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ORIGINAL RESEARCH

Elemental and Polyphenol Contents of Green Coffee Beans from Central Gondar Zone, Ethiopia

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ABSTRACT

Variations in the major (Ca and Mg) and trace (Cu, Zn, Fe and Mn) essential metals as well as in the total polyphenol contents of green coffee beans with the major growing districts of Central Gondar Zone, Ethiopia, (Gondar Zuria, Takusa, Tach Armachiho and Chilga) were assessed using flame atomic absorption spectroscopy and UV/Vis spectroscopy, respectively. A total of 23 samples were used for analysis. For the analysis of metals, a wet digestion procedure was optimized. Accordingly, a 0.5 g of green coffee bean powder was digested on a hot plate with HNO_3:$HClO_4$ (4:4 v/v) at 240 °C for 3.5 h. The accuracy of the optimized procedure was evaluated from recoveries of spiked samples, which varied in the range 85–116%. The coffee beans were found to contain considerable amounts of the essential elements, particularly Ca, and total polyphenols. The average concentration (mg/kg) of the elements were in the range of 2876–3515 Ca, 839–877 Mg, 71.8–140 Fe, 31.6–100 Cu, 21–24.2 Mn and 10.0–13.3 Zn. Significantly higher concentrations of Fe and Ca was found for green coffee beans from Takusa than the other districts. The polyphenol content of the coffee beans ranged from 31.5–52.5 mgGAE/g, across samples. Analysis of variance ($p < 0.05$) revealed that there were no significant differences in the concentrations of total polyphenols among coffee beans from the four districts.

Keywords/phrases: Green coffee beans, Metals, Total polyphenol, Central Gondar, Ethiopia

Introduction

Coffee is a plant that belongs to the family *Rubiaceae* and genus *Coffea*. Coffee is the most commercialized food product, and widely consumed beverage in the world. It is ranked as the second most traded global commodity, after petroleum (Mehari *et al*., 2016a). Among the several coffee species, only two, Arabica (*Coffea Arabica*) and Robusta (*Coffea Canephora*), account for 75% and 25%, respectively, of the world commercial production of coffee (Asfaw *et al*., 2019). Arabica is mainly produced in upland and mountain areas, such as in East Africa, while Robusta in lowland of Central and West Africa (Grzes-kowiak *et al*., 2014). Arabica coffee is favored by consumers compared to Robusta coffee (Sabah *et al*., 2019).

The name coffee is believed to be derived from the name of the province Keffa, Ethiopia, where a shepherd is believed to discover the coffee beans in the 6th century (Amamo, 2014). Coffee is a popular beverage in Ethiopia, and currently the annual local coffee consumption in the country is estimated to be more than 9.0 million ton (Sabah *et al*., 2019).

Coffee is composed of different chemicals, of which the most important bioactive compounds include phenolic compounds, such as chlorogenic acids (Mehari *et al*., 2016a), methylxanthines, such as caffeine, theophylline and theobromine (Mehari *et al*., 2016b), diterpenes, such as cafestol and kahweol, nicotinic acid (vitamin B3) and its precursor trigonelline, magnesium and potassium. Typical compounds in coffee, such as caffeine, trigonelline and chlorogenic acid, contribute to the acidity and confer astringency and bitterness, thereby influencing coffee flavor (Mehari *et al*., 2016b). For example, caffeine, an alkaloid, stimulates the central nervous system, heart rate and respiration; and chlorogenic acid, a family of esters formed between caffeic acid and quinic acid, exhibits various biological properties including anti-bacterial, anti-oxidant and anti-carcinogenic activities (Arai *et al*., 2015). Moreover, the flavor and aroma of coffee are believed to be affected by the presence of various volatile and nonvolatile chemical constituents such as proteins, amino acids, fatty acids, and phenolic compounds (Dechassa *et al*., 2018). Besides the genetic factors, the chemical composition of green coffee beans depends largely on growing environmental conditions (Mehari *et al*., 2016c).

Coffee is believed to have multifarious beneficial health effects, usually attributable to its high antioxidant activity. The antioxidant activity of coffee depends mainly on chlorogenic, ferulic, caffeic, and n-coumaric acids contained in it (Yashin *et al*., 2013). Recently, there has been growing evidence that oxidative stress and some human diseases can be prevented by including in the diet of plant foods that contain large amounts of antioxidants, such as polyphenols. This is because they can act as free radical scavengers, radical chain reaction inhibitors, metal chelators, oxidative enzyme inhibitors and antioxidant enzyme cofactors (Karadag *et al*., 2009). In this respect, coffee is one of the sources of polyphenolic species like chlorogenic, ferulic, caffeic and n-coumaric acids that can prevent the formation of free radicals, leading to bond breakage in DNA and deformation of genetic makeup (Yashin *et al*., 2013).

Coffee also contains various mineral elements, such as Na, B, Mg, Fe, Ca, and K, with good nutritional values (Mehari *et al*., 2016c). These elements are absorbed in various proportions from the soil and accumulated by the plant. In this respect, climate, elevation, underlying geology, type of soil and its chemical composition, and the application of fertilizers and pesticides are the factors, affecting the types and proportions of such elements (Habte *et al*., 2016). Hence, the determination of the concentrations of elements in coffee helps to assess its nutritive quality and judge its possible side effects to human health upon their presence at higher doses (Sabah *et al*., 2019).

The coffees produced in the different areas of Central Gondar Zone have distinct flavors, where some of the areas have the reputation for their exceptionally good flavor coffees. It is known that the final beverage quality of coffee depends on the biochemical composition of the green beans. Hence the coffees that are praised for distinct beverage qualities are expected to have distinct biochemical composition in the green beans. Despite this, no literature information is available concerning the biochemical characteristics of coffee beans from Central Gondar Zone. Therefore, the aim of this study was to determine the elemental and total polyphenol contents of green coffee beans from the major coffee producing areas of Central Gondar Zone, and investigate the effects of their geographic sources on the levels of the constituent chemicals.

Materials and methods

Coffee Samples

The coffee samples were collected from four different districts of Central Gondar Zone (Figure 1). A total of 23 samples, three from Takusa, two from Gondar Zuria, eight from Tach Armachiho, and ten from Chilga district were included in the study. These districts were included in the study. These districts were

were selected purposefully because of their relatively higher productivity. All the samples were from the 2019/2020 crop season, collected during December 2019 to February 2020. Each sample was 250 g of coffee cherries and was stored in polyethylene plastic bags under room temperature in the laboratory until analysis.

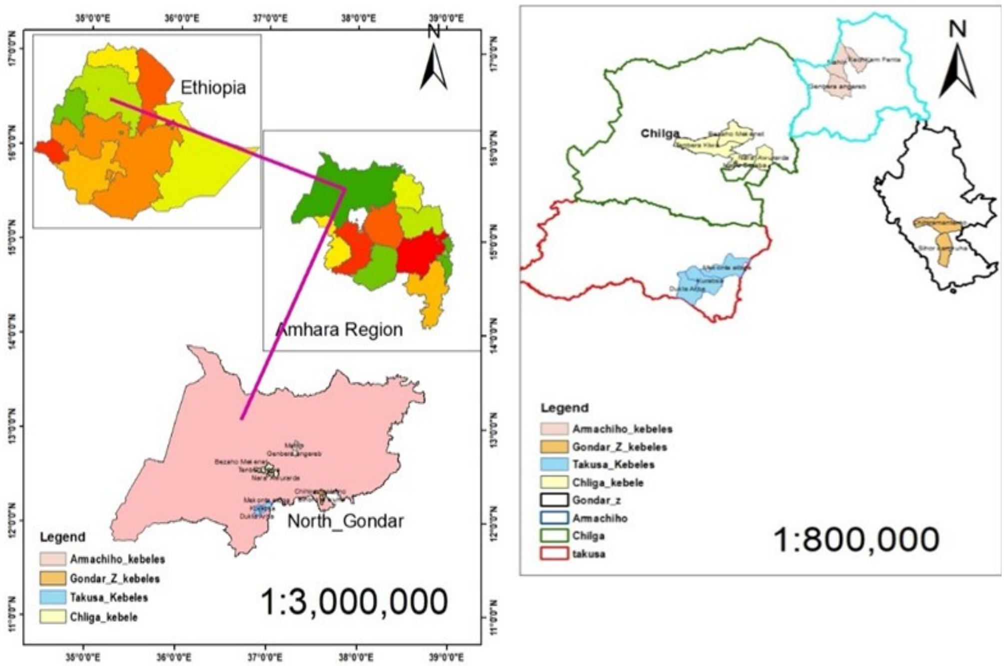

Figure 1: Map of Ethiopia showing the green coffee sample collection districts.

Coffee Sample Preparation

The coffee cherry samples were dried and the husks of the cherries were removed by using mortar and pistil to get the beans. The coffee beans were washed at first with tap water, followed by rinsing with distilled water. From each sample 50 g was weighed and powdered by using electrical grinder. The coffee powder was then sieved using a 200 µm mesh size sieve.

Chemicals and Equipment

Folin-Ciocalteu reagent, Gallic acid and methanol (Fine Chemicals, India), sulfuric acid (98%), potassium dichromate, nitric acid (65%) (BDH, India), standard solution of Ca, Mg, Zn and Mn perchloricacid and sodium carbonate (Blulux Laboratory, India), standard solution of Fe and Cu (Lova Chemie, India), UV-Vis spectrometer (Abron Instruments, China), and flame atomic absorption spectrometer (Buck Scientific, 210 BGP, USA) were used in the study.

Extraction of Polyphenols

The extraction was performed following a reported procedure (Geremu *et al*., 2016), with little modification. Briefly, a 0.5 g of powdered green coffee beans was macerated with 10 mL of 80% methanol in water as the solvent for 24 h at room temperature. The mixture was then stirred for 30 min by using magnetic stirrer. Finally the resulting solution was filtered with Whatman no.1 filter paper.

Determination of Total Polyphenols

The total polyphenol content of the green coffee extract was determined based on spectrophotometric method using Folin-Ciocalteu's reagent (Asfaw *et al*., 2019). Briefly, a 0.5 mL of extract was mixed with 0.25 mL of Folin-Ciocalteu reagent and 3 mL distilled water in a test tube. After 5 min, 1 mL of 7.5% sodium carbonate solution was added, covered by aluminum foil and incubated for 1:30 h in the dark. Blank samples were prepared side by side with the 0.5 mL of 80% methanol instead of the samples. The absorbance of the resulting solution was recorded at 760 nm against the blank solution. Quantitation was done using a calibration curve prepared with Gallic acid as a standard, and the total phenolic content was expressed as milligrams of Gallic acid equivalents per gram of dry coffee beans (mgGAE/g).

Sample Digestion for Metal Analysis

An optimized procedure was used for the digestion of green coffee beans prior to the analysis of metals using FAAS. Applying the optimized digestion procedure, 0.5 g green coffee bean powder was digested with 4 mL of HNO_3 (69%) and 4 mL $HClO_4$ (70%) mixture, at a digestion temperature of 240 °C for 3 h and 50 min. These optimum condition was selected based on clarity of sample digest, minimum reagent volume consumption, minimum digestion time, simplicity, and minimum temperature applied for complete digestion of sample. A 1% lanthanum chloride solution was aslso added in order to liberate calcium from phosphate. The digest was filtered in to a 50 mL volumetric flask and filled with distilled water to the mark. Blank samples were also prepared by taking a mixture of HNO_3 and $HClO_4$ treating in a similar procedure to that of the samples.

FAAS Analysis of Metals

For the analysis of the samples using FAAS calibration of the instrument with the known concentration of standards were done for each metal of interest. Calibration curves were plotted by five point standards for each of the metal. Standard solutions of Ca 0.5, 1.5, 2.5, 4.5, 5.5 mg/L; Mg 0.5, 2.5, 3.5, 4.5, 5.5, 6.5 mg/L; Mn 0.1, 0.2, 0.3, 0.5, 1.5, 2.5, 3.5 mg/L; Fe 15, 20, 25, 30, 35 mg/L; Cu 0.5, 1.5, 2.5, 3.5, 4.5 mg/L; and Zn 0.5, 1.5, 2.5, 3.5, 4.5 mg/L. Each of the sets of working standards was then aspirated one after the other by their increasing order of concentration into the atomic absorption spectrometry and their absorbance was recorded. The instrumental operating conditions used for the determination of metals using FAAS are shown in Table 1.

Table 1. Instrumental operating conditions used for the determination of metals with FAAS.

Metals	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Energy (erg)
Ca	422.7	0.7	2.0	3.96
Mg	285.2	0.7	1.0	3.726
Cu	342.7	0.7	1.5	3.728
Zn	213.9	0.7	2.0	3.022
Fe	248.3	0.2	7.0	3.093
Mn	279.5	0.7	3.0	4.045

Method Validation

In order to ascertain the accuracy of the method used for the analysis of metals in samples, spiking experiment was used.The method was judged good when the percentage recovery was within the range of 80–120% (Harvey et al., 2000). This was assessed by adding different volumes of standard solution of each metal (1000 mg/L), 1795.5 µL Ca, 424.5 µL Mg, 25 µL Cu, 1.65 µL Zn, 25 µL Fe and 110 µL Mn, to 0.5 g of powdered coffee beans. Triplicate samples were prepared similarly and digested in the same manner as for original samples. Finally the solution was analyzed by using FAAS. Precision was expressed as relative standard deviation (RSD) of the three replicate results. The precision of the method was regarded as good when the RSD did not exceed 15% (Skoog *et al.*, 2013). The limit of detection of the method was calculated as the concentration of analyte equal to three times the standard deviation of the blank signal.

Data Analysis

All sample measurements were performed in triplicates and results are reported as mean and standard deviation. Data were analyzed statistically using Microsoft Excel 2013. One-way ANOVA was used to assess the existence of significant differences among the means due to coffee growing districts.

Result and Discussion

Analytical Characteristics of the Method

Linearity

Calibration curves for all the analysed metals showed good linearity with coefficients of determination (R^2) ranged between 0.9960 and 0.9996 (Table 2), which showed good linear fit between absorbance and concentrations of the elements.

Table 2. The regression equation, correlation coefficient (R^2) and limits of detection (LOD, mg/kg) of the method used for the analysis of metals using FAAS.

Metal	Regression equation	r^2	LOD	%Recovery
Ca	A = 0.0045C + 0.0024	0.9980	31	85
Mg	A = 0.0893C + 0.8061	0.9960	55	116
Cu	A = 0.0466C - 0.0089	0.9996	0.7	108
Zn	A = 0.1032C + 0.0116	0.9969	0.06	95
Fe	A = 0.0013C + 0.0054	0.9963	1.8	92
Mn	A = 0.0367C - 0.0031	0.9972	0.4	93

*A is absorbance, C is concentration

Sensitivity

The sensitivity of the method was evaluated from the slope of the calibration curve. Hence the sensitivity of the method for the analysis of the metals was in the order of Zn > Mg> Cu> Mn > Ca > Fe.

Table 3. The concentrations of metals (mg/kg) and total polyphenols content (TPC) (mgGAE/g) determine in green coffee beans grown in four different districts of Centaral Gondar Zone, Ethiopia.

Precision

The precision of the method was assessed from the relative standard deviation (RSD) of triplicate analysis for each sample. The RSD values were in the range of 0.2–12%, across the different samples and metals.

Limits of detection

The limits of detection (LOD) of the method were in the range of 0.06 mg/kg for Zn to 55 mg/kg for Mg. The LOD values were sufficiently low to allow the analysis of the elements in the green coffee bean samples.

Accuracy

The accuracy of the optimized digestion procedure was assessed by spiking samples with standard solutions of metals, analyzing with FAAS and calculating the percentage recoveries. The recovery values of metals in the green coffee ranged from 85 to 116% (Table 2). These results indicated that the digestion and analysis method eployed was reliable with respect to each metal.

The concentrations of metals in the green coffee beans

All of the analyzed metals were detected in the green coffee beans collected from all the sampling districts. Calcium is the most abundant element in the green coffee beans, followed by Mg. Among the trace metals, Fe is found in relatively higher concentrations followed by Cu. The concentration (mg/kg) of metals in the green coffee beans ranged 1910–4465 Ca, 791–910 Mg, 40.5–251 Fe, 23.4–46.3 Cu, 18.4–28.4 Mn, and 3.0–30.1 Zn (Table 3).

Relatively higher concentration of Ca was recorded for coffee beans from Genbera (4465±105 mg/kg) in Tach Armachiho, followed by Nara-Awudarda (4273±71 mg/kg) in Chilga district. Higher magnesium concentration was found in green coffee beans from Dikularba (910±1.9 mg/kg) in Takusa district and Eyaho (875.2±3.4 mg/kg) in Chilga district. In contrast, lower Zn (3.0 ± 0.4 mg/kg) and Mn (18.4±1.6 mg/kg) values were recorded, respectively, for coffee bean samples from Mahin and Kanfenta in Tach Armachiho district.

Table 3. The concentrations of metals (mg/kg) and total polyphenols content (TPC) (mgGAE/g) determine in green coffee beans grown in four different districts of Centaral Gondar Zone, Ethiopia.

District	Sample code	Ca	Mg	Cu	Zn	Mn	Fe	TPC
Tach	Arm-1	2791±39	822±2.2	34.8±2.5	8.1±0.7	18.4±1.6	46.2±0.1	50.5±4.6
Amachiho	Arm-2	2154±34	791±2.3	37.0±2.5	12.0±1	18.4±1.6	71.8±4.4	42.9±2.8
	Arm-3	3228±34	852±1.1	37.7±1.2	8.8±1.2	19.3±0.1	71.8±4.4	47.3±3.2
	Arm-4	3176±90	830±2.3	37.7±1.2	11.4±1.1	21.2±1.6	251±10.3	49.0±4.8
	Arm-5	3324±200	864±14	38.4±2.1	6.5±0.9	22.1±0.1	71.8±9.8	42.2±3.4
	Arm-6	3532±181	872±2.6	43.4±1.2	13.0±1.7	23.9±3.1	123±6.9	48.2±4.4
	Arm-7	4465±105	838±5.6	34.8±2.5	16.9±1.0	22.1±0.1	46.2±0.5	43.7±3.8
	Arm-8	3324±267	847±1.7	46.3±1.2	3±0.4	22.2±1.6	46.2±0.5	48.5±3.1
Chilga	Chi-1	1910±136	808±3.2	29.8±2.1	3.3±0.4	20.3±1.6	46.2±0.5	47.2±3.6
	Chi-2	2569±22	842±3.0	36.0±0.1	9.4±1.3	22.1±0.1	46.2±0.5	43.7±3.0
	Chi-3	2999±13	875±3.4	37.0±1.2	5.9±0.7	26.6±1.6	71.8±2.4	39.6±2.2
	Chi-4	3495±34	857±3.4	33.4±3.3	10.4±1.5	23±1.6	46.2±0.5	51.9±4.3
	Chi-5	3110±90	845±4.0	33.4±3.3	9.1±0.7	20.3±2.1	46.2±0.5	51.5±4.5
	Chi-6	2495±90	849±4.0	34.8±1.2	6.2±0.9	19.3±2.7	46.2±0.5	47.8±3.3
	Chi-7	3228±46	871±1.1	34.1±0.1	11.0±1.0	23.0±1.6	46.2±0.5	48.5±4.1
	Chi-8	2984±13	867±1.7	38.4±3.7	11.0±1.0	21.2±1.6	97.4±8.8	46.8±3.7
	Chi-9	3147±44	849±2.2	34.1±2.1	10.4±1.5	23.0±3.1	46.2±0.5	45.4±2.8
	Chi-10	4273±71	858±3.9	36.3±0.1	30.1±0.6	22.1±0.5	405±44.4	44.9±2.9
Takusa	Tak-1	2910±51	852±0.6	34.1±2.1	10.1±1.0	23.9±1.6	46.2±0.5	31.5±1.9
	Tak-2	3636±102	910±1.9	234±3.7	14.6±2.0	28.4±1.6	185±13.3	47.0±3.7
	Tak-3	3999±46	870±1.9	32.7±1.2	13.6±0.6	20.3±1.6	190±17.8	52.5±5.0
Gondar	Gon-1	2873±22	842±2.3	29.1±1.2	9.1±1.1	20.3±1.6	71.8±8.1	43.6±3.1
Zuria	Gon-2	2880±22	849±1.9	34.1±2.1	17.5±0.6	23.0±1.6	71.8±8.5	42.6±2.8

The results obtained for the overall mean concentration of metals in green coffee bean samples corresponding to the four districts are presented in Table 4. Accordingly, the overall mean concentrations (mg/kg) ranged from 2876±22 (Gondar Zuria) to 3515±66 (Takusa) for Ca, 839.4±4.0 (Tach Armachiho) to 877±1.5 (Takusa) for Mg, 31.6±1.7 (Gondar Zuria) to 100±2.3 (Takusa) for Cu, 10.0±1.6 (Tach Armachiho) to 13.3±0.85 (Gondar Zuria) for Zn, 21±1.2 (Tach Armachiho) to 24.2±1.6 (Takusa) for Mn, and 71.8±8.3 (Gondar Zuria) to 140±10 (Takusa) for Fe. The concentrations of Fe and Ca in coffee beans from Takusa district were found to be significantly highe compared to those from the other districts. The observed variations in elemental contents green coffee beans among districts may be due to various factors. As suggested in different literatures, the chemical composition of green coffee beans differs with the growing region depending on variations in environmental conditions and genetic properties of the beans (Endaye *et al*., 2019).

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Table 4. The concentration (mg/kg) of elements and total polyphenols (mgGAE/g) determined in green coffee beans grown in different districts of Central Gondar zone.

District	Ca	Mg	Cu	Zn	Fe	Mn	Poly-phenol
Tach Arimachiho	*3249±42	839±1.4	38.8±0.64	10.0 ±0.35	91.1±1.6	21.0±0.42	46.5±3.1
Chilga	3021±15	852±0.95	34.7±0.54	10.7±0.28	53.3±1.8	22.1±0.50	46.7±3.6
Takusa	3515±38	877±1.2	30.1±1.3	12.8±0.69	140±5.8	24.2±0.92	43.7±11
Gondar zuria	2876±16	845±1.4	31.6±1.2	13.3±0.56	71.8±5.9	21.6±1.1	43.1±0.7

*Values are mean ± standard error of the mean.

Comparison of the elemental contents of green coffees with literature values

There are a number of studies from different countries on the analysis of metal contents in green coffee beans. The concentration (mg/kg) of metals in green coffee beans from Vietnam have been reported as Ca (768), Mg (683), Cu (17.4), Zn (5.97), Fe (42.98) and Mn (10.4) (van Cuong *et al*., 2014). These values are generally lower than the results of this study for green coffee from central Gondar Zone, Ethiopia. The concentration of metals in green coffee beans from the Sidama region of Ethiopia was reported as Mg (1670±20 mg/kg), Ca (880±10 mg/kg), Mn (19.0±1.0 mg/kg), Fe (26.2±1.5 mg/kg), Cu (22.9±2.2 mg/kg), and Zn (21.1±0.1mg/kg) (Gure *et al*., 2017). These values are generally lower than the measured values for green coffee beans of Central Gondar Zone, except the concentration of Mg and Zn. This indicats that green coffee beans from Central Gondar Zone are good sources of the analysed major and trace metals.

Total Polyphenols

The calibration curve used for the determination of total polyphenols was linear in the range 25-300 mg/L of galic acid (Figure 2), with a correlation coefficient of 0.9989, which implies a strong linear relationship between absorbance and concentration.

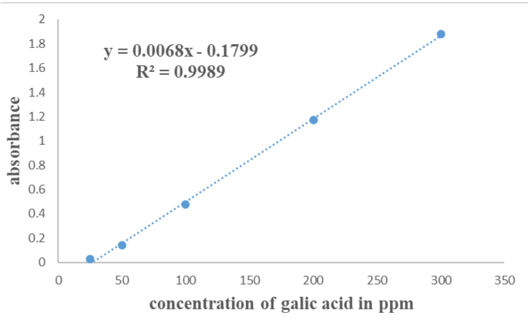

Figure 2. The calibration curve of Gallic acid.

Total Polyphenol Contents of Green Coffee beans

The total polyphenol content (TPC) of green coffee beans varied in the range of 31.5–52.5 mgGAE/g (Table 3). The highest TPC was found in green coffee from Takusa (52.5±5 mgGAE/g) followed by Chilga (51.9±4.3 mgGAE/g). The lowest TPC was recorded for green coffee beans from Takusa (31.5±1.9 mgGAE/g).

The average TPC of green coffee beans categorized in to the four districts is provided in Table 4. The mean values of TPC ranged from 43.1±2.95 mg GAE/g in green coffee beans from Gondar Zuria to 46.7±3.44 mg GAE/g from Chilga. The distribution pattern for mean total polyphenol values s was in the order of Chilga > Tach Armachiho > Takusa > Gondar Zuria.

The results of one-way ANOVA showed that sources of green coffee beans (sampling sites) had no significant effect ($p > 0.05$) on the mean total phenol concentrations in green coffee beans from the studied four districts (Table 5).

Table 5. ANOVA table obtained from the analysis of the variation of mean total polyphenol contents among green coffee beans from the four districts studied. Difference is significant when $p < 0.05$.

Source of Variation	**SS**	**df**	**MS**	**F**	**P**	**F_{crit}**
Between Groups	40.73097	3	13.57699	0.605765	0.619317	3.12735
Within Groups	425.8464	19	22.41297			

The determined TPCs of green coffee beans were compared with literature values. Accordingly, the highest mean value obtained in the present study (46.76 ± 3.44 mg GAE/ was lower than the values reported for other Ethiopian coffee beans from Yirgacheffe and

Kochere (55.45 ± 1.08 mgGAE/g), Sidama (49.19 ± 0.70 mg GAE/g), Yirgacheffe (54.53 ± 1.62 mg GAE/g) (Bobkova *et al*., 2020). Moreover, comparison of the results with those from other countries revealed that higher TPCs values were reported in Brazil (745.50 ± 10.5 mgGAE/g) (Nassar *et al*., 2019). In contrast, lower TPCs were reported for Jember coffee (33.21 mg GAE/g), Malang coffee (3.8619 mg GAE/ g), Bondowso (21.88 mg GAE/g) and Banyuwangi coffee (7.68 mg GAE/g) all from Indonesia (Perdani *et al*., 2019) than the results observed in this study. The variation in the total phenolic contents might be attributed to the geographical factors as well as the different cultivation methods. A previous study had reported that phenolic content was influenced by the origin of the coffee beans and extracting solvents (Daniel *et al*., 2017).

Conclusion

In this study, the levels of some major (Ca and Mg) and minor (Fe, Cu, Zn and Mn) metals and total polyphenols in green coffee beans from the major production areas of Central Gondar Zone were determined. Despite the initial hypothesis, no significant variation was observed in the determined concentrations of total polyphenols and metals, except Fe, with the different growing areas.

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