THE PHENOLIC COMPOSITION AND THE ANTIOXIDANT CAPACITY OF SERBIAN RED WINES

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The aim of this study was to determine the phenolic composition and free radical scavenging activity of some Serbian commercially red wines by using DPPH assays. The wines of red grape varieties and different vintage were analysed in June 2012. Standard spectrophotometric methods of the analysis for general wine components were used for the preliminary control of the selected wines. Anthocyanins in the wine were measured by the HPLC method. Among the red wines tested, "Medvedja krv" and "Vranac" contained the highest total phenolic content with 3080 ± 101 and 2850 ± 93 mg/l, respectively. All analyzed red wines exerted remarkable antioxidant activities. In all cases, malvidin-3-glucoside was a predominant anthocyanin. According to the obtained results, the red wines from Serbia could be a good dietary source of polyphenols.

Keywords: red wines, phenolic, antioxidant activities, spectrophotometric method, HPLC method

Introduction

Phenolic compounds contribute to many sensory attributes of the wine including color, astringency, structure and mouth feel [1]. The phenolic composition is largely determined by the grape cultivar and oenological practices, and the ranges between 1000 and 5000 mg/L of total phenols for young red wines [2]. Prior to or during fermentation, grape maceration and the skin contact are important determinants to the levels of phenols, as there are numerous opportunities for the extraction of tannins and flavonoids from the skins and seeds which contribute to astringency. Cold extraction, cap management, fermentation vessels and temperature are also known to significantly affect the phenolic composition [3]. The maturation of the wine in oak barrels allows the agaseous exchange and an influx of atmospheric oxygen which modifies itself and may assemble the phenolic components [2].

Anthocyanins are responsible for most of the red, blue, and purple colours of fruits, vegetables, flowers, and other plant tissues of the products. The amount and composition of anthocyanins in red wine grapes vary greatly in species, cultivar, maturity, season, region, and yield [4].

The anthocyanins extracted from the skins of grapes during crushing, pressing, and fermentation are the major components responsible for the red wine color. It is generally accepted that the color of the red wine changes during maturation and aging, due to interactions between anthocyanins and colorless phenols present in grapes including (+)-catechin, (-)-epicatechin, quercetin,

kaempferol, and phenolic acids [4, 5]. The contact with the oak wood of barrels or wood chips during maturation can result in the extraction of the additional phenolics from the wood into the wine. In addition to their direct role in the color, anthocyanins also contribute to the taste and chemical characteristics of the wine because of their interactions with other molecules such as colorless phenols, polysaccharides, metals, and anthocyanins themselves [5, 6]. The color components of the wine are important parameters that contribute to sensory characteristics (color and astringency) and the antioxidant properties of the wine [7]. With aging, the monomeric anthocyanins are thought to be gradually incorporated into polymeric pigments and this confers color stability of the wine. In relation to their antioxidant characteristics [7, 8, 9], the objective measurement of the components of the wine color is the essential part of the modern concept of winemaking- "Red Wine Color Management".

So, the aim of this study was to determine some basic chemical characteristics of the tested wines and, for the first time, to investigate the anthocyanin profile and the antioxidant capacity of several red wines produced in Serbia and Macedonia.

Experimental part

Wine samples

Representative, high-quality, Serbian red wines were tested (Table 1). All the wines examined were produced according to standard procedures and with a defined

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varietal composition. All samples were stored at 10 °C in the dark and analyzed shortly after opening. All the wines analyzed are frequently consumed in Serbia and Macedonia.

Table 1. Origin and varietal composition of the wines tested

Wine	Appellation	Cultivar(s)	Location
W1	Ždrepcëva krv	Vranac, Merlot, C. Sauvignon	Subotica-Serbia
W2	Medaš beli	Merlot, Game, Burgundac	Aleksandrovac-Serbia
W3	Medveda krv	Prokupac, Vranac	Kruševac-Serbia
W4	Kratošija	Kratošija	Aleksandrovac-Serbia
W5	Merlot	Merlot	Subotica-Serbia
W6	Game	Game	Kruševac-Serbia
W7	Cabernet Sauvignon	Cabernet Sauvignon	Kruševac-Serbia
W8	Cabernet Sauvignon	Cabernet Sauvignon	Tikveš-Macedonia
W9	Ždrebac	Vranac	Subotica-Serbia
W10	Vranac	Vranac	Aleksandrovac-Serbia
W11	Vranac	Vranac	Kruševac-Serbia
W12	Vranac	Vranac	Štip-Macedonia
W13	Vranac	Vranac	Ohrid-Macedonia
W14	Vranac	Vranac	Ovčepolsko-Macedonia
W15	Vranac	Vranac	Skopje-Macedonia

Chemicals

The 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, gallic acid and (+)-catechin were from Sigma-Aldrich (Steineheim, Germany). Sodium hydroxide, sodium nitrite, sodium carbonate, aluminum chloride hexahydrate, methanol, acetonitrile (HPLC grade) and formic acid (HPLC grade) were purchased from Merck® (KGaA, Darmstadt, Germany). All standards for HPLC determination were purchased from Sigma-Aldrich (Steineheim, Germany) and were of HPLC quality.

Instruments

An Agilent 8453 UV/Vis spectrophotometer with an optical cuvette of 1 cm was used for all antioxidant capacity measurements. In order to determine the individual anthocyanin compound, Agilent-1200 series HPLC with the UV-Vis photodiode array detector (DAD) was used.

Determination of the total phenolic content (TP)

The total phenolic content of the wines was determined spectrophotometrically according to the Folin-Ciocalteu method [10]. The samples of the wine were diluted with methanol (1:100). Then, an aliquot (1 ml) of the red diluted wine and 0.5 ml of Folin-Ciocalteu reagent were mixed into a 25 ml calibrated flask. Exactly 1 min. later, 5 ml of sodium carbonate (5 % v/v) was added, and the volume was made to 25 ml with deionized water. The absorbance was measured at 765 nm after the incubation for 1 h in the dark, at room temperature. The total polyphenolic concentration was calculated from a calibration curve (1-50 mg/L) using gallic acid as a standard, and the content was expressed as mg gallic acid equivalent (GAE)/L of the wine. All samples were analyzed in triplicate.

Determination of total flavonoid content (TF)

The measurement of the total flavonoid content in the investigated wines was determined spectrometrically [11] by using the method based on the formation of the complex flavonoid-aluminium. 0.5 ml (diluted 1:50 with methanol) of the wine was placed in a 10 ml volumetric flask, and 5 ml of distilled water and 0.3 ml of 5 % NaNO₂ was added and mixed. 5 min. later, 0.6 ml of 10 % AlCl₃ • 6H₂O was added. Two milliliters of 1 mol/L NaOH were added 5 min later, and then the volume was made with distilled water up to 10 ml. The solution was mixed well and the absorbance was immediately measured at 510 nm. The flavonoid contents were calculated by using a standard calibration curve, prepared from (+)-catechin (R² = 0.992).

Determination of total antioxidant activity (TAA)

The DPPH radical scavenging method [12] was based on the reduction of DPPH radical in the presence of a hydrogen-donating antioxidant, in the methanol solution. DPPH radical solution showed an absorption band at 515 nm and was of the intensively violet color. The absorption and color intensity decreased when DPPH was reduced by an antioxidant compound. The remaining DPPH radical corresponded inversely to the radical scavenging activity of the antioxidant. Each wine was diluted 1:10 with methanol immediately before the analysis. In the test tubes, 0.2 ml of the samples was added to 4.8 ml of the DPPH solution (5.2 × 10⁻⁶ mol/L in methanol) and the mixture was well mixed. The absorbance at 515 nm was measured at 30 min against a blank (0.2 ml methanol and 4.8 ml DPPH solution in methanol). All determinations were performed in triplicate.

The radical scavenging capacity (RSC), expressed in percentage, was calculated by the following equation [13]:

$$RSC (\%) = 100 \frac{A(\text{Blank}) - A(\text{sample})}{A(\text{Blank})} \quad (1)$$

The chart of the remaining DPPH concentration against the concentration of Trolox in standard samples was used to calculate the total antioxidant activity (TAA) of the wines. The antioxidant potency (AP) of total phenols for each cultivar wine was calculated as the ratio of TAA to total phenols (TP) [14]:

$$AP = \frac{TAA}{TP \cdot 100} \quad (2)$$

Determination of monomeric anthocyanins

The total monomeric anthocyanin content in the wine samples was determined by using the pH-differential method described by Guisti and Wrolstad (2003) [15]. Anthocyanins have the maximum absorbance at the wavelength of 520 nm and at the pH of 1.0. The color-oxonium form predominates at pH 1.0 and the color-less hemiketal forms at pH 4.5. The pH-differential method is based on this reaction and permits an accurate and rapid measurement of the total monomeric anthocyanins.

The result, considered as the monomeric anthocyanin

pigment, was calculated as mg of malvidin-3-glucoside, using a molar absorptivity (ϵ) of 28.000 L/mol·cm and a molecular weight of 493. The data presented are the averages of the three measurements.

Determination of the indices for the anthocyanin pigment degradation and polymeric color

Indices for the anthocyanin degradation in the wine can be derived by using the pH-differential method described by Guisti and Wrolstad (2003) [15]. The absorbance at 420 nm of the bisulfite treated sample served as an index for browning. The color density is defined as the sum of the absorbances at the maximum absorbance and at 420 nm. The ratio between the polymerization color and the color density was used to determine the percentage of the color. The data presented are the averages of the three measurements.

HPLC analysis of the individual anthocyanin compounds

For the determination of individual anthocyanin compounds, we used HPLC Agilent-1200 series with UV-Vis photodiode array (DAD). The column was thermostated at 30 °C. After injecting 5 μ L of the sample, the separation was performed in an Agilent-Eclipse XDB C-18 4.6×150 mm column. Peaks were identified by the comparison of retention times and UV spectra with commercial standards: delphinidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-glucoside, peonidin 3-glucoside and malvidin 3-glucoside, as well as with those found in literature [16]. The concentration of anthocyanins is expressed as mg/L malvidin 3-glucoside. The range of the linear calibration curve for malvidin 3-glucoside was from 0.5 to 50.0 mg/L ($R^2 = 0.991$). Two solvents were used for the gradient elution: A-(H₂O+5%HCOOH) and B-(80%ACN+5%HCOOH+H₂O). The elution program used was the following: from 0 to 28 min, 0.0 %B, from 28 to 35 min, 25 %B, from 35 to 40 min, 50 %B, from 40 to 45 min, 80 %B, and finally for the last 10 min again 0 %B.

Statistical analysis

All samples were reported and analyzed in triplicate. The statistical analysis was done with the one-way analysis of variance. The Pearson correlation coefficient (R) and p-value were used to show the correlations and their significance. Probability values of $p < 0.05$ and $p < 0.01$ were considered statistically significant and extremely significant, respectively [17].

Results and discussions

Total phenolic and flavonoid compound content

The results of the determination of the total phenolic content in red wines by the Folin-Ciocalteu method are presented in Table 2. The total phenolic content varies from 2381 to 3080 mg/L. According to these researchers, the most interesting sort with high levels of total phenols

is "Medvedja krv". The red wine "Medvedja krv" comes from ancient grape sorts "Vranac" and "Prokupac". "Vranac" originated in the Balkans and is the most common type in Serbia and Macedonia. This highly estimated sort yields intensely coloured, strong, full-bodied wines. The skin of "Prokupac" is of a dark blue colour, with characteristic dots. Unlike wines produced from some other red-berried varieties, the wines produced from Vranac and Prokupac are smooth and have a noticeable varietal flavour. These indigenous varieties are compared to the international wine varieties Cabernet Sauvignon, Merlot and Game.

Table 2. Total phenol (TP) and total flavonoid (TF) contents

Wine	TP ^a (mg GAE/L)	TF ^b (mg CE/L)	TF/TP
W1	2321 $\pm$ 81 ^c a	1578.9 $\pm$ 50.3 ^c a	0.68
W2	2738 $\pm$ 86 bc	1802.3 $\pm$ 68 bc	0.66
W3	3080 $\pm$ 101 bc	1950.2 $\pm$ 68.4 bc	0.63
W4	2800 $\pm$ 95 bc	1850.2 $\pm$ 61.6 bc	0.66
W5	2620 $\pm$ 95 ab	1762.3 $\pm$ 59.9 abc	0.67
W6	2665 $\pm$ 87 ab	1820.1 $\pm$ 62.7 bc	0.68
W7	2429 $\pm$ 82 a	1682.3 $\pm$ 57.2 ab	0.69
W8	2860 $\pm$ 105 bc	1998.4 $\pm$ 69.9 bc	0.7
W9	2377 $\pm$ 78 a	1580.2 $\pm$ 50.5 a	0.66
W10	2561 $\pm$ 84 ab	1772.3 $\pm$ 67.3 abc	0.69
W11	2668 $\pm$ 98 ab	1770.5 $\pm$ 60.2 abc	0.66
W12	2525 $\pm$ 84 ab	1720.2 $\pm$ 59.9 ab	0.68
W13	2850 $\pm$ 93 bc	1955.3 $\pm$ 66.5 c	0.69
W14	2379 $\pm$ 76 a	1735.8 $\pm$ 57.3 abc	0.73
W15	2850 $\pm$ 99 bc	1910.8 $\pm$ 63.8 c	0.66

^aThe level of total phenols is expressed as a gallic acid equivalent (GAE) and the data are reported as mean $\pm$ standard deviation (n=3)

^bThe level of total flavonoids is expressed as a catechin equivalent (CE) and the data are reported as mean $\pm$ standard deviation (n=3)

^cValues in the same columns sharing different letters expressed as significantly different ($p < 0.05$)

Significant differences were found in the total phenolic contents in comparisons among Vranac (Ohrid-Macedonia; Skopje-Macedonia), Vranac (Ovčepolsko-Macedonia) and Ždrebac (Subotica-Serbia) ($p < 0.05$); However, significant differences in total phenolic contents were not found between Vranac (Aleksandrovac-Serbia) and Vranac (Štip-Macedonia) and between Ždrebac (Subotica-Serbia) and Vranac (Aleksandrovac-Serbia) ($p < 0.05$). The total phenolic content of Cabernet Sauvignon (W7) and Game (W6), Merlot (W5) and Kratošija (W4) were similar ($p > 0.05$) but lower ($p < 0.05$) than that of Cabernet Sauvignon (W8), Vranac (W13) and Vranac (W15). It is well known that both genetic and agronomic or environmental factors play important roles in the phenolic composition and thus the nutritional quality of the crops.

While comparing these results with literature, similar values were reported for red wines which grow in Croatia (2193-3183 mg GAE/L, [18]) and Greece (1217-3772 mg GAE/L, [19]). Italian red wines have a higher value (3888-5860 mg GAE/L [20]), but Spanish red wines (1262-2389 mg GAE/L, [8]), Turkish red wines (1130-2410 mg GAE/L, [21]) and South African red wines (2016-2412 mg GAE/L, [13]) have similar values of the total phenolic content.

Furthermore, the results obtained from the evaluation of the total flavonoid content also indicate great variations (Table 2). The Cabernet Sauvignon (Tikveš-Macedonia) showed the highest flavonoid content (1998.4 ± 69.9 mg of the catechin equivalent/L of red wines), followed by Vranac (W13), Medveđa krv, Vranac (W15), Kratošia, Game, Medjaš blank, Vranac (W10), Vranac (W11), Merlot, Vranac (W14), Vranac (W12), Cabernet Sauvignon (W7), Ždrebac and Ždrepčeva krv. The flavonoid contents of Vranac (Ohrid-Macedonia) and Vranac (Skoplje-Macedonia) were significantly different from Ždrebac (Subotica-Serbia) ($p < 0.05$). However, any significant differences in the flavonoid content were not found in comparisons among Vranac (Ohrid-Macedonia), Vranac (Skoplje-Macedonia), Vranac (Ovčepolsko-Macedonia), Vranac (Kruševac-Serbia) and Vranac (Aleksandrovac-Serbia) ($p < 0.05$).

The average percentage of flavonoids in total phenols was 67.6 %. The high flavonoid content in the red vines contributes to its increased antioxidant potential in comparison to the white wine [18].

Free radical scavenging capacity (RSC)

The radical-scavenging capacity was evaluated by measuring the scavenging activity of the examined wine samples on 2,2-diphenyl-1-picrylhydrazyl (DPPH $\cdot$) radicals. All antioxidant results are summarized in Table 3. The percentage RSC for red wines was 74.8-87.9 %. The percentage for Croatian red wines [20] (after dilution 1:10, v/v) was 54.6-82.2 % and for Greek wines (after dilution 1:10, v/v) it was 42.2-60.0 % [21].

Table 3. Radical scavenging capacity (RSC), total antioxidant activity (TAA) and antioxidant potency (AP) in different red wines

Wine	RSC (%)	TAA ^a (mM Trolox)	AP ^b
W1	77.4 ± 0.9	13.5 ± 0.36^c a	5.82 ± 0.72
W2	83.7 ± 0.8	14.6 ± 0.48 a	5.33 ± 0.63
W3	85.9 ± 0.8	15.0 ± 0.48 a	4.87 ± 0.52
W4	83.7 ± 0.3	14.6 ± 0.45 a	5.21 ± 0.58
W5	81.8 ± 0.8	14.3 ± 0.47 a	5.46 ± 0.67
W6	80.3 ± 0.7	14.0 ± 0.43 a	5.25 ± 0.43
W7	78.5 ± 0.3	13.7 ± 0.46 a	5.64 ± 0.28
W8	87.9 ± 0.2	14.3 ± 0.46 a	5.00 ± 0.62
W9	77.8 ± 0.2	13.1 ± 0.43 a	5.51 ± 0.82
W10	79.5 ± 0.8	13.8 ± 0.52 a	5.41 ± 0.75
W11	84.6 ± 0.5	14.7 ± 0.48 a	5.53 ± 0.67
W12	82.6 ± 0.4	14.4 ± 0.46 a	5.71 ± 0.55
W13	83.8 ± 0.3	14.6 ± 0.38 a	5.13 ± 0.57
W14	79.5 ± 0.2	13.8 ± 0.39 a	5.80 ± 0.39
W15	86.3 ± 0.7	15.0 ± 0.40 a	5.28 ± 0.48

^aThe level of total antioxidant activity is expressed as a Trolox equivalent and the data are reported as mean $\pm$ standard deviation ($n=3$)

^bAP (antioxidant potency) = TAA (mM Trolox) $\times$ 1000 / total phenols (mg gallic acid equiv/L)

^cValues in the same columns sharing different letters expressed as significantly different ($p < 0.05$)

The total antioxidant activity (TAA) for wine samples was of the same order of magnitude using the DPPH scavenging assay when expressed in terms of Trolox. Any significant differences in TAA were not found between the analyzed red wines ($p < 0.05$). The TAA values of the red wines (13.1-15.0 mM Trolox) analyzed in this study were in the range of the wines from other wine producing countries such as France (9.6-29.9 mM Trolox, [22]), Italy (6.1-19.8 mM Trolox, [23]), Canada (7.5-28.6 mM Trolox, [24]) and Spain (14 mM Trolox, [25]).

Spectrophotometric analysis of anthocyanins

The monomeric anthocyanin content of the analyzed red wines is presented in Table 4. The mean monomeric anthocyanin content of the analyzed red wines was 63.3 mg/L. The highest concentration of the anthocyanin pigment was found in the young wine (Cabernet Sauvignon, 2010). The monomeric anthocyanin content of Cabernet Sauvignon (W8) was significantly different from all others ($p < 0.05$). However, any significant differences in the monomeric anthocyanin content were not observed among Vranac (Štip-Macedonia), Vranac (Ohrid-Macedonia) and Vranac (Skoplje-Macedonia) or among Ždrebac (Subotica-Serbia), Vranac (Aleksandrovac-Serbia) and Vranac (Kruševac-Serbia) ($p < 0.05$).

Table 4. Monomeric anthocyanins, percent polymeric color in different red wines determined by the spectrophotometric method

Wine	Monomeric anthocyanins ^a (mg/L)	Percent polymeric color (%) ^b
W1	62.2 ± 5.5^c bc	70.4 ± 0.9
W2	77.5 ± 6.2 c	65.2 ± 0.7
W3	46.6 ± 3.9 ab	76.9 ± 1.2
W4	43.1 ± 2.9 ab	77.1 ± 1.0
W5	38.8 ± 3.2 ab	79.9 ± 1.1
W6	63.5 ± 5.0 bc	70.1 ± 0.6
W7	30.5 ± 2.4 a	85.5 ± 0.8
W8	165.5 ± 9.9 c	36.5 ± 0.9
W9	54.1 ± 4.5 b	72.0 ± 1.2
W10	48.5 ± 4.2 ab	73.2 ± 1.1
W11	55.7 ± 4.2 bc	72.9 ± 0.7
W12	63.8 ± 5.3 bc	70.0 ± 0.6
W13	79.3 ± 5.4 c	66.2 ± 0.5
W14	50.2 ± 4.0 ab	80.0 ± 1.1
W15	70.3 ± 4.8 bc	70.1 ± 0.9

^aThe level of monomeric anthocyanins is expressed as a malvidin-3-glucoside equivalent and the data are reported as mean $\pm$ standard deviation ($n=3$)

^b%polymeric color = (polymeric color / color density) $\times$ 100

^cValues in the same columns sharing different letters expressed as significantly different ($p < 0.05$)

The polymeric anthocyanin pigments are stable compounds responsible for chromatic properties of the wine. These compounds are formed during the wine making process and in the time of aging through reactions which take place between free anthocyanins and tannin. During winemaking and aging, anthocyanins are thought

to react with tannins to give rise to polymeric pigments, the stable color compounds in the wine. In our case, the

polymeric color was 36.5-85.5 % depending on the age of the wine and the variety of the grapes used.

Table 5. Individual anthocyanins (mg/L) of red wines determined by HPLC

Wine	Merlot, W5	Vranac, W11	Vranac, W15
Delphinidin-3-glucoside	1.22 ± 0.02	4.75 ± 0.20	3.85 ± 0.09
Cyanidin-3-glucoside	0.13 ± 0.01	0.43 ± 0.05	1.27 ± 0.05
Petunidin-3-glucoside	1.52 ± 0.04	4.82 ± 0.18	5.72 ± 0.17
Peonidin-3-glucoside	0.54 ± 0.02	3.84 ± 0.11	6.75 ± 0.21
Malvidin-3-glucoside	15.47 ± 0.15	26.52 ± 0.35	33.29 ± 0.56
Delphinidin-3-glucoside acetate	0.76 ± 0.09	0.26 ± 0.03	0.28 ± 0.05
Cyanidin-3-glucoside acetate	1.09 ± 0.08	0.14 ± 0.02	0.38 ± 0.06
Petunidin-3-glucoside acetate	1.26 ± 0.03	0.28 ± 0.02	0.29 ± 0.03
Peonidin-3-glucoside acetate	nd	0.78 ± 0.07	1.01 ± 0.18
Malvidin-3-glucoside acetate	7.47 ± 0.10	2.34 ± 0.10	4.67 ± 0.12
Delphinidin-3-glucoside coumarate	0.62 ± 0.05	nd	0.46 ± 0.09
Cyanidin-3-glucoside coumarate	0.26 ± 0.01	0.10 ± 0.02	0.28 ± 0.07
Petunidin-3-glucoside coumarate	0.14 ± 0.02	0.09 ± 0.03	0.17 ± 0.06
Peonidin-3-glucoside coumarate	0.16 ± 0.02	0.64 ± 0.05	0.84 ± 0.12
Malvidin-3-glucoside coumarate	0.87 ± 0.02	2.68 ± 0.06	3.11 ± 0.18
Malvidin-3-glucoside coumarate-cis	nd	0.36 ± 0.03	0.43 ± 0.08
Vitisin A	1.62 ± 0.08	1.23 ± 0.10	1.36 ± 0.06
Malvidin-3-glucoside-vinylphenol	nd	0.07 ± 0.10	0.21 ± 0.03
Total ^a	33.13	49.65	64.23

^aThe value is expressed as malvidin-3-glucoside equivalent and the data are presented as mean ± standard deviation (n=3)

Figure 1. Chromatogram of the red wine. Peaks identification: Delphinidin-3-glucoside (1), Cyanidin-3-glucoside (2), Petunidin-3-glucoside (3), Peonidin-3-glucoside (4), Malvidin-3-glucoside (5), Delphinidin-3-glucoside acetate (6), Vitisin A (7), Cyanidin-3-glucoside acetate (8), Petunidin-3-glucoside acetate (9), Delphinidin-3-glucoside coumarate (10), Peonidin-3-glucoside acetate (11), Malvidin-3-glucoside acetate (12), Cyanidin-3-glucoside coumarate (13), Malvidin-3-glucoside coumarate-cis (14), Petunidin-3-glucoside coumarate (15), Peonidin-3-glucoside coumarate (16), Malvidin-3-glucoside coumarate (17), Malvidin-3-glucoside-vinylphenol (18). Chromatogram was recorded at 520 nm. Chromatographic separations were obtained on a Agilent-Eclipse XDB C-18 column (5.0 µm particle size, 4.6×150 mm) using a binary gradient (mobile phase A, 5 % HCOOH in water; mobile phase B, 80 % ACN and 5 % HCOOH in water). The operating conditions were: flow rate 0.8 ml/min; column temperature 30 °C.

HPLC analysis of individual anthocyanins in red wines Vranac and Merlot

In Table 5 the results of individual anthocyanin compounds in the red wine Vranac (Serbia and Macedonia) and Merlot (Serbia) are reported. Fig. 1 shows the chromatogram of the wine samples monitored at 520 nm. In all cases, the predominant anthocyanin was malvidin-3-glucoside (mean value 25.09 mg/L), accounting for approximately 45.7 % of all pigments in contrast. Cyaniding-3-glucoside had the lowest mean content (0.61 mg/L), with its percentage to the overall concentration being only 1.1 %. Malvidin-3-glucoside was the main anthocyanin of all varieties. Therefore, its derivatives were also the main anthocyanins in the acetylated and coumaroylated forms, and the anthocyanins in acetylated forms were significantly higher for Merlot than for Vranac

Correlations

In this research, red wine varieties showed a high TP and TF content and high TAA. A significant correlation between TAA and TP ($R^2 = 0.85$) (Fig. 2) and TAA and TF content ($R^2 = 0.77$) (Fig.3) was found. Further, a good correlation ($R^2 = 0.16$) between TAA and MA content was not found (Fig. 4). This suggests that anthocyanins may not be the most powerful radical scavengers that occur in grape berry skins. Recently, many authors [26, 27] found a linear correlation between the phenolic content, antioxidant activity and the level of anthocyanins. On the other hand, other researchers [19, 27, 28] determined that the correlation of total phenols to antiradical or antioxidant activity was even higher but poor with the total anthocya-

Figure 2. Correlation between the total phenolics and total antioxidant activity

Figure 3. Correlation between the total flavonoids and total antioxidant activity

Figure 4. Correlation between monomeric anthocyanins and the total antioxidant activity

Conclusion

This study showed that, although all the analyzed red wines contained the phenolic compounds, flavonoids and anthocyanins and their contents were significantly different among the grape varieties. All red wine varieties represent a potential source of natural antioxidants, but the variety Medvedja krv showed a better performance. Serbian and Macedonian red wines, produced from indigenous varieties Prokupac and Vranac, can be considered to be a rich source of dietary antioxidants. Our results also showed a significant correlation between the TAA with the TP and TF.

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Izvod

FENOLNI SASTAV I ANTIOKSIDATIVNA AKTIVNOST CRVENIH VINA IZ SRBIJE

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Cilj ovog rada je bio da se ispita fenolni sastav i slobodno-radikalna hvatačka aktivnost, koristeći DPPH metodu, odabranih crvenih vina iz Srbije. Vina od crvenih sorti grožđa, različitih berbi, su analizirana u junu 2012. Standardna spektrofotometrijska metoda je korišćena za analizu glavnih grupa jedinjenja u odabranim vinima. Nakon analize je utvrđeno da vina "Medveđa krv" i Vranac imaju najveći sadržaj totalnih fenola sa 3080 ± 101 i 2850 ± 93 mg/l, respektivno. Sva vina su pokazala izuzetnu antioksidativnu aktivnost. U svim slučajevima malvidin-3-glukozid je najzastupljeniji antocijanin. Uskladu sa dobijenim rezultatima, crvena vina iz Srbije mogu da budu dobar izvor polifenola u ishrani.

Ključne reči: crvena vina, antioksidativna aktivnost, spektrofotometrijska metoda, HPLC metoda.