Bull. Chem. Soc. Ethiop. **2011**, 25(2), 169-180.
Printed in Ethiopia

ISSN 1011-3924

CONCENTRATION LEVELS OF ESSENTIAL AND NON-ESSENTIAL ELEMENTS IN SELECTED ETHIOPIAN WINES

Daniel Minilu Woldemariam[1] and Bhagwan Singh Chandravanshi[2*]

[1]Department of Chemistry, Hawassa University, P.O. Box 05, Hawassa, Ethiopia
[2]Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

(Received December 6, 2010; revised February 27, 2011)

ABSTRACT. The concentrations of thirteen metals (K, Na, Ca, Mg, Fe, Zn, Mn, Pb, Co, Ni, Cd, Cu and Cr) were determined in four brands of Ethiopian wines by flame atomic absorption spectrometer (FAAS) after digesting the wine samples with HNO_3-H_2O_2 mixture. Among the major metals potassium (694–767 mg/L) was found to be at the highest level, followed by magnesium (58.1–79.2 mg/L), calcium (28.4–37.1mg/L) and sodium (24.0–24.4 mg/L). From the minor metals iron, zinc and manganese were in the ranges of 1.42–3.16, 1.82–2.70, and 1.04–1.88 mg/L, respectively, followed by copper (0.5–1.5 mg/L), nickel (0.18–0.20 mg/L), cobalt (<0.065–0.091 mg/L) and chromium (<0.075–0.192 mg/L). The toxic metal lead was in the range of 0.14–0.31 mg/L while cadmium was below detection limit of the analytical procedure used in this study (<0.01 mg/L). The levels of metals in the Ethiopian wines were found to be comparable with wines of some other countries.

KEY WORDS: Essential element, Non-essential element, Acid digestion, Flame atomic absorption Spectrometry (FAAS), Ethiopia, Wine

INTRODUCTION

The term wine describes an alcoholic beverage that contains products of fermentation of the juice from the grapes [1]. Grapes are not the only fruits used to make wine, though they are the most commonly used; wine is also made from the fermented juice of pears, apples, berries, and even flowers such as dandelions [1]. Wine naturally contains about 85−89% water, 10−14% alcohol, less than 1% fruit acids, and hundreds of aroma and flavor components in very small amounts. Wine character, its taste and smell, is derived from many factors including the grapes it is made from, where they were grown, and the production techniques applied by the wine maker, or enologist [2].

Wine is one of the most common alcoholic beverages consumed in all over the world. Wine is also widely produced and consumed in Ethiopia. Awash Winery is the first commercial winery in Ethiopia, established in 1943. The winery produces a range of well-made wines, including Gouder Red, Dukem Red, Awash Crystal White, Axumite Red, Kemila White as well as a Sparkling wine [3].

The content of metals in wine can be attributed to the natural sources, the atmospheric deposition of airborne particulate matter on grapes and transfer of metals from the soil via the roots to the grapes and finally to wine, and to the contamination during the winemaking process [4]. Metals of primary, natural origin come from soil on which wines are grown and reach wine through grapes. The concentration of primary metals is characteristic and comprises the largest part of the total metal content in wine. It is connected with the maturity of the grapes, their variety, the type of soil in the vineyard, and the climatic conditions during their growth. The contribution of metals of a secondary origin is associated with external impurities that reach wine during growth of grapes or at different stages in winemaking (from harvesting to bottling and cellaring). During the growth of grapes in a vineyard, contaminations can be classified as geogenic (originating in the soil), from protection and growing practices, or from environmental pollution [5].

---

*Corresponding author. E-mail: bhagnan@chem.aau.edu.et

Accordingly, wines from vineyards in the vicinity of sea or ocean result in a higher Na content compared to wines from other regions [6]. Differences in K, Ca and Cu content can be due to fertilizers used for cultivation [7]. Application of pesticides, fungicides and fertilizers containing Cd, Cu, Mn, Pb and Zn compounds during the growing season of vines leads to increases in the amounts of these metals in wine [8, 9]. Wines from vineyards located close to road traffic or situated in industrial areas contain higher levels of Cd and Pb because of vehicle-exhaust fumes or other emissions to air, water and soil [8, 9]. Finally, there is an enological (winemaking) source of metals, as contamination may occur at different steps of wine production. The reason for this is the long contact of wine with materials (aluminum, brass, glass, stainless steel, and wood) from which wine-making machinery and pipes, casks and barrels used for handling and storing wine are made. This is the usual source of Al, Cd, Cr, Cu, Fe and Zn [10, 11]. Contamination with Na, Ca or Al can be associated with fining and clarifying substances (flocculants, such as bentonites) added to wine to remove suspended solids after fermentation and to reduce turbidity [7, 11]. Ca concentration can also be affected by adding $CaCO_3$ or $CaSO_4$ for de-acidification of must and wine or enhancement of acidity of grape juices, respectively [8, 11].

Daily consumption of wine in moderate quantities contributes significantly to the requirements of the human organism for essential elements such as K, Ca, Mg, Cr, Co, Fe, F, I, Cu, Mn, Mo, Ni, Se, Zn. On the other hand, several metals, such as Pb, Cd and As, are known to be potentially toxic. At the same time, the analysis for certain elements in 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 wine [9, 11]. A typical example is copper, which is both an essential and a potentially toxic element for humans when in excess. Several elements, including Cu, Fe, Al and Zn, contribute to haze formation and sometimes taste effects. Determination of other elements, such as Pb, As or Cd is of considerable importance due to their potential toxic effects. Moreover, the content of some metals can be used to identify the geographic region in which the grapes were grown due to the direct relationship with soil composition [4].

Generally, the metallic composition of wine depends on many factors, some of which are related to the specific production area: grape varieties, soil and climate, culture and wine making practices, yeasts, etc. Due to the influence of all these factors a great variability in the metal content in wines from different areas, regions and countries is observed. Determination of typical levels of metal in wines is a very useful tool to differentiate wines from different geographic origins and different Certified Brands of Origin (CBO) as well as to detect adulterations and falsifications of wines [10].

Several reviews on the determination of trace metals in wines and alcoholic beverages have been reported in the literature [12-15]. These reviews also report that there are several methods for the determination of trace metals in wines and alcoholic beverages including the frequently used atomic absorption spectrometry (FAAS) [4, 16-28] and atomic emission spectrometry (AES) [26-29]. Graphite furnace AAS (GF-AAS) has found application to determination of trace and sub-trace metals [4, 11, 17, 19]. Inductively coupled plasma AES (ICP-AES) facilitates multi-elemental analysis [8, 19, 27, 28, 30]. The most frequently used atomic absorption spectrometry (FAAS) was used in the present study because of its availability, simplicity, convenience and high precision.

A direct determination is seldom used because of the significant interferences of the wine matrix [4, 19]. The interferences of sugar and alcohol can be partially compensated by the standard addition method or by the standard solutions prepared in a matrix similar to the wine matrix [29]. However, most workers recommended some pretreatment of the wine samples: evaporation of alcohol, extraction of metals by complexing agents into organic solvents or decomposition of organic matter [9, 15, 21, 28].

Normally, it is necessary to decompose the wine because of possible matrix interferences [9, 15]. This can be done by wet digestion on a hot plate or in a microwave oven using concentrated HNO_3 [28], a mixture of concentrated HNO_3 and 30% H_2O_2 [19, 20, 26], 30% H_2O_2 [25] or 30% H_2O_2 followed by adding concentrated H_2SO_4 [31, 32] or HNO_3 [28, 34]. Ca, K, Mg and Na are usually measured only after dilution of original samples with water [16, 29]. Al, Cd, Cu, Fe, Ni, Pb and Zn can be determined without dilution [20, 34], after dilution with HNO_3 and HCl solutions and/or water [19], or following acidification with concentrated HNO_3 [34].

There are no reports in the literature about any study on the metal contents in the Ethiopian wines. This study reports the levels of essential and non-essential metals in the Ethiopian wines. Thus the objectives of the present investigation were to: (i) determine the levels of essential and non-essential elements (K, Mg, Ca, Na, Fe, Zn, Mn, Cu, Cr, Co, Ni, Cd, and Pb) in the export quality Ethiopian red and white wines (Axumite red wine, Gouder red wine, Awash Crystal white wine, and Kemila white wine), (ii) compare the levels of minerals among the four brands of wines, and (iii) compare the levels of minerals in the Ethiopian wines with data obtained from other countries. The results of this study may provide useful baseline data on the levels and temporal variations of some nutrients in the Ethiopian wines.

EXPERIMENTAL

Instrumentation

A refrigerator (Samsung Hitachi, Japan) was used to keep the wine samples cool till the analysis. 250 mL round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus (Kjeldahl, Germany) hot plate to digest the wine samples. Buck Scientific Model 210 VGP (East Norwalk, USA) flame atomic absorption spectrophotometer equipped with deuterium ark background corrector was used for analysis of the analyte metals (K, Mg, Ca, Na, Fe, Zn, Mn, Cu, Cr, Co, Ni, Cd, and Pb) using air-acetylene flame. A micropipette (100 μL–1000 μL) was used for the volume measurement of reagents and standards.

Reagents and chemicals

Reagents used in the analysis were all analytical grades. HNO_3 (69–72%) (Spectrosol, BDH, England) and H_2O_2 (30%) (BDH Chemicals limited, Poole, England) were used for the digestion of wine samples. Lanthanum nitrate hydrate (99%) (Riedel-de-Haën, Germany) was used as molecular suppressor (to release calcium and magnesium from their common phosphates). Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals (K, Mg, Ca, Na, Fe, Zn, Mn, Cu, Cr, Co, Ni, Cd, and Pb) (Buck Scientific Puro-Graphic^{tm}) were used for preparation of calibration standards and in the spiking experiments. Working standard solutions were obtained by suitable dilution from stock solution. Iron(III) chloride anhydrous (99%, Avocado Research Chemicals Ltd., England) was used for preparation of calibration standards and spiking of iron. Distilled-deionized water was used throughout the experiment for sample preparation and dilution, and rinsing of apparatus prior to analysis.

Sampling

For this study, four most popular bottled Ethiopian wine brands (Axumite Red Wine, Gouder Red Wine, Awash Crystal White Wine, and Kemila White Wine) were selected. Five bottles from each brand were collected randomly from five supermarkets at different sites of Addis Ababa, the capital city of Ethiopia. By taking 100 mL from each bottle of the same brand four bulk samples (500 mL each) were prepared. The bulk samples were shaken slightly to ensure mixing.

Digestion of the wine samples

From the bulk samples prepared, 10 mL wine samples were wet ashed (digested) to decompose the organic substances and make clear solution in triplicate according to the HNO_3/H_2O_2 procedure described by Lazos and Alexakis [19] and Sanllorente *et al.* [35]. The digestion procedure was optimized by taking the following points in to consideration: (i) the amount of H_2O_2 and HNO_3 added to the sample, (ii) the digestion time, (iii) the maximum temperature required, and (iv) the color and clearness of the digest.

A 10 mL aliquot of the bulk sample was taken and quantitatively transferred to a 250 mL digestion flask. To this flask a freshly prepared 7 mL HNO_3/H_2O_2 mixture in the ratio of 2:5 was added. The flask was shaken slightly to mix the mixture. The flask with the acid-sample mixture was then placed on the hot plate of the Kjeldahl apparatus and fitted with the condenser. By setting the temperature first to 60 ^{o}C and slightly increasing up to 180 ^{o}C, the heating continued (for one hour) until the solution becomes clear and colorless. After the digestion was completed the flask with the digest was removed from the hot plate and was left to cool (for 10 min). The cooled digest was transferred quantitatively to 50 mL volumetric flask and diluted to the volume with distilled-deionized water. The digest was kept in the refrigerator until the analysis by AAS.

Digestion of the blank samples

During the determination of method detection limits, the procedure proposed by Galani-Nikolakaki *et al.* [9] was used. Six blank samples were prepared. Absolute ethanol was added to the distilled-deionised water in order to achieve a final alcohol concentration similar to that of the wine samples (12% v/v). The six blank samples were digested following the same procedure as the samples and each of the samples were determined for the elements of interest (K, Mg, Ca, Na, Fe, Zn, Mn, Cu, Cr, Co, Ni, Cd, and Pb) by atomic absorption spectrophotometer.

Recovery test

The accuracy of the analytical procedure was investigated by spiking a suitable known amount of the analyte metals into a test portion of the sample having a known concentration of the analyte, and analyzing the spiked test portion along with the original sample. While the precision of the method was expressed as percent relative standard deviation (% RSD) of the triplicate analyses.

For this research, in order to demonstrate the validity of whole analytical procedure, the recovery test was done as follows: 200 mg/L of K, 25 mg/L Mg, 10 mg/L Ca, 8 mg/L Na from the 1000 mg/L were spiked at once in to 10 mL wine sample and the remaining metals (1.5 mg/L of Fe, 1.05 mg/L Zn, 0.5 mg/L of Cu and Mn and Zn; 0.075 mg/L of Ni and Cr; and 0.01 mg/L of Cd were spiked at once in to another round bottomed flask containing 10 mL wine. Then same digestion procedure was followed for non-spiked and spiked wine samples side by side. Each sample was analyzed for their respective spiked metals by atomic absorption spectrophotometer. The recovery test for all samples was performed in triplicates.

Determination of the metals in wine samples by FAAS

Na, K, Ca, Mg, Fe, Cu, Zn, Co, Ni, Pb, Cd, Cr and Mn were determined by AAS using an air/acetylene flame. Major elements (Na, K, Ca and Mg) were analyzed by further diluting the digested wine samples. Dilution was required to bring the concentration within the linear range of the calibration. Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Na, K, Ca, Mg, Mn, Fe, Cu, Zn, Ni, Co, Pb, Cd, and Cr were used for the preparation of calibration standards and in the spiking experiments. All the analyses were carried out using flame atomic absorption spectrophotometer at the wavelengths specific for each metal. Sodium and potassium were analyzed in the emission mode of the instrument. For the preparation of calibration curve, a series of four working standards were prepared for each metal. The working standards were prepared daily from the intermediate standards, that were earlier prepared from the stock solutions (1000 mg/L).

Statistical analysis

Mean values obtained for the metals studied in the four brands of wine samples were compared by One-Way ANOVA at 95% level using SPSS 13 for windows (SPSS Inc. 1989–2004) assuming that there were significant differences among them when the statistical comparison gives $p < 0.05$.

RESULTS AND DISCUSSION

Figures of merit

The analytical wavelengths, the correlation coefficients, and the correlation equations of the calibration curves for the determination of metals in wine samples by FAAS are given in Table 1. The correlation coefficients of all the calibration curves were > 0.999 and these correlation coefficients showed that there was very good correlation (relationship) between concentration and absorbance.

Table 1. Analytical wavelengths, detection limits, correlation coefficients and correlation equations of the calibration curves for the determination of metals in wine samples by FAAS.

Metal	Wavelength (nm)	Instrument detection limit (mg/L)	Method detection limit (mg/L)	Correlation coefficient of calibration curve	Equation for calibration curve
K	766.5	0.010	0.09	0.9991	Y = 0.07566X
Na	279.5	0.002	0.10	0.9999	Y = 0.0121 + 0.39725X
Ca	422.7	0.010	0.10	0.9998	Y = 0.000802 + 0.01193X
Mg	285.2	0.001	0.10	0.9996	Y = 0.18298X
Cu	324.7	0.020	0.03	0.9998	Y = 0.0511 X
Zn	213.9	0.005	0.05	0.9997	Y = 0.159X
Mn	279.5	0.001	0.02	0.9998	Y = 0.0526X
Ni	232.0	0.04	0.10	0.9953	Y = 0.00845X
Fe	248.3	0.030	0.10	0.9997	Y = 0.00637X
Co	240.7	0.050	0.06	0.9998	Y = 0.0245X
Cr	357.9	0.050	0.07	0.9997	Y = 0.0452X
Cd	228.9	0.005	0.01	0.9997	Y = 0.1599X
Pb	283.2	0.100	0.10	0.9995	Y = 0.0085X

The method detection limits were calculated as the concentrations that give signals equal to three times the pooled standard deviations of the six blanks [36] and are given in Table 1. The method detection limits for all the metals were < 0.1 mg/L which clearly indicate that the method is applicable for the determination of metals at trace levels. The percentage recovery lies within the range 93–107% (with RSD 2–5%) which are within the acceptable range for all metals (Table 2). This confirms that the method is of good precision and accuracy. The precision of the analytical method was evaluated in terms of pooled standard deviations and relative standard deviation (% RSD) of the eighteen measured values of each metal in each of the bulk sample. The % RSD were found to be < 8% which indicate that the precision of the analytical method is good (Table 3).

Table 2. Recovery test results for the metals (mean ± SD).

Metal	Concentration (mg/L) in			% Recovery
	un-spiked sample	spiked amount	spiked sample (mg/L)	
K	767 ± 15	200	961 ± 14	97 ± 3
Mg	79.2 ± 5.5	25	103.4 ± 6	97 ± 3
Ca	37.1 ± 2.8	10	46.8 ± 2.0	97 ± 2
Na	24.4 ± 2.1	8	32.0 ± 2.5	95 ± 5
Fe	3.16 ± 0.25	1.5	4.60 ± 0.22	96 ± 3
Zn	2.14 ± 0.15	1.05	3.15 ± 0.14	96 ± 4
Cu	1.46 ± 0.03	0.5	1.97 ± 0.04	102 ± 4
Mn	1.50 ± 0.08	0.5	1.97 ± 0.04	94 ± 5
Pb	0.25 ± 0.02	0.1	0.35 ± 0.02	100 ± 3
Cr	0.19 ± 0.01	0.075	0.26 ± 0.02	93 ± 4
Ni	0.19 ± 0.01	0.075	0.27 ± 0.01	107 ± 5
Cd	<0.01	---	----	----
Co	<0.10	---	----	----

Levels of essential and non-essential metals in the wine samples

The levels of essential and non-essential elements (K, Mg, Ca, Na, Fe, Zn, Mn, Cu, Cr, Co, Ni, Cd, and Pb) were determined by FAAS. The results are given in Table 3. The concentrations of the metals analyzed vary widely in the wine samples. Each brand differs from the other in its metal content. The macroelements were present at higher concentrations than microelements as described below.

Table 3. Mean concentrations (mg/L) of metals in the Ethiopian Red and White wines.

Metal	Ethiopian wine brand			
	Axumite red wine	Gouder red wine	Awash Crystal white wine	Kemila white wine
K	767 ± 15	694 ± 15	764 ± 19	735 ± 13
Mg	79.2 ± 5.5	66.0 ± 5.0	77.6 ± 5.6	58.1 ± 4.3
Ca	37.1 ± 2.8	28.4 ± 2.2	31.6 ± 2.4	28.4 ± 2.2
Na	24.4 ± 1.9	24.3 ±1.7	24.4 ± 1.6	24.0 ± 1.9
Fe	3.16 ± 0.25	1.49 ± 0.06	1.42 ± 0.07	2.33 ± 0.15
Zn	2.14 ± 0.15	2.70 ± 0.15	2.40 ± 0.18	1.82 ± 0.12
Mn	1.46 ± 0.03	1.56 ± 0.12	1.88 ± 0.15	1.04 ± 0.02
Cu	1.50 ± 0.08	0.55 ± 0.04	0.61 ± 0.04	0.50 ± 0.03
Pb	0.25 ±0.02	0.16 ± 0.01	0.31 ± 0.02	0.14 ± 0.01
Cr	0.19 ± 0.01	< MDL	< MDL	< MDL
Ni	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.2 ± 0.01
Cd	< MDL	< MDL	< MDL	< MDL
Co	< MDL	< MDL	0.091 ± 0.007	< MDL

MDL = Method detection limit.

Axumite red wine contains K in highest amount of the macroelements with concentration 767 ± 15 mg/L followed by Mg (79.2 ± 5.5 mg/L) and Ca (37.1 ± 2.8 mg/L). Na (24.4 ± 1.9 mg/L) was found to be present at the lowest concentration of the macroelements analyzed. Fe (3.16 ± 0.25 mg/L) was found in higher amounts than other trace microelements. It was followed by Zn (2.14 ± 0.15 mg/L), Mn (1.46 ± 0.03 mg/L) and Cu (1.50 ± 0.08 mg/L). Cr (0.19 ± 0.01 mg/L) and Ni (0.19 ± 0.01 mg/L) were found to be at the same level perhaps by coincidence. While Co was below the method detection limit. Pb (0.25 ± 0.02 mg/L) was found at appreciable concentration while Cd was below the method detection limit. The level of metals in the Axumite red wine was found in the decreasing concentration order of metals K > Mg > Ca > Na > Fe > Zn > Mn > Cu > Pb > Co = Cr.

Gouder red wine contained lowest concentration of the macroelements K (694 ± 15 mg/L). Mg (66.0 ± 5.0 mg/L) was found at much higher concentration than Ca (28.4 ± 2.2 mg/L) and Na (24.3 ± 1.7 mg/L). Gouder red wine also contained Zn (2.70 ± 0.15 mg/L) at higher concentration than other microelements, followed by Mn (1.56 ± 0.12 mg/L) and Fe (1.49 ± 0.06 mg/L) and Cu (0.55 ± 0.04 mg/L). Only a small amount of Ni at a concentration of 0.19 ± 0.01 mg/L was detected. Cr and Co were below the method detection limits. The non-essential metal Pb was detected at trace level of 0.16 ± 0.01 mg/L while Cd was below the method detection limit. The concentrations of metals determined in Gouder red wine was in different decreasing concentration order than the Axumite red wine: K > Mg > Ca > Na > Zn > Mn > Fe > Cu > Ni > Pb.

Awash Crystal white wine was also much rich in its content of K (764 ± 19 mg/L) than other macroelements followed by Mg (77.6 ± 5.6 mg/L), Ca (31.6 ± 2.4 mg/L) and Na (24.4 ± 1.6 mg/L). The concentration of Zn (2.40 ± 0.18 mg/L) was higher than Mn (1.88 ± 0.15 mg/L) followed by Fe (1.42 ± 0.07 mg/L) and Cu (0.61 ± 0.04 mg/L). Ni (0.18 ± 0.01 mg/L) was present at low concentration and Co (0.091 ± 0.007 mg/L) was only at trace level. Cr was below the method detection limit. The toxic metals Pb (0.31 ± 0.02 mg/L) was present at the highest concentration than the other brands of the Ethiopian wines. Cd was below the method detection limit. The concentrations of metals determined in Awash Crystal white wine was found in the decreasing concentration order of metals K > Mg > Ca > Na > Zn > Mn > Fe > Cu > Pb > Ni > Co.

Kemila white wine was also much rich in its content of K (735 ± 13 mg/L) than other macroelements followed by Mg (58.1 ± 4.3 mg/L), Ca (28.4 ± 2.2 mg/L) and Na (24.0 ± 1.9 mg/L). The concentration of Fe (2.33 ± 0.15 mg/L) was the highest among the microelements. The concentration of Zn (1.82 ± 0.12 mg/L) was higher than Mn (1.04 ± 0.02 mg/L) followed by Cu (0.50 ± 0.03 mg/L). Ni (0.20 ± 0.01 mg/L) was present at low concentration. Cr and Co were below the method detection limits. The toxic metals Pb (0.14 ± 0.01 mg/L) was present at the lowest concentration than the other brands of the Ethiopian wines. Cd was below the method detection limit. The concentrations of metals determined in Kemila white wine was found in the decreasing concentration order of metals K > Mg > Ca > Na > Fe > Zn > Mn > Cu > Ni > Pb >.

Analysis for significance of differences between means (ANOVA)

Comparing the means of all the four brands for their potassium content, at the 95% confident level, the means were significantly different ($p < 0.05$). The K content of Axumite red wine was significantly higher than any of the other wines K level. Also Gouder red wine has significantly lowest level of potassium. The mean values of magnesium were significantly different ($p < 0.05$) in the four brands of Ethiopian wines. There existed statistically significant differences ($p < 0.05$) among the means of Ca content of the four wines. The statistical analysis for sodium content, presented the absence of significant differences among all the four wine brands and between any pair of the four wine brands studied ($p > 0.05$). That means all the four brands of Ethiopian wines contained significantly equal concentrations of sodium.

The mean values of iron of four brands of Ethiopian wines were statistically different ($p < 0.05$), where as iron in Axumite red wine was statistically higher than it was in other three brands ($p < 0.05$). Mean values of Zn showed significant difference between the four wine brands ($p < 0.05$). And it was determined that the zinc level in Gouder red wine to be the highest, Kemila white wine contained significantly the lowest amount of zinc from the other wines analyzed in this study. The mean values of Mn showed no significant difference between Axumite red wine and Gouder red wine. Axumite red wine had mean Cu content of significantly highest level from the others. The analysis for the difference in mean values of Ni presented no significant differences among the four brands of the Ethiopia wanes ($p > 0.05$). Pb analysis showed that there was statistically significant difference between the four brands of wines ($p < 0.05$).

In summary, when the mean contents of the different metals of the Ethiopian red and white wines were compared, it was found that Axumite red wine had significantly higher mean contents in potassium, calcium, magnesium, and zinc ($p < 0.05$), and Awash Crystal white wine contain significantly higher level of Pb ($p < 0.05$).

Comparisons of the levels of the metals between the four brands of Ethiopian wines

The level of metals in wines is highly affected by the soil type on which the grapes are cultivated, which means on the geographical location of the grapes from which the wines are produced. Others like usage of fertilizers, pesticides, and herbicides also affect the metallic content of the wines. During vinification, the wines' metal content could be affected by the type of water, the components of the pipes, barrels and other materials involved in the process.

From the four brands of wines, Axumite red wine contains the highest level of K (767 ± 15 mg/L), followed by Awash Crystal white wine (764 ± 19), Kemila white wine (735 ± 13 mg/L) and Gouder red wine (694 ± 15 mg/L). So the order is Axumite red wine > Awash Crystal white wine > Kemila white wine > Gouder red wine. The mean values for magnesium in the four wines studied were in a range of 58.2‒79.2 mg/L. The order of magnesium content of the wine brands is Axumite red wine (79.2) > Awash Crystal white wine (77.6) > Gouder red wine (66.0) > Kemila white wine (58.1), all in units of mg/L. In their calcium content the four wine brands can be arranged in the following order of Axumite red wine (37.1) > Awash Crystal white wine (31.6) > Gouder red wine = Kemila white wine (28.4) all in mg/L. The sodium levels found in wines from the four brands varied inconsiderably, with an average value of 24 mg/L. And the order was Axumite red wine (24.4) = Awash Crystal white wine (24.4) > Gouder red wine (24.3) > Kemila white wine (24.0) all in mg/L.

All the wines studied were found to contain iron below 7 mg/L (mean value was 2.1 mg/L), considered to be the very low level to form ferric cases. The order in the iron content of the wines analysed was Axumite red wine (3.16 mg/L) > Kemila white wine (2.33 mg/L) > Gouder red wine (1.49 mg/L) > Awash Crystal white wine (1.42 mg/L). The order in the level of copper in the four wines is Axumite red wine (1.50) > Awash Crystal white wine (0.61) > Gouder red wine (0.55) > Kemila white wine (0.50) in the units of mg/L. All the wine samples except for Axumite red wine have Cu contents below the 1 mg/L recommended by OIV to prevent formation of cupric cases. In the case of zinc, all the samples presented a content of below 2.7 mg/L, much lower than 5 mg/L, the amount recommended by the OIV. In this study the level of zinc was in the range 1.82‒2.70 mg/L. The order in the level of zinc in the wines studied is Gouder red wine (2.7 mg/L) > Awash Crystal white wine (2.4 mg/L) > Axumite red wine (2.14 mg/L) > Kemila white wine (1.82 mg/L). The values for manganese in the Ethiopian wines were between 1.04 mg/L and 1.88 mg/L. Awash Crystal white wine was found to contain the highest level of manganese (1.88 mg/L), followed by Gouder red wine (1.56 mg/L), Axumite red wine (1.46 mg/L) and Kemila white wine 1.04 mg/L, respectively.

The level of lead was the highest in Awash Crystal white wine (0.31), followed by Axumite red wine (0.25), Gouder red wine (0.16), and Kemila white wine (0.14) in mg/L. The level of Ni was similar in the four brands (0.18‒0.2 mg/L). Chromium in the studied Ethiopian wines was below the method detection limit for the three brands (i.e. Gouder, Awash Crystal, and Kemila). But it was determined in small amounts (0.19 mg/L) in Axumite red wine. Cadmium was below the method detection limit in all the four Ethiopian wines studied (< give the MDL value).

Comparison of the level of the metals in Ethiopian wines with data on the level of metals in wines from other countries

The comparison of the metals in Ethiopian wines and published data on wines of different origins is given in Table 4. The K levels in Ethiopian wines were in range of 694–766 mg/L. The Ethiopian wines had a slightly larger interval to that reported for French wines. The Ethiopian wines had lower levels of K compared to the Czech, German, Greek, Hungarian, Italian, Spanish and American wines. The magnesium levels in the Ethiopian wines were in the range of 58–79 mg/L and are comparable to that in French, German and Italian wines. But the Ethiopian wines contain lower amount of Mg than that reported for Czech, Greek, Hungarian, Spanish and American wines. The calcium concentration range in Ethiopian wines was 28–37 mg/L and is comparable to that in French and German wines but lower than that Czech, Greek, Hungarian, Italian, Spanish and American wines. The lower content (24–25 mg/L) of Na in Ethiopian wines in comparison with literature data can be attributed to the considerably lower influence of sea salts in Ethiopian vineyards. The sources of sodium in wines, apart from natural concentration, are the addition of sodium salts such a sulfite, sorbate and (in some countries) sulfide [10].

Table 4. Comparison of the concentrations of metals in wines of different origin.

Metal	Concentration of metals (mg/L) in different wine origin								
	Czech	French	German	Greek	Hungarian	Italian	Spanish	American	Ethiopian
K	493-3056	265-426	480-1860	955-2089	489-1512	750-1500	338-2032	462-1147	694-767
Na	2.0-110	7.7-14.6	6-25	5.5-150	18.6-81.1	3.4-200	3.5-300	7-106	24-25
Ca	40-100	65-161	58-200	14.0-47.5	51-164	30-151	12-241	17-94	28-37
Mg	7.8-138	55-96	56-105	82.50-122.5	72-174	53-115	50-236	100-245	58-79
Zn	--	0.44-0.74	0.3-1.5	0.05-8.9	0.6-1.9	0.135-4.8	ND-4.63	0.75-3.60	1.82-2.70
Cu	0.012-6.827	ND-0.48	0.02-0.71	0.2-1.65	0.15-2.57	0.001-1.34	ND-3.1	0.05-0.58	0.5-1.5
Fe	0.9-5.2	0.81-2.51	0.4-4.2	0.7-7.3	2.03-23.7	1.35-27.8	0.4-17.4	1.2-6.6	1.42-3.16
Mn	0.28-3.26	0.63-0.96	0.5-1.3	ND-2.3	0.12-2.9	0.67-2.5	0.1-5.5	0.81-4.08	1.04-1.88
Ni	0.019-0.034	ND-0.052	--	ND-0.5	--	0.015-0.21	0.005-0.079	--	0.18-0.25
Pb	0.010-1.253	0.006-0.023	--	ND-0.62	--	0.01-0.35	0.001-0.096	--	0.14-0.31
Cd	0.000055-0.0033	ND-0.0002	--	ND-0.03	0.00014-0.054	0.0012-0.0016	ND-0.019	--	<0.01
Co	ND-0.018	0.004-0.011	0.004-0.005	ND–0.04	0.003–0.009	0.003-0.006	ND-0.04	--	ND-0.09
Cr	0.032-0.037	0.006-0.09	0.01-0.41	ND-0.41	0.032-0.062	0.02-0.05	0.025-0.029	--	ND-0.09
Ref.	[15, 29, 37]	[15, 39]	[15]	[9, 15, 19]	[15, 37]	[15, 28, 40, 41]	[15, 25, 38, 40, 42]	[43]	Present study

Iron concentration in wines depends on several factors, mostly on the soil, however iron levels may increase in wines due to the usage of steel devices during production. At concentrations > 10 mg/L, Fe(III) creates insoluble suspensions with tannin and phosphates which are known as hazes or "casses" [44]. Iron is of importance to the wine maker because when it is present at > 7–10 mg/L, it may cause cloudiness or colour change; the content depends upon iron levels in soil and dust, and contamination during harvesting, transportation and processing [19]. The Fe contents in the Ethiopian wines were in the range 1.42–3.16 mg/L and are comparable to that in Czech, French, German, Greek and American wines but lower than that in Hungarian, Italian and Spanish wines.

Copper content of wines may be mainly originated from residues of copper-based pesticides in addition to transport from the soil [37]. The copper contents in Ethiopian wines were in the range 0.5-1.5 mg/L and are comparable to that in German, Greek, Italian wines. The Cu levels in Ethiopian wines are higher French and American wines but lower than that in Czech, Hungarian and Spanish wines. The comparison made for zinc levels in the Ethiopian wines and wines of different origins showed that the Ethiopian wines are among the intermediate levels. The Zn levels in Ethiopian wines are higher than in French, German and Hungarian wines but lower than in Greek, Italian, Spanish and American wines. The values for manganese in the Ethiopian wines were between 1.04 mg/L and 1.88 mg/L and are comparable to Greek, Hungarian and Italian wines. These levels are higher than reported for German and French but lower than that in Czech, Spanish and American wines. The chromium level in the Ethiopian wines was found to be in the range of ND–0.09 mg/L. The Cr levels in Czech, Hungarian, Italian, and Spanish wines are lower than in Ethiopian wines while comparable in French wines and higher in Greek and German wines. The levels of Ni in Ethiopian wines are comparable to that in Greek and Italian wines but higher than that in Czech, French and Spanish wines. The Co levels in Ethiopian wines are slightly higher than in wines of other origin.

The Pb levels in the Ethiopian wines were in the range 0.14–0.31 mg/L. These levels are higher than that reported for French and Spanish wines. But some studies also showed higher lead levels in comparison with the Ethiopian wines, for example Czech, Greek and Italian wines have lead contents in the ranges of 0.01–1.25, ND–0.62, and 0.01–0.35 in mg/L, respectively. Cadmium was not detected in all the four brands of Ethiopian wines. Some studies have shown higher contents of Cd in wine compared with the present data. Traces of Cd have been reported in Czech, French and Italian wines while the Cd levels up to 0.02, 0.03 and 0.05 mg/L have been reported in Spanish, Greek and Hungarian wines, respectively.

Comparison of the metal concentrations with limits set by International Organization of Wine

Many countries have set maximum permissible level of some metals in wines considering both the enological and toxicological effects of the metals in wines. The tolerable limits for some metals in wines are also set by the International Organization for Grapes and Wine (OIV). The maximum permissible levels in Australia, Germany, Italy and by OIV are given in Table 5 [18, 45].

Table 5. Comparison of the levels of metals in the Ethiopian wines to permissible levels of some metals (mg/L) in some countries and by OIV*.

Country	Concentrations of metals (mg/L)				
	Na	Cu	Zn	Pb	Cd
Australia		5	5	0.2	0.05
Germany		5	5	0.3	0.01
Italy		10	5	0.3	
OIV*	60	1	5	0.2	0.01
Ethiopian wine	24–25	0.5–1.5	1.8–2.7	0.1–0.3	< 0.01

*Office International de la Vigea et du Vin (International Organisation for Grapes and Wine).

The Ethiopian wines contained lower concentration of sodium (24–25 mg/L) than the limit set for sodium (60 mg/L) by OIV. The concentrations of copper (0.5–1.5 mg/L) and zinc (1.8–2.7 mg/L) were comparable to the limit set by OIV and other countries. The content of iron did not present risks of "casses". The Ethiopian wines contained iron concentration lower than 3.5 mg/L, and therefore posing no risk of ferric casses, which is formed after iron concentration of greater than the range 10 mg/L [44]. Some of the Ethiopian wines contained lead to levels slightly higher than the upper limit established by OIV, while cadmium was not detected in Ethiopian wines.

CONCLUSIONS

The wet digestion method and the determination of selected metals at trace levels in wines by flame atomic absorption method were found to be efficient, precise and accurate. The most popular Ethiopian wines were found to contain potassium in the highest level followed by magnesium and calcium, which shows that the wines could be good sources of the essential elements mentioned. It was also found that the level of sodium is below the maximum limit set by the International Organization for Wine and Grapes (OIV). In general, moderate wine consumption contributes for the daily nutritional requirements of many essential metals, including K, Mg, Ca, Na, Fe, Zn, Mn, and Cu. The toxic metal cadmium was not detected in the Ethiopian wines revealing that the Ethiopian wines contain either very low concentration (< 0.01 mg/L) of Cd or may be free from this metal. However the other toxic metal lead concentrations in the Ethiopian wines were found in the range of 0.14–0.31 mg/L; these values are some how larger than the limit set by OIV as 0.2 mg/L. In general the values found are in line with previously reported data on wines from other origin.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Department of Chemistry, Addis Ababa University, Ethiopia, for providing the laboratory facilities.

REFERENCES

1. Bisson, L.F.; Butzke, C.E. *Wine*, Microsoft® Encarta® Online Encyclopedia **2008** http://encarta.msn.com, accessed on March **2008**.
2. German, J.B.; Walzem, R.L. *Annu. Rev. Nutr.* **2000**, 20, 561.
3. Feleke, M. *Addis Fortunate* **2007**, 353, 17.
4. Pyrzynska, K. *Chem. Spec. Bioav.* **2007**, 19, 1.
5. Dugo, G.; La Pera, L.; Pellicanó, T.M.; Di Bella, G.; D'Imperio, M. *Food Chem.* **2005**, 91, 355.
6. Frias, S.; Perez Trujillo, J.P.; Pena, E.M.; Conde, J.E. *Eur. Food Res. Technol.* **2001**, 213, 145.
7. Diaz, C.; Conde, J.E.; Estevez, D.; Perez Olivero, S.J.; Perez Trujillo, J.P. *J. Agric. Food Chem.* **2003**, 51, 4303.
8. Alvarez, M.; Moreno, I.M.; Jos, A.M.; Camean, A.M.; Gonzalez, A.G. *J. Food. Comp. Anal.* **2007**, 20, 391.
9. Galani-Nikolakaki, S.; Kallithrakas-Kontos, N.; Katsanos, A.A. *Sci. Total Environ.* **2002**, 285, 155.
10. Núñez, M.; Peña, R.M.; Herrero, C.; García-Martín, S. *Analusis* **2000**, 28, 432.
11. Lara, R.; Cerutti, S.; Salonia, J.A.; Olsina, R.A.; Martinez, L.D. *Food Chem. Toxicol.* **2005**, 43, 293.

12. Stafilov, T.; Karadjova, I. *Macedonian J. Chem. Chem. Eng.* **2009**, 28, 17.
13. Ibanez, J.G.; Carreon-Alvarez, A.; Barcena-Soto, M.; Casillas, N. *J. Food Comp. Anal.* **2008**, 21, 672.
14. Saurina, J. *Trends Anal. Chem.* **2010**, 29, 234.
15. Pohl, P. *Trends Anal. Chem.* **2007**, 26, 941.
16. Pyrzynska, K. *Crit. Rev. Anal. Chem.* **2004**, 34, 69.
17. Mozaz, S.R.; Sotro, A.G.; Segovia, J.G.; Azpilicueta, C.A. *Food Res. Int.* **1999**, 32, 683.
18. Benitez, P.; Castro, R.; Barroso, C.G. *Anal. Chim. Acta* **2002**, 458, 197.
19. Lazos, E.S.; Alexakis, A. *Int. J. Food. Sci. Technol.* **1989**, 24, 39.
20. Cvetkovič, J.; Apradjan, S.; Karadjova, I.; Stafilov, T. *Acta Pharm.* **2006**, 56, 69.
21. Pereira-Filho, E.R.; Berndt, H.; Arruda, M.A.Z. *J. Anal. At. Spectrom.* **2002**, 17, 1308.
22. Matusiewicz, H.; Kopras, M. *J. Anal. At. Spectrom.* **1997**, 12, 1287.
23. Giokas, D.L.; Paleologos, E.K.; Tzouwara-Karayanni, S.M.; Karayannis, M.I. *J. Anal. At. Spectrom.* **2001**, 16, 521.
24. Ukhun, M.E.; Okolie, N.P.; Oyerinde, A.O. *Afr. J. Biotechnol.* **2005**, 4, 829.
25. Rebolo, S.; Peña, R.M.; Latorre, M.J.; García, S.; Botana, A.M.; Herrero, M.C. *Anal. Chim. Acta* **2000**, 417, 211.
26. Brescia, M.A.; Caldarola, V.; De Giglio, A.; Benedetti, D.; Fanizzi, F.P.; Sacco, A. *Anal. Chim. Acta* **2002**, 458, 177.
27. Aceto, M.; Abollino, O.; Bruzzoniti, M.C.; Mentasti, E.; Sarzanini, C.; Malandrino, M. *Food Addit. Contam.* **2002**, 19, 126.
28. La Torre, G.L.; La Pera, L.; Rando, R.; Lo Turco, V.; Di Bella, G.; Saitta, M.; Dugo, G. *Food Chem.* **2008**, 110, 729.
29. Kment, P.; Mihaljevič, M.; Ettler, V.; Šebek, O.; Strnad, L.; Rohlova, L. *Food Chem.* **2005**, 91, 157.
30. Bujake, J.E. *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 4, 4th ed., John Wiley and Sons: New York; **1992**; pp 153-182.
31. Rodriguez Mozaz, S.; Garcia Sotro, A.; Garrido Segovia, J.; Ancin Azpilicueta, C. *Food Res. Int.* **1999**, 32, 683.
32. Taylor, A.; Branch, S.; Halls, D.J.; Owen, L.M.W.; White, M. *J. Anal. At. Spectrom.* **1999**, 14, 717.
33. Kallithraka, S.; Arvanitoyannis, I.S.; Kefalas, P.; El-Zajouli, A.; Soufleros, E.; Psarra, E. *Food Chem.* **2001**, 73, 501.
34. Kim, M. *Food Addit. Contam.* **2004**, 21, 154.
35. Sanllorente, S.; Ortiz, M.C.; Arcos, M.J. *Analyst* **1998**, 123, 513.
36. Gustavo Gonza'lez, A.; Angeles Herrador, M. *Trends Anal. Chem.* **2007**, 26, 227.
37. Sass-Kiss, A.; Kiss, J.; Havadi, B.; Adányi, N. *Food Chem.* **2008**, 110, 742.
38. Frías, S.; Conde. J.E.; Rodríguez, M.A.; Dohnal, V.; Pérez-Trujillo, J.P. *Nahrung/Food* **2002**, 46, 370.
39. Cabrera-Vique, C.; Teissedre, P.L.; Cabanis, M.T.; Cabanis, J.C. *J. Agric. Food Chem.* **1997**, 45, 1808.
40. Sauvage, L.; Frank, D.; Stearne, J.; Millikan, M.B. *Anal. Chim. Acta* **2002**, 458, 223.
41. Marengo, E.; Aceto, M. *Food Chem.* **2003**, 81, 621.
42. Álvarez, M.; Moreno, I.M.; Jos, Á.M.; Cameán, A.M.; González, A.G. *J. Food Comp. Anal.* **2007**, 20, 391.
43. Li, P.; Hardy, J.K. *J. Wine Res.* **1999**, 10, 197-206.
44. Paleologos, E.K.; Giokas, D.L.; Tzouwara-Karayanni, S.M.; Karayannis, M.I. *Anal. Chim. Acta* **2002**, 458, 241.
45. Azenha, M.A.G.O.; Vasconcelos, M.T.S.D. *Food Chem. Toxicol.* **2000**, 38, 899.