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LEVELS OF ESSENTIAL AND NON-ESSENTIAL METALS IN ETHIOPIAN OUZO

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ABSTRACT: The levels of selected metals (Na, Ca, Mg, Fe, Zn, Mn, Cr, Co, Cu, Ni, Cd and Pb) in five different brands (Kokeb, National, Liyu Addis, Victoria and Balezaf) of Ethiopian ouzo sampled from different supermarkets in the capital city Addis Ababa were determined. 10 mL of ouzo samples were digested by using 2 mL of HNO_3 and 5 mL of H_2O_2 for 90 minutes at a temperature of 180°C and the levels of the minerals in the digests analyzed by flame atomic absorption spectrometer. The following results were recorded in mg/L for each five ouzo brands Na (8.529–18.194), Ca (8.330–12.830), Mg (1.345–10.977), Fe (0.942–2.881), Zn (0.642–2.215), Mn (0.015–0.225), Cr (0.054–0.121), Co (0.072–0.130). Cu (0.212 mg/L) was detected only in Kokeb brand while Ni and Cd were below the method detection limit in all the five brands of ouzo analyzed. The toxic metal Pb (0.127–0.507 mg/L) was detected in all the five brands of ouzo samples. The level of Na was the highest in all the brands followed by Ca and Mg respectively except for Kokeb ouzo in which the level of Mg was higher than Ca. Among the trace elements, Zn was found to be highest next to Fe followed by Mn and Co. The levels of metals were higher in Liyu Addis ouzo compared to the other brands. Generally, ouzo contains some nutritionally essential minerals in large quantity so that it can be used as one source of minerals. Non-essential and trace metals were either not detected or found only in smaller quantity. But non-essential metal Pb was detected in all ouzo brands which ranged from 0.13 to 0.51 mg/L. Thus, periodic determinations are advisable in view of the potential medium and long-term risks associated with Pb contamination.

Key words/phrases: Alcoholic beverage, Ethiopia, essential metal, non-essential metal, ouzo

INTRODUCTION

Alcoholic beverages are any fermented and distilled liquids such as wine, beer, whisky, ouzo, gin, etc. that contain ethyl alcohol as intoxicating agent. Fermentation is a widely practiced ancient technology and fermented foods are an essential part of diets in all regions of the world. However, developing countries cannot continue to depend on historical methods of food processing. Traditional fermentation processes and the potential for their modernization are increasingly attracting the attention of scientists and policy makers as a vital part of food security strategies and commercial use (Mogessie Ashenafi, 2000). Some of the known Ethiopian traditional fermented foods and beverages are injera, dabo, ambasha, kocho, bulla, ergo, siljo, tella, tej, borde, cheka, shamita, korefe, keribo, bukire, kineto araki, redistilled araki, ouzo, and merissa (Kebede Abegaz *et al.*, 2002). Ouzo is one type of distilled alcoholic beverage with alcoholic content of 40 to 43% flavoured by anethole extracted from anise, star anise or fennel plant. Ouzo contains mainly water, ethanol and trans-anethole. Trans-anethole, an aromatic compound, is extracted from anise, star anise and fennel flowering plant (WHO, 2004). The distinctive smell of ouzo comes from the addition of anise as flavorant (Lazarakis, 2006; Cardoso *et al.*, 2004).

It was reported that the distilled beverages, whiskey, gin and brandy, (which are relatively with high alcoholic content) were conspicuously less poisonous in both sets of experiments than either the wines or malt beverages (Bujake, 1992). Hence, it can be concluded that the poisonousness of the alcoholic beverage cannot depend on the alcoholic content and it occurs due to the presence of other components including essential and non-essential metals. In November 1985, the Canadian Government indicated that it had detected ethyl carbamate, $C_3H_7NO_2$, which is developed naturally during the fermentation of alcoholic beverages a suspected carcinogen, in some distilled spirits and wines (Karadjova *et al.*, 2007).

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The presence of trace metallic ions in alcoholic beverage could be attributed to the so-called primary sources (transfer of metal from the soil/groundwater to the grapes and finally to the alcoholic beverage, or atmospheric deposition of airborne particulate matter on grapes) and secondary sources including the making process (clarification and refining agents). In the specific case of ouzo the trace metal ions come from processing equipment (valves, pipes, pumps, bronze tanks) and bottles (cork capsules used for sealed bottles) and from the water used for processing (Inhant, 2003; Cvetkovic *et al.*, 2006).

Waste incineration, phosphate fertilizer manufacture, wood, coal, oil and gasoline combustion, iron and steel production, industrial metal applications and non-ferrous metals mining are also source for metallic ion in alcoholic beverage (Cvetkovic *et al.*, 2006; Nunez *et al.*, 2000). Mineral elements can generally be classified as nutritionally essential major elements, such as Ca, K, Mg and Na, nutritionally essential minor (or trace) elements, like Fe and those regarded as toxic or with an essential/toxic duality: As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Pd, Se, Sn, Tl, V, and Zn; and non-essential metals like As, Pb, Cd and Hg; all can exist in alcoholic beverages. Some heavy metals are indispensable for humans in very small quantities, but become toxic at higher doses (Inhant, 2003; Cvetkovic *et al.*, 2006). At excessive levels, even nutritionally essential elements may exhibit toxicity (Wang *et al.*, 2006). If heavy metals are present in alcoholic beverage, they may lead to severe effects that include reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death. Exposure to some metals, such as Hg and Pb, may also cause development of autoimmunity, in which a person's immune system attacks its own cells. This can lead to joint diseases such as rheumatoid arthritis, and diseases of the kidneys, circulatory system, and nervous system. Mn, Pb and Cd are recognized as neurotoxic metals (Bhatia, 2005).

Metallic ions content of different alcoholic beverages has been reported from different countries and compared with the standard values. The levels of fourteen metal ions were determined in 113 commercial Greek wines by atomic absorption and emission spectroscopy and the values were within acceptable ranges (Nascentes *et al.*, 2005). Trace element determination in wines and other alcoholic beverages have also been reported by different methods (Baluja *et al.*, 1996; Lara *et al.*, 2005).

Similarly, in Spain levels of Cu, Zn, Ca and Mg were measured in alcoholic beverages (whiskies, gins, rums, liquors, brandies, wines and beers) using flame atomic absorption spectrometry (FAAS) (Navarro-Alaroon, 2007). The measurements showed that mineral levels were found to be significantly different. In distilled alcoholic beverages, levels measured in rums and brandies were statistically lower than those determined in gins and alcoholic liquors. For Cu, measured levels were statistically different for each of the five groups of distilled alcoholic beverages studied. In fermented beverages, Zn, Ca and Mg levels were significantly higher than those levels determined in distilled drinks. Contrarily, Cu levels were statistically lower. Wines designated as sherry had significantly higher Ca and Mg levels.

Differentiation of Spanish brandies according to their metal content has been reported by (Cameán *et al.*, 2001). Galani-Nikolakaki *et al.* (2002) investigated the elements Al, As, Cd, Cu, Cr, Fe, Pb, Mn, Ni and Zn in Greek wine. The levels for all the elements that were determined were almost in all cases, well below the maximum permissible levels by the Greek and the European Union legislation. A review on the determination of metals in wines with atomic spectroscopy (Flame-AAS, GF-AAS and ICP-AES) has also been reported (Aceto *et al.*, 2002). The determination of metallic ions in palm wine, popular traditional alcoholic beverage in Africa, from Nigeria has also been reported (Ukhun *et al.*, 2005).

However, no study on the metal contents of Ethiopian alcoholic beverages has been reported in the literature. Hence, it was essential to determine the levels of metals in the Ethiopian ouzo alcoholic beverage.

There are different brands of this common local alcohol beverage in Ethiopia. For this particular study only five different brands which are most commonly consumed were selected, namely Liyu Addis, National, Balezaf, Kokeb and Victoria. Except Balezaf, which is produced near Addis Ababa (at Sebeta), all are produced in Addis Ababa. Liyu Addis at Kotebe, Kokeb around Saris, National at Mekanisa and Victoria at Akaki. The locations of the factories, which produce the four brands of studied ouzo alcohol beverage in Addis Ababa, are shown in Figure 1.

There is no agreed standard level of metallic ions in ouzo alcoholic beverage in Ethiopia. The determination of these metals in ouzo is relevant because they might be essential or toxic to the human body. Therefore, it was necessary to determine the levels of some essential and non-essential metallic ions due to toxicity of certain metals in case of excess intake. The metal contents were compared with other international alcoholic beverages.

MATERIALS AND METHODS

Equipment

Round bottomed flasks (250 mL) fitted with reflux condenser were used in Kjeldahl apparatus hot plate to digest the ouzo samples. Buck Scientific Model 210 VGP (East Norwalk, USA) flame atomic absorption spectrophotometer equipped with deuterium ark background correctors (Department of Chemistry, Addis Ababa University) was used for analysis of the analyte metals (Na, Ca, Mg, Fe, Zn, Mn, Cr, Co, Cu, Ni, Cd and Pb) using air-acetylene (99.8%) flame.

Reagents and chemicals

Reagents that were used in the analysis were all analytical grade. HNO_3 (69–72%) (Spectrosol, BDH, England) and 30% H_2O_2 (BDH Chemicals, England) were used for digestion of ouzo sample. Lanthanum nitrate hydrate (98%, Aldrich, USA) was used to avoid refractory interference (for releasing Ca and Mg from their phosphates). Stock standard solutions containing 1000 mg/L in 2% of HNO_3, the metals Na, Ca, Mg, Fe, Zn, Mn, Cr, Co, Cu, Ni, Cd and Pb (Buck Scientific Puro-Graphic^{tm}) were used for preparation of calibration standards and in the spiking experiments. Deionized water was used throughout the experiment for sample preparation, dilution and rinsing apparatus prior to analysis.

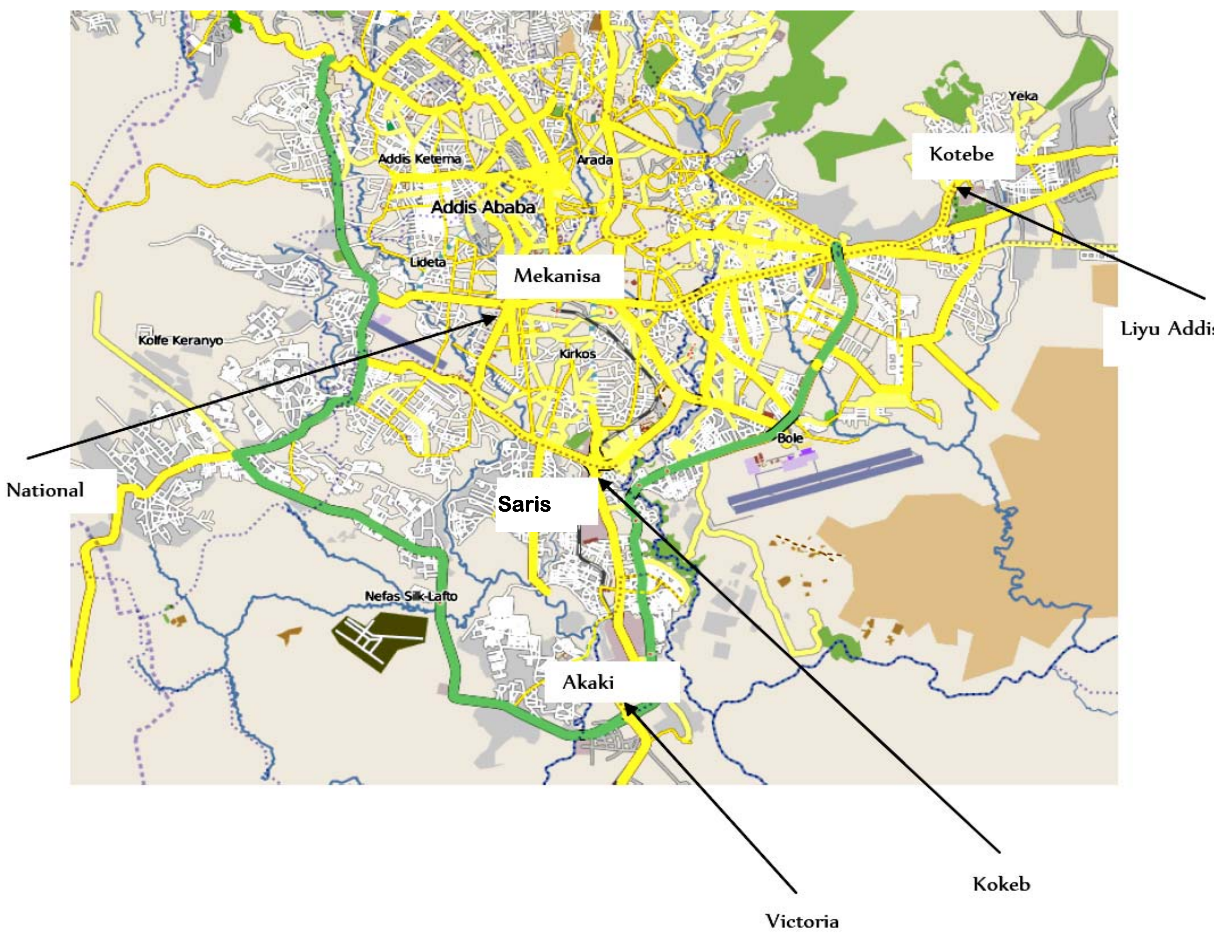

Fig. 1. Locations of ouzo distiller industries in Addis Ababa.

Sampling and sample preparation

Five brands of ouzo beverage namely, National, Balezaf, Kokeb, Victoria and Liyu Addis were collected from different supermarkets and factories in Addis Ababa. Ouzo alcoholic beverage is sold in a sealed glass bottle. For the analysis, five bottles (each containing 700 mL) of each brand was purchased from different parts of Addis Ababa. For sample collection, the supermarkets were selected randomly. The collected samples were kept in a laboratory safely and neat. 100 mL of ouzo beverage was taken from each of the five bottles to prepare 500 mL of bulk sample of each brand. The prepared bulk sample was kept in a refrigerator until digestion. Finally, 10 mL of ouzo alcoholic beverage was taken from each bulk sample for the digestion.

Method for ouzo sample digestion was optimized and the levels of essential and non-essential elements such as Na, Ca, Mg, Fe, Zn, Mn, Cr, Co, Cu, Ni, Cd and Pb in Ethiopian local gin "ouzo" was determined by flame atomic absorption spectrometry (FAAS).

The basis for selecting the 12 elements is that these elements are frequently present and determined in common alcoholic beverages. The reason for the exclusion of some important toxic elements such as As and Hg is that these two elements have lower sensitivity and higher detection limit and can not be determined at trace levels by FAAS. The determination of As and Hg in alcoholic beverages requires special accessories (hydride generator) in the spectrometer. Hence, these two elements were not determined in the present study.

RESULTS AND DISCUSSION

Digestion of ouzo sample

Even though the ouzo sample was colourless solution sample matrix should be broken down to avoid interference during analysis by flame atomic absorption spectroscopy. To do these different methods were tried using the HNO_3, H_2SO_4, H_3PO_4 and H_2O_2 under different volume and reagent, digestion temperature and different digestion time. The results are given in Table 1. The procedure involving less volume of reagents, lower temperature, and shorter time was taken as optimum digestion procedure.

Table 1. Different trials attempted during optimization procedure for digestion of ouzo alcoholic beverage.

No	Volume of ouzo alcoholic beverage (mL)	Volume of 5:2 HNO_3, $HClO_4$ or H_2SO_4 and H_2O_2 (mL)	Digestion temperature (°C)	Digestion time (min)	Observation
1	25	7(5:2 H_2O_2 and HNO_3)	120	180	Yellow clear solution with no suspended matter
2	25	7(5:2 $HClO_4$ and HNO_3)	120	180	Brown solution with suspended matter
3	25	7(5:2 H_2SO_4 and HNO_3)	120	180	Clear yellow solution with no suspended matter
4	50	7(5:2 H_2O_2 and HNO_3)	180	180	Clear yellowish solution with no suspended matt
5	50	7(5:2 $HClO_4$ and HNO_3)	180	180	Clear yellowish solution with no suspended matter
6	50	7(5:2 H_2SO_4 and HNO_3)	180	180	Clear yellowish solution with no suspended matter
7	10	7(5:2 H_2O_2 and HNO_3)	180	90	Clear colourless solution with no suspended matter
8	10	7(5:2 $HClO_4$ and HNO_3)	180	90	Deep yellow solution with no suspended matter
9	10	7(5:2 H_2SO_4 and HNO_3)	180	90	Deep brown solution with suspended matter
10	25	7(5:2 H_2O_2 and HNO_3)	180	180	Clear light yellowish solution with no suspended matt
11	25	7(5:2 $HClO_4$ and HNO_3)	180	180	Light brown solution with suspended matter
12	25	7(5:2 H_2SO_4 and HNO_3)	180	180	Deep brown solution with suspended matter

Digestion for the ouzo sample was done using the optimized procedure. Exactly 10 mL of ouzo sample transferred into a 250 mL round bottom digestion flask. 2 mL of HNO_3 and 5 mL of H_2O_2 were added to digestion flask and the mixture was digested on a micro Kjeldahl digestion apparatus by setting the temperature to dial at 6 (180°C) for 90 min; then after the digested solution was allowed to cool for 10 min without dismantling the condenser from the flask and for 10 min after removing the condenser. The cooled solution was filtered through Whatman® (110 mm, diam) filter paper then the round bottom flask was rinsed subsequently with 5 mL of deionised water. The filtrate was collected in a 50 mL volumetric flask and the solution was diluted to the mark (50 mL) with deionised water. Triplicate digestions were carried out for each bulk sample. The digested samples were kept in the refrigerator until the level of all the metals in the sample solutions were determined by flame atomic absorption spectroscopy. Six blank solutions, which were prepared by diluting 96% of ethanol to 41% by deionised water, were prepared following the same digestion procedure as the sample.

Method detection limit

The limit of detection is the smallest amount or concentration of analyte in the test sample that can be reliably distinguished, with stated significance, from the background or blank level. Detection limit can be made based on 3 times the standard deviation of the concentration in a matrix blank (McNaught and Wilkinson, 1997). For the present study, six blank samples were digested following the same procedure as the samples and each of the samples were determined for the elements and the pooled standard deviation of the six blank reagents was calculated. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three. The detection limits of analyte metals are given in Table 2. The method detection limits for all the analyte metals were < 0.05 mg/L except Pb for which it was 0.1 mg/L which indicate that the method used is applicable for the determination of analyte metals at trace levels in Ethiopian ouzo.

Determinations of essential and non-essential elements in ouzo samples

Standard solutions of metals of interest (10 mg/L each) were prepared from 1000 mg/L of the atomic absorption spectroscopy standard stock solutions. Each of this solution was then diluted with deionised water to obtain four working standards for each metal ion. Twelve macro- and micro-elements; Na, Ca, Mg, Mn, Cd, Co, Cr, Zn, Ni, Pb, Fe, and Cu were analyzed with FAAS using their respective hallow cathode lamp as radiation source in which the sample was aspirated to the flame through nebulizer. FAAS instrumental parameters were optimized for maximum signal intensity of the instrument. Four points calibration curves were constructed for each metal. Three replicate determinations were carried out on each sample after the instrument has been calibrated. The analytical wavelength used, correlation coefficient and equation for the calibration curves for the metal are given in Table 2.

Table 2. Analytical wavelengths, method detection limits, concentration of standards, absorbance of standard solutions, correlation coefficients and equations of the calibration curves for determinations of metals using FAAS.

Metal	Wave-length (nm)	Method detection limit (mg/L)	Equation of calibration curve	Correlation coefficient of calibration curve
Na	589.0	0.020	Y = 0.00138 + 0.39738X	0.99893
Ca	422.7	0.040	Y = 0.00128 + 0.01181X	0.99956
Mg	285.2	0.010	Y = -0.01649 + 0.1884X	0.99956
Fe	248.3	0.040	Y = 0.00029971 + 0.00819X	0.99917
Zn	213.9	0.010	Y = -0.00246 + 0.16413X	0.99993
Mn	279.5	0.010	Y = 0.00377+ 0.0309X	0.99909
Cr	357.9	0.010	Y = 0.0000120435+ 0.0081X	0.99978
Co	240.7	0.050	Y = 0.000378325+ 0.05069X	0.99999
Cu	324.7	0.020	Y = -0.000675071 + 0.00681X	0.99986
Ni	232.0	0.050	Y = 0.000364391 + 0.04397X	0.99993
Cd	228.9	0.005	Y = 0.000503605 + 0.14649X	0.99971
Pb	283.2	0.100	Y = -0.000404069+ 0.03202X	0.99991

Recovery test

The efficiency of the optimized digestion procedure used for the analysis of metals in this study was checked by adding known level of each metal to the 10 mL ouzo sample. The following procedure was followed for spiking: 3000 µg of Na, 300 µg of Zn, 5 µg of Mn and 10 µg of Co and Cr were spiked at once into 10 mL of ouzo sample and the remaining metals (2000 µg of Na and Ca, 300 µg Fe and 100 µg Pb and 45 µg Cu) were spiked at once into another round bottomed flask containing 10 mL of ouzo sample. The amount of metal spiked was 20 to 30% of the amount detected. 5 mL of H_2O_2 and 2 mL of HNO_3 were added to both spiked samples and the same digestion procedure as that of sample was followed. Each sample was determined for their respective spiked metals by the atomic absorption spectrophotometer. Each recovery test was performed in triplicates. The percentage of the recovery test was between 95 and 109% (Table 3), which indicates that it was within the acceptable range.

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

The total metal content of five different brands of ouzo varied widely. Actually the metal level varies based on the sources of metal. All manufacturers may not use the same raw material with the same level. Even the water treatment procedure will differ from one to other. Thus the metal level may somewhat varied. Even though the metal level of each brand differs, the order of increasing or decreasing levels of metal are almost the same. All the five brands of ouzo contain major elements in higher level [Na (8.529–18.194), Ca (8.330–12.830), Mg (1.345–10.977) mg/L)] and minor elements in lower level [Fe (0.942–2.881), Zn (0.642–2.215), Mn (0.015–0.225), Cr (0.054–0.121), Co (0.072–0.130), Cu (0.212 mg/L)]. Also, some non-essential and toxic metals were below the method detection limit. This indicates that Ethiopian ouzo beverages contain major and essential elements in appreciable amount, minor elements in lower amount and do not contain Cd but contain high levels of Pb (0.13–0.51 mg/L). The levels of metals in each of five different brands of ouzo are given in Table 4.

Table 3. Recovery test results of ouzo samples.

Metal	*Level in sample mg/L	Amount added mg/L	*Level in spiked sample mg/L	% Recovery
Na	17.71 ± 0.036	3.00	20.64 ± 0.1040	97.5 ± 6.3
Ca	8.83 ± 0.073	2.00	10.88 ± 0.0900	102.4 ± 0.9
Mg	10.21 ± 0.045	2.00	12.17 ± 0.0280	97.9 ± 4.0
Fe	1.01 ± 0.068	0.30	1.340 ± 0.0380	109.2 ± 6.3
Zn	0.68 ± 0.020	0.30	0.996 ± 0.0170	105.7 ± 9.0
Mn	0.015 ± .0002	0.005	0.021 ± 0.0003	94.4 ± 5.2
Cr	0.054 ± .0004	0.010	0.070 ± 0.0010	108.7 ± 9.3
Co	0.072 ± 0.003	0.010	0.080 ± 0.0030	97.6 ± 5.0
Cu	0.21 ± 0.020	0.045	0.260 ± 0.0040	96.9 ± 4.6
Ni	ND	-	-	-
Cd	ND	-	-	-
Pb	0.51 ± 0.004	0.10	0.630 ± 0.0060	109.0± 2.0

ND = Not detected, - = Indicates null; * =Values are mean ± SD of triplicate readings of triplicate analyses in µg/L.

Table 4. Levels of selected elements in Kokeb, National, Victoria, Liyu Addis and Balezaf ouzo drink.

Metals	*Levels of metals in different ouzo brands (mg/L)				
	Kokeb	National	Victoria	Liyu Addis	Balezaf
Na	17.711 ± 0.136	10.079 ± 0.074	8.529 ± 0.076	18.194 ± 0.163	14.002 ± 0.170
Ca	8.834 ± 0.073	8.330 ± 0.053	10.583± 0.085	11.130 ± 0.075	12.830 ± 0.063
Mg	10.208 ± 0.045	1.345 ± 0.017	2.140 ± 0.010	10.977 ± 0.080	1.502 ± 0.020
Fe	1.010 ± 0.068	0.942 ± 0.010	1.346 ± 0.039	2.881 ± 0.020	1.075 ± 0.021
Zn	0.679 ± 0.020	0.642 ± 0.020	0.660 ± 0.004	1.368 ± 0.011	2.215 ± 0.011
Mn	0.0153 ± 0.0002	0.130 ± 0.002	0.086 ± 0.002	0.085 ± 0.002	0.225 ± 0.002
Cr	0.0544 ± 0.0004	0.120 ± 0.003	0.121 ± 0.003	0.102 ± 0.003	0.071 ± 0.0005
Co	ND	ND	0.123 ± 0.002	0.130 ± 0.002	0.072 ± 0.001
Cu	0.212 ± 0.020	ND	ND	ND	ND
Ni	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND
Pb	0.507 ± 0.004	0.127 ± 0.005	0.167 ± 0.003	0.323 ± 0.007	0.193 ± 0.003

* = Values are mean ± SD of triplicate readings of triplicate analyses in mg/L, ND = Not detected.

Comparison of the levels of metals

The comparison of the levels of metals in the five brands of ouzo is shown in Figure 2. Even though the trends of levels of essential and non-essential metals are the same, the total metal content of each brand was quite different. It has been observed that Liyu Addis, Balezaf, and Victoria ouzo contained the highest levels of major elements and National ouzo contained the least level of major elements and some trace metals. While Liyu Addis and Balezaf ouzo had relatively higher level of trace metal, Kokeb and National ouzo had less trace metallic content. Co was detected only in Victoria, Liyu Addis, and Balezaf ouzo while it was below the method detection limit in the Kokeb and National ouzo. Cu was detected only in Kokeb ouzo; it was below the method detection limit in the other four brands. Ni and Cd were not detected in all the five brands of Ethiopian ouzo. Pb was detected at higher levels in all the five brands of Ethiopian ouzo.

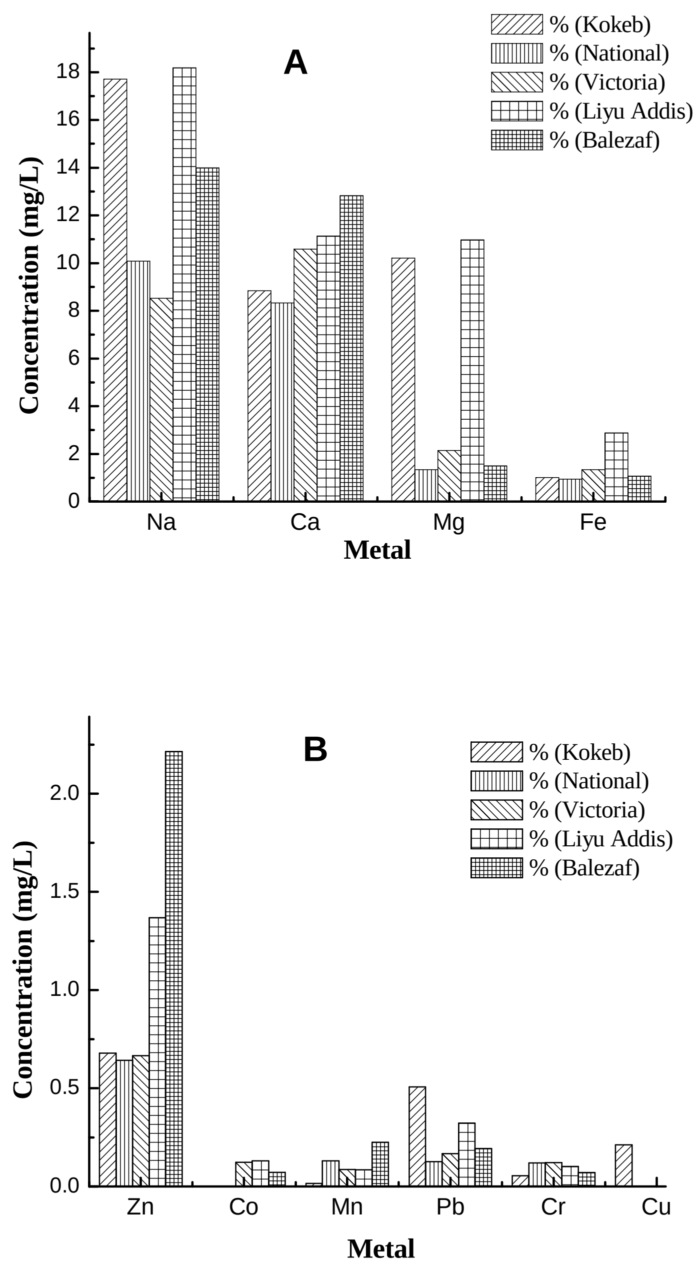

Fig. 2. The comparison of the levels of metals in the five brands of ouzo (a) Na, Ca, Mg and Fe and (b) Zn, Co, Mn, Pb, Cr and Cu.

Comparison of the levels of metals with other alcoholic beverages

The level of metals in Ethiopian ouzo has been compared with that of the other alcoholic beverages from different countries (Table 5). The levels of metals in Ethiopian ouzo are almost comparable with reported metal contents of different alcoholic beverages like wine, beer and other distilled beverages. Ouzo beverage can be compared with distilled spirits since the category and method of preparation is similar.

Statistical analysis of data (ANOVA)

The significance of variation between the samples was studied using one-way ANOVA. Minitab (computer program) was used to calculate the presence or absence of significant difference in mean level of each metal between five brands of ouzo, namely Kokeb, National, Liyu Addis, Victoria and Balezaf for each metal.

There was no significant difference in the levels of each particular metal in the five brands of ouzo at $p > 0.05$. Significant differences were observed at $p < 0.05$ for some metals in different brands of ouzo while no significant differences were observed at $p < 0.05$ for other metals in different brands of ouzo. The differences might be due to the type and level of flavouring used and environmental conditions under which the plant grows. Not only this, the type of water used for dilution will determine the levels of elements in Ethiopian ouzo alcoholic beverage.

Table 5A. Comparison of levels of major metals in Ethiopian ouzo with that of beer, wine, and distilled spirit from different countries.

Alcoholic beverage	Level of major metals (mg/L) in alcoholic beverage						
	Na	Ca	Mg	Fe	Mn	Zn	Reference
Beer	NR	5.000–10.000	2.000–5.000	NR	0.110–0.348	0.053–0.166	Cvetkovic *et al.* 2006; Dawson-Hughes *et al.* ,1997
Wine	8.000–24.200	27.700–68.100	21.000–75.100	0.700–7.300	0.500–4.400	0.050–1.800	Shin *et al.* 2007; Nunez *et al.*, 2000
Distilled spirits	17.800–161.800	8.000–14.800	1.410–4.140	0.350–2.030	NR	NR	Lazos and Alexakis, 1989
Ethiopian ouzo*	8.529–18.194	8.330–12.830	1.345– 10.977	0.942–2.881	0.015–0.225	0.642–2.215	Current study

* = Results are the average of three readings of triplet analysis, NR = Not reported, ND = Not detected.

Table 5B. Comparison of levels of trace metals in Ethiopian ouzo with that of beer, wine, and distilled spirit from different countries.

Alcoholic beverage	Level of trace metals (mg/L) in alcoholic beverage						
	Cr	Co	Cu	Ni	Cd	Pb	Reference
Beer	NR	NR	0.038–0.144	NR	ND	0.065–0.105	Karadjova *et al.* 2007; Nunez *et al.* 2000
Wine	0.010–0.260	ND–0.040	0.010–1.650	ND–0.130	ND–0.030	0.050–0.500	Barbeira *et al.* 1995; Karadjova *et al.* 2007
Distilled spirits	0.085–0.183	NR	ND–0.105	ND–0.022	ND	0.032–0.548	Karadjova *et al.* 2007; Nunez *et al.* 2000
Ethiopian ouzo*	0.054–0.121	ND–0.130	ND–0.212	ND	ND	0.127–0.507	Present study

* = Results are the average of three readings of triplet analysis, NR = Not reported, ND = not detected.

CONCLUSION

The levels of selected essential and non-essential elements (Na, Ca, Mg, Fe, Zn, Mn, Cr, Co, Cu, Ni, Cd and Pb) in five different brands (Kokeb, National, Victoria, Liyu Addis and Balezaf) of ouzo were determined by FAAS. The optimized wet digestion method for the analysis of ouzo was found to be efficient for the majority of the metals and it was evaluated through the recovery experiment and a good percentage recovery was obtained (100 ± 10) for the majority of the metals identified. The results showed that the levels of metals in all the five Ethiopian ouzo brands were in appropriate level. Generally, the levels of metals were higher in Liyu Addis compared to the rest. The ANOVA results suggest that there were significant variations in the levels of some elements in different brands of ouzo, which result from different factors. This may be due to different precautions taken during processing, fermentation and storage conditions, amount of flavouring added and environment through which flavouring plants grow and are extracted. In all the five brands of ouzo the levels of major elements were in higher level, minor elements in intermediate level; Ni and Cd were not detected but Pb was detected at higher level. Some trace metals (Mn, Cr and Co) were found at lower level. Cu was detected only in Kokeb ouzo beverage and it was below detection limit in the other four brands of ouzo.

Except for Zn and Co, the results obtained in this study are comparable with reported literature values analyzed from other parts of the world for other alcoholic beverages like beer, wine and other distillate. Thus, ouzo can be a source of essential metals to our body. The results indicate that ouzo either do not contain or contain lower amount of non-essential metals. However, Pb was detected in all the five brands. The optimum levels of Pb in wine ranges from 0.4 to 0.5 mg/L. The current data for Pb in ouzo ranges from 0.13 to 0.51 mg/L. This could be a problem for people consuming ouzo daily in larger quantity. Therefore, periodic determinations are advisable in view of the potential medium and long term risks associated with Pb contamination.

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REFERENCES

1. Aceto, M., Abollinzo, O., Bruzzonit, M.C., Mentastiz, E., Sarzaniniz, C. and Malanndrinoz, M. (2002). Determination of metals in wines with atomic spectroscopy (Flame-AAS, GF-AAS and ICP-AES): a review. *Food Addit. Contam.* **19**:126–133.
2. Baluja, C., López, A., Basadre, M.I. and González, A. (1996). Determination of lithium in alcoholic beverages by inductively coupled plasma atomic emission spectometry (ICP/AES). *Fresenius' J. Anal. Chem.* **355**:240–241.
3. Barbeira, P.J.S., Mazo, L.H. and Stradiotto, N.R. (1995). Determination of trace amounts of zinc, lead and copper in sugar cane spirits by anodic stripping voltammetry. *Analyst* **120**:1647–1650.
4. Bhatia, S.C. (2005). *Environmental Pollution and Control in Chemical Process Industries*. Khanna Publisher, New Delhi.
5. Bujake, J.E. (1992). *Kirk-Othmer Encyclopedia of Chemical Technology*. Vol. 4. John Whiley and Sons, New York.
6. Cameán, A.M., Moreno, I., López-artíguez, M., Repetto, M. and Gonzalez, A.G. (2001). Differentiation of Spanish brandies according to their metal content. *Talanta* **54**:53–59.
7. Cardoso, D.R., Andrade Sobrinho, L.G., Lima-Neto, B.S. and Franco, D.W. (2004). A rapid and sensitive method for dimethylsulphide analysis in Brazilian sugar cane sugar spirits and other distilled beverages. *J. Braz. Chem. Soc.* **15**:277–281.
8. Cvetkovic, J., Arpadjan, S., Karadjova, I. and Stafilov, T. (2006). Determination of cadmium in wine by electrothermal atomic absorption spectrometry. *Acta Pharm.* **56**:69–77.
9. Dawson-Hughes, B., Harris, S.S., Krall, E.A. and Dallal, G.E. (1997). Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *New Engl. J. Med.* **337**:670–676.
10. Galani-Nikolakaki, S., Kallithrakas-Kontos, N. and Katsanos, A.A. (2002). Trace element analysis of Cretan wines and wine products. *Sci. Total Environ.* **285**:155–163.

11. Inhant, M. (2003). A survey of methods of analysis for minerals in feedstuffs. *J. Anim. Sci.* **81**:3218–3225.

12. Karadjova, I.B., Lampugnani, L., D'Ulivo, A., Onor, M. and Tsaley, D.L. (2007). Determination of lead in wine by hydride generation atomic fluorescence spectrometry in the presence of hexacyanoferrate(III). *Anal. Bioanal. Chem.* **388**:801–807.

13. Kebede Abegaz, Fekadu Beyene, Langsrud, T. and Narvhus, J.A. (2002). Indigenous processing methods and raw materials of Borde, an Ethiopian fermented beverage. *J. Food Technol. Africa* **7**:59–64.

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

15. Lazarakis, M.W. (2006). *Greek Food, Wine and Travel Magazine*. http://www.greekgourmet traveler.gr. Accessed on October 22, 2007.

16. Lazos, E.S. and Alexakis, A. (1989). Metal ion content of some Greek wines. *Int. J. Food Sci. Technol.* **24**:39–46.

17. McNaught, A.D. and Wilkinson, A. (1997). *The Orange Book, IUPAC Compendium of Chemical Terminology*. Blackwell Scientific Publications, London.

18. Mogessie Ashenafi (2000). Traditional brewing in Ethiopia. *Proceeding of Workshop on Modern and Traditional Brewing in Ethiopia*. The Chemical Society of Ethiopia, Addis Ababa.

19. Nascentes, C.C., Kamogawa, M.Y., Fernandes, K.G., Arruda, M.A., Nogueira, A.R.A. and Nobrega, J.A. (2005). Direct determination of Cu, Mn, Pb, and Zn in beer by thermospray flame furnace atomic absorption spectrometry. *Spectrochim. Acta Part B* **60**:749–753.

20. Navarro-Alaroon, M., Velasco, C., Tesrres, C., Olalla, M., Lopez, H. and Lopez, M. C. (2007). Copper, zinc, calcium and magnesium content of alcoholic beverages and by-products from Spain: nutritional supply. *Food Addit. Contam.* 24:685-694.

21. Nunez, M., Pena, R.M., Herrero, C. and Garcia-Martin, S. (2000). Analysis of some metals in wine by means of capillary electrophoresis. Application to the differentiation of Ribeira Sacra Spanish red wines. *Analusis* **28**:432–437.

22. Shin, Y.-J., Jung, M.-J., Kim, N.-S., Kim, K. and Lee, D.-S. (2007). Characterization of fennel flavors by solid phase trapping-solvent extraction and gas chromatography-mass spectrometry. *Bull. Korean Chem. Soc.* **28**:2389–2395.

23. Ukhun, M.E., Okolie, N.P. and Oyerinde, A.O. (2005). Some mineral profiles of fresh and bottled palm wine – a comparative study. *Afr. J. Biotechnol.* **4**:829–832.

24. Wang, X., Yang, Y., Wang, X. and Xu, S. (2006). The effect of occupational exposure to the metal on the nervous system function in welders. *J. Occupat. Health* **48**:100–106.

25. World Health Organisation (2004). *WHO Global Status Report on Alcohol* 2004. WHO, Geneva.