DETERMINATION OF SELECTED METALLIC IONS IN CROATIAN WHITE WINES BY ICP-OES METHOD

R. Bukovčan [1], V. Kubanović [1], M. Banović [2], N. Vahčić [2], Lj. Gašparec-Skočić [1]

[1] Croatian Institute of Viticulture and Enology, Jandrićeva 42, 10000 Zagreb, Croatia
e-mail: renata.bukovcan@zg.t-com.hr
[2] Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

ABSTRACT

Metallic ions take important place in enological practice. Usually all metallic ions are naturally present in wine in nontoxic level. Heavy metals (Pb, Cd, As, Hg) are toxic to biological systems because of their negative impact on physiological functions of cell. Maximum acceptable levels in wine have been established by the Office International de la Vigne et du Vin (OIV), while in Croatia maximum permissible levels are given by Official Gazette 2/05 and 16/05. The goal of this work was to determine the content of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn and Zn, in 43 selected white wine samples from continental wine-growing region of the Republic of Croatia by ICP-OES method. Average values for each metal were 0.99 mg/L Al, 0.05 mg/L As, 0.0005 mg/L Cd, 0.0029 mg/L Co, 0.016 mg/L Cr, 0.18 mg/L Cu, 2.68 mg/L Fe, 1.25 mg/L Mn, 0.045 mg/L Ni, 0.046 mg/L Pb, mg/L 0.074 Sn and 0.716 mg/L Zn. The obtained results in all studied cases were lower than limits given by official regulation that indicates application of good vitiviniculture practice in that region.

Gli ioni metallici hanno un ruolo molto importante nella produzione enologica. I metalli pesanti (Pb, Cd, As, Hg) sono tossici per i sistemi biologici perchč hanno un effetto negativo sulle funzioni fisiologiche delle cellule. L’Organizzazione internazionale della vigna e del vino (OIV) ha fissato i livelli massimi ammissibili nei vini, mentre in Croazia il livello massimo permissibile di stato stabilito dai Gazzetta Ufficiale 2/05; 16/05. Lo scopo di questo lavoro č stato quello di determinare il contenuto di Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn e Zn in 43 campioni di vino di una zona vinicola della Croazia continentale tramite ICP-OES. I valori medi ottenuti per ogni metallo sono stati 0.99 mg/L Al, 0.05 mg/L As, 0.0005 mg/L Cd, 0.0029 mg/L Co, 0.016 mg/L Cr, 0.18 mg/L Cu, 2.68 mg/L Fe, 1.25 mg/L Mn, 0.045 mg/L Ni, 0.046 mg/L Pb, mg/L 0.074 Sn e 0.716 mg/L Zn. I valori ottenuti in tutti i casi studiati sono stati inferiori ai limiti stabiliti dai regolamenti ufficiali; ciň dimostra la presenza di buone pratiche vitivinicole in questa regione.

INTRODUCTION

Metallic ions take important place in enological practice, some of them are necessary for the correct course of the fermentation, they influence clarity, aroma and sensorial properties of wine, and are important for toxicological and nutritional properties (Zoecklein et. al., 1999; Thiel and Danzer, 1997). Certain metal ions, such as Fe, Cu and Mn participate in destabilization of wine and in their oxidative evolution (McKinnon and Scollary, 1997). Several elements, including Cu, Fe, Al, Zn and Ni, contribute to haze formation and undesirable changes of aroma and taste. All metallic ions are naturally present in wine in nontoxic level. Some of them (Pb, Zn, Sn, Hg) may occur in higher level as a result of agricultural treatments in vineyard. Fertilizers and pesticides are responsible for anion (phosphates and nitrates) and cation (Cu, Zn, and Mn) level increase (Ramachandran and D`Souza, 1998; Pietrzak and McPhail, 2004). Besides, wine and must are due to their acidity able to dissolve Cu, Ni, Zn and even Cr from winemaking equipment such as pumps and taps (Ribereau-Gayon et. al., 2006). Heavy metals are toxic to biological systems because of their negative impact on physiological functions of some cell enzymes (Zoecklein et.al., 1999). Determination of elements such as Pb, As and Cd is of considerable importance due to their potential toxic effects (Rebolo et. al., 2000; Galani-Nikolakaki et. al., 2002). Maximum acceptable limits in wine have been established by the Office International de la Vigne et du Vin (OIV) for Pb, Cd, As, Cu, Na, F, Br and B (Tab. 1), while in Croatia, maximum permissible levels are given for Pb, Cd, As, Cu, Zn, Sn, Fe, Ni, Ag, St, Al, Cr, Na, F, Br and B, by Croatian Official Gazette 2/05 and 16/05.

Table 1. Maximum acceptable limits of metallic ions in wine (mg/L)

ELEMENT	OG 16/05	OG 2/05	OIV
Pb	0.2	0.3	0.15
Cd	0.01	0.01	0.01
As	-	0.2	0.2
Cu	1.0	1.0	1.0
Zn	5.0	5.0	-
Sn	10.0	-	-
Fe (white wine)	10.0	10.0	-
Fe (rosé wine)	15.0	15.0	-
Fe (red wine)	20.0	20.0	-
Ni	-	0.1	-
Ag	-	0.1	-
St	-	0.2	-
Al	-	10.0	-
Cr	-	0.1	-
Na	-	20.0	60.0
F	-	1.0	1.0
Br	-	1.0	1.0
B	-	80.0	80.0

The aim of this study was to determine the content of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn and Zn, in 43 white wine samples from different continental wine-growing regions of the Republic of Croatia by ICP-OES method.

MATERIALS AND METHODS

Instrumentation

Multi-element determinations were carried out with Perkin-Elmer Optima 2000 DV instrument equipped with a Meinhard spray chamber, nebulizer and peristaltic sample delivery system. The instrument was controlled by the ICP WinLab 1.35 Perkin Elmer software. The operating conditions and the wavelength used for the analysis of each element are given in Tab. 2. The flow conditions for plasma gas, auxiliary gas and nebulizer gas were 15.0, 0.2 and 0.8 L/min, respectively. Power was set at 1300 Watt. Ultrapure water (18 MΩ/cm) was obtained from Easy pure RF (Barnstead, Dubuque, IA, USA).

Table 2. Operating conditions and wavelengths used for the quantification of each element by ICP-OES method

Element	Wavelength (nm)	Points/Peak	Background Correction (No. of points)	Plasma View
Al	396.153	5	2	Axial
As	193.696	4	2	Axial
Cd	226.502	1	2	Axial
Co	230.786	4	2	Axial
Cr	267.716	4	2	Axial
Cu	327.393	5	2	Axial
Fe	238.204	5	2	Axial
Mn	257.610	5	2	Axial
Ni	232.003	4	2	Axial
Pb	220.353	1	2	Axial
Sn	235.485	5	1	Axial
Zn	206.200	7	2	Axial

Samples

43 white wine samples from continental winegrowing region of Croatia were analyzed.

Sample preparation

The sample of wine (50 mL) was put in a beaker and 4 mL of 60 % nitric acid was added.

Open beaker was placed in a water bath at 90-95 °C, to reduce the volume to approximately 30 mL in order to remove ethanol and therefore minimize matrix interferences during analysis and diminish plasma instability caused by introduction of organics into the plasma. The sample residue was than quantitatively transferred to volumetric flask and volume was set to 50 mL with 2 % nitric acid (Bukovčan, 2007).

Blanks and standards

2 % HNO_3 blank solution was prepared from 60 % HNO_3, suprapure, obtained from Merck (Darmstadt, Germany) and ultrapure water (18 MΩ/cm). Standard solutions containing 2 % HNO_3 were prepared with appropriate dilutions to cover the concentration range of each element in the wines. Standards for Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn, were obtained as multielement solution (109480), standard for Sn (170362), K (170342), Ca (170308), Mg (170331), and Na (170353) from Merck (Darmstadt, Germany).

Method

The validation parameters of method used for determination of 16 elements is fully described by Bukovčan et. al., (2008).

RESULTS AND DISCUSION

The results of 16 elements concentration in studied wine samples are summarized and presented as minimum, maximum and average values for each element in Tab 3. The Al content ranged from 0.49 to 2.01 mg/L (average 0.99 mg/L). These values are lower than results in Croatian wines presented by Šeruga et. al., (1998). They found Al concentration in range from 0.62 to 8.60 mg/L. Lower Al concentration can be interpreted by improvement of vinification process during the last decade. In general, Al levels are considerably lower than the value of 10 mg/L, which is considered to be the limit for aluminum haze formation in wine (McKinnon et. al., 1992).

Table 3. Concentrations of metallic ions in white wines from continental part of Croatia

Metallic ions	**Concentration (mg/L)**		
	min.	**max.**	**average**
Al	0.49	2.01	0.99
As	n.q.	0.034	0.005
Cd	n.q.	0.003	0.0005
Co	n.q.	0.008	0.0029
Cr	0.005	0.032	0.016
Cu	0.028	0.721	0.18
Fe	0.59	9.5	2.68
Mn	0.287	3	1.25
Ni	0.003	0.167	0.045
Pb	0.007	0.122	0.046
Sn	n.q.	1.357	0.074
Zn	0.102	3.07	0.716

(n.q. – not quantified)

In this study the highest As concentration was 0.034 mg/L, with average value of 0.005 mg/L, yet 19 samples had As concentration below the quantification limit 0.001 mg/L. These low As concentration indicates that arsenate pesticides was not used to spray the vineyards during grape production. According to maximum acceptable limit for As of 0.2 mg/L (OIV and OG 02/05), it can be speculated that determined As concentration does not perform risk in term of toxicological effect on consumer health.

The same trend was observed in case of Cd concentration. Average Cd concentration was 0.0005 mg/L. Only 11 analyzed samples contained Cd at quantifiable level and the highest determined concentration was far below of maximum acceptable limit.

Maximum acceptable level for Co is not defined by regulation and its effect on wine technology or consumer safety is not established. The Co is essential element and constituent of vitamin B12. Therefore, its concentration in Croatian wines (8 µg/L max.), leads to assumption that wine is a potential nutritive source of Co.

According to OG 2/05 maximum acceptable level for Cr is 0.1 mg/L. In this study it was found an average of only 0.016 mg/L Cr, which clearly indicates absence of asbestos filters usage (main source of Cr in wine) in wine production (Ough and Amerine, 1988). Kristl et. al., (2003) found the same Cr concentration average value for Slovenian white wines.

The concentration of Cu in wines may be of exogenous (spraying grapes with copper sulfate) or endogenous origin (Carvalho et. al., 1996). The average of Cu concentration in studied wines was 0.18 mg/L. This value is far below of Cu concentration that causes wine browning or clouding (Benitez et. al., 2002). The highest Cu level was 0.72 mg/L which is also far below of maximum acceptable level according to OIV and Croatian regulation (1.0 mg/L).

The Fe concentration of grapes and wines depends upon several factors, the most important being soil where the grapes are grown. The Fe derived from soil reaches wine in two ways: it either covers the berries as a part of dust or it is absorbed from the vine through its roots. Knowing Fe level in wine is important because of its effect on undesirable process of oxidation. Wine clouding usually occurs when wine contains more than 10 mg/L of Fe (Galani-Nikolakaki et. al., 2002). In this study Fe concentration ranged from 0.59 to 9.5 mg/L (average 2.68 mg/L).

Legal limitation for Mn concentration is not defined, but it is important to know the concentration because of a great influence of Mn on wine oxidation and development of acetaldehyde during oxidation (Cacho et. al., 1995). In small quantities this element is a natural constituent of grape berries. The concentration of Mn ranged from 0.287 to 3.00 mg/L (average 1.25 mg/L). These values are in some degree lower in comparison to values obtained by Šebečić et. al., (1998) which were from 0.73 to 4.23.

Maximum acceptable level for Ni is 0.1 mg/L. The concentration of Ni in studied wine samples ranged from 0.003 to 0.167 mg/L (average 0.045 mg/L). The use of nickel alloys in stainless steel is a potential source of Ni in wine and also contact with asbestos or nickel-containing enamels (Ough and Amerine, 1988).

Knowledge of the Pb concentration in wine is important because of its toxic impact on consumer’s health. Therefore, its maximum acceptable level is regulated by legislative (Tab.1). The values varied between regulative from 0.15 mg/L (OIV), 0.2 mg/L (OG 16/05) and 0.3 mg/L (OG 02/05). In this study Pb concentrations were below maximum acceptable level and they ranged from 0.007 to 0.122 mg/L (average 0.046 mg/L). The results indicate that studied wines were produced on vineyards far from high traffic roads and environmental pollution from car exhausts.

Wine can be considered as a nutritive source of Sn which is an essential element for human body (Dunne, 1996). The average value of Sn concentration in this study was only 0.074 mg/l, far below maximum acceptable level of 10.0 mg/L defined by regulation (OG 2/05).

Zinc is essential element with maximum acceptable level in wine (5.0 mg/L) defined by domestic regulation (OG 16/05, OG 2/05). In study of Croatian wines conducted by Šebečić et. al., (1998) Zn concentration ranged from 0.23 to 1.70 mg/L. In present study Zn concentration ranged from 0.102 to 3.07 mg/L (average of 0.716 mg/L).

CONCLUSION

The contents of analyzed elements did not exceed the limits given by the Croatian regulations, which lead to conclusion that organic and mineral fertilizers, inorganic pesticides and other means of vine growing and winemaking practice are not misused. Besides that, quality important to emphasize is absence of environment pollutants such as heavy industry and automobiles exhausts gases around vineyards where grape for wine production were grown.

BIBLIOGRAPHY

1. Benitez P., Castro R., Barroso C.G. (2002) Removal of iron, copper and manganese from white wines through ion exchange techniques: effects on their organoleptic characteristics and susceptibility to browning, *Anal. Chim. Acta*, **458**, 197-202

2. Bukovčan R. (2007) Implementation of inductively coupled plasma – optical emission spectrometry (ICP-OES) method for macro- and microelements determination in Croatian wines, University of Zagreb, Faculty of Food Technology and Biotechnology, Master thesis.

3. Bukovčan R., Kubanović V., Gašparec-Skočić Lj., Banović M. (2008) Validation of ICP-OEC method for wine macro and microelements determination, *Proceedings the 2008 Joint Central European Congress, 4th Central European Congress on food and 6th Congress of foodtechnologists, biotechnologists and nutritionists,* **Vol. 2**., 169-174.

4. Cacho J., Castells, J.E., Esteban, A., Laguna, B., Sagrista, N. (1995) Iron, Copper, and Manganese Influence on Wine Oxidation. *Am. J. Enol. Viticult.*, **43**, 380-384.

5. Compendium of international methods of analysis (2001) Maximum acceptable limits of various substances contains in wine, OIV, *Resolutions adopted in Adelaide* (Australia).

6. Carvalho M., Barreiros M., Costa M., Ramos M., Marques I. (1996) Study of heavy metals in Madeira wine by total reflection XRF analysis, *X_Ray Spectrom*, **25**, 29-32.

7. Dunne L.J. (1996) Sve o zdravoj prehrani. MATE d.o.o. Zagreb

8. Galani-Nikolakaki S., Kallithrakas-Kontos N., Katsanos A.A. (2002) Trace element analysis of Cretan wines and wine products, *Sci. Total Environ.*, **285**(1-3), 155-163.

9. Kristl J., Veber M., Slekovec M. (2003) The contents of Cu, Mn, Zn, Cd, and Pb at different stages of the winemaking process, *Acta Chim. Slov.,* **50**, 123-136.

10. Larcher R., Nicolini G. (2001) Survey of 22 mineral elements in wines from Trentino (Italy) using ICP-OES, *Ital. J. Food Sci.*, **13**(2), 233-241.

11. McKinnon A.J., Catlrall R.W., Scollary G.R. (1992) Aluminium in wine-its measurement and identification of major sources, *Am. J. Enol. Viticult.*, **43**, 166-170.

12.McKinnon A.J., Scollary G.R. (1997) Size fractionation of metals in wine using ultrafiltration, *Talanta*, **44**, 1649-1658.

13. Ough, C.S., Amerine, M.A. (1988) Methods for analysis of Musts and Wines, University of California, John Wiley & Sons, New York.

14. Pietrzak U., McPhail, D.C. (2004) Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia, *Geoderma*, **122**, 151-166.

15. Ramachandran V., D`Souza T.J. (1998) Plant uptake of cadmium, zinc, and manganese in soils amended with sewage sludge and city compost, *Bulletin of Environmental Contamination and Toxicology*, **61**, 347-354.
16. Rebolo S., Pena, R.M., Latorre, M.J., Garcia, S., Botana, A.M., Herrero C. (2000) Characterisation of Galician (NW Spain) Ribeira Sacra Wines using pattern recognition analysis, *Anal. Chim. Acta*, **417**, 211-220.D
17. Regulation on wine production, No 2, (2005) *Official Gazette*, Zagreb (Croatia).
18. Regulation on toxins, metals, metalloids and other harmful substances that can be found in food, No 16, (2005) *Official Gazette,* Zagreb (Croatia).
19. Ribereau-Gayon P., Glories Y., Maujean A., Dubourdieu D (2006), Volume 2: The Chemistry of Wine and Stabilization and treatments, *Handbook of Enology*, John Wiley & Sons Ltd.
20. Šebečić B., Pavišić-Strache D., Vedrina-Dragojević I. (1998) Trace elements in wine from Croatia, *Deut. Lebens. Rundsch*., **94**, 341-344.
21. Šeruga M., Grgić J., Grgić Z., Šeruga B. (1998) Aluminium content of some Croatian wines, *Deut. Lebens. Rundsch*., **94**, 336-340.
22. Thiel G., Danzer K. (1997) Direct analysis of mineral components in wine by inductively coupled plasma optical emission spectrometry (ICP-OES), *Fresen. J. Anal. Chem.*, **357**(5), 553-557.
23. Zoecklein B.W., Fugelsang K.C., Gump B.H., Nury F.S. (1999) Wine analysis and production, Kluwer Academic/Plenum Publishers, New York.