Evaluation of Mineral and Heavy Metal Contents in Croatian Blackberry Wines

Daniela AMIDŽIĆ KLARIĆ[1], Ilija KLARIĆ[2], Darko VELIĆ[3] and Irena VEDRINA DRAGOJEVIĆ[1]

[1]Department of Food Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia; [2]Department of Public Health, Brčko, Bosnia and Herzegovina; [3]Department of Process Engineering, Faculty of Food Technology Osijek, Osijek, Croatia

Abstract

Amidžić Klarić D., Klarić I., Velić D., Vedrina Dragojević I. (2011): **Evaluation of mineral and heavy metal contents in Croatian blackberry wines**. Czech J. Food Sci., **29**: 260–267.

The mineral and heavy metal contents in 17 commercially available Croatian blackberry wines were determined by FAAS/FAES and GFAAS. The concentrations of potassium, sodium, calcium, magnesium, iron, copper, manganese, zinc, cobalt, chromium, and cadmium were between (in mg/l) 924–1507, 11.81–120.10, 86.4–457.1, 183.4–381.2, 0.082–6.273, 0.058–0.767, 1.47–11.53, 0.247–6.645, and (in μg/l) 3.21–11.89, 10.08–15.88, and 0.55–9.9, respectively. A negative correlation was found between the concentrations of macro (Mg) and micro (Fe) minerals. Furthermore, positive correlations were observed between the concentrations of manganese, cadmium, and cobalt that indicated the origin of these elements in the anthropogenic source. Multivariate analyses (PCA/LDA) showed that the distinct patterns of the metal contents in blackberry wines could be identified with quite satisfactory accuracy (sensitivity and specificity) with the subregion of the origin. In regard to the results obtained, Croatian blackberry wines could be considered as safe from the health risk point of view and as a good additional source of the essential nutrients investigated such as manganese, magnesium, and potassium.

Keywords: blackberry wine; minerals; heavy metal; multivariate analyses; PCA; LDA

Blackberry (*Rubus fruticosus*, *Rosaceae*) is grown in continental Croatia on small farms using traditional techniques of cultivation and harvesting by hand in summers (July–August). Thornless Logan, Thornfree, Black Satin, and Tayberry are the most common blackberry cultivars in Croatia. A significant proportion of fresh fruits is directly processed into blackberry products – jam, juice, or wine. Blackberry wine is a product of yeast fermentation of natural sugars present in blackberry juice. This popular fruit wine is mainly produced in the continental part of the country, which is divided into a number of subregions: from the Danube Basin, through Slavonia, Moslavina, Pokuplje, Plešivica, to Prigorje-Bilogora and Zagorje-Međimurje (Figure 1). Slavonia is a geographical and fertile agricultural area in eastern Croatia and the plantations in this subregion are located on the slopes of the central Slavonian mountain range. Prigorje-Bilogora subregion offers favourable conditions for the cultivation of all kinds of fruits and vegetables, and the plantations are located on attractive hills. Zagorje and Međimurje are situated in the north-western part of the country. This subregion features a wide range of different landscapes and types of vegetation.

The quality of wine depends on numerous factors related to the specific production area, such as the plant variety, soil and climate, culture, yeast,

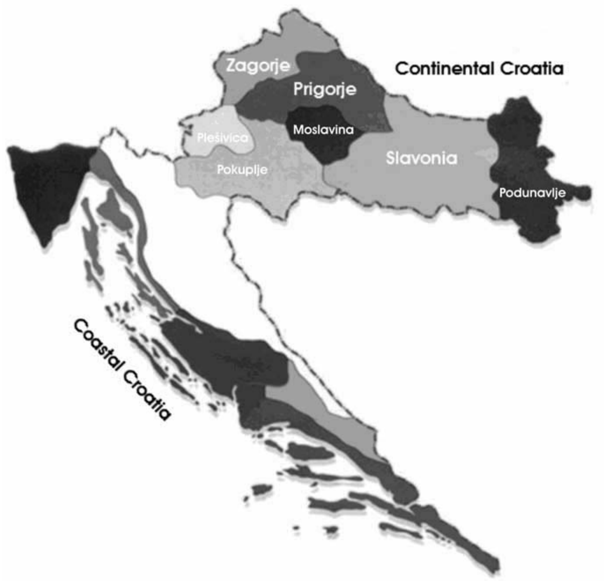


Figure 1. Wine-growing (sub) regions in Croatia

winemaking practices, transport, and storage. It was also shown that all listed factors determine the mineral content in wines, so that the concentration of minerals can be used as a useful tool for the characterisation of wines and their classification according to the geographical origin. (Zoecklein *et al.* 1995; Murányi & Zsuzsanna 2000; Frías *et al.* 2003; Marengo & Aceto 2003; Kment *et al.* 2005; Moreno *et al.* 2007). Today, the evaluation of wine quality is important for manufacturers, merchants, and consumers. Blackberry wine is usually served as a dessert wine and is enjoyed with meals in moderate quantities. Daily consumption of blackberry wine in recommended quantities (about 250 ml) can be a significant dietary source of essential minerals and could improve health. Traditionally, blackberry wine has been in the use as a popular medicine for anaemia and iron deficiency, and for increasing the absorption of minerals and reducing the occurrence of sleep disorders. On the other hand, several metals in wine, such as Cd, Pb, and As can be toxic and harmful. Therefore, the metal content in wine is regulated according to the national legislation and the legislation of the European Union (Croatian Regulation No. 96/96 1996; Galani-Nikolakaki *et al.* 2002; Lara *et al.* 2005; OIV 2008).

The production of blackberry wine has been increasing in Croatia for many years, but there is no available information or data on mineral and heavy metal contents. For this reason, the aims of this work were: (*i*) to analyse the metal content in Croatian blackberry wines, (*ii*) to compare the metals concentrations with the maximum acceptable levels in wines, (*iii*) to investigate this kind of wines as a good source of essential mineral nutrients, (*iv*) to correlate the concentrations of metals, and (*v*) to study the relationship between the metal content and the geographical origin of the wines using univariate and multivariate statistics with principal components analysis (PCA) and linear dicriminant analysis (LDA).

MATERIAL AND METHODS

Samples. Seventeen commercially available samples of Croatian blackberry wine (BW) were collected during years 2000–2004 and divided into three groups according to their growing subregions: Slavonia (SL, five samples: BW 1–BW 5), Prigorje-Bilogora (PB, six samples: BW 6–BW 11), and Zagorje-Međimurje (ZM, six samples: BW 12–BW 17). The alcoholic contents were reported on the bottles for all samples and the labelled values ranged from 11.6% to 15.6% ethanol (v/v).

Chemicals and reagents. Standard solutions of elements (concentration 1000 ± 0.002 mg/l) for FAAS and FAES were purchased from Panreac (Barcelona, Spain), while GFAAS mixed standard and the matrix modifiers were obtained from Perkin Elmer (Shelton, USA). $LaCl_3 \times 7H_2O$ (99.9%), used as a molecular suppressor, was obtained from Roth (Karlsruhe, Germany) and nitric acid (TraceSelect, for the trace analysis, ≥ 69.0% (T)), used as a reagent and cleaning agent, was delivered by Sigma-Aldrich (St. Louis, USA). Ethanol (≥ 99.8% HPLC grade) was purchased from Carlo Erba (Rodano, Italy), while hydrochloric acid (36.5% (w/w), density 1.18 g/cm^3, p.a.) came from Kemika (Zagreb, Croatia). Other reagents used in this work were of analytical reagent grade or better. Double deionised water (DDW) was used for all dilutions.

Determination of metals in blackberry wines. A Perkin-Elmer AAnalyst 100 atomic absorption spectrometer equipped with HGA-800 graphite furnace and with deuterium background corrector was used for the determination of metals in blackberry wines. The contents of potassium, calcium, magnesium, iron, manganese, copper, and zinc in the samples were analysed by FAAS, the sodium content by FAES, while the concentrations of cobalt, chromium, and cadmium were measured by GFAAS. The main analytical parameters for the determination of metals in the studied wines were those recommended by the manufacturer (Perkin-Elmer 2000). Immediately after opening, the wine samples were filtered through 0.45 μm filter and analysed in triplicate without pre-treatment. All samples were stored in plastic sealed bottles at 2–8°C in the dark.

Statistical methods. The variables with the normal distribution were described by the arithmetic mean and standard deviation, and those not showing the normal distribution were presented by the median and interquartile range. The univariate characterisation of blackberry wines by their geographical origin was carried out using ANOVA for variables with the normal distribution and non-parametric statistics – Median test and Kruskal-Wallis ANOVA by ranks for others. Pearson product-moment correlation coefficient and Spearman rank correlation coefficient were performed to examine potential relationships between the concentrations of metals. $P < 0.05$ were considered statistically significant and $P < 0.01$ as very significant.

Principal component analysis (PCA). Principal component analysis was used to provide a new set of variables (the least possible number) that were calculated in such way as to keep most of the present information in the original data set. Principal components (PCs) derived from the original data set with the eigenvalues >1.0 were used for further analysis with linear discriminant analysis.

Linear discriminant analysis (LDA). Linear discriminate analysis was carried out using the original data set with the standard procedure (using all variables) and was compared with the same analysis done with PCs. A classification model for subregional separation was provided for both analyses. As classifications for the subregions gave significantly worse results when using LDA with forward stepwise procedure for both original data sets and PCs, these data are not shown.

The statistical package, STATISTICA ver. 7.1 from Statsoft was used for the analyses of all data.

RESULTS

Mineral and heavy metals contents in blackberry wines

The values obtained by skewness and kurtosis statistical tests showed that the concentrations of the investigated metals in the samples of blackberry wines were not normally distributed (except Mn, Cu, and Co). The corresponding descriptive basic statistics for the wines studied is shown in Table 1.

It is clear that the major mineral in blackberry wines, potassium, varied over the range from 924 mg/l to 1507 mg/l (RSD 0.08–0.70%), and the median value was similar in all subregions. It had been observed that blackberry wines from Croatia had a narrower range than Czech wines (493–3056 mg/l) (Kment *et al.* 2005). The potassium content in the studied wines was higher than the potassium content in French wines (Pohl 2007), red wines from Galicia (Rebolo *et al.* 2000), and commercial wines from the Canary Islands (Frías *et al.* 2003). The results obtained are in accordance with the data reported by Moreno *et al.* (2007) for Tacoronte and Orotava red wines from the Canary Islands.

Moreover, the concentrations of sodium found in the blackberry wines ranged from 11.81 mg/l to 120.10 mg/l (only BW 13 > 50 mg/l) and the sodium content did not change significantly in the dependence on the locality. The mean concentration of sodium (29.53 mg/l) and the range are in conformity with the data reported by Sauvage *et al.* (2002) for white wines from Australia.

As can be seen in Table 1, the detected calcium concentrations varied between 86.4 mg/l and 457.1 mg/l (RSD 0.10–2.09%). The calcium content of blackberry wines was higher than in Argentinian white and red wines (Lara *el al.* 2005) and Italian red wines (Marengo & Aceto 2003). These results, except for the sample BW 6 (457.1 mg/l), are close to those reporting calcium contents of French (65–161 mg/l) and Italian wines (88–151 mg/l) (Pohl 2007).

Magnesium is a natural component of blackberry fruit and the concentrations of this macro nutrient in blackberry wines were between 183.4 mg/l to 381.2 mg/l (RSD 0.06–0.57%). These wines had higher contents of magnesium than wines from other countries (Marengo & Aceto 2003; Moreno *et al.* 2007; Pohl 2007) but lower than the highest value of 718 mg/l reported for Australian white wines, which had the widest range of magnesium (78–718 mg/l) (Sauvage *et al.* 2002).

It should be also noted that the range of iron concentrations in the investigated blackberry wines was found to be rather wide (0.082–6.273 mg/l). It was observed that blackberry wines had lower

Table 1. Metal content in Slavonia samples (n = 5), Prigorje-Bilogora samples (n = 6) and Zagorje–Međimurje samples (n = 6)

Metal	Slavonia				Prigorje–Bilogora				Zagorje–Međimurje			
	mean ± SD (mg/l)	median (mg/l)	range of quantified values (mg/l)	interquartil	mean ± SD (mg/l)	median (mg/l)	range of quantified values (mg/l)	interquartil	mean ± SD (mg/l)	median (mg/l)	range of quantified values (mg/l)	interquartil
K	1296 ± 235	1369	924–1507	1221–1460	1389 ± 58	1387	1311–1461	1351–1439	1350 ± 62	1330	1289–1427	1289–1403
Na	26.91 ± 12.48	23.46	11.81–45.14	22.04–32.08	22.13 ± 6.65	21.80	14.61–32.59	15.97–25.99	39.56 ± 45.32	19.63	12.59–120.10	18.42–36.89
Ca	119.4 ± 26.0	120.4	86.4–150.3	101.7–138.1	188.4 ± 132.1	141.1	117.2–457.1	127.0–146.3	132.2 ± 22.6	131.0	109.1–160.7	111.7–148.7
Mg	319.9 ± 81.0	350.8	183.4–381.2	311.1–372.9	331.3 ± 24.6	334.4	296.3–367.4	312.7–342.4	340.5 ± 27.1	335.7	301.8–373.4	334.3–357.5
Fe	1.236 ± 1.881	0.615	0.082–4.568	0.218–0.696	2.171 ± 2.141	1.664	0.369–6.273	0.663–2.392	1.130 ± 0.762	1.268	0.256–1.946	0.430–1.751
Cu	0.237 ± 0.177	0.168	0.079–0.508	0.113–0.318	0.359 ± 0.296	0.241	0.099–0.767	0.112–0.696	0.230 ± 0.136	0.253	0.058–0.406	0.092–0.297
Mn	5.53 ± 3.58	5.07	2.11–11.53	3.73–5.18	6.24 ± 2.76	5.93	2.82–10.98	4.62–7.16	6.08 ± 3.45	8.12	1.47–9.20	3.33–9.10
Zn	0.569 ± 0.291	0.635	0.247–0.858	0.283–0.823	2.398 ± 2.320	1.997	0.382–6.645	0.500–2.871	0.845 ± 0.653	0.739	0.277–1.872	0.312–1.023
Co*	7.49 ± 3.11	7.07	3.21–11.89	6.96–8.31	6.71 ± 1.43	6.63	5.29–9.31	5.50–6.94	5.77 ± 1.28	6.31	3.48–6.41	6.29–6.41
Cr*	13.05 ± 0.74	13.25	12.21–13.91	12.36–13.53	13.30 ± 0.69	13.44	12.36–14.04	12.61–13.89	12.86 ± 2.07	12.74	10.08–15.88	12.38–13.64
Cd*	3.73 ± 3.51	2.64	1.40–9.94	1.96–2.73	3.16 ± 2.06	2.85	0.88–6.91	2.02–3.42	2.35 ± 1.31	3.22	0.55–3.40	1.36–3.40

*Cr, Co and Cd values were expressed in µg/l

concentrations of iron than red and white wines from Croatia (Šebečić *et al*. 1998), Italian red wines (Marengo & Aceto 2003), or Spanish and Hungarian wines (Pohl 2007) but higher when compared with Argentinian white and red wines (Lara *et al.* 2005).

It is noteworthy that all blackberry wines had the copper content below the internationally established maximum allowed values (1 mg/l). The concentration of copper was between 0.058 mg/l and 0.767 mg/l (RSD 0.15–2.68%) and these values are similar to those reported by Šebečić *et al*. (1998) for white and red wines from different regions of Croatia. The range of the copper content in blackberry wines was narrower then the same range in Czech, Spanish, and Jordanian wines (Kment *et al.* 2005; Pohl 2007).

Furthermore, the manganese content in the investigated samples of blackberry wines was in the range from 1.47 mg/l to 11.53 mg/l and the average value of 6.15 mg/l was elevated when compared with Croatian red (0.82–4.23 mg/l) and white (0.73–2.73 mg/l) wines (Šebečić *et al.* 1998). It was observed that the studied wines had higher manganese contents than all the wines summarised in Pohl's article (Pohl 2007).

The concentrations of zinc in blackberry wines are partly due to the natural contents in fruits (0.5 mg/100 g raw), partly to artificial sources (fungicidal treatments and the containers used for the processing and ageing stages) (Galani-Nikolakaki *et al.* 2002; Marengo & Aceto 2003; NutritionData 2008). The obtained results showed that the concentration of zinc varied from 0.247 mg/l to 6.645 mg/l (RSD 0.24–1.88%). This range was wider than the concentration ranges of red (0.23 to 1.70 mg/l) and white (0.26–0.96 mg/l) Croatian wines (Šebečić *et al.* 1998), but narrower than that of Greek wines (Pohl 2007). In this study, the concentrations of zinc in 11 samples were below 1 mg/l and in only one sample (BW 10) was the zinc content over 5 mg/l. In comparison to the maximum allowed values postulated by wine regulations (5 mg/l), the concentrations of zinc in blackberry wines were considerably lower, except for the sample BW 10 (6.645 mg/l).

Moreover, the level of cobalt found in the studied wines ranged between 3.21–11.89 µg/l, and the average value was 6.82 µg/l. These wines did not have as wide a range as reported in the data for Austrian (8–45 µg/l) and Spanish (below 40 µg/l) wines and this value agrees with the data reported for French wines (Pohl 2007).

The literature data show that chromium content in wines may increase with the prolonged contact of wines with materials such as stainless steel, glass utensils, and bottles during the ageing process (Galani-Nikolakaki *et al.* 2002; Lara *et al.* 2005; Pohl 2007). The established chromium content was in a very narrow range of 10.08–15.88 µg/l (RSD: 1.14–6.00%) and it was lower compared to Croatian red and white wines (Šebečić *et al.* 1998), Greek, German, Hungarian, Czech, and Italian wines (Marengo & Aceto 2003; Kment *et al.* 2005; Pohl 2007). All analysed wine samples contained chromium in lower levels than the maximum allowed by Croatian legislation (100 µg/l) (Croatian Regulation No. 96/96 1996).

The presence of cadmium, a non-essential heavy metal, was detected in all samples and the values of the cadmium content in blackberry wines were in the range of 0.55–9.94 µg/l. Italian red wines (Marengo & Aceto 2003), Argentinian red and white wines (Lara *et al.* 2005; Pohl 2007), Macedonian (Cvetković *et al.* 2006), and Slovenian wines (Kristl *et al.* 2002) had lower contents of cadmium than the studied Croatian wines. The cadmium concentration range for Hungarian wines (Pohl 2007) was wider than the concentration range established in this study. The concentrations of cadmium found in all seventeen blackberry wines from Croatia were below the limits established by OIV for this element.

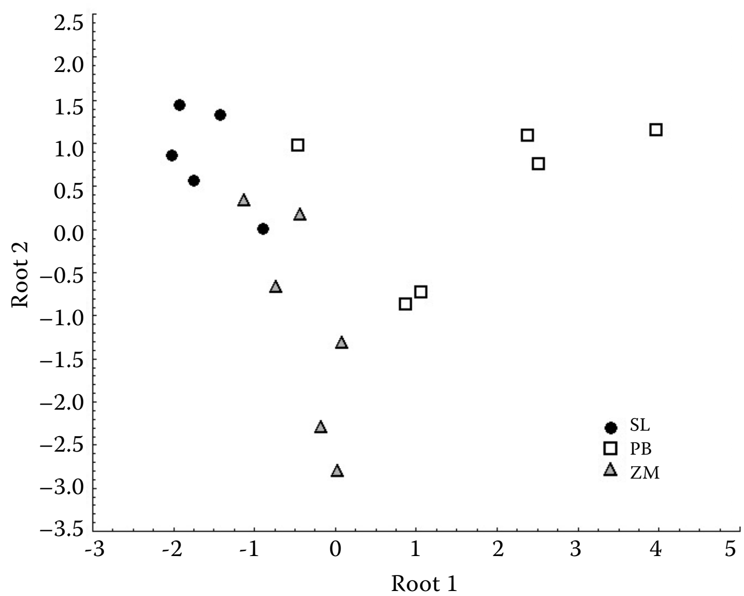
Multivariate analysis

PCA revealed that the first five (of 11) PCs explain 99.98% of total variance (Table 2). It can also be seen that the first seven PCs had eigenvalues > 1.0 and explain 99.99% of total variance in our data set. These seven PCs were used for further analysis.

As one of the aims was to try to relate the metal content to the geographical origin of the blackberry wine, LDA was used to determine the discriminant power of both original data set and PCs determined previously using standard procedure. The non error rate (NER%) of the linear discriminant model for the original data set was 76.47% and for PCs model

Table 2. Variance explained by 11 PCs

PC	Eigenvalue	Variance explained (%)	Cumulative variance explained (%)
1	18 486.62	68.21	68.21
2	6 784.97	25.03	93.24
3	1 225.67	4.52	97.77
4	585.87	2.16	99.93
5	14.66	0.05	99.98
6	1.77	0.006	99.99
7	1.28	0.004	99.99
8	0.98	0.004	99.998
9	0.34	0.001	99.999
10	0.21	0.001	99.9999
11	0.02	0.0001	100.00



SL – Slavonia; PB – Prigorje-Bilogora; ZM – Zagorje-Međimurje

Figure 2. Scatter plot of canonical scores for two roots for each sample according to the subregion

Table 3. Confusion matrix

	Sensitivity (%)	SL	PB	ZM
SL	80.00	4	0	1
PB	83.33	0	5	1
ZM	83.33	1	0	5
Specificity (%)		80.00	100.00	71.43

SL – Slavonia; PB – Prigorje-Bilogora; ZM – Zagorje-Međimurje

82.35%, giving a slightly better discriminate power. The model based on PCs derived from the original data set on the metal content of the investigated wines gave a quite satisfactory separation of the three subregions from which these wines originated. The data for sensitivity and specificity (Table 3) associated with LDA model for PCs show good results with sensitivity and specificity exceeding 70%. Canonical scores for both roots for each sample from the three subregions are shown in Figure 2.

DISCUSSION

It is interesting to note that statistical analyses have demonstrated significant correlations (Spearman coefficient $R \geq 0.6$ and $P < 0.01$) between the concentrations of Fe and Mg ($R = -0.8848$; $P = 0.000002$), Cd and Mn ($R = 0.7647$; $P = 0.0004$), and Cd and Co ($R = 0.6210$; $P = 0.008$). Also, Pearson correlation coefficient indicated a relationship between the concentrations of Mn and Co ($R = 0.7741$; $P = 0.0003$).

Primarily, Mg and Fe contents in wines depend on their concentrations in the soil on which the blackberries are grown (natural source). The data in the literature show antagonistic competition between Mg and Fe in their absorption from soil through the roots of the plants. However, iron compounds released from machinery, piping, storage tanks, and dust, which can be found on blackberries, may have a strong influence on the iron content in wines. Furthermore, a significant amount of iron is precipitated during the fermentation process, while the additions of carbonates during the production cycle enhance Mg concentrations in wines (Galani-Nikolakaki *et al.* 2002; Marengo & Aceto 2003; Kment *et al.* 2005). It was observed that high concentrations of Mg and low concentrations of Fe were present in the same samples (e.g. BW 1, BW 3, BW 11, and BW 14), except for the sample BW 9 (Mg 296.3 mg/l and Fe 6.273 mg/l). For all reasons stated it can be concluded that this negative correlation is probably connected with the natural source and fermentation process of blackberries. Our investigation showed that the concentrations of manganese positively correlate with the concentrations of cadmium and cobalt in blackberry wines. The amount of manganese in wine is a result of the natural and anthropogenic sources of raw materials. The available literature data show that blackberry fruits are a very good source of manganese, which the plants absorb from the ground. In the subregions studied, soil and water have high concentrations of this lithophile element, and the drip irrigation of the plantations is also used. Furthermore, the concentrations of manganese, cadmium, and cobalt in wines increase due to the use of agrochemical products (application of pesticides, fungicides, and fertilisers which contain these elements) during the plant growth, environmental contamination (road traffic and industrial complexes close to the plantations), and contamination during winemaking (the apparatus in wine production and packaging process) (Galani-Nikolakaki *et al.* 2002; Kment *et al.* 2005; Pohl 2007). Very high concentrations of these elements are present in the same samples (extreme: BW 5 and outlier: BW 9), positive correlations existing between manganese, cadmium, and cobalt (Cd/Mn, Cd/Co, and Co/Mn) that indicate the origin of elements in the anthropogenic source.

The results obtained by ANOVA and Kruskal-Wallis test indicated that there were no significant differences in the metal content of blackberry wines related to the subregion of origin.

Although univariate characterisation of wine samples by subregion did not yield significant results, multivariate analyses (PCA/LDA) showed that the distinct patterns of metal content in blackberry wines could be related with a quite satisfactory accuracy (sensitivity and specificity) to the subregions of origin.

The evaluation of mineral and heavy metal intake from blackberry wines

Based on the daily consumption of wine in recommended quantities (about 250 ml) and the mean concentrations of macro minerals in all samples, the average daily intake of potassium, sodium, calcium, and magnesium through ingestion of blackberry wine would be 336 mg, 7 mg, 37 mg, and 82 mg, respectively. The amounts of macro minerals in all samples were lower than the recommend daily allowances and varied over the range from 11–19% of the reference dose for K (RDA 2 g), 2–14% for Ca (RDA 0.8–1.2 g), 13–32% for Mg (RDA 0.30–0.35 g), and about 1% for Na (RDA 2.4 g). Obviously, blackberry wine could be a good source of K and Mg. Furthermore, a low amount of sodium in blackberry wine is an advantage of this food product because this macro mineral is found in abundance in other types of food and the excessive intake of sodium can be harmful to human health.

The intake of iron was evaluated according to the RDA (14 mg for man/28 mg for woman), and it was observed that daily consumption of this fruit wine in recommended quantities could cover up to 11% of the RDA for this essential mineral. Furthermore, blackberry wine contains dietary compounds such as ascorbic, citric and malic acids, carotenoids, fructose, and alcohol that enhance iron absorption (Reilly 2004; García-Casal 2006; Péneau *et al.* 2008). Because of that, blackberry wine is often called "ferrous wine" and has been used as a popular medicine for anaemia and the iron deficiency.

The daily dietary intake of micro minerals due to blackberry wine consumption could cover up to 11% of the RDA for zinc (15 mg for adult) and less than 10% of the RDA for copper (2–3 mg for adult). This study shows that blackberry wine is a significant source of manganese (0.37–2.89 mg/day) similarly to fresh blackberry fruits (0.6 mg/100 g raw; 32% of daily values). The intake of cobalt was evaluated according to the RDA (100 µg), and the average intake of cobalt through blackberry wine consumption was 2 µg (range 1–3% of the RDA) (Reilly 2004; Nutrition Data 2008).

The average daily intakes of chromium and cadmium were 3 µg (range: 2–4 µg) and less than 1 µg, which is well below the minimum suggested as safe daily intake (Reilly 2004).

CONCLUSION

The present study brings the first data on the metal contents in blackberry wines coming from Croatia, which is in accordance with the already published data for wines made from grapes. However, it was observed that the amount of manganese in blackberry wines was higher than the content of Mn in other wines. Furthermore, the obtained data provide evidence that the contents of metals in blackberry wines were well below the permissible limits, except for the concentration of zinc in one sample. Thus, the concentrations of manganese and zinc found in blackberry wines indicate the necessity for more extended investigations of these elements in this very popular fruit wine, because their increased contents could be the result of contamination.

Seventeen blackberry wines were classified into three groups according to the growing subregion, but no statistically significant differences in the metals contents were observed. It was noted that the significant correlations between the concentrations of metals (Fe/Mg, Cd/Mn, Co/Mn, and Cd/Co) could be affected by two major sources of metals.

From our point of view, it was established that the growing subregion of blackberry wines could play a dominant role in their metal content pattern.

Also, this finding implies that a better standardisation of the production process and the more uniform quality of raw material are necessary to promote the high quality of blackberry wine.

In regard to the results obtained, Croatian blackberry wines could be considered safe from the health risk point of view and as a good additional source of the essential nutrients investigated such as manganese, magnesium, and potassium.

Reference

Cvetković J., Arpadjan S., Karadjova I., Stafilov T. (2006): Determination of cadmium in wine by electrothermal atomic absorption spectrometry. Acta Pharmaceutica, **56**: 69–77.

Frías S., Conde J.E., Rodríguez-Bencomo J.J., García-Montelongo F., Pérez-Trujillo J.P. (2003): Classification of commercial wines from the Canary Islands (Spain) by chemometric techniques using metallic contents. Talanta, **59**: 335–44.

Galani-Nikolakaki S., Kallithrakas-Kontos N., Katsanos A.A. (2002): Trace element analysis of Cretan wines and wine products. The Science of the Total Environment, **285**: 155–63.

García-Casal M.N. (2006): Carotenoids increase iron absorption from cereal-based food in the human. Nutrition Research, **26**: 340–44.

Croatian Regulation No. 96/96 (1996): Government of the Republic of Croatia – Ministry of Agriculture, Fisheries and Rural Development, Zagreb.

Kment P., Mihaljevič M., Ettler V., Šebek O., Strnad L., Rohlová L. (2005): Differentiation of Czech wines using multielement composition – A comparison with vineyard soil. Food Chemistry, **91**: 157–165.

Kristl J., Veber M., Slekovec M. (2002): The application of ETAAS to the determination of Cr, Pb and Cd in samples taken during different stages of the winemaking process. Analytical and Bioanalytical Chemistry, **373**: 200–204.

Lara R., Cerutti S., Salonia J.A., Olsina R.A., Martinezc L.D. (2005): Trace element determination of Argentine wines using ETAAS and USN-ICP-OES. Food and Chemical Toxicology, **43**: 293–297.

Marengo E., Aceto M. (2003): Statistical investigation of the differences in the distribution of metals in Nebbiolo-based wines. Food Chemistry, **81**: 621–630.

Moreno I.M., González-Weller D., Gutierrez V., Marino M., Cameán A.M., González A.G., Hardisson A. (2007): Differentiation of two Canary DO red wines according to their metal content from inductively coupled plasma optical emission spectrometry and graphite furnace atomic absorption spectrometry by using Probabilistic Neural Networks. Talanta, **72**: 263–268.

Murányi Z., Zsuzsanna K. (2000): Statistical evalution of aroma and metal content in Tokay wines. Microchemical Journal, **67**: 91–96.

NutritionData: [Internet]. New York; CondéNet; c2003–2008. Available at http://www.nutritiondata.com (accessed Oct 13, 2008)

OIV – Organisation Internationale de la Vigne et du Vin (2008): Compendium of International Méthods of Wine and Must Analysis. Edition 2007, Vol. 2, Annex C, Paris: 1–3.

Perkin-Elmer (2000): Analytical methods for atomic absorption spectrometry. Part No. 0303-0152., Norwalk.

Péneau S., Dauchet L., Vergnaud A., Estaqio C., Kesse-Guyot E., Bertrais S., Latino-Martel P., Hercberg S., Galan P. (2008): Relationship between iron status and dietary fruit and vegetables based on their vitamin C and fiber content. The American Journal of Clinical Nutrition, **87**: 1298–305.

Pohl P. (2007): What do metals tell us about wine? Trends in Analytical Chemistry, **26**: 941–949.

Rebolo S., Peña R.M., Latorre M.J., García S., Botana A.M., Herrero C. (2000): Characterisation of Galician (NW Spain) Ribeira Sacra wines using pattern recognition analysis. Analytica Chimica Acta, **417**: 211–220.

Reilly C. (2004): The Nutritional Trace Metals. 1st Ed. Blackwell Publishing, Oxford: 12–223.

Sauvage L., Frank D., Stearne J., Millikan M.B. (2002): Trace metal studies of selected white wines: an alternative approach. Analytica Chimica Acta, **458**: 223–230.

Šebečić B., Pavišić-Strache D., Vedrina-Dragojević I. (1998): Trace elements in wine from Croatia. Deutsche Lebensmittel-Rundschau, **94**: 341–344.

Zoecklein B., Fugelsang K., Gump B., Nury F. (1995): Wine analysis and production. Chapman & Hall, New York: 53–230.

Received for publication June 27, 2009
Accepted after corrections August 7, 2010

Corresponding author:

Dr. Daniela Amidžić Klarić, University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Food Chemistry, Domagojeva 2, 10000 Zagreb, Croatia
tel.: + 385 146 126 90, e-mail: daniela.admidzic@gmail.com, danami@net.hr