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Determination of Selected Essential and Non-Essential Elements in Different Beer Brands of Ethiopia

*Marye Mulugeta[1] Tamiru Gashaw[2]
1.Department of Chemistry, College of Natural and Computational Sciences, University of Gondar, Ethiopia
2. Department of Chemistry, College of Natural and Computational Sciences Wolaita Sodo University, Ethiopia

Abstract
The determine amount of selected Mg, Ca, Fe, Cu, Cr, Cd and Pb elements present in different brands of Ethiopian beer available in Ethiopian markets were surveyed, in April 2011. Beer samples were randomly collected from Gondar, Bahir Dar and Woreta town. Detection was performed by FAAS after degassing and digestion of beer samples. Average content of Mg 44.291 to 59.122 mg/L, Ca 7.379 to 51.307 mg/L, Fe 0.023 to 0.079 mg/L, Cu 0.051 to 0.074 mg/L, Pb ND to 0.056 mg/L, Cd ND to 0.013 mg/L and Cr ND to 0.052 mg/L for all brands were determined and significantly different ($P<0.05$) except Mg for all brands, this study indicated that beer may contribute heavy metals to the diet. The values determined comparable to the literature values from different beer brands different countries indicate.
Keywords: Ethiopian beers, essential and non-essential elements**,** FAAS

INTRODUCTION

Beer is most widely consumed and probably oldest of alcoholic beverages in the world (Robert, S. and Walter, L., 2000), different types of starchy plants have been used for brewing, including maize (in South America), soy (in India and Persia), millet and sorghum (in Africa), rice (in the Far East) and barley malt is the most common brewing process worldwide (Inna P.and D'mitry P., (2009**)**. Most beer is flavored with hops, which add bitterness as a natural preservative, though other flavorings such as herbs or fruit may occasionally be included (Alemayehu A. and Grethe W., (2005, Clesia C., et al, 2005 and Čejka p., ea al, 2011). These cereals can supply sufficient qualities of carbohydrates, fat, protein and many minerals (Adebayo G., ea al, 2010) intended for brewing and it uptake from the environment, cereal varieties, cultivars, organic matter composition of soil, chemicals applied to agricultural practices, the type of enterprises established on a territory (Donadini G., ea al, 2008).
The important nutrients, including vitamins, amino acids, and minerals of metals depends on their content in the raw materials (malt, hops, brewer's yeast and water) and the ability to transfer into solution during the brewing process (Čejka p., *et al* 2011, Adebayo G., *et al* 2010, Donadini G., *et al* 2008, Getachew L., *et al* 2007, Viñas P., *et al* 2002 and Yonkova G., *et al* 2007). Since, depending on the concentration, various metals might be essential or toxic to the human body (Pawel P., 2007).

In Ethiopia there are five breweries: BGI Ethiopia (St. George), Dashen, Harar, Meta and Bedele. Ethiopians currently drink 4 liters of beer each year. The Ethiopian brewery's major raw materials are barley malt, hop, yeast and water (spring, river, Borehole & municipal). The Malt is obtained from Assela Malt Factory in Ethiopia small quantities of malt, yeast, hops and chemicals that are imported from abroad (Heidi M., *et al* 2003).

The aim of this study is to determine the amount of selected elements present in different brands of Ethiopian beer and to compare the determined value in the different beer brands with each other and literature values. It is very advantageous to the brewers and to the beer consumers.

MATERIALS AND METHOD

Sampling Sites and Methodology of sampling: Beer samples were obtained from retail Hotels, Bars, Restaurants, and local stores found in Bahir Dar, Woreta and Gondar area, Ethiopian. Sampling of beer was done on April 4-15, 2011 from brands BGI (St. George), Dashen, Meta, Harar and Bedele factory seven glass bottled beer samples in each brand were randomly collected from three sites of Ethiopia. The samples differ and/or same from each other dates and factories for filling up.
Methods: Apparatus (beakers, pipettes, volumetric flasks), were washed by soaked in 50% nitric acid for one day, rinsed with distilled water five times, dried and kept in dust free place until analysis begins. Hot plate, Whatman type # 41 filter paper, selected elements hollow cathode lamps and a Bulk Scientific Model 210 VGP atomic absorption spectrometer equipped with air-acetylene flame atomizer was used.
Reagents and Standard Solutions: 69-72% HNO_3 (laboratory Rasayana, Mumbai), 30% H_2O_2 (HDH limited Poole, England) and Buck Scientific puro graphics calibration standards (UK) for Mg, Ca, Cu, Fe, Pb, Cd and Cr were used as received. Distilled water was used for all sample preparation.
Beer Samples Pre-treatments: The collected glass bottled beer samples of each brand were mixed to form a bulk sample. Storing open through overnight and adding 5 ml HNO_3 in to 20 ml of beer sample and then heated for 6 min (Robert S. and Walter L., 2000).
Digestion of Samples: it involves dissolution of samples on the hot plate with different acid combination.

Therefore, acid digestion involves dissolving of metals into solution for chemical analysis. However, the efficiency of the digestion procedure strongly influences the accuracy of the final result. One of the basic requirements for sample preparation for the analysis is to get an optimum condition for digestion. In order to develop an optimum procedure for the analysis of samples, different digestion parameters were tested and the procedures that produce clear residue solution, consumed minimal reagent volumes, and required shorter digestion time was selected from the different alternatives. During the optimization of the procedure 20 ml of degassed beer was transferred to three 100 mL Griffin beaker and various acid combinations were added in table 1. For the beaker A, B and C; 4, 8 and 10 mL of concentrated HNO_3; 2, 4 and 5 mL of concentrated H_2O_2 was added to beaker respectively. Then the mixture of the beaker were closed and heated at 160^0C.

After cooling, the beaker A and B were not clear solution, while clear solution was obtained in beaker C. Therefore, beaker C was selected and digested degassed beer sample triplicate was filtered using Whatman type Grade 597 (4-7 µm) and diluted to 50 mL volumetric flask using distilled water and kept at 4 ^{0}C until further analysis.

Metals Determinations: The digested beer samples were analyzed for selected metals using FAAS at Department of Chemistry Laboratory, University of Gondar. Working standard solutions containing 100 mg/L for Mg and Ca for other 10 mg/L were prepared from 1000 mg/L stock solutions. These working standards were diluted with distilled water to obtain five working solutions for each metal of interest. Mg, Ca, Fe, Cu, Pb, Cd and Cr were analyzed with FAAS equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve. The parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument.

Method validation (Accuracy)

A. Analytical Performance of Optimized Digestion Method (Recovery): The performance of the digestion procedure was estimated using recovery test. A known solutions 15 mg/L of Mg, 10 mg/L of Ca, 0.08mg/L of Cu, 0.05mg/L of Fe, 0.05mg/L of Pb, 0.005 mg/L of Cd and 0.05mg/L of Cr were spiked at once into 20 mL of beer samples. The recovery test for all samples was performed in triplicates. Percent recovery for each metal analyze were shown Table 2.

To assure the accuracy of the data reported, recovery experiments were performed. The experiments were performed in five replicate measurements, all steps in the sample preparation procedures were carried out and reagent blanks were prepared and measured in the same way as the samples. The recoveries for all elements were obtained in the ranges of 86.174 ± 0.399 to 98.237 ± 0.338 % which shows the procedure had good accuracy to state that the digestion method is valid. The results of the percentage recovery are 80 - 115% was acceptable by literature.

B. Analytical Procedures for Metal Analysis

Instrument Calibration: The instrument was calibrated using five series of working standards. The working standard solutions of each metal were prepared freshly by diluting the series standard solutions. The value of correlation coefficient of the calibration graphs for each of the metals is listed in Table 2. Standards are adjusted concentration for each element in order to cover the concentration range of the beer samples. The intensities of the standards were fitted using linear regression.

Method Detection Limit (MDL): MDLs for beer samples analysis were tried to be determined using blank reagent (distilled water/ HNO_3/H_2O_2) that was digested in the same condition as beer samples and five replicate measurements of reagent water were measured. Each of the metal MDL is listed in Table 2.

Limit of quantification (LOQ): is the smallest amount of analyte in a sample, which is quantitatively determined with suitable precision and accuracy. To determine the LOQ is 10 times the standard deviation of the blank and list in Table 2.

RESULTS AND DISCUSSION

The samples were analyzed in order to examine mean distribution of the average concentration of the Mg, Ca, Cu, Fe, Pb, Cd and Cr in the beer brands as shown in table 3 and figure 1.a and 1.b. The variations mean concentrations (± standard deviation) of metals in the different brands of bottled beers table 3 could be due to factors such as heterogeneity of the samples, different sources of raw materials, origin, manufacturing and packing processes.

The concentrations of Mg is smallest, Ca, Cu and Fe are comparable, Pb, Cd and Cr are highest than to the levels reported in beers from other parts of the world. The concentration of Cd and Pb in all bottled beer samples were below the maximum permissible limit by Brazilian regulations (Cd 14.3 µg/mL and Pb 290 µg/L) (Donadini G., *et al* 2008 & Lucia M. and Adriana M. 2003).

Analysis of variance (ANOVA) data analysis was used to least significant difference between the element concentrations of different brands. The following results were obtained from table 5; whereas: A = Meta beer, B = Bedele beer, C =Dashen beer, D = Harar and E = St. George beer. Negatives values the means are no significantly different at the 0.05 level. Positive values the means are significantly different at the 0.05 level. * shows undefined (one of the brand the elements is ND

CONCLUSION

The mean concentration levels of the selected elements of Mg 44.952 to 50.555 mg/L; Ca 7.774 to 46.729 mg/L; Cu 0.055 to 0.072 mg/L; Fe 0.028 to 0.074 mg/L; Pb ND to 0.051 mg/L; Cd ND to 0.011 mg/L and Cr ND to 0.044 mg/L in the Ethiopian brewery factories beer were determined. There were significant variation in the level of essential elements (Mg, Ca, Cu & Fe) and non-essential elements (Cr, Cd & Pb) between all beer brands. The beer does not pose a serious risk for consumers' health on the basis of the detected levels of non-essential elements. The detection level of calcium, magnesium, iron and copper have sufficient amount for human with other parts of world.

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Tables

Table 1. Optimization procedures for beer sample digestion.

Beaker	Reagent used	Time min.	Condition of digested solution	Remark
A	4 ml of conc. HNO_3 2 ml of 30 % H_2O_2	20”	yellow	Rejected
B	8 ml of conc. HNO_3 4 ml of 30 % H_2O_2	30”	Light yellow	Rejected
C	10 ml of conc. HNO_3 5 ml of 30 % H_2O_2	40”	Colorless	Selected

Table 2. Percentage recovery, confidence limits, correlation coefficient (R), method detection limits and limit of quantification of metal.

Elements	Percentage Recovery	R	MDL in mg/L	LOQ in mg/L
Mg	91.787±0.626	0.9994	0.014	0.047
Ca	96.074±0.331	0.9993	0.018	0.060
Cu	98.237±0.338	0.9989	0.026	0.087
Fe	92.000±0.398	0.9995	0.024	0.080
Pb	86.174±0.399	0.9993	0.017	0.057
Cd	86.000±0.545	0.9998	0.003	0.006
Cr	90.763±0.322	0.9991	0.022	0.074

Table 3. The repeatability mean of metal concentrations in different beer brand available in Ethiopia, analysis spectroscopy by flame atomic absorption spectroscopy (Mean values ± 95% confidence limits are given (n = 3))

Beer Brands	Mg	Ca	Cu	Fe
	mg/L			
Meta	44.952±0.661	14.637±1.334	0.062±0.007	0.052±0.005
Bedele	50.942±.354	14.343±1.810	0.072±0.002	0.028±0.005
Dashen	50.555±3.567	46.729±4.578	0.059±0.002	0.043±0.005
Harar	47.899±3.827	7.774±0.395	0.055±0.004	0.074±0.005
St. George	48.140±4.240	41.010±5.413	0.072±0.002	0.052±0.005
Beer Brands	**Cd**	**Cr**	**Pb**	
	mg/L			
Meta	0.005±0.001	ND	0.051±0.006	
Bedele	0.008±0.001	0.029±0.008	0.047±0.010	
Dashen	0.004±0.002	ND	0.025±0.010	
Harar	0.011±0.002	0.024±0.000	ND	
St. George	ND	0.044±0.008	0.029±0.006	

*ND = below detection limit

Table 4. The simple comparison of different country literature values of metals with present study.

Metals	Present Study in mg/L	Literature value	Country	References
Mg	44.130 to 55.000	65.1 mg/L 83.7 mg/L 84.3 mg/L 67.9 mg/L 61.7 mg/L	South Africa China Belgium Mexico Spain	(Don P., 2002)
Ca	7.480 to 49.735	45.3 mg/L 33.4 mg/L 42.9 mg/L 80.2 mg/L 25.4 mg/L	South Africa China Belgium Mexico Spain	(Don P., *et al* 2002)
Cu	0.050 to 0.071	47 ± 2 µg/L 25 – 137 ng/mL 28 - 48 µg/L 7 - 49 ng/mL 38.0 - 155 µg/L 0.04-0.10 µg/mL	Norway (2000) Spain Italy Norway (2005) Brazil Nigeria	Clesia C., *et al* 2005, Čejka p., *et al* 2011, Viñas P., *et al* 2002, Robert S., 2000, Heidi M., *et al* 2003 & Chukwujindu M., 2010
Fe	0.213 to 0.769	35–175 ng/mL 58 ± 2 µg/L 0.31 - 0.73 µg/mL 0.33-1.59 mg/L	Norway (2005) Norway (2000) Nigeria Romania	(Čejka p., *et al* 2011, Robert S. and Walter L., 2000 & Chukwujindu M., 2010)
Pb	ND to 0.058	37µg/L 13.0 - 32.9 µg/L 1.83 ± 3.24 µg/L 0.001 - 0.047 µg/mL	Brazil (2003) Brazil (2005) Italy in Nigeria	(Clesia C., *et al* 2005, Donadini G., *et al* 2008, Chukwujindu M., 2010 & Lucia M., *et al* 2003)
Cd	ND to 0.013	1.6 µg/L 0.16 ± 0.15 µg/L	Brazil Italy	(Donadini G., *et al* 2008, Getachew L., *et al* 2007)
Cr	ND to 0.054	6-8 µg/L 0.31–0.73 µg/mL	Poland Nigeria	(Chukwujindu M., 2010, Wiesawa R., *et al* 2005]

Table 5. The ANOVA-Minitab output significant different concentration between beer brands of each element, at the 0.05 level.

Between brands	Mg	Ca	Cu	Fe	Pb	Cd	Cr
A & B	-14.794	-9.384	-0.018	0.103	-0.020	-0.005	*
A & C	-14.408	-41.769	-0.005	-0.051	0.002	-0.002	*
A & D	-11.751	-2.815	0.002	-0.360	*	-0.009	*
A & E	-11.992	-36.050	-0.018	-0.144	-0.001	*	*
B & C	-8.417	-42.063	0.005	-0.298	-0.001	0.001	*
B & D	-5.761	-3.109	0.012	-0.607	*	-0.007	-0.012
B & E	-6.002	-36.344	-0.008	-0.390	-0.005	*	-0.037
C & D	-6.147	29.276	-0.001	-0.453	*	-0.010	*
C & E	-6.389	-3.959	-0.021	-0.236	-0.027	*	*
D & E	-9.045	-42.913	-0.027	0.073	*	*	-0.047

Whereas: A = Meta beer, B = Bedele beer, C =Dashen beer, D = Harar and E = St. George beer. Negatives values the means are no significantly different at the 0.05 level. Positive values the means are significantly different at the 0.05 level. * shows undefined (one of the brand the elements is ND

Figures:

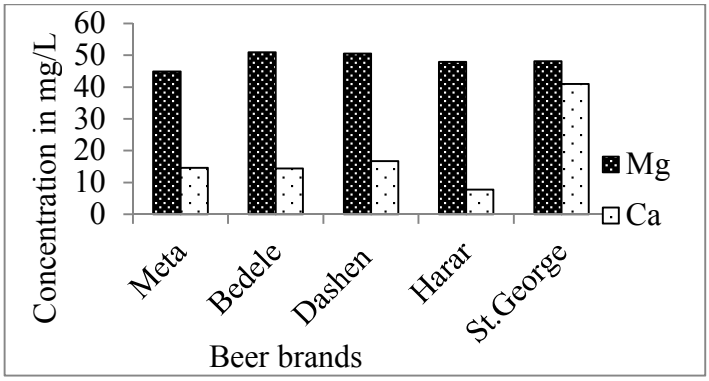

Figure 1.a The mean concentration distributions of Mg and Ca metals in bottled beer samples

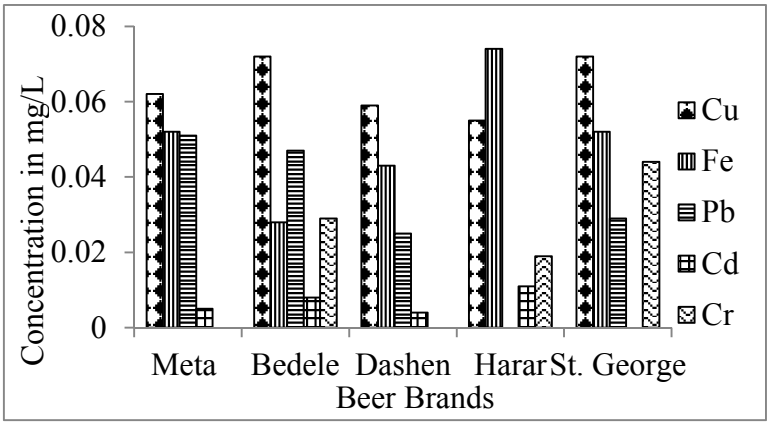

Figure 1.b**:** The mean concentration distributions of Cu, Fe, Pb, Cd and Cr metals in bottled beer samples