Free Radical Scavenging Activity and Phenolic Profile of Selected Serbian Red Fruit Wines

MILAN N. MITIC, MIRJANA V. OBRADOVIC, SNEZANA S. MITIC, ALEKSANDRA N. PAVLOVIC,* JOVANA LJ. PAVLOVIC, BRANKA T. STOJANOVIC

Faculty of Sciences and Mathematics, Department of Chemistry, University of Niš, Višegradska 33, P.O.Box 224, 18000 Niš, Serbia

The objective of the study was to determine the total phenolic content (TPC), the total antioxidant activity (TAA) and the anthocyanin, hydroxycinnamic acid and flavonol profile in three categories of fruit wines (sour cherry, blackberry ant raspberry wines). Among the wines of different fruit sources, TAA ranged from 3.91 to 17.74 mmol TE (Trolox Equivalent)/L. TPC were the highest in blackberry wines. The amount of anthocyanins determined by HPLC method ranged from 103.20 to 465.73 mg/L in raspberry and blackberry wines, respectively. The major anthocyanins in blackberry wines were cyanidin-3-glucoside, in sour cherry wines cyanidin-3-glucosyl-rutinoside and raspberry wines cyanidin-3-sophoroside. Caffeic acid was the most abudant hydroxycinnamic acid in fruit wines, followed by p-coumaric acid. Also, the amounts of flavonols (quercetin and kaempferol) were determined in fruit wines (0.98-6.51 mg/L). A high correlation was observed between the antioxidant activity and the phenolic content of the investigated red fruit wines.

Keywords: fruit wines, phenolic composition, antioxidant activity, anthocyanins, hydroxycinnamic acids, flavonols

The consumption of fruits and vegetables results in protection against cancer [1], cardiovascular diseases [2], and cerebrovascular diseases [3]. Their antioxidant constituents seem to be responsible for these health effects.

Berries and red fruits are two of the most important dietary sources of polyphenols such as anthocyanins, flavonols, flavan 3-ols and benzoic and cinnamic acid derivatives [4]. Numerous in vitro studies have now reported various health effects that these fruits have when they are included in the human diet, among those being the high antiradical activity of berries [5] and the capacity to inhibit the human low-density lipoprotein and liposome oxidation [6]. Many reports have been written about the phenolic profile in different fruit and berry samples - from fresh, freeze-dried, and frozen fruits [7, 8], as well as from juices and fruit extracts as raw materials [9]. However, only a few reports have been based on fruit wines [10, 11].

Wines consist of different phenolic compounds, so the antioxidant and the biological activities of wine are connected with the synergy of these compounds. Recent studies indicate that the consumption of small amounts of red wine on a regular basis reduces the risk of coronary heart diseases and atherosclerosis, and this benefit is ascribed to the antioxidant properties of the polyphenolic compounds [12].

For the production of berry and fruit wines, the pressed juice is made from the fruit and berries such as apples, cherries, red currants, cranberries, raspberries [13]. In general, the berry and fruit wine-making process is the same as the making of wine from grapes; that is, firstly, the berry or fruit mash is pressed, and the pressed juice is then fermentated. Berry and fruit wines are produced industrially in many countries, e.g. apple wine (cider) in France, the United Kingdom, and the United States and pear wine known as “poire” in France [13]. Other berry and fruit wines are produced mainly for domestic use in some European Union countries. There is such a tradition of producing wines from fruits in Serbia.

Therefore, the objective of this work is to evaluate various red fruit wines (sour cherry, blackberry and raspberry wines) regarding the amount of polyphenols and the antioxidant activity and to determine the individual polyphenolic compounds.

Experimental part

Materials and methods

Chemicals

Standards of quercetin, kaempferol and the phenolic acid standards, such as gallic, ferulic, p-coumaric and caffeic acids, were purchased from Sigma Chemicals Co. (Saint Louis, Mo, USA). Cyanidin-3-glucoside and cyanidin-3-rutinoside were purchased from Extrasynthese S.A.S (Ganay, France). 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). 6-hydroxy-2,5,7,8-tetramethyl-chromancarboxylic acid (Trolox) and Folin-Ciocalteu’s phenol reagent were obtained from Merck KGaA (Darmstadt, Germany). Other chemicals and solvent were of analytical grade.

Fruit wines

The totals of 6 commercial and 6 domestic fruit wines were analyzed. The commercial fruit wines belonging to different commercial trade marks were purchased from the local markets. The domestic wines were made by the following procedure: sugar was added to the measured amount of fruit prior to fermentation. The wine fermentation was set at room temperature for 40 days. The samples were prepared according to the method of Escarpa and Gonzalez [14]: 10 mL of samples was extracted with 25 mL methanol containing 1% HCl, using an ultrasonic bath.

Determination of the total phenolic compounds

Folin-Ciocalteu reagent was used to determine the total phenolic compounds [15]. A volume of 1 mL of fruit wine

*email: aleksandra.pavlovic@pmf.edu.rs; Tel. : +381 18 533-015

extract, previously diluted 5-6 times with methanol was mixed with 0.5 mL of Folin-Ciocalteu reagent, previously diluted with distilled water (1:2). A volume of 2 mL of 20% sodium carbonate solution was added to the mixture, shaken thoroughly and diluted to 10 mL by adding distilled water. The mixture was to stand for 120 min and the formed blue color was measured at 760 nm with a spectrophotometer (UV/Vis spectrometer Agilent 8454). Gallic acid was used as the standard for the calibration curve. The concentrations of gallic acid in the solution from which the curve was prepared were 0, 50, 100, 150, 250 and 500 mg/L (R^2 = 0.998). The content of TP was expressed as mg of gallic acid equivalent (GAE) per 1L of fruit wines. All the measurements were carried out in three repetitions.

Measurement of the DPPH· scavenging activity

The free radical scavenging capacity of fruit wine extracts was determined according to the previously reported procedure using the stable DPPH radicals [16]. The method was based on the reduction of stable DPPH nitrogen radicals in the presence of antioxidants. An aliquot (2.5 mL) of fruit wines extracts or methanol solution of Trolox (10-30 mM) was mixed with 2.5 mL of 0.1 mM DPPH methanolic solution. The mixture was thoroughly vortexed, kept in the dark for 30 min, and after that the absorbance was measured at 515 nm against a blank of methanol without DPPH. The results were calculated according to the calibration curve for Trolox (R^2 = 0.996). DPPH values, derived from triplicate analyses, were expressed as mmol of TE per 1L of fruit wines.

HPLC-DAD determination of polyphenolic composition

The individual phenolics were analyzed by the direct injection of the extracts (previously filtered through a 0.45 mm pore size membrane filter) into an Agilent 1200 chromatographic system equipped with a quaternary pump, and Agilent 1200 photodiode array detector with radiofrequency identification tracking technology for flow cells and automatic injector and ChemStation software. The column temperature was 30^{0}C. After injecting 5 mL of sample extract, the separation was performed in the Agilent-Eclipse XDBC-18 4.6 × 150 mm column. Two solvents were used for the gradient elution: A (H_2O + 5% HCOOH) and B (80% ACN + 5% HCOOH + H_2O). The elution program used was as follows: from 0 to 10 min 0% B, from 10 to 28 min gradually increases 0-25% B, from 28 to 30min 25% B, from 30 to 35 min gradually increases 25-50% B, from 35 to 40 min gradually increases 50-80% B, and finally for the last 5 min gradually decreases 80-0% B. The detection wavelengths were 320, 360, and 520 nm. The identification and quantization of the various phenolic compounds were performed by means of calibration curves obtained with standard solutions of cyanidin-3-glucoside, cyanidin-3-rutinoside, quercetin, kaempferol, caffeic acid, p-coumaric acid, and ferulic acid. The results are expressed as mg per liter of fruit wines.

Statistical analysis

The data were reported as mean ± standard deviation (SD) for triplicate determinations. The significance of inter-group differences was determined by the analysis of variance (ANOVA). The p value of $p < 0.05$ was considered statistically significant.

Results and discussions

The total phenolics and the total antioxidant capacity

The results of determining the total phenolic content (TPC) of the selected monovarietal red fruit wines from Serbia are presented in table 1. The TP content was the highest in blackberry wines of the both samples (2792.8 and 2715.3 mg GAE/L), followed by sour cherry wines (2680.2 and 2275.2 mg GAE/L), and finally raspberry wines. The phenolic content of the blackberry wines (1,2) was significantly different from the other blackberry wines (3,4) ($p < 0.05$). However, no significant differences in the total phenolic content were found among the raspberry wine (1), raspberry wine (3) and raspberry wine (4). Also, the phenolic content of the commercial sour cherry wine (2) was significantly different from other sour cherry wines ($p < 0.05$); the commercial blackberry wine (2) was significantly different from other blackberry wines and finally the commercial raspberry wine (2) was significantly different from other raspberry wines ($p < 0.05$), respectively. It is well known that the genetic, agronomic or environmental factors and different wine-making techniques play an important role in the phenolic composition of wines. In this study, there was a 1.76-fold difference in the total content between the highest and lowest ranked blackberry wine samples, commercial (1) and commercial (2); 1.73-fold difference measured sour cherry wine samples, and 1.42-fold difference measured raspberry wine samples, respectively.

Comparing these results with the literature, the similar values were reported for red fruit juices and red fruit wines grown in other countries [10, 17-20].

The total antioxidant activity (TAA) of the 12 fruit wines, expressed in millimoles (mmol) of Trolox equivalent per L of fruit wine, is shown in table 1. The commercial blackberry wine (1) had the highest antioxidant activity, followed by the domestic blackberry wine (4), the domestic sour cherry (4), and finally the commercial raspberry wine (2). A significant difference was found between the commercial blackberry wine (1) and the commercial blackberry wine (2) ($p<0.05$). Also, a significant difference was found between the domestic blackberry wine (4) and the domestic wine (3). However, no significant differences in the total antioxidant activity were found between the domestic raspberry wines (3 and 4). The samples containing high total phenolic content had higher antioxidant activities. The present study reveals a very good correlation between the total antioxidant activity and the total phenolics (R^2 = 0.96) (fig. 1).

HPLC analysis

For a better a description of Serbian commercial and domestic fruit wines, the profiles of individual anthocyanin compounds were studied. In order to separate and determine individual antocyanic compounds present in red fruit wines, HPLC method was applied. The HPLC chromatograms of red fruit wines recorded at 520 nm are presented in figure 2. The amounts of anthocyanins in red fruit wines are shown in table 2.

The concentration of total anthocyanins of the investigated fruit wines ranged from 103.2 (commercial raspberry wines, 2) to 465.7 (commercial blackberry wines, 5). Among all the fruit wines, blackberry wine had the highest anthocyanin content, followed by sour cherry wines and raspberry wines. The concentration of the total monomeric anthocyanins were found to correlate with the total antioxidant activity of wines (R^2 = 0.84) (fig. 3). Acording to the present study, the antioxidant activity correlated better with the polyphenol content than with the anthocyanin content. It is well known that berries contain a large amount of phenolic compounds that act as antioxidant beside anthocyanins [21, 22]. Another possible reason for this observation could be the different antioxidant

Wine	Number of samples	Company, Location	Total polyphenols[a)] (mgGAE/l)	Total antioxidant activity[b)] (mMTE/l)
Sour cherry				
commercial	1	Vinarija Jović, Knjaževac	2250.20 ± 56.66[a]	13.17 ± 0.20[a]
commercial	2	Vino Župa, Aleksandrovac	1533.20 ± 15.15[b]	4.58 ± 0.53[b]
domestic	3	Knjaževac	2037.77 ± 19.35[c]	12.52 ± 0.78[a]
domestic	4	Stara planina	2651.87 ± 32.10[d]	16.89 ± 0.51[c]
Blackberry				
commercial	1	Blumen R. G., Beograd	2836.17 ± 44.71[a]	17.74 ± 0.95[a]
commercial	2	Foodland, Brus	1607.93 ± 43.54[b]	10.36 ± 0.38[b]
domestic	3	Knjaževac	2176.07 ± 25.78[c]	12.06 ± 0.53[b]
domestic	4	Stara planina	2752.03 ± 33.93[a]	17.41 ± 0.51[a]
Raspberry				
commercial	1	Vino Župa, Aleksandrovac	1466.47 ± 14.18[a]	7.91 ± 0.82[a]
commercial	2	Foodland, Brus	1051.90 ± 10.58[b]	3.91 ± 0.15[b]
domestic	3	Knjaževac	1490.40 ± 10.06[a]	6.05 ± 0.13[c]
domestic	4	Stara planina	1405.40 ± 30.38[c]	7.10 ± 0.18[ac]

Table 1
CONCENTRATION OF TOTAL POLYPHENOL CONTENT (TPC) AND TOTAL ANTIOXIDANT ACTIVITY (TAA) OF FRUIT WINES

[a)] The level of total phenolics is expressed as gallic acid equivalent (GAE) and the data are reported as mean ± standard deviation (n = 3)
[b)] The level of total antioxidant activity is expressed as Trolox equivalent (TE) and the data are reported as mean ± standard deviation (n = 3)
[a-c] Bars with no letters in common are significantly different ($p < 0.05$) in the same column for each type of fruit wines

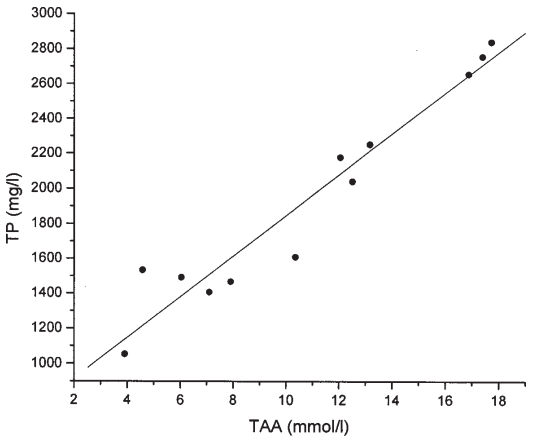

Fig. 1. Relationship between the total phenolics and total antioxidant activity of the selected fruit wine samples.

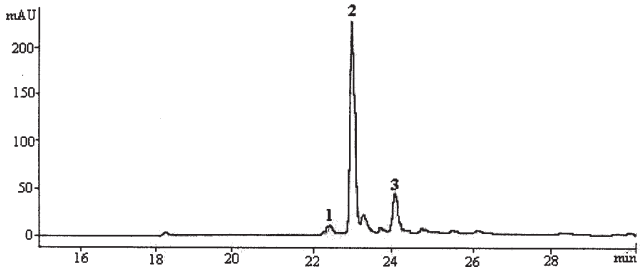

Fig. 2a. HPLC profile of sour cherry wine. Identification peaks: cyanidin-3-sophoroside (1); cyanidin-3-glucosyl-rutinoside (2) and cyanidin-3-rutinoside (3)

potential of different anthocyanins which is determined by other structural characteristics [22, 23].

Cyanidin-3-glucoside was the major anthocyanin in blackberry wines (209.3-403.8, 80.6-86.7% of the total anthocyanin content) (except for the commercial blackberry wine, 2). Cyanidin-3-rutinoside was found only in the commercial blackberry wine (2). Generally, blackberry wines contained higher levels of the total anthocyanins (table 2). According to the data in the literature, we identified cyanidin-derivative as cyanidin-3-xyloside. These data are in accordance with those found in literature [17, 24].

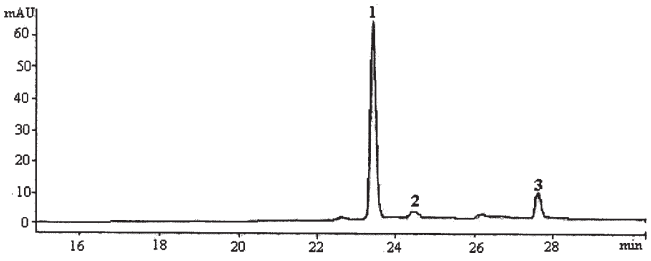

Fig. 2b. HPLC profile of blackberry wine. Identification peaks: cyanidin-3-glucoside (1); cyanidin-3-rutinoside (2) and cyanidin-3-xyloside (3)

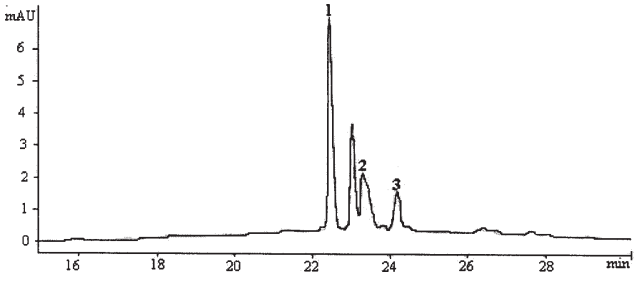

Fig. 2c. HPLC profile of raspberry wine. Identification peaks: cyanidin-3-sophoroside (1); cyanidin-3-glucoside (2) and cyanidin-3-rutinoside (3)

Sour cherry wines contained only cyaniding based pigments. Sour cherry wines contained a mixture of three different cyaniding-glycosides: cyanidin-3-glucosyl-rutinoside, cyanidin-3-rutinoside and cyanidin-derivative. According to the data in the literature we identified cyanidin-derivative as cyanidin-3-sophoroside [17]. The main anthocyanin found in sour cherry wines which represent 74.2-80.8% of the total anthocyanin content, was cyanidin-3-glucosyl-rutinoside. The concentration of cyanidin-3-sophoroside was low (0-3.6%). The previous study confirmed that the anthocyanins present in sour cherry wines are cyanidin-3-glucosyl-rutinoside, cyanidin-3-rutinoside and cyanidin-3-sophoroside, which is in accordance with our results [17, 25- 27].

Raspberry wines contained a mixture of three different cyanidin-glycosides: cyanidin-3-sophoroside, cyanidin-3-

Wines	Concentration mg/l (total anthocyanins [%])[a]			
Sour Cherry	1	2	3	4
Cy-3-sopho	/	/	9.1 ± 0.7(3.6)	8.5 ± 0.4(3.1)
Cy-3-glu-rut	208.5 ± 1.3(74.2)	86.8 ± 1.2(80.8)	196.1±1.6(77.8)	215.3 ± 1.7(76.6)
Cy-3-rut	72.3 ± 0.9(25.8)	20.6 ± 0.8(19.2)	46.9 ± 0.9(18.6)	57.3 ± 1.2(20.3)
total	280.8 ± 2.2(100.0)	107.4 ± 2.0(100.0)	252.1 ± 3.2(100.0)	281.1 ± 3.3(100.0)
Blackberry	1	2	3	4
Cy-3-glu	403.8 ± 2.0(86.7)	35.4 ± 0.2(20.3)	209.3 ± 0.7(80.6)	305.7 ± 1.8(87.7)
Cy-3-rut	/	39.3 ± 0.5(22.5)	/	/
Cy-3-xyl	61.9 ± 1.1(13.3)	99.7 ± 1.1(57.2)	50.3 ± 0.4(19.4)	42.8 ± 0.4(12.3)
total	465.7 ± 3.1(100.0)	174.4 ± 1.8(100.0)	259.6 ± 1.1(100.0)	348.5 ± 2.2(100.0)
Raspberry	1	2	3	4
Cy-3-sopho	178.9 ± 1.5(69.5)	91.8 ± 1.6(88.9)	153.7 ± 1.2(73.0)	201.5 ± 1.0(83.8)
Cy-3-glu	45.8 ± 0.3(17.8)	3.5 ± 0.1(3.4)	38.2 ± 0.4(18.1)	14.3 ± 0.5(5.9)
Cy-3-rut	32.8±0.3(12.7)	7.9±0.2(7.7)	18.5±0.3(8.9)	24.5±0.3(10.3)
total	257.5±2.1(100.0)	103.2±1.9(100.0)	210.4±1.9(100.0)	240.3±1.8(100.0)

[a] The data are reported as mean ± standard deviation (n = 3)

Table 2
CONCENTRATION OF ANTHOCYANINS IN FRUIT WINES (mg/L) DETERMINED BY HPLC METHOD AND PERCENTAGE DISTRIBUTION OF ANTHOCYANINS

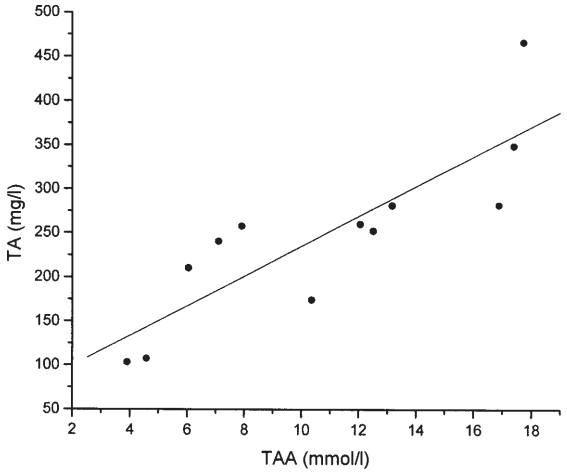

Fig. 3. Relationship between the total anthocyanins and total antioxidant activity of the selected fruit wine samples

Fruit wines	Concentration (mg/L) [a]			
Sour cherry	1	2	3	4
Quercetin	3.74 ± 0.22	1.13 ± 0.12	2.63 ± 0.22	3.05 ± 0.18
Kaempferol	2.76±0.18	0.82 ± 0.08	1.58 ± 0.18	2.16 ± 0.16
Total	6.51 ± 0.20	1.95 ± 0.10	4.21± 0.20	5.21 ± 0.17
Blackberry	1	2	3	4
Quercetin	4.07 ± 0.28	3.87 ± 0.19	4.90 ± 0.32	4.72 ± 0.19
Kaempferol	0.45 ± 0.05	0.30 ± 0.05	0.40 ± 0.05	0.85 ± 0.07
Total	4.52 ± 0.16	4.17 ± 0.12	5.30 ± 0.18	5.57 ± 0.13
Raspberry	1	2	3	4
Quercetin	0.98 ± 0.05	1.20 ± 0.09	1.32 ± 0.08	1.80 ± 0.08
Kaempferol	nd	nd	nd	nd
Total	0.98 ± 0.05	1.20 ± 0.09	1.32 ± 0.08	1.80 ± 0.08

[a] The data are reported as mean ± standard deviation (n = 3)

Table 3
CONCENTRATION OF FLAVONOLS IN FRUIT WINES (mg/L) DETERMINED BY HPLC METHOD

glucoside and cyanidin-3-rutinoside. The mean anthocyanin in the raspberry wines, which measured 69.5-88.9% of the total anthocyanin content, was cyanidin-3-sophoroside (91.8-201.5 mg/L). Cyanidin-3-glucosyde and cyanidin-3-rutinoside were found in relatively lower amounts (3.5-45.8 and 7.9-32.8 mg/L, respectively). However, the anthocyanin profiles of various raspberry wines were heterogeneous: the two samples contained cyanidin-3-glucoside > cyanidin-3-rutinoside, whereas for the other two samples cyanidin-3-glucoside < cyanidin-3-rutinoside.

Flavonols and hydroxycinnamic acids were determined by using HPLC method (table 3 and 4). The highest concentration of flavonols (4.89 mg/L) was found in blackberry wines. The dominant flavonol in blackberry wines was quercetin (4.39 mg/L), where as kaempferol was found at considerably lower concentration (0.5 mg/L). Myricetin was no detected in all blackberry wines. The data presented by other authors also confirmed that the major flavonol in blackberry is quercetin [23, 28]. Hydroxycinnamic acids in our sample of blackberry wines were caffeic, as the dominant one (3.83-9.86 mg/L), p-coumaric (0.80-3.01 mg/L) and ferulic acid (1.79-2.68 mg/L). These hydroxycinnamic acids were identified in blackberries in the previous studies as well [29].

In sour cherry wines, the concentration of hydroxycinnamic acid was higher than the concentration of flavonols. The main hydroxycinnamic acid found in sour cherry was caffeic acid (9.85-13.79 mg/L), followed by p-

Fruit wines	Concentration (mg/L) [a]			
Sour cherry	1	2	3	4
Caffeic acid	13.79 ± 0.38	11.52 ± 0.32	12.83±0.42	9.85 ± 0.30
p-coumaric acid	10.38 ± 0.29	1.88 ± 0.22	9.63±0.18	7.38 ± 0.25
Ferulic acid	nd	nd	nd	nd
Total	98.81 ± 0.56	73.84±0.48	80.96 ± 0.42	77.95 ± 0.46
Blackberry	1	2	3	4
Caffeic acid	7.62 ± 0.36	3.83 ± 0.28	9.86 ± 0.25	8.32 ± 0.35
p-coumaric acid	1.08 ± 0.15	0,80 ± 0.06	2.08 ± 0.12	3.01 ± 0.22
Ferulic acid	nd	nd	2.68 ± 0.09	1.79 ± 0.10
Total	21.08 ± 0.30	6.42±0.17	28.17 ± 0.17	30.94 ± 0.30
Raspberry	1	2	3	4
Caffeic acid	5.31 ± 0.16	2.99 ± 0.12	7.38 ± 0.52	6.82 ± 0.24
p-coumaric acid	nd	nd	1.08 ± 0.15	1.85 ± 0.20
Ferulic acid	nd	6.42 ± 0.18	3.28 ± 0.18	1.05 ± 0.16
Total	28.13 ± 0.50	9.41 ± 0.15	24.12 ± 0.32	20.35 ± 0.24

[a] The data are reported as mean ± standard deviation (n = 3)

Table 4
CONCENTRATION OF HYDROXYCINNAMIC ACIDS IN FRUIT WINES (mg/L) DETERMINED BY HPLC METHOD

Value	PCA1	PCA2	PCA3
Eigenvalue	2.620	0.296	0.084
% Total variance	87.349	9.865	2.786
Cumulative %	87.349	97.214	100.0

Table 5
EXPLAINED VARIANCE AND EIGENVALUES

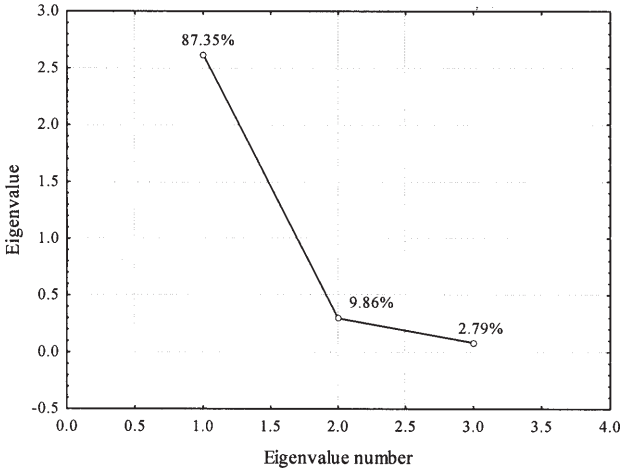

Fig. 4. Score plot of successive eigenvalues

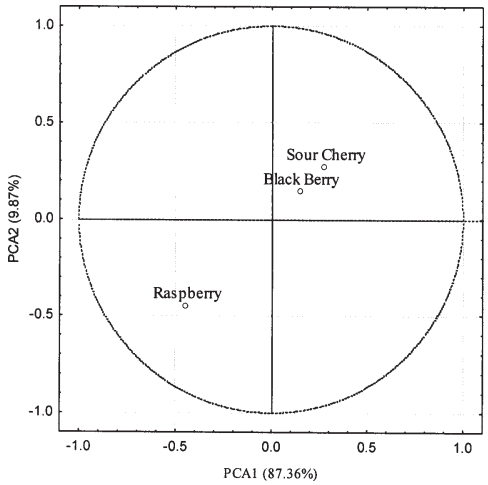

Fig. 5. Principal component score plot (PCA1 and PCA2) of the studies of fruit wines based on spectrophotometric data for the total phenols

coumaric acid (1.88-10.38 mg/L). Ferulic acid was not detected in all sour cherry wines. Flavonols found in low concentration in sour cherry wines were quercetin (1.13-3.74 mg/L) and kaempferol (0.82-2.76 mg/L). These results are in accordance with those reported by Jakobek et al. [17].

Like sour cherry wines, red raspberry wines were characterized by a relatively higher concentration of hydroxycinnamic acids in comparison to flavonols. Caffeic acid (2.99-7.38 mg/L) was the dominant hydroxycinnamic acid whereas p-coumaric and ferulic acid were found at lower concentration. The only flavonol found in red raspberry wines was quercetin (0.98-1.80 mg/L) which agrees with the previous study [30].

Principal component analysis

Visualization method such as principal component analysis (PCA) has been applied to the data set. From table 5 and figure 4, it can be seen that the first two eigenvalues and their corresponding principal components should be retained. The third component explains only a small amount of the variance (~2.8%).

The relationship between PCA1 (which explained 87.36% of the total variance) and PCA2 (which explained a further 9.87%) is shown, along with graphical representation of the contributions of the original variables to both PCA1 and PCA2 on figure 5.

Fruit wines from Sour Cherry and Blackberry have a small contribution to PCA1 and PCA2, as seen from their small positive components on the PCA1 and PCA2 axes. Fruit wine from Raspberry has a small negative contribution to PCA axes. In other words, Sour Cherry and Blackberry fruit wines at the right-hand side of the score plot contain a higher total polyphenol content than the Raspberry sample at the left-hand side.

Conclusions

The results suggest that the investigated red fruit wines contain a high content of different group of polyphenols, which have a potent antioxidant capacity. Generally, the healthiest fruit wines are blackberry wines. The red fruit wines evaluated in this study show significant variations in the anthocyanins content and profile. Some wines have a

low amount of anthocyanins, as sour cherry wines, whereas the amount of these phytochemicals is very high in blackberry wines. The concentrations of flavonols and hydroxycinnamic acids in red fruit wines are under investigation. The predominant phenolic acid is neochlorogenic acid. The predominant flavonol is quercetin. Myricetin is not detected in any of the samples. This data are in accordance with those found in the literature.

Acknowledgements: This work was supported by the Serbian Ministry of Education and Science (grant numbers 174007 and 31060).

References

1.DRAGSTED, L.O., STRUBE, M., LARSEN, J.C., Pharmacol. Toxicol., **72**, no. 1, 1993, p. 116
2.NESS, A.R., POWEL, J.W., Int. J. Epidemiol., **26**, no. 1, 1997, p. 1
3.JOSEPH, J.A., SHUKITT-HALE, B., DENISOVA, N.A., BIELINSKI C., MARTIN, A., MCEWEN, J.J.J., Neurosci., **19**, no. 18, 1999, p. 8114
4.MACHEIX, J.J., FLEURIET, A., BILLOT, J., Fruit phenolics, CRC, Boca Ration, FL, 1990.
5.WANG, S.Y., JIAO, H., J. Agric. Food Chem., **48**, no. 11, 2000, p. 5672
6.HEINONEN, I.M., MEYER, A.S., FRANKRL, E.N., J. Agric. Food Chem., **46**, no. 10, 1998, p. 4107
7.HAKKINEN, S.H., HEINONEN, I.M., KARENLAMPI, S., MYKKANEN, H., RUUSKANEN, J., TORRONEN, A.R., Food Res. Int., **32**, no. 5, 1999, p. 345
8.HAKKINEN, S.H., KARENLAMPI, S.O., HEINONEN, I.M., MYKKANEN, H.M., TORRONEN, A.R., J. Agric. Food Chem., **47**, no. 6, 1999, p. 2274
9.NETZEL, M., STRASS, G., KAUL, C., BITSCH, I., DIETRICH, H., BITSCH, R. Food Res. Int., **35**, no. 2-3, 2002, p. 213
10.DEY, G., NEGI, B., GANDHI, A., Nat. Prod. Rad., **8**, no. July-August, 2009, p. 314
11.TARKO, T., DUDA-CHODAK, A., SROKA, P., SATORA, P., JURASZ, E. Acta Sci. Pol., Techol. Aliment., **7**, no. 3, 2008, p. 35
12.RENAUD, S., de LOREGIL, M., The Lancet, **339**, no. 8808, 1992, p. 1523
13.BELITZ, H.D., GROCH, W., Food Chemistry, Springer-Yerlag Berlin Heidelberg, Berlin, 1987, P. 774
14.ESCARPA, A., GONZALEZ, M.C., J. Chromatogr. A., **897**, no. 1-2, 2000, p. 161
15.SINGLETON, V.L., ORTHOFER, R., LAMUELA-RAVANTOS, R.M., Meth. Enzym., **29**, 1999, p. 152
16.LIU, Q., YAO, H., Food Chem., 102, no. 3, 2007, p. 732
17.JAKOBEK, L., ŠERUGA, M., MEDVIDOVIĆ-KOSANOVIĆ, M., NOVAK, I., Dtsch. Lebensm.-Rundsch., **103**, no. 2, 2007, p. 58
18.HEINONEN, M.I., LEHTONEN, P.J., HOPIA, A.I., J. Agric. Food Chem., **46**, no. 1, 1998, p. 25
19.RUPASINGHE, V.H.P., CLEGG, S., J. Food Compos. Anal., **20**, no. 2, 2007, p. 133
20.POPA, C.V., DANET, A.F., JIPA, S., ZAHARESCU, T., Rev. Chim. (Bucharest), **61**, no. 1, 2010, p. 11
21.PRIO, R.L., CAO, G., MARTIN, A., SOFIC, E., McEWEN, J., O'BRIEN, C., LISCHNER, N., EHLENFELDT, M ., KALT, W., KREWER, G., MAINLAND, C.M., J. Agric. Food Chem., **46**, no. 7, 1998, p. 2686
22.DENEV, P., CIZ, M., AMBROZOVA, G., LOJEK, A., YANAKIEVA, I., KRATCHANOVA, M., Food Chem., **123**, no. 4, 2010, p. 1055
23.ZHENG, W., WANG., S.Y., J. Agric. Food Chem., **51**, no. 2, 2003, p. 502
24.FAN-CHIANG, H.J., WROLSTAD, R.E., J. Food Sci., **2005**, **70**, 2005, p. c198
25.BLANDOI, F., GERARDI, C., NICOLETTI, I., J. Biomed. Biotech., **2004**, no. 5, 2004, p. 253
26.BLANDO, F., SCARDINO, A.P., De BELLIS, L., NICOLETTI, I.; GIOVINAZZO, G., Food Res. Int., **38**, no. 8-9, 2005, p. 937
27.MULABAGAL, V., LANG, G.A., DeWITT, D.L., DALAVOY, S., NAIR, M.G., J. Agric. Food Chem., **57**, no. 4, 2009, p. 1239
28.TURKBEN, C, SARIBURUN, E., DEMIR, C., UYLASER, V., Food Anal. Method., **3**, no. 3, 2010, p. 144
29.SELLAPPAN, S., AKOH, C.C., KREWER, G., J. Agric. Food Chem., **50**, no. 8, 2002, p.2432
30.HAKKINEN, S., HEINONEN, M., KARENLAMPI, S., MYKKANEN, H., RUUSKANEN, J., TORRONEN., R., Food Res. Int., **32**, no. 5, 1999, p.345

Manuscript received: 8.01.2012