Investigation of Total Polyphenol, Antioxidant Activity and Levels of Metals in Ethiopian Commercial Beers

Molla Tefera (molla.tefera@uog.edu.et)
University of Gondar College of Natural and Computational Sciences https://orcid.org/0000-0003-4469-5388
Daniel Ayele
University of Gondar College of Natural and Computational Sciences

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Abstract

Total polyphenol, antioxidant activity and heavy metal contents in selected Ethiopian beers (Dashen= B1, Harar = B2, Habesha= B3, Saint George= B4 and Walya= B5) were investigated. The average concentration of total phenolic content in B1, B2, B3, B4 and B5 were found to be 219.80, 206.09, 190.24, 193.00 and 212.10 mg GAE/L, respectively. The obtained results showed that B1 contained significantly highest total polyphenol content (219.80±2.83 mg/L) and showed the highest radical scavenging capacities (84.16±0.65). According to Pearson's correlation between polyphenol extract with antioxidant activities, suggested that phenolic compound in beer were contributor to antioxidant activity. Similarly, all the beer samples were analyzed for Cd, Cr, Cu, Ni and Pb with flame atomic absorption spectrophotometer. The total metal concentrations of Cd, Cr, Cu Ni and Pb were found to be in the range of (ND–0.022 mg/L), (0.02 -0.041), (0.023-0.054), (ND-0.064) and (ND-0.022 mg/L), respectively. The efficiency of the procedure was validated by spiking and the percent recovery of the metals varied from 90.9-104.3%.

Introduction

Beer is the third most popular beverage in the world next to tea and water [1]. In recent decades, beer consumption has been steadily increasing even in countries where alcoholic beverages are not traditional. Since beer is brewed from water, yeast, malt, and hops, beer contains a broad range of different chemical components that may react and interact at all stages of the brewing process [2]. Beers consumed in Ethiopia are produced locally from barely and are sold in restaurants, bars, and shops, or served at as festivals, funerals, weddings and marriage ceremonies.

As long as beer is consumed moderately in responsible manner, it can have significant effect on human health as a source of essential vitamins (B2, B6 and B12), carbohydrates, amino acids, phenolic compounds and minerals [3]. Phenolic compounds are widely and naturally abundant in fruits, vegetables, and cereals crops. Many studies have shown that regular consumption of foods and beverages abundant in polyphenols have antibacterial, antifungal, anti-inflammatory, antitumor activities and prevent diseases related to aging [4]. In beverages, polyphenols arise from the raw materials such as malt and hops during brewing process, which play a key role in antioxidant activity and they can also influence the flavor, color and sensory stability of the beer [5, 6].

The concentrations of inorganic components may vary depending on raw materials, brewing processes, equipment, bottling and adulteration. Application of pesticides, fungicides, and fertilizers containing metals like Cd, Cu and Pb in crops also increase the amounts of metals in beverages [7]. Depending on the types and levels, metals may be essential or toxic to human body and can also affect the brewing process and beer quality such as taste, appearance, product stability and haze formation. Identifying the content of metal in beer is considered as valuable for differentiation, classification of beer and essential issue for consumer safety in order to develop effective and efficient risk communication channels in the brewery industry [8, 9]. Labels in most beer products do not provide information on the mineral content and acceptable concentration of specific elements, and there is no obligation to inform customers about the levels of the elements. In Ethiopia, limited studies were carried out on the total polyphenol, antioxidant activities and mineral contents of beers produced and consumed in Ethiopia.

Therefore, the objectives of this study were to evaluate the total polyphenol, antioxidant activities and levels of metals in Ethiopian commercial beers.

Materials And Methods

Sampling

For this study, five Ethiopian commercial beers (Dashen= B1, Harar = B2, Habesha= B3, Saint George= B4 and Walya= B5) originated from various producers were chosen. A total of 60 bottled beers (twelve from each brand) were randomly purchased in different Hotels and Groceries from Gondar Town and stored in a refrigerator at 4°C till further analysis.

Chemicals

All chemicals used in this study were all analytical grade unless otherwise stated. The chemicals that were used for this study are carboxymethyl cellulose sodium salt (CMC, Aldrich, USA), ethylene diamine tetra acetic acid disodium salt dehydrate (Aldrich, USA), ammonium iron citrate reagents (Aldrich, USA), ammonia reagent (Neolab, USA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), HNO_3(Blulux, India), calred indicator, H_2O_2 (Carel Abmed, India), standards of Pb, Cd and Ni (Loba Chemie, India) and Cr and Cu (Blulux, India). Standard solutions of elements were used for calibration were prepared by diluting 1000 mg/L of stock solutions of each element by deionized water.

Instruments

The instruments used in this study were turbidity meter (Type Vos Rota), density meter (DMA 4500), pH meter (Metler Toledo), Hazimeter cuvette, and UV-VIS spectrophotometer (thermo scientific model Evolution 201, USA), flame atomic absorption spectroscopy (Buck Scientific Model 210 VGP, USA) equipped with deuterium arc background corrector and standard air-acetylene flame system.

Determination of total polyphenols

The total polyphenol content of commercial beers were determined by using EBC method. Briefly, 10 mL of degassed beer and 8 mL of CMC/EDTA reagent were added to 50 mL volumetric flask with stopper and thoroughly mixed. Then, 0.5 mL of ferric reagent (ammonium iron citrate) was added to the sample and homogenized. Then after, 0.5 mL of ammonia were added to the solution and mixed again. Finally, distilled water was added and then the sample solution was allowed to stand for 10 min. The absorbance was measured by UV-Vis spectrophotometer at 600 nm [10].

Determination of Antioxidant activity

DPPH scavenging activity of beer was determined according to the method described by Zhao et al. [6] with minor modification. Brefiely, 0.5 mL of diluted beer was mixed with 2.5 mL of 1 mM DPPH in methanol and the mixture was allowed to stand for an incubation time of 1 h at room temperature. The blank sample was run in the same conditions as the beer samples. Finally, the absorbance was measured at 517 nm using UV-Vis spectrophotometer [8]. The radical scavenging activity was calculated as follows:

$\%I = [(A_o - A_s)/A_o)] * 100$,

where, A_o is the absorbance of blank and A_s is the absorbance of sample. The measurements were performed in triplicate.

Determination of metals

In order to select an optimum procedure for digestion, reagent volume, digestion temperature and digestion time were optimized on varying one parameter by keeping other parameters constant until clear and colorless solution was obtained. The procedure which consumed minimum reagent volume at lower temperature and shorter digestion time was chosen as optimal procedure. To determine the concentration of metals, 10 mL of degassed beer and various volumes of HNO_3 and H_2O_2 were added into round bottomed flask and digested at different digestion time and temperature. After digestion was completed, the solution was cooled and filtered through Whatman No. 42 filter paper and diluted with deionized water. Finally, it was kept in refrigerator until analysis. Digestion of a reagent blank was also performed following similar digestion procedure as that of the sample [11].

Finally, 3 mL of HNO_3 and 4 mL of H_2O_2 at a temperature of 150^oC for 50 min were chosen as the optimal digested procedure for determination of the concentration of metals in beer samples. For flame atomic absorption spectroscopy measurements, acetylene was used as inert gas. The operating parameters for the determination of metals were given in Table 1.

Table 1.

Data Analysis

Physico-chemical properties and levels of trace metals in beer were expressed as mean ± SD and one-way ANOVA was employed to examine the statistical significance of elements concentration in beer samples at 95% confident interval. The quantifications and statistical analysis were done with IBM SPSS Statistical software Version 20. Pearson correlation coefficients were applied to investigate the correlations between total phenolic content and antioxidant capacities.

Results And Discussion

Physicochemical Analysis

The physicochemical properties of Ethiopian commercial beers are described in the Table 2. The value of AE was ranged from 1.74 to 2.51^oP and generally found in the order of B5 > B2> B4 > B1 > B3. The highest AE value may be related to the presence of unfermented sugars (oligosaccharides) by yeasts, while the least AE value was due to fermentation of lower molecular weight sugars present in the wort like glucose, fructose and maltose basically come from the malt. Similarly, B5 showed the highest RE value, followed by B2, B4, B1 and B3. Among the investigated beers, B2 displayed the highest OE value (11.28 ^0P), while B4 was the least (10.74 ^0P), this attributed to the quality and amount of malt used; and the nature of brewing process used for beer production [12].

The maximum value of ADF and RDF were observed in B3, whereas the least amount were found in beer sample B5 and B4, respectively (Table 2). The highest RDF indicates that the extract originally present in the wort was converted in to ethanol and CO_2 in better degree than the one which contained least RDF value [10].

The alcoholic (ethanol) content was found in the range of 4.58 to 4.88 (%, v/v), the highest alcoholic content was found in B2 and the least one was in B4. The results of this investigation are consistent with researches carried out by Pai et al. [13], Mitic et al. [14]. The difference in alcoholic content among all beer samples may be attributed to the difference in brewing temperature, yeast cell count, aeration, composition of wort (i.e. amount of original extract, pH, dissolved oxygen, metals, etc) [15]. Many studies demonstrated that the alcoholic content of most beers were found in the range of 3%–6% (v/v) [13].

The pH of the beer samples ranged from 4.20 to 4.38. B4 was the most acidic among all the beer samples, while B1 was the least acidic. The variation attributed to the difference in raw material used, water and the amount of acid added in the mashing step. The pH of a beer strongly influences physiological parameters such color, odour, taste, biological and chemical stability of beer. The pH range of beers identified in this study are consistent with previous reports [13].

On the other hand, the haziness of this study followed the order B3 > B1 > B4> B2 > B5, this attributed to residual starch, oxalate from calcium deficient worts, carbohydrate and protein from autolysed yeast, lubricants from can lids, and dead bacteria from malt [16]. Concerning bitterness, B2 had the highest bitter with a value of 27.68 IBU, while B4 had the least bitter with a value of 14.76 IBU. This difference is due to variation in the type and amount of hope added in the brewing process. The physicochemical properties Ethiopian commercial beers that was studied here are in agreement with other reports [13, 17, 18].

Total polyphenols content

Phenolic compounds play critical roles in both flavour and colloidal stability of beer [6]. The total phenolic contents of beer depend on the raw materials for brewing mainly malt and hop; and the type of beer produced [5]. Studies showed that beers with high phenolic content showed better quality, more stable sensory properties and longer shelf life than beers with low phenolic content [14, 19].

As shown in Table 3, the total phenolic content significantly varied with the type of beer, which ranged from 190.24 to 219.8 mg/L, of which B1 contained highest amount of total phenolic content and B3 the least total phenolic content (190.24±5.63 mg/L). The result indicated that significant differences were observed ($p < 0.05$) on total phenolic content in all analyzed beer samples except between B3 and B4 ($p > 0.05$). The amount of TPC variation comes from the difference in raw material used mainly from malt and hope and the nature of beer production process [20, 21]. The results obtained in this study were in agreement with reported values [1, 5, 6, 20].

So, the difference in amount of total polyphenols can cause difference on their antioxidant activities, flavour, color and sensory properties and also may contribute to maintain the endogenous redox balance in humans [9].

Antioxidant activity

The values of antioxidant activity for the analyzed beers was expressed as percent of inhibition (Table 3). Among the beers, B1 had significantly ($p<0.05$) highest percent of inhibition (84.16 %), whereas B3 had the least percent of inhibition with a value of 72.7%. Like with the total polyphenol content, the antioxidant activity of Ethiopian commercial can be ranked as follows: B1 > B5 > B2 > B4 > B3, attributed to the difference in the amount of raw materials and brewing process. A beer which had greater DPPH radical scavenging activity could have enhanced flavour stability because beer staling can occur as a result of the formation of trans-2-nonenal and other saturated and unsaturated aldehydes as a result of oxidation [13]. The antioxidant activity of Ethiopian beers are found to be in agreement with studies reported in literature [1, 13, 17, 21].

As shown in Table 4, the total phenolic content of beers showed positive correlations with the antioxidant capacities of the beers analyzed. This indicating that total phenolic content could be used as an indicator in evaluating the antioxidant capacity of beers which may preliminarily applied as natural sources of antioxidant functional beverages [22].

Levels of metals

The efficiency of the analytical method that used to determine the levels of each metal in commercial beers was validated through linearity, recovery, precision and limit of detection. The validated data of the methods are presented in Table 5. The correlation coefficients of the calibration curves of the analyzed metals were found above 0.9979.

The recovery test was performed for the analysis of metals in beer samples through spiking experiments, in which known volumes of standard solutions of elements of interest were added in to beer samples. The spiked samples were digested following the same procedures as utilized for digesting the beer samples. As shown in Table 5, the percentage recovery were 99.2±5.4%, 90.9±8.9%, 95.2±10.5%, 96.5±8.7 and 104.3±9.1% for Cd, Cr, Cu, Ni and Pb, respectively. The recovery results indicated that the method was applicable for determining the levels of metals in beer samples.

The limit of detection (LOD) was obtained from three times its standard deviation of the blank signals divided by the slope of the calibration equation, whereas limit of quantification (LOQ) was calculated by multiplying LOD by a factor of three [23] and the values for LOD and LOQ of all studied elements are given in Table 5.

As shown in Table 6, Cd was only detected in B2, B3 and B4, whereas in B1 and B5 it was under the limit of detection of the method applied.

The trend of Cr concentration (mg/L) in beers was B3 (0.47±0.002) > B5 (0.23±0.004) > B4 (0.05±0.001) > B2 (0.035±0.002) > B1 (0.023±0.001). Cr levels in beers have been reported in the range of 0.0256-0.4417 mg/L [24] and 0.17–0.34 mg/L [25], which suggest that the values obtained in this study are within range reported in literatures.

Copper content of beers ranged between 0.020 and 0.041 mg/L. B4 had the highest level (0.041 ± mg/L) of Cu, while B1 had the smallest level (0.020 ± mg/L) of Cu. This is within ranges of beer studies by Voica et al. [24] and Izah et al. [25] who reported a range of 0.0259-0.736 mg/L and 0.04–0.08 mg/L, respectively.

The overall concentration of Ni ranged from 0.026 to 0.064 mg/L, the highest being from B4 and the lowest from B3. However, Ni metal were found to below detection limit in B1 and B2. Of all the studied beers, B2 and B4 were contained highest values of Ni. These results were in agreement with reported by Voica et al. [24]. Lead metal was detected only in B3, B4 and B5. However, Pb was found below of detectable limit in B1 and B2. These results are comparable with the results reported by Voica et al. and Izah et al. [24, 25].

In general, Cu was found to be highest in B1, B3 and B5, while Ni was the highest among other metals in B2 and B4. However, lead was the least detected.

Conclusions

The results of the present study indicated that the Ethiopian beers contained substantial amount of phenolics contents and correspondingly exhibited high antioxidant activity. The average content of total polyphenol and antioxidant activities were represented in the order of B3 < B4 < B2 < B5 < B1. Therefore, beer can be used as an important sources of polyphenols. The Pearson correlation analysis revealed that the TPC showed strong positive correlations with DPPH radical scavenging activity.

Depending on the concentration, metals may be essential or toxic to human body for health with a profound effect on well-being. Metals compositions in beer are important for yeast growth and influence yeast metabolism and also advantageous to the consumers. In the present study, heavy metal concentrations in Ethiopian beers showed considerable variations. Highest amounts of Cr, Cu, Ni and Pb were investigated in B4. However, B2 contained highest amount of Cd. Generally, Cu showed the highest mean value, followed by Ni, Cr, Pb and Cd.

The results obtained are in agreement with data reported in literature and all the beer samples analyzed were found to be good potentials sources in minerals.

Declarations

Authors’ contributions

MT and DA involved in the conception of the research idea and design of the experiments. DA involved in the sampling, sample preparation and data analysis and also drafted the paper.MT provided guidance, corrections and supervision to the entire research, critically reviewed and amended the manuscript. All authors read and approved the final manuscript.

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Availability of supporting data

The data sets used and analysed during the study are available to readers as in the manuscript. All the data are included in the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. FAAS instrumental operating conditions of investigated elements

Element	Wave length (nm)	Lamp current (mA)	Slit width (nm)
Cr	357.9	2.0	0.7
Cu	324.8	0.7	0.7
Fe	248.3	7.0	0.2
Ni	232.0	7.0	0.2
Pb	283.3	2.0	0.7

Table 2. Characteristics of Ethiopian beers (n=3, mean± SD)

Parameter	B1	B2	B3	B4	B5
AE (^{O}p)	1.96±0.12	2.06 ±0.02	1.74±0.06	2.04±0.10	2.51±0.13
RE (^{O}p)	3.7±0.10	3.84±0.15	3.51±0.06	3.72±0.17	4.2±0.12
OE (^{O}p)	10.98±0.09	11.28±0.06	10.96±0.01	10.74±0.11	11.24±0.14
ADF (%)	82.07±1.33	81.71±1.4	84.09±0.47	80.94±1.19	79.84±2.83
RDF (%)	67.56±1.07	67±1.16	69.17±0.36	66.62±0.95	68.48±6.88
Alcohol	4.76±0.11	4.88±0.06	4.86±0.04	4.58±0.11	4.62±0.06
pH	4.38±0.08	4.28±0.23	4.29±0.12	4.2±0.11	4.33±0.09
Bitterness	18.23±0.99	27.68±1.13	16.33±0.2	14.76±1.07	17.73±0.11
Haze	0.38±0.02	0.77± 0.02	0.38±0.13	0.57±0.02	0.92±0.005

Where AE= apparent Extract, RE= real Extract, OE= original Extract, ADF= apparent degree of fermentation, BU= bitterness

Table 3. The total polyphenol content and antioxidant activity of Ethiopian beers

Sample	TPC (mg/L)±SD	% Inhibition
B1	219.80±2.83^{a}	84.16±0.65^{a}
B2	206.09±2.53^{b}	76.70±1.85^{b}
B3	190.24±5.63^{c}	72.70±1.09^{b}
B4	193.00±0.94^{c}	76.62±2.26^{b}
B5	212.10±3.94^{b}	83.10±4.52^{c}

Different letters in the same column indicates means that are significantly different at $p < 0.05$

Table 4. Pearson's correlation coefficient of total phenolic content and antioxidant activity in beer samples

Sample	AOA	
B1	TPC	0.89
B2		0.40
B3		0.91
B4		0.45
B5		0.83

Table 5. Analytical parameters of metals measurement by atomic absorption spectroscopy

Cd	Cr	Cu	Ni	Pb	
Reg. Eq.	A=0.0372C+ 0.0052	A=0.0424C+0.0028	A=0.0452C+0.0042	A=0.0468C+0.0018	A=0.0412C+ 0.0032
R^2	0.9993	0.9979	0.9995	0.9996	0.9994
LOD	0.002	0.013	0.008	0.023	0.009
LOQ	0.006	0.039	0.024	0.069	0.027
Recovery[a]	99.9±2.5	90.9±3.1	95.23±4.5	96.5±6.9	104.3±1.8

[a] in%

Table 6. Concentration of metals (mg/L), LoD and recovery (mean ±SD) in beers

Beer type	Cd	Cr	Cu	Ni	Pb
B1	ND	0.020±0.001[a]	0.023±0.001[a]	ND	ND
B2	0.022±0.002[a]	0.028±0.003[b]	0.035±0.002[b]	0.04±0.003[a]	ND
B3	0.013±0.001[b]	0.025±0.002[a]	0.047±0.005[b]	0.026±0.001[a]	0.013±0.001[a]
B4	0.014±0.001[b]	0.041±0.002[c]	0.054±0.003[a]	0.064±0.002[a]	0.022±0.001[b]
B5	ND	0.023±0.001[a]	0.034±0.004[b]	ND	0.016±0.001[c]
LOD	0.002	0.013	0.013	0.023	0.009
Recovery (%)	99.2±5.4	90.9±8.9	95.2±10.5	96.5±8.7	104.3±9.1

Different letters in the same column indicates means that are significantly different at $p < 0.05$