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Original article

Determination of heavy metals in wine products in Montenegro in order to protect consumer health

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Summary. Background/Aim: Wine may contain essential macronutrients and mircoelements, but it can also be a source of heavy metals. Heavy metals significantly affect the quality and shelf-life of wine, even when present in micro- quantities. The aim of this study is to investigate the possibility of determining the content of heavy metals, zinc, cadmium, lead, copper, in the red and rosé wines samples, produced by the Montenegro, using potentiometric stripping analysis. Methods: Testing 12 samples of the red and rosé wines, produced by Montenegro, in five replications, were performed using the potentiometric stripping analysis (PSA) technique, whereas comparative analyses were performed using atomic absorption spectrometry (AAS) by flame and graphite technique. Results: The results of testing a total of 12 red and rosé wine samples using potentiometric striping analysis (PSA) and flame atomic absorption analysis (AAS) and graphite technique, indicate that the heavy metals content of Zn, Cd, Pb and Cu was in the range (values are expressed in ppb) of: 277.14 - 305.5; 8.98 - 13.83; 45.87 - 59.94 for the red wine; and 14.21 - 19.02: 321.88 - 414.58; 3.05 - 4.41; 36.88 - 44.56; 19.48 - 22.17 for the rosé wine. Conclusion: The results obtained in this study, as well as the complexity and the duration of the analysis, lead to the conclusion that it is justified to employ potentiometric stripping analysis in determining the heavy metal content in wine.

Key words: Potentiometric stripping analysis; Heavy metals; Red and Rosé wines; Human health protection

Introduction

To protect human health, it is of vital importance that the food products ingested are of good quality, safe and free of heavy metals, which can have detrimental effects on human health. Inadequate and contaminated products are an important causative agent of disease in modern society. The goal of modern medicine is to reduce mortality rates and extend life expectancy; however, various contaminants such as heavy metals, ingested through food and beverages, contribute to the emergence of modern- day diseases, which are increasingly difficult to eradicate. From a consecrated drink to a staple beverage, wine has become an indispensable part of our civilisation.

Wine is a complex mixture of water and ethanol, which contains a large number of organic and inorganic compounds [1]. It is a source of antioxidants which have a positive effect on the heart, reduce the risk of blood clots, and have a protective effect on the brain and nerve cells as they cross the blood-brain barrier [2]. Consuming this drink is associated with a decrease in the incidence of oxidative stress related neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease [3].

Wine may contain essential macronutrients and mircoelements, but it can also be a source of heavy metals [4]. Cellular components such as cell membranes, mitochondria, lysosomes, endoplasmic reticulum, nucleus, enzymes involved in metabolism,

detoxification and repair, are all affected by heavy metals [5]. There are many reasons for analysing the content of heavy metals in wine: bioavailability/toxicity, quality assurance/control, challenge, origin and authenticity [6]. The needs of the human body for essential elements such as calcium, zinc, chromium, copper, iron, magnesium, selenium, among others, can be met by a moderate consumption of wine [6,7]. Excessive intake of these elements puts health at risk. In order to protect human health, analyses which can detect the presence of heavy metals in wine have been introduced. The laws of almost all countries in the world prescribe the need to detect the presence of hazardous substances (e.g. lead, arsenic, cadmium, mercury, sulfur, various organometallic compounds lead, arsenic). The interest in assessing toxicity, bioavailability, bioaccumulation and transfer of the specific elements (e.g. lead, arsenic), is on the rise. It is estimated that about 70% of the intake of lead in the human body comes from the consumption of food and drink, and wine is the alcoholic beverage with the highest lead content [8]. Excess lead can have adverse effects on health, affecting both the nervous system and the hemoglobin biosynthesis [8]. With chronic exposure, cadmium accumulates in the epithelial cells of the proximal tubule and exerts a toxic effect on the kidneys [9]. The significant amount of cadmium present in wine comes from its contact with the apparatus used in the production and packaging process, as well as other means used in production [10]. The main source of copper in the finished product comes from the use of CuSO4, which is used to remove both aqueous sulfides and other sulfide compounds of uncharacteristic odours. It is recommended to maintain the copper level below 1 mg/L [11]. Due to its low natural content (5- 50 μg/l), lithium is a metal ion used in Italy as a means of denaturing and marking non-consumable wines [12]. Technological characteristics (pressing), chemical factors (alcohol content, acidity) and physical parameters, such as temperature, can also cause metal extraction [13]. The content of the elements is subject to change during the production process and therefore the analysis of the elements at different stages of production can result in significant information [14]. Analytical control of heavy metals during the technological process itself, as well as of the end product, is very important for the protection of health of consumers. One of the main requirements is that the method should be very fast and easy to perform. There are several methods for determining the content of heavy metals in wine, mainly using atomic spectrometry techniques and electrochemical methods. Most elements in wine can be determined using these methods in concentrations of mg/l to μg/l. Potentiometric stripping analysis (PSA) is the youngest of all stripping techniques. It was first presented in 1976, when Jagner and Granelli pointed to the possibility of using chemical oxidation ($Hg^{2}+$) in the analytical step of electrochemical stripping analysis [15]. Electrochemical stripping analysis (ESA) is a specific technique that is performed in 4 successive steps. The first step involves electrolysis during which the material being analysed is concentrated onto or in the working electrode, with reproducible stirring of the solution, i.e. mass transfer from the solution. During the second step, the rest period, the stirring of the solution is interrupted to provide conditions for mass transfer by one of the diffusion techniques (voltammetry, potentiometry, chronopotentiometry, chronoamperometry). In the third step, the potentiostatic control is terminated and the substance stripped is dissolved by changing the potential of the working electrode, communicating a constant current or potential, or by a chemical reaction. The fourth step is necessary in case of a repeated analysis of the same solution [16]. The PSA electrolytic step is the same as for voltammetric stripping techniques. After the expiration of the electrolysis time, the potentiostatic control is terminated and the change of the potential of the working electrode, which occurs due to the chemical oxidation of the collected deposit, is registered. The response signal is in the form of a classical potentiogram, with the oxidation time as the quantitative and the mean value of the dissolution potential or the potential of the inflection point of the function, as a qualitative characteristic [17].

The aim of this study was to investigate the possibility of determining the content of heavy metals, Zn, Cd, Pb, Cu, in red and rosé wines samples, using potentiometric stripping analysis. In order to achieve this goal, a comparative examination of a series of the wine samples (both red and rosé) was performed under standard laboratory conditions using potentiometric

striping analysis (PSA) and atomic absorption spectrophotometry (AAS).

Materials and Methods

Materials and Reagents. The materials to be analyzed were twelve different bottled wines (six red wines and six rosé wines) produced in Montenegro and commercially available. Once opened, each wine was kept at 2°C until analyzed. Standard stock solutions of zinc, cadmium, lead and copper were prepared by diluting "Titrisol" (Merck) to a content of 1 g/L. Working standard solutions were prepared by diluting the stock solutions with triply-distilled water to a content of about 100 mg/L. Hydrochloric acid ("Suprapur" Merck) was used as the supporting electrolyte. All tests were performed using a M1 computerised stripping analyser („Elektrouniversal", Leskovac). The M1 Striping Analyzer enables the communication of electrolysis (deposition) potentials of -2V to 2V and a constant deposition or dissolution current in the range of -50 *μA to 50 μA. The accuracy of parameter setting is defined by an error of* $\Delta E < 2$ mV and $\Delta I < 0.2$ *μA, respectively.* During the analytical step, the potential values of the working electrode are sampled at a frequency of approximately 81 s^{-1}, stored, and finally recorded on a dot matrix printer. The quantitative characteristic is determined by measuring the time between two consecutive turning points of the response signal with an accuracy defined by an error of $\Delta t = 50$ ms. The quantitative characteristic of an analyte is determined on the basis of its dissolution potential, as the mean of the plateau potential of the potentiogram or chronopotentiogram. The resolution when measuring the dissolution potential is $\Delta E = 2$ mV. The mechanical mixer enables highly reproducible mixing of the solution at fixed rpm speeds of 1000, 2000, 4000, 5000, or 6000 min^{-1}. The working electrode (thin-film mercury electrode carrier) is made by imprinting a carbon glass cylinder ("SIGRADUR" G) at elevated temperatures into a Teflon tube, outside diameter d = 8 mm. The surface of the disc (active electrode surface) is 7.07 mm^2. Ag/AgCl, KCl (3.5 mol / dm^3) and platinum auxiliary wire (d = 0.7 mm, l = 7 mm) were used as the reference electrode. Comparative tests were carried out using atomic absorption spectrophotometry employing a flame and graphite technique, performed on an AA-7000 atomic absorption spectrophotometer (Shimadzu).

Pre-treatment of wine. Determining the content of lead, copper and cadmium using the PSA analysis techniques was performed directly after a simple acidification. For the testing of the zinc content, the sample was first diluted and then acidified. For the comparative tests by atomic absorption spectrophotometry, samples were prepared according to ISO 5515: 2003, which involves treating the samples with nitric and sulfuric acid with uniform boiling [18].

Determination. We tested 6 samples of the red wine in five replications, and 6 samples in five replications. During the potentiometric stripping analysis, the standard addition method was used in all tests, and the quantitative determination of the heavy metal content in the samples tested was calculated according to the formula:

$$Cx = \frac{\tau_1 \times CsVs \times Vsm_1}{Vu(Vsm_2 \times \tau_2 - Vsm_1 \times \tau_1}$$

$Vsm_1 = Vuz + V$ of supporting electrolyte

$Vsm_2 = V\ sm_1 + Vs$

Vs – volume of standard addition

Cs – concentration of standard addition

τ_1 – oxidation time of the metal analysed in the solution without standard addition

τ_2 – oxidation time of the metal analysed in the solution with standard addition

Vuz – sample volume

Zinc was determined directly from 5ml of the sample which was diluted 4 times due to the fact that high values could not be recorded with the apparatus described (5 ml of sample + 15ml of H_2O) and after the addition of 20 *μl HCl (supporting electrolyte).*

Cadmium was determined directly from 15 ml of the sample without dilution and with the addition of 20*μl HCl (supporting electrolyte).*

Lead and copper were determined directly from the same solution from a 15 ml sample with the addition of 80 *μl HCl (supporting electrolyte).*

Flame and graphite techniques and the calibration curve method were used in comparative tests using atomic absorption spectrometry.

Statistical analysis. When it comes to statistical indicators, standard deviation was calculated in order to test whether there existed a significant difference in the precision between the two sets of results obtained using two different methods, and this was performed using the F-test and by calculating the means [19].

Results and Discussion

The concentrations of Zn, Cd, Pb and Cu in the red wine samples were obtained using potentiometric stripping analysis (PSA) and atomic absorption spectrophotometry (AAS); the means, standard deviation and maximum permitted levels are presented in Table 1.

When it comes to determining the zinc content in the samples, the electrolysis potential was - 1147mV, and the electrolysis time was 120 s. The dissolution of Zn from the solution without the standard addition started at – 907 mV and lasted for $\tau_1 = 0.2$ s.

The dissolution of Zn from the solution with the standard addition (Vs = 0.018 ml; Cs = 99999 *μg/ l) started at* – 929 mV and lasted for $\tau_2 = 0.6$ s. When it comes to determining the content of cadmium in the samples, the electrolysis potential was - - 1095 mV, and the electrolysis time was 480 s. The dissolution of Cd from the solution without the standard addition started at – 895 mV and lasted for $\tau_1 = 0.2$ s. The dissolution of Cd from the solution with the standard addition (Vs = 0.004 ml; Cs = 60114 *μg/l) began at* – 895 mV and lasted for $\tau_2 = 1$ s. The determination of lead and copper content was performed simultaneously from the same solutions; the electrolysis potential was – 905 mV and the electrolysis time was 480 s. The dissolution of Pb from solutions without the standard addition started at – 376 mV and lasted for $\tau_1 = 0.1$ s. The dissolution of Pb from solutions with the standard addition (Vs = 0.008 ml; Cs = 90063 *μg/l) started at* -376 mV and lasted for $\tau_2 = 0.3$ s. The dissolution of Cu from the solution without the standard addition started at – 179 mV and lasted for $\tau_1 = 1.3$ s. The dissolution of Cu from the solution with the standard addition (Vs = 0.003 ml; Cs = 99999 μg/l) started at – 163 mV and lasted for $\tau_2 = 3.1$ s. The concentrations of Zn, Cd, Pb and Cu in the rosé wine samples were obtained using potentiometric stripping analysis (PSA) and atomic absorption spectrophotometry (AAS); the means, standard deviation and maximum permitted levels are presented in Table 2.

When it comes to the zinc content in the rosé wine samples, the electrolysis potential was - 1130 mV and the electrolysis time was 120 s. The dissolution of Zn from the solution without the standard addition started at – 960 mV and lasted for $\tau_1 = 0.6$ s. The dissolution of Zn from the solution with the standard addition (Vs = 0.02 ml; Cs = 99999 μg/l) started at - 980 mV and lasted for $\tau_2 = 1.3$ s. As for cadmium in the rosé wine samples, the electrolysis potential was - 1905 mV and the electrolysis time was 0.7 s. The dissolution of Cd from the solution without the standard addition started at - 946 mV and lasted for $\tau_1 = 0.7$ s. The dissolution of Cd from the solution with the standard addition (Vs = 0.02 ml; Cs = 60114 μg / l) started at - 946 mV and lasted for $\tau\tau_2 = 0.9$ s. The determination of lead and copper content was performed simultaneously from the same solutions; the electrolysis potential was - 905 mV and the electrolysis time was 360 s. The dissolution of Pb from the solution without the standard addition started at - 365 mV and lasted for $\tau_1 = 0.2$ s. The dissolution of Pb from the solutions with the standard addition (Vs = 0.007 ml; Cs = 90063 μg/l) started at - 385 mV and lasted for $\tau_2 = 0.5$ s. The dissolution of Cu from the solution without the standard addition started at - 189 mV and lasted for $\tau_1 = 1.4$ s. The dissolution of Cu from the solution with the standard addition (Vs = 0.003 ml; Cs = 99999 μg/l) started at - 177 mV and lasted for $\tau_2 = 2.8$ s.

Discussion

The results of measurements of heavy metals in red and rosé wine are summarized in Tables 1 and 2. The results of an average of five measurements for each sample are presented. According to Montenegrin regulation, the maximum permitted content of certain metals in wines are: 5 mg Zn / kg, 1 mg Cd / kg, 0.3 mg Pb / kg and 3 mg Cu / kg [20]. The results of testing a total of 12 red and rosé wine samples using potentiometric striping analysis (PSA) and flame atomic absorption analysis (AAS) and graphite technique, indicate that the heavy metals content of Zn, Cd, Pb and

Table 1. Concentrations of Zn, Cd, Pb and Cu in the red wine samples obtained via potentiometric stripping analysis (PSA) and atomic absorption spectrophotometry (AAS); the means, standard deviation and maximum permitted levels.

Samples of the red wine "Vranac" (13-jul Plantaže) "Vranac" Plantaže 13.Jul		Content (μg/kg) Ppb							
Sample no.	Replication no.	Zn		Cd		Pb		Cu	
		PSA	AAS	PSA	AAS	PSA	AAS	PSA	AAS
1	1	285.4	291.08	12.61	9.58	53.95	45.87	15.18	14.21
	2	290.5	295.42	10.42	9.05	50.48	50.15	18.92	12.33
	3	283.6	288.52	15.48	12.08	58.15	41.33	20.15	15.38
	4	287.8	295.82	12.88	8.48	54.05	52.16	11.48	10.11
	5	288.6	292.45	13.42	7.52	55.61	48.88	12.88	16.21
2	1	268.0	239.46	13.55	12.62	59.94	50,91	18.98	19.02
	2	272.2	242.52	11.88	15.04	65.15	48.33	20.16	21.15
	3	260.5	231.56	14.21	10.14	53.88	53.41	16.66	17.48
	4	263.4	238.41	12.88	11.58	61.48	55.16	21.18	22.16
	5	265.8	240.15	13.01	8.99	60.33	49.55	19.61	18.48
3	1	305.5	300.41	13.83	11.13	47.96	46.21	15.19	17.27
	2	310.6	305.81	13.01	10.88	41.58	48.58	14.88	20.15
	3	307.8	302.88	15.05	14.28	43.42	42.16	20.16	14.28
	4	303.4	299.52	10.15	9.52	51.16	45.16	13.16	21.16
	5	301.5	296.56	11.28	10.21	49.88	49.61	15.02	18.95
4	1	246.9	281.66	10.01	10.92	49.81	52.16	18.80	16.53
	2	252.6	285.42	11.52	8.55	52.66	55.88	15.66	21.15
	3	240.8	290.54	9.48	12.01	45.88	48.92	19.90	18.41
	4	253.4	275.59	8.58	13.48	50.16	54.16	20.16	17.99
	5	248.8	283.16	12.61	15.41	53.41	59.81	14.22	13.58
5	1	276.5	256.92	12.18	12.02	51.10	49.81	17.80	18.19
	2	281.4	250.81	15.61	9.51	55.88	43.62	15.66	20.16
	3	272.6	260.48	9.21	15.41	45.64	45.88	18.01	16.55
	4	285.7	253.16	8.99	13.02	49.18	53.61	19.52	17.28
	5	282.5	258.14	10.52	9.21	52.33	52.82	18.88	16.21
6	1	268.0	277.14	10.01	8.98	53.20	48.14	17.32	15.28
	2	271.5	272.81	13.42	7.52	58.41	52.18	20.15	11.28
	3	262.4	273.52	12.62	10.45	47.52	44.21	18.16	15.48
	4	270.3	280.61	9.88	12.11	55.16	45.22	14.52	16.28
	5	269.4	282.51	10.48	8.52	54.28	49.58	15.92	17.99
Mean		275.91	274.76	11.96	10.24	52.72	49.45	17.27	17.02
Standard deviation		18.68	22.15	1.97	2.27	5.37	4.27	2.63	2.95
Maximum permitted levels		5000		100		200		3000	

Table 2. Concentrations of Zn, Cd, Pb and Cu in the rosé wine samples obtained via potentiometric stripping analysis (PSA) and atomic absorption spectrophotometry (AAS); the means, standard deviation and maximum permitted levels

Samples of the rosé wine		Content (μg/kg) Ppb							
Sample no.	Replication no.	Zn		Cd		Pb		Cu	
		PSA	AAS	PSA	AAS	PSA	AAS	PSA	AAS
1	1	414.0	321.88	3,97	3.08	38.16	36.88	21.45	22.17
	2	408.1	329.65	4.80	3.87	39.52	37.99	22.38	22.99
	3	420.2	315.61	3.75	3.78	40.14	35.45	20.08	21.87
	4	415.4	320.48	3.98	3.99	36.28	36.52	21.99	23.04
	5	427.2	319.88	3.99	2.92	39.14	38.02	22.45	23.41
2	1	380.0	414.58	3.53	3.99	41.97	42.14	19.62	19.64
	2	369.5	420.61	3.99	4.15	42.88	43.17	20.51	20.41
	3	381.4	410.20	4.52	3.62	40.03	41.58	18.72	19.02
	4	372.5	413.41	4.01	4.28	43.15	43.02	20.99	18.92
	5	388.6	412.28	3.54	3.88	39.99	42.55	19.03	21.02
3	1	379.3	358.45	4.41	4.21	41.97	39.81	19.48	19.88
	2	382.5	360.58	5.12	4.15	42.99	39.02	19.99	20.47
	3	365.6	354.21	4.02	3.99	39.02	40.15	20.52	21.02
	4	385.6	362.88	4.21	4.81	43.04	38.99	18.92	19.02
	5	387.5	359.21	5.15	3.95	40.14	39.51	19.05	18.99
4	1	336.9	388.41	4.24	3.77	38.16	40.15	21.45	20.51
	2	345.1	379.52	3.88	4.15	39.58	41.03	20.88	21.04
	3	321.5	359.88	4.88	3.03	40.00	40.99	23.07	20.02
	4	348.5	390.45	4.15	4.22	37.18	41.58	20.45	19.61
	5	349.8	396.51	3.95	3.02	39.13	38.99	21.02	21.58
5	1	381.1	405.24	3.65	4.08	41.59	40.88	20.22	20.88
	2	385.4	410.12	3.95	4.15	40.02	39.15	22.01	22.04
	3	372.8	401.88	4.12	3.52	43.07	42.01	20.88	20.02
	4	378.9	407.66	3.45	3.99	42.14	40.45	19.87	19.28
	5	382.4	406.21	3.81	4.13	40.27	38.99	19.04	19.99
6	1	379.3	369.97	3.92	3.92	44.56	41.62	21.00	21.61
	2	390.5	375.41	4.15	3.88	45.78	43.03	22.07	22.72
	3	384.8	372.58	4.18	4.01	42.17	40.99	21.48	20.21
	4	372.6	365.14	3.51	3.15	45.02	40.88	20.99	20.99
	5	389.5	364.15	3.93	4.21	44.12	41.28	20.51	20.12
Mean		379.88	375.57	4.09	3.86	41.04	40.22	20.67	20.74
Standard deviation		23.75	32.02	0.44	0.44	2.35	1.96	1.16	1.29
Maximum permitted levels		5000		100		200		3000	

Cu was in the range (values are expressed in ppb) of: 277.14 – 305.5; 8.98 – 13.83; 45.87 – 59.94; 14.21 – 19.02 for the red wine; and 321.88 – 414.58; 3.05 – 4.41; 36.88 – 44.56; 19.48 – 22.17 for the rosé wine.

In all wines the zinc content was lower then allowed 5 mg/kg (Table 1; Table 2). Arcos et al. [21] analyzed content of heavy metals in wine using pulse anodic stripping voltrammetry. The results they obtained show that the heavy metal content was also highest for Zn and lowest for Cd. Zinc was found in higher amounts in rosé wine than red wine, but within the allowed concentrations.

From the analyzed heavy metals, the lowest values of cadmium were observed. The accuracy of the PSA technique was confirmed through a good agreement of its results with the results obtained using the AAS technique. After comparing the standard deviations of the concentrations obtained by testing with the PSA and the AAS techniques, using the F-test [17], it can be concluded that there is no significant difference in accuracy at the confidence level of 95%.

Lead content in Montenegrin wines, determined using PSA technique, is similar to lead content in Italy, determined using atomic absorption spectrometry [22], square-wave anodic stripping voltammetric technique [23], atomic fluorescence spectrometry [24] and derivative stripping chronopotentiometry [25].

The copper concentration ranged from 0.01421 to 0.02217 mg/kg (Table 1; Table 2). Simões da Costa [26] analyzed copper content in wine using potentiometric chemical sensors. Results obtained in the study [26] are equivalent to those acquired using potentiometric stripping analysis.

The content of all tested metals is below the maximum permitted levels prescribed by the applicable regulations [20]. As for the PSA technique, it was found that wine as a matrix did not significantly affect the direct tests of the Cd, Pb and Cu contents, while it was necessary to dilute the samples before adding acid to test for the Zn content. In order to obtain valid test results using the AAS technique, it was necessary to first prepare the samples by destruction, which took 2 hours. Therefore, taking into account the complexity and the duration of the analysis, it can be argued that potentiometric stripping analysis may have an advantage over AAS techinique and is accurate to use for the determination of heavy metals in wine.

Conclusions

It can be concluded that electrochemical stripping analysis, in general, and, within it, potentiometric stripping analysis, represent a technique that today is one of the most commonly used micro-analytical techniques, bearing in mind that it largely meets almost all analytical requirements.

Its most significant advantages are its exceptional sensitivity, selectivity in terms of different oxidation states of the analyte, mobility of instrumentation and cheap exploitation.

The results obtained in this study, as well as the complexity and the duration of the analysis, lead to the conclusion that it is justified to employ potentiometric stripping analysis in determining the content of heavy metals in wine.

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