Blaufraenkisch showed higher contents of spectrophotometrically-determined phenolic compounds (except TA) and all the examined phenolic acids, flavan-3-ols and stilbenes than Zweigelt wines. Vranac, an important autochthonous Montenegrin variety, showed a typical chemical and phenol composition: medium to high levels of spectrophotometrically-determined phenolic groups, phenolic acids and flavan-3-ols and a moderate stilbene content. The antioxidant activity was high in all wines and showed high correlation with the TP, LMP and HMP contents. Wines from the vintage with best maturity (2015) contained the highest TP content estimated spectrophotometrically and the lowest level of phenolic acids and stilbenes.

Our findings contribute to improving knowledge of the phenolic composition of Austrian and Montenegrin autochthonous wines. Furthermore, the comparative study of Cabernet-Sauvignon wines from the two countries increases our understanding of the influence of specific terroirs on the phenolic characteristics of this international variety.

Acknowledgements

This research was conducted with the support of the bilateral cooperation program “Scientific and Technological Co-operation Program Austria-Montenegro, 2015-16” announced by the Ministry of Science of Montenegro and the Ministry of Science of Austria

—project title: “Phenolic content and antioxidant activity of Austrian and Montenegro red wines”.

References

Aleixandre-Tudo, J. L., Buica, A., Nieuwoudt, H., Aleixandre, J. L., & du Toit, W. (2017). Spectrophotometric analysis of phenolic compounds in grapes and wines. *Journal of Agricultural and Food Chemistry*, *65*(20), 4009-4026. https://doi.org/10.1021/acs.jafc.7b01724

Baiano, A., Terracone, C., Gambacorta, G., & La Notte, E. (2009). Phenolic content and antioxidant activity of Primitivo wine: Comparison among winemaking technologies. *Journal of Food Science*, *74*(3), C258-C267. https://doi.org/10.1111/j.1750-3841.2009.01101.x

Bavaresco, L., Pezzutto, S., Gatti, M., & Mattivi, F. (2007). Role of the variety and some environmental factors on grape stilbenes. *Vitis*, *46*(2), 57-61.

Bavaresco, L., Vezzulli, S., Battilani, P., Giorni, P., Pietri, A., & Bertuzzi, T. (2003). Effect of ochratoxin A-producing aspergilli on stilbenic phytoalexin synthesis in grapes. *Journal of Agricultural and Food Chemistry*, *51*(21), 6151-6157. https://doi.org/10.1021/jf0301908

Broadhurst, R. B., & Jones, W. T. (1978). Analysis of condensed tannins using acidified vanillin. *Journal of the Science of Food and Agriculture*, *29*(9), 788-794. https://doi.org/10.1002/jsfa.2740290908

Burns, J., Gardner, P. T., O'Neil, J., Crawford, S., Morecroft, I., McPhail, D. B., Lister, C., Matthews, D., MacLean, M. R., Lean, M. E., Duthie, G. G., & Crozier, A. (2000). Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. *Journal of Agricultural and Food Chemistry*, *48*(2), 220-230. https://doi.org/10.1021/jf9909757

Cheynier, V., Duenas-Paton, M., Salas, E., Maury, C., Souquet, J. M., Sarni-Manchado, P., & Fulcrand, H. (2006). Structure and properties of wine pigments and tannins. *American Journal of Enology and Viticulture*, *57*(3), 298-305. https://doi.org/10.5344/ajev.2006.57.3.298

Chira, K., Schmauch, G., Saucier, C., Fabre, S., & Teissedre, P. L. (2009). Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet-Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). *Journal of Agricultural and Food Chemistry*, *57*(2), 545-553. https://doi.org/10.1021/jf802301g

Costa, E., Cosme, F., Rivero-Pérez, M. D., Jordão, A. M., & González-SanJosé, M. L. (2015). Influence of wine region provenance on phenolic composition, antioxidant capacity and radical scavenger activity of traditional Portuguese red grape varieties. *European Food Research and Technology*, *241*, 61-73. https://doi.org/10.1007/s00217-015-2434-x

De Beer, D., Harbertson, J. F., Kilmartin, P. A., Roginsky, V., Barsukova, T., Adams, D. O., & Waterhouse, A. L. (2004). Phenolics: A comparison of diverse analytical methods. *American Journal of Enology and Viticulture*, *55*(4), 389-400. https://doi.org/10.5344/ajev.2004.55.4.389

Di Stefano, R., Cravero, M. C., & Gentilini, N. (1989). Methods for the study of wine polyphenols. *L’enotecnico*, *25*(5), 83-89.

Di Stefano, R., & Guidoni, S. (1989). The analysis of total polyphenols in musts and wines. *Vignevini*, *1*(2), 47-52.

Eder, R. (2019). Phenole. In R. Wittkowski, H. Otteneder, & H. Dietrich, H. (Eds.), *Analytik des Weines: Untersuchen und bewerten* (pp. 90-102). Eugen Ulmer.

Eder, R., Beyer, B., Patzl-Fischerleitner, E., Wendelin, S., & Hann, S. (2014). Determination of pyranoanthocyanine and malvidin-3-glucoside content in red wine of different vintages via LC-MS/ESI. *Mitteilungen Klosterneubg*, *64*, 183-192.

Eder, R., Oswald, B. & Wendelin, S. (2004). Einfluss von pektolytischen Enzympräparaten mit Acetylaseaktivität sowie Botrytisbefall und Maischeerhitzung auf Anthocyanzusammensetzung und Qualität von Rotweinen. *Mitteilungen Klosterneuburg*, *52*, 204-221.

Eder, R., Wendelin, S., & Barna, J. (1994). Classification of red wine cultivars by means of anthocyanin analysis. Pt. 1. Application of multivariate statistical methods for differentiation of grape samples. *Mitteilungen Klosterneuburg* 44, 201-212.

Eder, R., Wendelin, S., Kalchgruber, R., Rosenthal, F., & Barna, F (1992). Untersuchungen über den Einfluss von Hefe – und Enzympräparaten auf die Rotweinfarbe. *Mitteilungen Klosterneuburg*, *42*, 148-157.

Eder, R., Wendelin, S., & Vrhovšek, U. (2001). Resveratrolgehalte von Trauben und Rotweinen in AbhaČngigkeit von Lesejahrgang und Lesetermin. *Mitteilungen Klosterneuburg*, *51*, 64-78.

Fernández-Pachón, M. S., Villano, D., Garcıa-Parrilla, M. C., & Troncoso, A. M. (2004). Antioxidant activity of wines and relation with their polyphenolic composition. *Analytica Chimica Acta*, *513*(1), 113-118. https://doi.org/10.1016/j.aca.2004.02.028

Frankel, E. N., Waterhouse, A. L., & Teissedre, P. L. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry*, *43*(4), 890-894. https://doi.org/10.1021/jf00052a008

Goldberg, D. M., Ng, E., Karumanchiri, A., Diamandis, E. P., & Soleas, G. J. (1996). Resveratrol glucosides are important components of commercial wines. *American Journal of Enology and Viticulture*, *47*(4), 415-420. https://doi.org/10.5344/ajev.1996.47.4.415

Goldberg, D. M., Yan, J., Ng, E., Diamandis, E. P., Karumanchiri, A., Soleas, G., & Waterhouse, A. L. (1995). A global survey of trans-resveratrol concentrations in commercial wines. *American Journal of Enology and Viticulture*, *46*(2), 159-165. https://doi.org/10.5344/ajev.1995.46.2.159

Granato, D., Katayama, F. C. U., & de Castro, I. A. (2011). Phenolic composition of South American red wines classified according to their antioxidant activity, retail price and sensory quality. *Food Chemistry*, *129*(2), 366-373. https://doi.org/10.1016/j.foodchem.2011.04.085

Gris, E. F., Mattivi, F., Ferreira, E. A., Vrhovšek, U., Pedrosa, R. C., & Bordignon-Luiz, M. T. (2011). Proanthocyanidin profile and antioxidant capacity of Brazilian *Vitis vinifera* red wines. *Food Chemistry*, *126*(1), 213-220. https://doi.org/10.1016/j.foodchem.2010.10.102

Ivanova-Petropulos, V., Ricci, A., Nedelkovski, D., Dimovska, V., Parpinello, G. P., & Versari, A. (2015). Targeted analysis of bioactive phenolic compounds and antioxidant activity of Macedonian red wines. *Food Chemistry*, *171*, 412-420. https://doi.org/10.1016/j.foodchem.2014.09.014

Jaitz, L., Siegl, K., Eder, R., Rak, G., Abranko, L., Koellensperger, G., & Hann, S. (2010). LC–MS/MS analysis of phenols for classification of red wine according to geographic origin,

grape variety and vintage. *Food Chemistry*, *122*(1), 366-372. https://doi.org/10.1016/j.foodchem.2010.02.053

Kallithraka, S., Mohdaly, A. A. A., Makris, D. P., & Kefalas, P. (2005). Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera* sp.): Association with antiradical activity. *Journal of Food Composition and Analysis*, *18*(5), 375-386. https://doi.org/10.1016/j.jfca.2004.02.010

Kallithraka, S., Tsoutsouras, E., Tzourou, E., & Lanaridis, P. (2006). Principal phenolic compounds in Greek red wines. *Food Chemistry*, *99*(4), 784-793. https://doi.org/10.1016/j.foodchem.2005.07.059

Kennedy, J. A., & Waterhouse, A. L. (2000). Analysis of pigmented high-molecular-mass grape phenolics using ion-pair, normal-phase high-performance liquid chromatography. *Journal of Chromatography A*, 866(1), 25-34. https://doi.org/10.1016/S0021-9673(99)01038-9

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 Science and Technology*, 1(1), 7-15. https://doi.org/10.13189/fst.2013.010102

Liszt, K. I., Eder, R., Wendelin, S., & Somoza, V. (2015). Identification of catechin, syringic acid, and procyanidin b2 in wine as stimulants of gastric acid secretion. *Journal of Agricultural and Food Chemistry*, 63(35), 7775-7783. https://doi.org/10.1021/acs.jafc.5b02879

Mattivi, F., & Nicolini, G. (1997). Analysis of polyphenols and resveratrol in Italian wines. *BioFactors*, 6(4), 445-448. https://doi.org/10.1002/biof.5520060415

Mattivi, F., Zulian, C., Nicolini, G., & Valenti, L. (2002). Wine, biodiversity, technology, and antioxidants. *Annals of the New York Academy of Sciences*, 957(1), 37-56. https://doi.org/10.1111/j.1749-6632.2002.tb02904.x

Minussi, R. C., Rossi, M., Bologna, L., Cordi, L., Rotilio, D., Pastore, G. M., & Durán, N. (2003). Phenolic compounds and total antioxidant potential of commercial wines. *Food Chemistry*, 82(3), 409-416. https://doi.org/10.1016/S0308-8146(02)00590-3

Monagas, M., Suárez, R., Gómez-Cordovés, C., & Bartolomé, B. (2005). Simultaneous determination of nonanthocyanin phenolic compounds in red wines by HPLC-DAD/ESI-MS. *American Journal of Enology and Viticulture*, 56(2), 139-147. https://doi.org/10.5344/ajev.2005.56.2.139

Muñoz, F., Urvieta, R., Buscema, F., Rasse, M., Fontana, A., & Berli, F. (2021). Phenolic characterization of Cabernet-Sauvignon wines from different geographical indications of Mendoza, Argentina: Effects of plant material and environment. *Frontiers in Sustainable Food Systems*, 5, 700642. https://doi.org/10.3389/fsufs.2021.700642

Pajovic, R., Raicevic, D., Popovic, T., Sivilotti, P., Lisjak, K., & Vanzo, A. (2014a). Polyphenolic characterisation of Vranac, Kratosija and Cabernet-Sauvignon (*Vitis vinifera* L. cv.) grapes and wines from different vineyard locations in Montenegro. *South African Journal of Enology and Viticulture*, 35(1), 139-148. https://doi.org/10.21548/35-1-994

Pajović, R., Wendelin, S., Forneck, A., & Eder, R. (2014b). Varietal differentiation of grapes cv. 'Vranac', 'Kratošija' and 'Cabernet-Sauvignon' from Montenegro according to their polyphenolic composition. *Mitteilungen Klosterneuburg*, 64, 9-19.

Pajović-Šćepanović, R., Krstić, M., Savković, S., Raićević, D., & Popović, T. (2016). Wine quality in Montenegro. *Agriculture & Forestry*, 62, 223-244. https://doi.org/10.17707/AgricultForest.62.3.19

Pajović-Šćepanović, R., Wendelin, S., & Eder, R. (2018). Phenolic composition and varietal discrimination of Montenegrin red wines (*Vitis vinifera* var. Vranac, Kratošija, and Cabernet-Sauvignon). *European Food Research and Technology*, 244, 2243-2254. https://doi.org/10.1007/s00217-018-3133-1

Pajović-Šćepanović, R., Wendelin, S., Forneck, A., & Eder, R. (2019a). Suitability of flavan-3-ol analysis to differentiate grapes from Vranac, Kratošija and Cabernet-Sauvignon (*Vitis vinifera* L.) grown in Montenegro. *Australian Journal of Grape and Wine Research*, 25(4), 376-383. https://doi.org/10.1111/ajgw.12406

Pajović-Šćepanović, R., Wendelin, S., Raičević, D., & Eder, R. (2019b). Characterization of the phenolic profile of commercial Montenegrin red and white wines. *European Food Research and Technology*, 245, 2233-2245. https://doi.org/10.1007/s00217-019-03330-z

Popîrdă, A., Luchian, C. E., Cotea, V. V., Colibaba, L. C., Scutarașu, E. C., & Toader, A. M. (2021). A review of representative methods used in wine authentication. *Agriculture*, 11(3), 225. https://doi.org/10.3390/agriculture11030225

Radović, B., Tešević, V., Kodžulović, V., & Maraš, V. (2015). Resveratrol concentration in 'Vranac' wines. *Vitis*, 54, 169-171.

Raičević, D., Popović, T., Ivanova-Petropulos, V., Stanoeva, J. P., & Maraš, V. (2020). HPLC-DAD-ESI/MS monitoring of stilbenes content in Vranac red wines produced with traditional and modern fermentation methods. *Macedonian Journal of Chemistry and Chemical Engineering*, 39(1), 49-58. https://doi.org/10.20450/mjcce.2020.1970

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20(7), 933-956. https://doi.org/10.1016/0891-5849(95)02227-9

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. *Journal of Agricultural and Food Chemistry*, 48(6), 1996-2002. https://doi.org/10.1021/jf991203d

Rodrigo, R., Miranda, A., & Vergara, L. (2011). Modulation of endogenous antioxidant system by wine polyphenols in human disease. *Clinica Chimica Acta*, 412(5-6), 410-424. https://doi.org/10.1016/j.cca.2010.11.034

Šćepanović, R. P., Madžgalj, V., & Vukoslavljević, V. (2019). Assay of polyphenols in Montenegrin Vranac wines. *Mitteilungen Klosterneuburg*, 69(1), 65-75.

Scutarașu, E. C., Luchian, C. E., Vlase, L., Colibaba, L. C., Gheldiu, A. M., & Cotea, V. V. (2020). Evolution of phenolic profile of white wines treated with enzymes. *Food Chemistry*, 340, 127910. https://doi.org/10.1016/j.foodchem.2020.127910

Singleton, V. L., Zaya, J., & Trousdale, E. K. (1986). Caftaric and coutaric acids in fruit of Vitis. *Phytochemistry*, 25(9), 2127-2133. https://doi.org/10.1016/0031-9422(86)80078-4

Sterneder, S., Stoeger, V., Dugulin, C. A., Liszt, K. I., Di Pizio, A., Korntheuer, K., Dunkel, A., Eder, R. Ley, J. P., & Somoza, V. (2021). Astringent gallic acid in red wine regulates mechanisms of gastric acid secretion via activation of bitter taste sensing receptor TAS2R4. *Journal of Agricultural and Food Chemistry*, 69(36), 10550-10561. https://doi.org/10.1021/acs.jafc.1c03061

Tonietto, J., & Carbonneau, A. (2004). A multicriteria climatic classification system for grape-growing regions worldwide. *Agricultural and Forest Meteorology*, 124(1-2), 81-97. https://doi.org/10.1016/j.agrformet.2003.06.001

Vanzo, A., Terdoslavich, M., Brandoni, A., Torres, A. M., Vrhovšek, U., & Passamonti, S. (2008). Uptake of grape anthocyanins into the rat kidney and the involvement of bilitranslocase. *Molecular Nutrition & Food Research*, 52(10), 1106-1116. https://doi.org/10.1002/mnfr.200700505

Vrhovšek, U. (1998). Extraction of hydroxycinnamoyltartaric acids from berries of different grape varieties. *Journal of Agricultural and Food Chemistry*, 46(10), 4203-4208. https://doi.org/10.1021/jf980461s

Vrhovšek, U., Vanzo, A., & Nemanić, J. (2002). Effect of red wine maceration techniques on oligomeric and polymeric proanthocyanidins in wine, cv. Blaufränkisch. *Vitis*, 41(1), 47 - 51.

Vrhovšek, U., Wendelin, S., & Eder, R. (1997a). Effects of various vinification techniques on the concentration of cis- and trans-resveratrol and resveratrol glucoside isomers in wine. *American Journal of Enology and Viticulture*, 48, 214-219 https://doi.org/10.5344/ajev.1997.48.2.214

Vrhovšek, U., Wendelin, S., & Eder, R. (1997b). Quantitative Bestimmung von Hydroxyzimtsäuren und Hydroxyzimtsäurederivaten (Hydroxycinnamaten) in Weißweinen mittels HPLC. *Mitteilungen Klosterneuburg*, 47,164-172.

Zoechling, A., 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. *American Journal of Enology and Viticulture*, 60(2), 223-232. https://doi.org/10.5344/ajev.2009.60.2.223