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# Proceedings

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# Zbornik radova

Vinogradarstvo, vinarstvo i voćarstvo

# Phenolic compounds evaluation in wines of Serbian autochthonous and local grapevine varieties

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## Abstract

Qualitative and quantitative content of phenolic compounds in monovarietal wines of seven Serbian and two international varieties was determined and compared. The results showed that the variety influences the composition and content for most phenolic components determined in wines. The largest number of phenolic compounds (15) was detected in 'Prokupac' wine, while the smallest number (10) was found in 'Smederevka' wine. Hidroxybenzoic and hidroxycinnamic acids had the strongest discriminating effect. PCA indicated that phenolic composition depends on both the variety and the vintage, while in some cases their interaction was also manifested. From Serbian variety 'Prokupac' wine had the most specific phenolic profile.

**Keywords**: *Vitis vinifera* L., antioxidant activity, phenolic profile, PCA

## Introduction

Wine is traditional and the most popular alcoholic beverage consumed worldwide. It is a rich source of phenolic compounds, which contribute to the mouth-feel sensations (Ma et al., 2014), color, flavor and aroma of wine (Morata et al., 2020). Biosynthesis of these compounds is determined by genes controlling the production of the individual enzymes involved in the relevant biosynthetic pathways (Makris et al., 2006). Further, several factors such as soil condition, climatic conditions and seasonal variation during grape development (Rodríguez-Montealegre et al., 2006), vineyard cultural and applied agronomical practices (Bešlić et al., 2015), can be affected the polyphenolic profile. The technological process and winemaking techniques can also play an important role in the final phenolic composition of the obtained wines (Zhang et al., 2021).

According to Peixoto et al. (2018), phenolic compounds found in wine can be classified in three main groups: phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids (catechins, flavonols and anthocyanins) and proanthocyanidins. Phenols are not only closely related to wine quality but have also been shown to have health-promoting properties. Numerous studies shown that moderate consumption of wine is healthy, including antioxidant (Gutiérrez-Escobar et al., 2021; Nemzer et al., 2022; Radeka et al., 2022) and cardioprotective effects (Haseeb et al., 2019; Weaver et al., 2021).

Serbia has a long tradition in wine production. Due to its favorable climate and geological characteristics, it is an ancient wine growing region hosting a wide range of indigenous and/or traditional local grapevine varieties, most of which are not internationally recognized (Bešlić et al., 2012). In recent decades, the area under vineyard has significantly increased resulting in an increase in wine production, and a higher presence of these wines in the domestic and worldwide markets. This has led to renewed interest in indigenous grapevine varieties, because monovarietal wines produced from these varieties gain attention on the wine market, and with their authenticity contribute to the recognition of the region of their origin. In order to protect the authenticity of these wines, characterization and discrimination is an important issue, and evaluation of the phenolic profile seems to be a very suitable method of defining the authenticity of the individual wines (Kallitraka et al., 2007). According to Garrido and Borges, (2013) the phenolic composition of wines, which determines their organoleptic properties and provides information about their primary characteristics, can be used as a fingerprint to distinguish them according to their origin in terms of grapevine variety, region and vintage.

Considering this, the aim of the present study was to determine total phenolic content (TPC), radical scavenging activity (RSA) and phenolic profile in wines of Serbian autochthonous or local grapevine varieties and to evaluate possible differences based on the detected polyphenols and PCA multivariate analysis. To the best of knowledge, this is the first comparative study of the phenolic profile of the Serbian monovarietal wines and well-known international wine varieties.

## Material and methods

As a material in this two-year trial wine samples from 7 Serbian autochthonous and local and 2 international varieties from two vintages were investigated (Table 1). The wines were produced in the experimental winery of Faculty of Agriculture in Belgrade using small batches of 50 l. Grapes were harvested at the technological ripeness for each variety individually. The grape samples of each variety were processed immediately using small steel vats of 80 l capacity and 50 l double jacketed stainless steel fermenters. The vinification techniques were the same for all the red and white wines, respectively. Wine samples was prepared by procedure descript by Pantelić et al. (2018)

Table 1. Description of the wines studied

| Variety | Type of wine | Type of variety | Vineyard location |
|---|---|---|---|
| Cabernet Sauvignon | Red | International | South Serbia |
| Prokupac | Red | Autochthonous | South Serbia |
| Crna Tamjanika | Red | Autochthonous | East Serbia |
| Plovdina | Red | Local | South Serbia |
| Začinak | Red | Autochthonous | East Serbia |
| Chardonnay | White | International | South Serbia |
| Smederevka | White | Local | Central Serbia |
| Bela Tamjanika | White | Local | South Serbia |
| Kreaca | White | Autochthonous | North Serbia |

In obtained wines total phenolic content (TPC), radical scavenging activity (RSA), and individual polyphenols were determined. Before the analysis all wine samples were filtered through a 0.45-μm PTFE filters and appropriately diluted. All procedures were described in Šuković et al. (2020). Spectrophotometric measurements (determination of TPC, and RSA) were carried out in triplicate on a GBC Cintra 6 UV-VIS spectrophotometer (GBC Scientific Equipment Ltd.). TPC were determined using Folin-Ciocalteu method, while RSA was evaluated using DPPH$^{\cdot}$ assay. TPC and RSA results were expressed as equivalents of gallic acids and Trolox, respectively. Identification and quantification of the individual polyphenols was performed using a Thermo Scientific ultra-high performance liquid chromatography (UHPLC) system consisting of a quaternary Accela 600 pump and Accela Autosampler, connected to a linear ion trap-orbitrap (LTQ Orbitrap XL) hybrid mass spectrometer with heated-electrospray ionization probe (HESI-II, ThermoFisher Scientific, Bremen, Germany). Syncronis C18-column (100 × 2.1 mm, 1.7 μm particle size) was used for the separations of compounds. The mobile phase consisted of water and acetonitrile (both with 0.1% formic acid). For the instrument control, data acquisition and analysis, Xcalibur software (version 2.1, Thermo Fisher Scientific, Waltham, MA, USA) was used. ChemDraw, the molecule editor program, (version 12.0, CambridgeSoft, Cambridge, MA, USA), was used as a reference library to calculate the exact (monoisotopic) masses of compounds of interest.

To establish the significance of differences between the varietal wines, analysis of variance (ANOVA) and Tukey's test for post host comparison et significance level of $P < 0.05$ was applied. The obtained data (44 variables) were used in a principal component analysis (PCA) to differentiate and discriminate the wine samples according to the variety, vintage and type of grape (white vs. red). All statistical analyses were performed using "Statistica" (Stat Soft software Inc., Tulsa, OK, USA) program package.

## Result and discussion

As expected, red wines had a higher TPC and RSA, than white wines. Differences between years also manifested (Table 2). Both TPC and RSA values were found to higher in 2019 than in 2018. On the average, TPC and RSA in the samples of red wines were several times higher than in the white wines (Table 3). Differences for these two parameters were determined between red varieties (0.33-1.70 mg GAE $g^{-1}$ for TPC and 1.26-10.85 µmol TE $g^{-1}$ for RSA) and it was fairly uniform across the white varieties (0.13-0.22 mg GAE $g^{-1}$ for TPC and 0.46-0.64 µmol TE $g^{-1}$ for RSA). The strong linear relationship between TPC and RSA, pointing phenolics as the major contributors to the antioxidant potential of the wine samples studied.

Table 2. Total phenolic content (TPC) and radical-scavenging activity (RSA) in wines of studied grapevine varieties

| Variety | TPC (mg GAE $g^{-1}$) | | RSA (µmol TE $g^{-1}$) | |
|---|---|---|---|---|
| | 2018 | 2019 | 2018 | 2019 |
| Cabernet Sauvignon | 1.29±0.01 | 2.10±0.02 | 7.29±0.22 | 14.40±0.03 |
| Prokupac | 1.01±0.02 | 1.29±0.02 | 6.36±0.03 | 8.39±0.10 |
| Crna Tamjanika | 0.57±0.00 | 1.01±0.01 | 2.69±0.14 | 5.64±0.05 |
| Plovdina | 0.79±0.02 | 0.81±0.01 | 4.42±0.01 | 5.13±0.05 |
| Začinak | 0.24±0.00 | 0.42±0.01 | 1.05±0.05 | 1.47±0.05 |
| Chardonnay | 0.18±0.00 | 0.18±0.00 | 0.68±0.03 | 0.50±0.01 |
| Smederevka | 0.14±0.00 | 0.12±0.00 | 0.54±0.01 | 0.37±0.01 |
| Bela Tamjanika | 0.18±0.00 | 0.21±0.00 | 0.68±0.01 | 0.48±0.01 |
| Kreaca | 0.20±0.00 | 0.23±0.00 | 0.63±0.00 | 0.65±0.00 |

A total of 20 phenolic compounds in the wine samples were detected by the UHPLC-DAD method and divided into seven classes based on their structure (Table 3). The largest number of phenolic compounds (15) was detected in the wine of the 'Prokupac', while the smallest number (10) was found in the wine of the 'Smederevka'. In general, in our study, the red wines contained the highest concentrations of phenolics, while the white wines had lower values. Thus, our results are in line with expectations, considering that the pulp, skin, and seeds of grapes contain different classes and amounts of phenolic components (Pantelić et al., 2016), and that red wines are exposed to all parts of the grape during winemaking, while polyphenols in white wines mostly originating from the pulp (Gutiérrez-Escobar et al., 2021).

Table 3. Mean values of TPC (mg GAE $g^{-1}$), RSA (μmol TE $g^{-1}$) and phenolics (mg/$l^{-1}$) in monovarietal wines studied

| Compounds | Red grapevine varieties | | | | | White grapevine varieties | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cabernet Sauvignon | Prokupac | Crna Tamjanika | Plovdina | Začinak | Chardonnay | Smederevka | Bela Tamjanika | Kreaca |
| TPC | 1.70$^{a}$ | 1.15$^{b}$ | 0.79$^{b}$ | 0.80$^{b}$ | 0.33$^{c}$ | 0.18$^{c}$ | 0.13$^{c}$ | 0.20$^{c}$ | 0.22$^{c}$ |
| RSA | 10.85$^{a}$ | 7.38$^{b}$ | 4.17$^{c}$ | 4.78$^{bc}$ | 1.26$^{d}$ | 0.59$^{d}$ | 0.46$^{d}$ | 0.58$^{d}$ | 0.64$^{d}$ |
| **Hydroxybenzoic acids** | | | | | | | | | |
| 1. Gallic acid | 12.38$^{a}$ | 11.57$^{a}$ | 2.68$^{b}$ | 2.37$^{b}$ | 3.26$^{b}$ | 0.29$^{c}$ | 4.85$^{b}$ | 4.38$^{b}$ | 0.32$^{c}$ |
| 2. Protocatechuic acid | 0.81$^{ab}$ | 0.93$^{ab}$ | 0.53$^{bcd}$ | 0.39$^{cd}$ | 1.33$^{a}$ | 0.05$^{d}$ | 0.64$^{bc}$ | 0.34$^{cd}$ | 0.09$^{d}$ |
| 3. Gentisic acid | 0.68$^{a}$ | 0.48$^{abc}$ | 0.17$^{de}$ | 0.27$^{cd}$ | 0.15$^{de}$ | 0.53$^{ab}$ | 0.36$^{bcd}$ | 0.03$^{e}$ | 0.35$^{bcd}$ |
| 4. Ellagic acid | 4.44$^{a}$ | 3.41$^{a}$ | 0.15$^{b}$ | 0.33$^{b}$ | 0.32$^{b}$ | - | 0.31$^{b}$ | 0.21$^{b}$ | 0.07$^{b}$ |
| 5. Vanillic acid | - | - | 0.28$^{a}$ | - | - | - | - | 0.06$^{b}$ | - |
| **Hydroxycinnamic acids** | | | | | | | | | |
| 6. Chlorogenic acid | - | - | - | 0.005 | - | - | - | - | - |
| 7. Caffeic acid | 0.89$^{bc}$ | 1.11$^{ab}$ | 0.40$^{bc}$ | 0.49$^{bc}$ | 1.01$^{b}$ | 1.84$^{a}$ | 0.29$^{c}$ | 0.40$^{bc}$ | 0.30$^{c}$ |
| 8. Ferulic acid | - | - | - | - | - | 0.01 | - | - | - |
| 9. p-Coumaric acid | 0.09$^{bc}$ | 0.12$^{ab}$ | - | 0.03$^{c}$ | 0.07$^{bc}$ | 0.16$^{a}$ | 0.06$^{bc}$ | - | - |
| 10. Sinapic_acid | - | 0.44$^{ab}$ | - | - | - | - | - | 0.13$^{b}$ | 0.92$^{a}$ |
| **Coumarins** | | | | | | | | | |
| 11. Aesculin | - | - | - | 0.005 | - | - | - | - | - |
| 12. Aesculetin | - | 0.38$^{a}$ | - | 0.09$^{b}$ | - | - | - | - | - |
| **Flavan-3-ols** | | | | | | | | | |
| 13. Catechin | 5.15$^{a}$ | 1.75$^{bc}$ | 2.33$^{b}$ | 0.37$^{cd}$ | 0.47$^{cd}$ | 0.22$^{cd}$ | 1.06$^{bcd}$ | 4.08$^{a}$ | 0.11$^{d}$ |
| 14. Gallocatechin gallate | 0.05 | 0.08 | 0.05 | - | - | 0.09 | - | - | 0.04 |
| 15. Gallocatechin | - | 0.045 | - | - | - | - | - | - | - |
| 16. Epigallocatechin gallate | - | 0.20$^{ab}$ | 0.13$^{b}$ | - | - | 0.48$^{a}$ | - | - | 0.06$^{b}$ |
| **Flavonols** | | | | | | | | | |
| 17. Quercetin 3-galactoside (Hyperoside) | 0.02$^{c}$ | 0.09$^{c}$ | 0.33$^{a}$ | 0.01$^{c}$ | 0.23$^{ab}$ | - | 0.03$^{c}$ | 0.12$^{bc}$ | - |
| **Flavons** | | | | | | | | | |
| 18. Cynaroside | - | - | - | - | - | - | - | 0.02 | - |
| **Dehydroflavonol** | | | | | | | | | |
| 19. Phlorizin | 0.120$^{a}$ | 0.085$^{ab}$ | 0.055$^{ab}$ | 0.020$^{b}$ | 0.120$^{a}$ | 0.010$^{b}$ | 0.035$^{b}$ | 0.040$^{ab}$ | 0.005$^{b}$ |
| **Stilbens** | | | | | | | | | |
| 20. Oxyresveratrol | 0.165$^{b}$ | 0.470$^{a}$ | 0.300$^{a}$ | 0.175$^{b}$ | - | 0.195$^{ab}$ | 0.075$^{b}$ | 0.120$^{b}$ | 0.175$^{b}$ |

[a] *Different letters in the same row denote a significant difference among varieties according to Tukey's test, $p < 0.05$.*

Significant differences for most phenolic components were determined (Table 3). Hydroxybenzoic acids were the most abundant in analyzed red wines, whereby the highest concentration was found in wines of autochthonous variety 'Prokupac' and international variety 'Cabernet Sauvignon'. From this group, vanillic acid was found only in 'Tamjanika Crna' and 'Tamjanika Bela' wines, which can be related to their muscat aroma. From the group of hydroxycinnamic acids caffeic acid and p-coumaric acid were the dominant compounds present in highest concentration in 'Chardonnay' wine (1.84 mg $l^{-1}$and 0.16 mg $l^{-1}$, respectively). Of the remaining phenolic compounds, only catechin and phlorizin were present in all monovarietal wines, while the others showed varietal specificity. Our results are consistent with some previous studies (Mitić et al., 2010; Zhu et al., 2014; Ivanova-Petroleus et al., 2015; Šćepanović et al.,2019; ‚Merkyté et al., 2020; Kropek et al., 2023), which found that specific phenolic profile are suitable for the characterization of wines according to variety.

For clearer interpretation of the results, Principal Component Analysis (PCA) was performed presenting differences among grapevine varieties and years of vintage. With respect to PCA, the first two components obtained explained 47.1% of the total variability of the original data (27.4% was assigned to the first factor and 19.7% to the second factor). Besides TPC and RSA out of 20 phenolic components detected in wines, PC 1 was mostly described by hydroxybenzoic acids (gallic, protocatechuic and ellagic) and phlorizin (positive loading), while hydrpxycinnamic acids (caffeic, ferulic and p-coumaric) and epigallocatechin gallate (negative loading) had high contributions within PC2 (Figure 1).

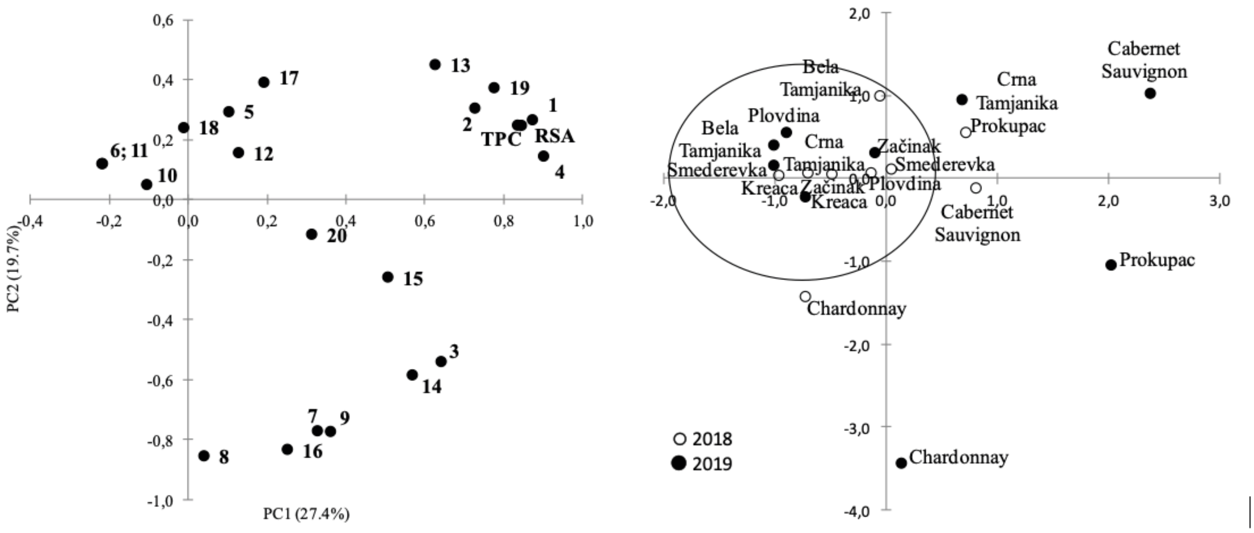


*Figure 1. Loading plot representing phenolic compounds (represented by number from Table 3) and scatter plots representing variety and vintages, based on PCA*

As can be seen in Figure 1, the distribution of samples into the score plot did not show any important grouping. Contrary to the results of Pantelić et al. (2018) and Šćepanović et al. (2019) in present study PCA not separated red vs. white wines. The wine of most varieties in both years showed little variability in terms of the phenolic profile and was closely located on the scatter plot forming one common group. From this group, the wines of 'Prokupac', 'Cabernet Sauvignon' and 'Chardonnay' stood out in both years and 'Crna Tamjanika' wine in 2019 year. Also, PCA showed that in addition to genetic potential of the variety, content of phenolic compounds in wines depends on vintage while in some cases interaction of these factors was manifested, which is in agreement with the results of Vilanova et al. (2009) and Lampíř and Pavloušek (2013). The influence of environmental factors was more pronounced in the varieties outside the group, while the varieties in the group showed greater stability in relation to the phenolic profile.

## Conclusion

The phenolic composition of wines from some autochthonous and local Serbian varieties and its comparison with international varieties 'Cabernet Sauvignon' and 'Chardonnay' is reported for the first time. Obtained results give important information for researchers, but also for the winemaking industry and consumers to understand the nature and content of phenolic compounds in different wine from Serbian varieties, as the most important components that influence the color, stability as well as sensorial properties of wines. Also, results are indicative of the polyphenolic richness of wine of autochthonous 'Prokupac' compared with the other varieties. This can be important information for winemakers in making decisions about the length of certain processes in winemaking, such as maceration time.

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