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Determination of Iron, Copper and Zinc in the Wine by FAAS

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Abstract

Objectives: The purpose of this paper has been to determine quantitatively the presence of some heavy metals (Fe, Cu, and Zn) which are of particular importance for the wine quality and human health. A total of 41 samples have been analyzed during different stages of winemaking. Methods: The determination of heavy metals is done by an analytical technique known as Flame Atomic Absorption Spectroscopy (FAAS). The limit set by the EU directive EC 606/2009 and International Office of Vine and Wine (OIV) has been taken as reference values. Results and Discussion: None of the analyzed samples has exceeded the limits set by the EU and OIV, excluding the type of white wine known as Rhine Riesling. In this sample the Cu concentration exceeds several times the permissible values. Findings: It has been observed that heavy metals immediately after the alcoholic fermentation process probably continue to remain even for a while in the upper part until wine sediment forms at the bottom of the tank. A higher concentration of heavy metals was observed in the samples taken from the bottom of the wine tank. Conclusion: The analyzed wines samples do not pose a risk in the future to human health.

Keywords:

Flame Atomic Absorption Spectroscopy;

Heavy Metals;

Iron, Copper, Zinc ;

Wine.

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1- Introduction

Without any doubt, wine is widely consumed beverage in the world with very obvious commercial value and social importance. The mineral content of wines may be influenced by many factors such as mineral composition of soil, viticultural practices, environmental conditions, processing, clarification procedures or storage conditions [1]. Metals in wine can originate from both natural and anthropogenic sources, and its concentration can be a significant parameter affecting consumption and conservation of wine. Metallic ions have an important role in oxide-reductive reactions, resulting in the wine browning, turbidity, cloudiness, astringency, and wine quality depends greatly on its metal composition [2].

Several elements (especially Cd, Cu, Fe, Mn, Sn and Zn) when present in excessively high concentration in wines, adversely affect the organoleptic quality and the stability of the wine [3]. Several metals and metalloids, such as Cd, Pb, Sn, Hg, and As, are known to be potentially toxic. At the same time, the analysis for certain elements in wines and fruit wines is of special interest due to their toxicity in case of excessive intake and also the effect they seem to have on the organoleptic properties of these alcoholic beverages. A typical example is copper which is an essential as well as a potentially toxic element for humans when in excess [4]. In contrast, the excessive presence of the elements like Al, Cu, Fe, and Zn has a definite negative effect on the organoleptic properties of the different kinds of wine [5].

Class A metals are essential for life in relatively high amounts (for example iron), class B includes metals that have no known biological function but are not particularly toxic at low concentrations (for example strontium), class C includes metals that are essential in very low concentrations (copper, molybdenum, nickel, manganese, and zinc), but which at higher than certain threshold concentrations may become toxic, and finally class D, which includes metals

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that are toxic even at low levels and their biological function is unclear (cadmium, mercury, and lead). The concentration of heavy metals can vary during different technological stages of winemaking. The mineral content of wines may be influenced by many factors such as mineral composition of soil, viticultural practices, environmental conditions, processing, clarification procedure or storage conditions. Heavy metals, decreased during fermentation, resulting in 0–50% of original amount. Increased concentrations in wine resulted from contamination during post-fermentation processing [6]. Increased concentration of heavy metals in the wine may result from long maceration times, and long contact times with metal-containing equipment. Yeasts also consume metals such as copper, iron, and zinc.

A large number of symptoms/ailments, comprising anemia, depressed growth, dermatitis, dwarfism, electrolyte-imbalance, gastro-intestinal and neurological disorders, lethargy and nausea, have been associated with Cu and Zn deficiency in humans, as well as with toxicity due to excessive intake [7].

2- Materials and Methods

The method we have used has been described by the Thermo-Scientific Company (Part of Thermo Fisher Scientific) [1]. Sampling was done at the Agrokosova Holding Company, the winery which operates in southern part of Kosovo, and three other wine samples for comparison have been taken from Macedonia. International standards of wine sampling have been applied, and sampling has been done during different technological stages of winemaking. A total of 41 samples were analyzed. Sample analyzes were performed in the laboratories of the University of Business and Technology – UBT (Prishtina - Kosovo). The analytical technique which has been used to determine heavy metals concentration in the wine has been *Flame Atomic Absorption Spectroscopy* (FAAS).

The Organisation International de la Vigne et du Vin (OIV) recommends a maximum Cu content of 1.0 mg/L for wines. The International Office of Vine and Wine (OIV) has also set minimum limits for As (0.2 mg/L), Cd (0.01 mg/L), Pb (0.2 mg/L) and Zn (5 mg/lL. The OIV also recommends that wines should contain Fe below 10 mg/l.

2-1- Standards and Sample Preparation

The type of flame atomic absorber that we have used was Perkin Elmer AA 300. In the beginning, the stock standard solution for iron, copper and zinc with known concentration of 1000 mg/L was prepared. All certified reference materials (standards) are obtained from the company C.P.A. Chem. Then, iron, copper, and zinc stock standard solutions containing 1000 mg/L were diluted with a pre-mixed solution of de-ionized water and analytical grade concentrated nitric acid (HNO3), to provide working standard solution of known concentrations in 2% (w/v) HNO3. The blank solution used for calibration was a 2% (w/v) HNO3. For zinc, working standards solutions were 0.5 mg/L, 1mg/L and 2 mg/L, while for iron and copper the working standard solutions were 1mg/L, 5mg/L, and 10 mg/L. From each wine sample 10 ml was accurately measured and transferred into a 40 ml volumetric flask. After that, 0.8 ml of analytical grade HNO3 was added and then made up to the final volume of 40 ml with Ultra-Pure DI Water. Iron, copper and zinc were determined by AAS in the air-acetylene flame using standard calibration curves.

Table 1. The instrument settings of Flame Atomic Absorber – Perkin Elmer AA 300.

	Fe	**Zn**	**Cu**
Wavelength (nm)	248.3	213.9	324.8
Fuel flow rate	0.9 L/min	1.1 L/min	1.2 L/min
Correlation coefficient	0.9925	0.9468	0.904
Replicates	3	3	3
Read time	4s	4s	4s
Unit	mg/L	mg/L	mg/L
Flame type	Air-Acetylene	Air-Acetylene	Air-Acetylene

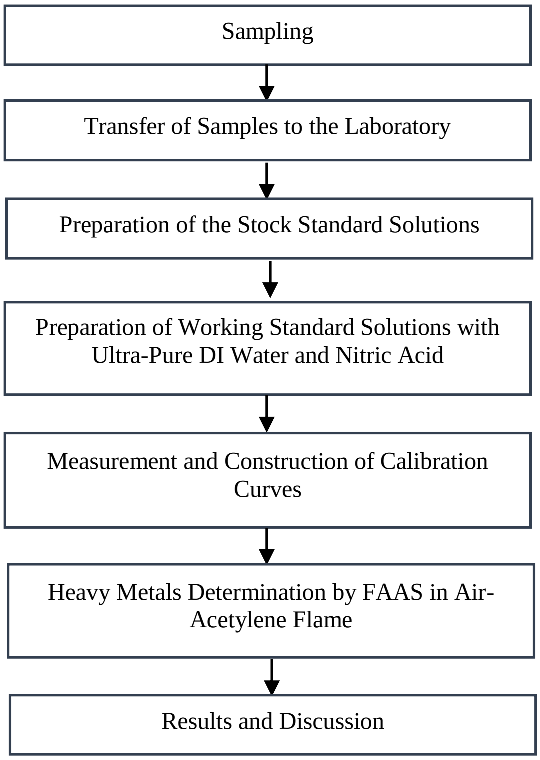

Figure 1. Flowchart of the research methodology.

3- Results and Discussion

The limit set by the European Union for iron in the wine is less than 30 mg/L, or according to the International Organisation of Vine and Wine (OIV) less than 10 mg/L. Most of the heavy metals in the grapes precipitate during fermentation into the sediments, which is the reason for their significantly lower content in the wine [8]. The application of fining agents, such as bentonite, copper sulfate (Al, Ca, Cd, Cr, Cu, Fe, Zn) and other environmental pollution (Cd, Co, Cr, Hg, Ni, Pb, V) may contribute also to wine contamination [9]. From the obtained results (Table 2) we can see that in the samples taken immediately after the alcoholic fermentation process there may still be relatively high concentrations of heavy metals in the upper part of the wine tank. This is due to impurity precipitation process which has been probably underway. As an example we can take the results of the wine type known as Pinot Noir (Table 2). We can see that in the upper part of the wine tank, the concentration of iron is higher compared to the bottom, a process which probably changes over time, up to in wine purification and sediment formation. The clarification and filtration are two common technological processes during winemaking. The potential negative impact of wine clarification and filtration has also been observed.

The application of these two technological processes it seems likely to induce increased concentration of iron in the wine. All this probably as a result of the wine passage through the technological equipment used for this purpose, or even passing through the special oenological pipes which are widely used during winemaking. Probably the wine pipes contain the impurity or even the composition of the equipment may affect the increase of iron concentration in the wine. As an example, it is worth analyzing the results of the wine type Pinot Blanc (Table 2). We can see that after applying the filtration process, the increased concentration of iron in the wine has been observed. We can support and compare these findings with the different articles of our colleagues who point out that one of the real possibilities of increasing the concentration of heavy metals in the wine may also be the use of oenological practices such as machinery, piping, use of fining agents, additives, etc. [2]. Regarding the Fe in wines, the main problem that appears is instability of wines when Fe concentration is greater than 10 mg/l. However, from the obtained results we can conclude that none of the analyzed samples poses a risk for significant wine instability in the future, especially after wine bottling and aging. These wines do not pose a risk for human health too.

Table 2. The Iron (Fe) concentration in the wine.

Type of Wine	Year of Production	Country	The Sampling Part in the Wine Tank	The Technological Stage During Winemaking	Fe (mg/L) after three replication
Pinot Blanc	2017	Kosovo	The bottom	After fermentation	0.464
Pinot Blanc	2017	Kosovo	Upper part	After fermentation	0.503
Pinot Blanc	2017	Kosovo	Upper part	After filtration	1.470
Rhine Riesling	2017	Kosovo	The bottom	After fermentation	2.483
Rhine Riesling	2017	Kosovo	Upper part	After fermentation	0.821
Rhine Riesling	2017	Kosovo	Upper part	After clarification	1.148
Franconia	2017	Kosovo	The bottom	After fermentation	0.958
Franconia	2017	Kosovo	Upper part	After fermentation	1.011
Pinot Noir	2017	Kosovo	The bottom	After fermentation	1.227
Pinot Noir	2017	Kosovo	Upper part	After fermentation	1.362
Sauvignon Blanc	2017	Kosovo	Upper part	After clarification	1.570
Sauvignon Blanc	2017	Kosovo	Upper part	After filtration	1.721
Merlot	2017	Macedonia	Upper part	After fermentation	1.346
Vranac	2017	Macedonia	Upper part	After fermentation	1.576
Cabernet Sauvignon	2017	Macedonia	Upper part	After fermentation	1.506

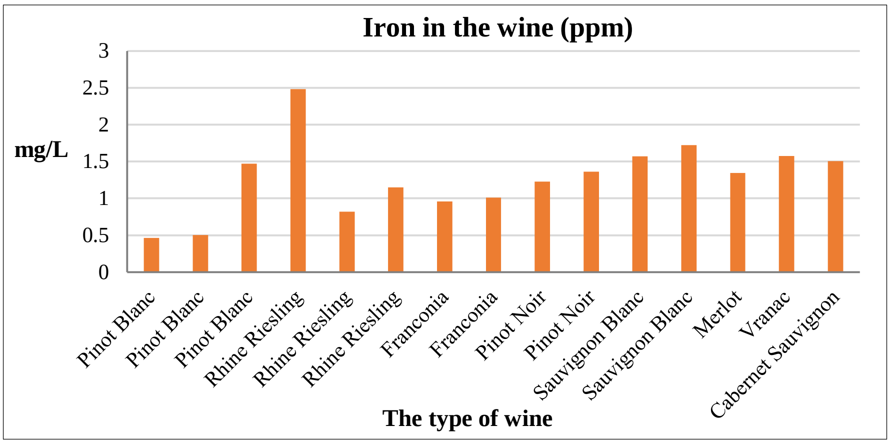

Figure 2. The iron concentration (mg/L) in the wine.

Table 3. The concentrations of cupper (mg/L) in the wine.

Type of Wine	Year of Production	Country	The Sampling Part in the Wine Tank	The Technological Stage During Winemaking	Cu (mg/L) after three replication
Pinot Blanc	2017	Kosovo	The bottom	After fermentation	0.037
Pinot Blanc	2017	Kosovo	Upper part	After fermentation	0.037
Pinot Blanc	2017	Kosovo	Upper part	After filtration	0.030
Rhine Riesling	2017	Kosovo	The bottom	After fermentation	5.668
Rhine Riesling	2017	Kosovo	Upper part	After fermentation	0.066
Rhine Riesling	2017	Kosovo	Upper part	After clarification	0.056
Franconia	2017	Kosovo	The bottom	After fermentation	0.088
Franconia	2017	Kosovo	Upper part	After fermentation	0.121
Pinot Noir	2017	Kosovo	The bottom	After fermentation	0.103
Pinot Noir	2017	Kosovo	Upper part	After fermentation	0.124
Merlot	2017	Macedonia	Upper part	After fermentation	0.158
Vranac	2017	Macedonia	Upper part	After fermentation	0.127
Cabernet Sauvignon	2017	Macedonia	Upper part	After fermentation	0.149

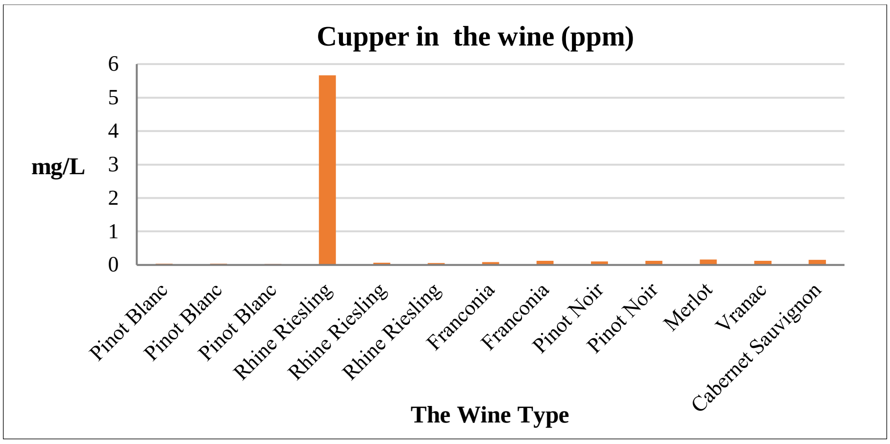

Figure 3. **The cupper concentration (mg/L) in the wine.**

Copper is also one of the most frequently occurring heavy metals in wine and can reach the wine via pesticides, brass fittings, and as copper sulphate for treatment of reductive off-flavors. High residual copper can contribute to an enhanced rate of oxidative spoilage, which ultimately results in the browning of the wine, particularly white ones. To minimize the incidence of these problems, it is generally recommended to maintain copper concentration below 0.3 – 0.5 mg/L [10].

European Union has sets limit regarding the cupper concentration in the wine, which is less than 0.5 mg/L, while according to the International Organisation of Vine and Wine (OIV) it should be less than 1.0 mg/l. From the obtained results we can see that in contrast to iron, the concentrations of copper in white wines at the bottom of the tanks are larger, although the differences are extremely small, compared to red wines. In red wines as in the case of iron, we can see a higher concentration of copper in the upper part compared to the bottom of the tank. The result that is worth analyzing and discussing is that of the white wine known as Rhine Riesling (Table 3). The obtained result indicates a cupper concentration several times higher (5mg/L) than the reference values. At the time of sampling, the sample taken from the bottom of the wine tank is characterized by large impurities. The same sample taken from upper part of the same tank turned out to be at significantly lower values compared to the bottom of the tank. Declines in values for the same sample have also been seen after the clarification process. Sun et al. (2019) [17] indicates that the copper ion concentration falling down was consistent with the trend of yeast growth rising up, meanwhile the copper content was still much less than the initial concentration after fermentation. In terms of wine quality and human health, this is very important because the wine from bottom of the tank during winemaking is dropped out, together with the excessive quantity of heavy metals. Approximately the same values in relation to the cupper concentration in the wine have been reported by other colleagues as well. According to a study in which 72 wines were analyzed, the average copper content was 0.18 mg/L with a maximum of 0.55 mg/L [11, 17]. Maciel et al. (2019) [12] indicates that the concentration of copper in all analyzed wine samples has been below (from 1.0 to 10.3 µg L−1) the maximum acceptable limit set by the OIV (1000 µg L−1). As a conclusion, all the analyzed samples in relation to the cupper concentration do not pose a risk to the wine stability and human health as well.

Table 4. **The concentrations of zinc (mg/L) in the wine.**

Type of Wine	Year of Production	Country	The Sampling Part in the Wine Tank	The Technological Stage During Winemaking	Zn (mg/L) after three replication
Pinot Blanc	2017	Kosovo	The bottom	After fermentation	0.004
Pinot Blanc	2017	Kosovo	Upper part	After fermentation	0.000
Pinot Blanc	2017	Kosovo	Upper part	After filtration	0.000
Rhine Riesling	2017	Kosovo	The bottom	After fermentation	1.609
Rhine Riesling	2017	Kosovo	Upper part	After fermentation	0.000
Rhine Riesling	2017	Kosovo	Upper part	After clarification	0.000
Franconia	2017	Kosovo	The bottom	After fermentation	0.000
Franconia	2017	Kosovo	Upper part	After fermentation	0.000
Pinot Noir	2017	Kosovo	The bottom	After fermentation	0.000
Pinot Noir	2017	Kosovo	Upper part	After fermentation	0.000
Merlot	2017	Macedonia	Upper part	After fermentation	0.291
Vranac	2017	Macedonia	Upper part	After fermentation	0.000
Cabernet Sauvignon	2017	Macedonia	Upper part	After fermentation	0.266

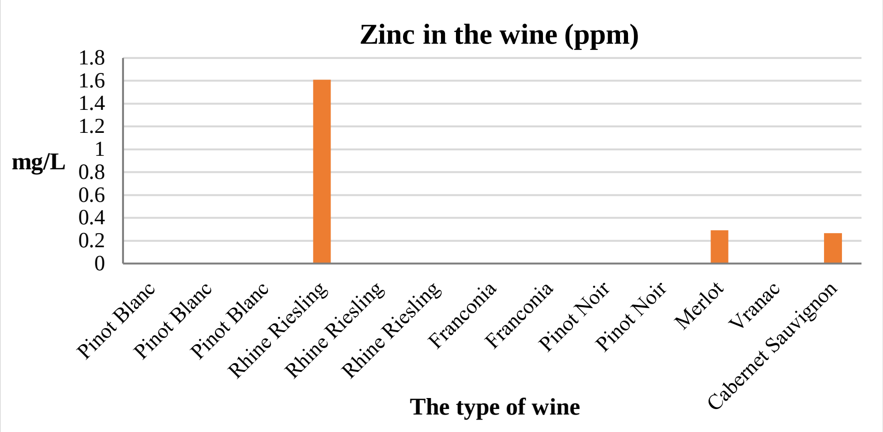

Figure 4. **The zinc concentration (mg/L) in the wine.**

Phytosanitary treatments using Bordeaux mixture or zinc (Zn) fungicides are often used in vineyards, which increase soil copper (Cu) and Zn contents [13, 18]. The authors found that bentonite fining significantly lowered concentrations of K, Cu, Rb, and Zn [14, 19]. The values set by the European Union for zinc concentration in the wine are less than 30mg /L, while according to International Organisation of Vine and Wine (OIV) they should be lower than 5mg /L. As in the previous two cases, zinc concentrations were within the permissible values. From the obtained results (table 4) we can see that zinc concentrations are at minimum values or virtually nonexistent, excluding any sample where low zinc concentrations has been found. Compared to the wines produced in Kosovo, red wines from Macedonia have been observed to have a slightly higher concentration of zinc, except sample no. 4 (Rhine Riesling). Approximately similar results from the Balkan region have been published by our colleagues as well. In white wine the highest concentration showed Zn 0.62mg/L, and in red-black wine Cu 0.238mg/L and Mn 2.12mg/L [15]. The Cu, Fe and Zn contents in twenty selected wine samples produced in the South-East region of Serbia were determined by flame atomic absorption spectrometry. The Cu concentrations varied from 0.07 to 0.57 mg/L in wines, and the Fe concentrations fluctuated from 2.93 to 36.2 mg/L, while the Zn levels were in the range from 0.21 to 0.67 mg/L [7, 20]. The concentrations of zinc found in the analyzed samples do not pose a risk to wine instability, nor do they pose a risk to human health as well.

4- Conclusion

Based on the results we have obtained, we can conclude that concentrations of the analyzed heavy metals (Fe, Cu and Zn) in the red wines are greater compared to the white wines, most likely due to the different mode of fermentation. The results show the potential influence of some technological processes during winemaking, such as clarification and filtration, which are carried out with special equipment, and so-called wine pipes which are widely used during winemaking process. Based on our findings and approximately similar results of our colleagues, we recommend that oenology equipments should be cleaned according to the standard cleaning protocols, including the wine pipes, in order to inhibit the induction of increased concentration of heavy metals in the wine. Probably in this process apart from the purity of the equipment, it seems to be very important, also the optimal time of sediment formation in both types of wines. Regarding to this, any movement of the wine can potentially disturb the wine sediment and thus can increase the concentration of heavy metals in the upper part of the wine tank. Special attention should also be paid to the process of grape and vineyard spraying with different chemical substances, then the proper use and according to the standards of clarifying agents, the proper use of different additives during winemaking, etc. All of these can lead to a potential increase in the concentration of heavy metals in the final product. We recommend colleagues to do as much as possible analysis and research of heavy metals in the wine, especially in relation to the metal oenological equipment used by different wineries. From the obtained results, as well as with a serious commitment during wine packaging and the storage, these wines will likely retain their optimal organoleptic properties. Finally we can conclude that all analyzed wine samples will not pose any risk in the future to human health

5- Acknowledgments

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6- Conflict of Interest

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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