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ORIGINAL RESEARCH ARTICLE

Physicochemical Properties and Levels of Selected Trace Metals in Honey from North Gondar, Ethiopia

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Abstract

In this study, physicochemical parameters and metal concentration of honey samples were collected from Beyeda, Dabat, Debark and Jan Amora Districts in North Gondar Administrative Zone and assessed. The mean values of physicochemical parameters were: moisture as 16.0–22.9% (w/w), ash content 0.48-0.74%, the electrical conductivity (EC) as 0.33–0.40 mS/cm and the pH as 3.59–4.65. Except the moisture content of honey from Beyeda and Debark districts which slightly exceeded the allowable limits, all quality parameters were found within the acceptable range given by Codex Alimentarius. For metal analysis, using flame atomic absorption spectrometer, honey samples were digested in HNO_3 and H_2O_2 (1:1, v,v) at temperature of 220 °C for 1:40 h. The mean concentrations (µg/g) of Cu, Fe, Mn, Ni and Zn were in the range of 0.40–1.62, 5.82–15.31, 0.20–1.41, 1.90–6.75 and 3.30–7.45, respectively. Among the physicochemical parameters investigated, a positive correlation was obtained between ash content, pH and EC. However, moisture content showed a negative correlation with ash content, pH, and EC. Copper, Fe, Mn, Ni and Zn showed positive correlation to each other. Analysis of variance at 95% confidence level indicated that there were only significant variations for Cu and Zn.

Keywords: Honey, Physicochemical, Trace Metal, North Gondar, Ethiopia

Introduction

Honey is a sweet food which contains varieties of valuable compounds like glucose, fructose, protein, and minor quantities of amino acids and minerals (Stankovska *et al*., 2008). The composition and physico-chemical properties of honey depend on the nature of the plants where bees collect the nectar, atmospheric conditions, time of ripening, mode of storage, harvest conditions and regions of production (Dobre *et al*., 2012). Honey is identified by its anti-bacterial and anti-inflammatory properties to treat skin wounds and number of gastrointestinal diseases (Conti *et al*, 2018). Naturally, honey honey also contains macro- and microelements, which play important roles in determining honey qualities.

Trace metals are toxic when found in appreciable amount with detrimental effect on human. They are capable of accumulate in human body particularly in liver and kidney Zhao *et al*., 2012). Elements like copper, iron, manganese, nickel and zinc are essential for humans by playing a very important role in number of biochemical processes. However, if present at higher levels, they will have a negative effect on human health (Tuzen *et al*., 2007). In general, metals enter to the human body through inhalation, dermal contact with soil, drinking and consumption of foods.

In general, metals enter to the human body through inhalation, dermal contact with soil, drinking and consumption of foods. Depending on the chemical form of the metal, the age and nutritional status, the amount of metal actually absorbed by the digestive tract can vary widely (Dhahir and Hemed, 2015).

The quality of honey is a basic parameter for both local and international markets, to enable attainment of competitive premium prices and ensure human health. The existing international honey market trend, regarding quality is more demanding (Se *et al*., 2019). The quality of honey is mostly dependent on the climatic condition, handling of honey, nectar source of the honey and chemical composition. The physicochemical properties and levels of heavy metals are relevant for quality control. According to the Codex Alimentarius Commission, a good quality honey should have a moisture content of not more than 20 g/100 g, ash content of not more than 0.6 g/100 g, pH between 3.2 and 4.5 and electrical conductivity of not more than 0.8 mS/cm (Codex Alimentarius Commission, 2001) and concentration of metals below the permitted levels set by international organization. However, these parameters may vary with the climatic condition, handling of honey, floral origin and chemical composition.

In Ethiopia is the most honey producer in Africa (21.7 %) and top ten producers globally (2.5%). Majority beekeepers follow different kinds of traditional method of beekeeping with different types of traditional hives without introducing improved techniques or technology (Haftu, 2015). Therefore, this study is aimed to evaluate selected physicochemical properties (moisture content, pH, ash content and electrical conductivity) and also the metallic content (copper, iron, manganese, nickel and zinc) of honey samples collected from different districts of North Gondar zone: Beyeda, Dabat, Debark, and Janamora.

Materials and Methods

Chemicals and instruments

All the reagents used were of analytical grade and de-ionized water was used as solvent for all dilutions throughout this study. Oxidizing agents 30% H_2O_2 and 70% HNO_3 (Brulux fine chem, India) were used for digestion of the honey samples. Stock solutions of Cu, Fe Mn, Ni and Zn (1000 mg/L, Buck Scientific Graphic™, USA) were used to prepare standard solutions for calibration curve and recovery test. Physicochemical properties were performed with oven (Gallenkamp Hot box oven, UK), conductometer (Jenway model 4330, UK), Abbe refractometer (60 DR, Bellingham and Stanley, USA), pH meter (MP 220, Switzerland). Atomic absorption spectrophotometer (BUCK SCIENTIFIC MODEL 210 VGP) equipped with deuterium arc as background corrector using air-acetylene flame was used as a fuel in this study for metals determination. The operating parameters for the determination of metals were given in Table 1.

Table 1. FAAS instrumental operating conditions of the investigated elements

Element	Wave length (nm)	Lamp current (mA)	Slit width (nm)	Energy (J)
Cu	324.7	0.7	0.7	3.84
Fe	248.3	7.0	0.2	3.00
Mn	279.5	3.0	0.7	4.06
Ni	232.0	7.0	0.2	3.00
Zn	213.9	2.0	0.7	3.24

Description of the study area

This research was carried out in four districts of North Gondar Administrative Zone, namely: Beyeda, Dabat, Debark and Jan Amora. (Fig. 1). Beyeda is located in the easternmost point of the North Gondar Zone, which is bordered on the south by the Wag Hemra Zone, on the west by Jan Amora, on the north by Tselemt, and on the east by the Tekeze River which separates it from the Tigray Region. Beyeda is found at a latitude of 41°24'12.2"N and longitude of 2° 10'26.5"E. Dabat is bordered by Tach Armachiho in west, Tegeda in northwest, Wegera in south, and Debark in northeast. It is situated at a latitude: 12° 59' 3.01"N and longitude: 37° 45' 54.00" E. Debark is bordered on the south by Dabat, on the west by Tegeda, on the northwest by the Tigray Region, on the north by Addi Arkay, and on the east by Jan Amora. It is located at a latitude of 30°15′20″ N and longitude of 30°15′20″ E. Jan Amora is bordered on the south by Misrak Belessa, on the southwest by Wegera, on the west by Debark, on the north by Addi Arkay and Tselemt, on the east by Beyeda, and on the southeast by Wag Hemra Zone. Jan Amora is situated at latitude of 13° 14'60.00" N and longitude: 38°00'0.00" E.

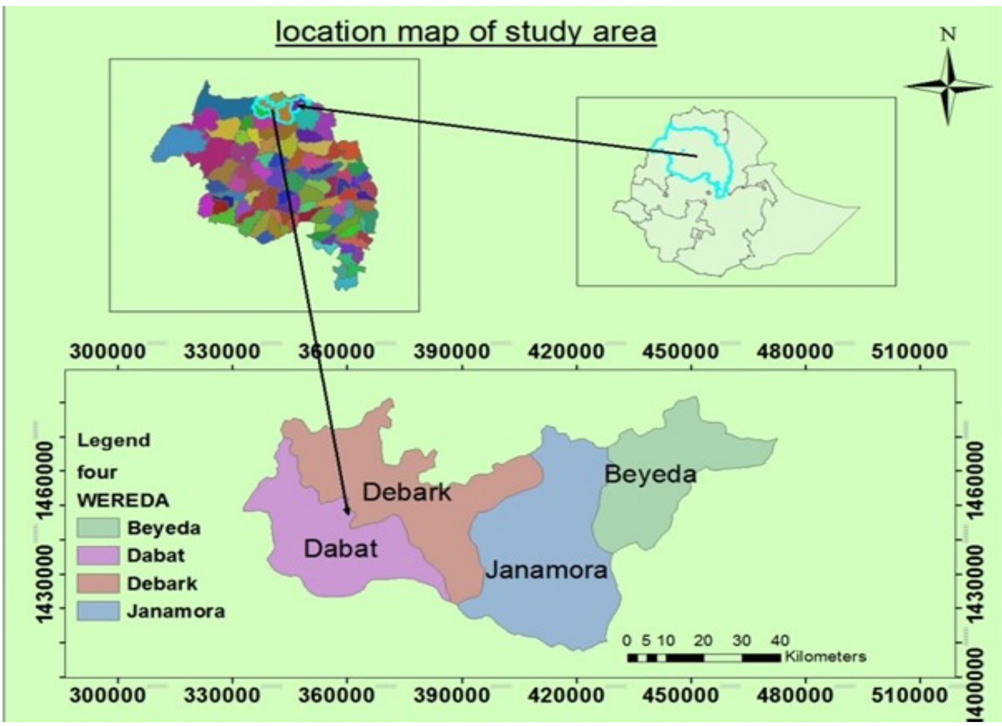

Figure 1. Map of sampling sites.

Honey sample collection and preparation

From the four districts a total of twelve sampling sites (three sites for every district) were chosen as a sampling area for collecting honey samples. From each sampling site, a 250 g of honey samples were collected from local beekeepers produced by traditional procedures and mixed together. The collected honey samples were stored in clean polyethylene bottles and protected from light, and kept at room temperature until analysis.

Determination of physicochemical parameters

The physicochemical parameters of the honey samples were determined using the methods of Association of Official Analytical Chemists (AOAC, 1999). The pH of honey samples was assessed in 133 g/L honey solution by means of pH meter. The pH meter was first calibrated with standard buffers of pH 4.0 and 7.0 before measuring the pH of the samples. The ash content of honey samples was determined by heating of 5.0 g of honey in a muffle furnace at 600 ^{o}C for about 4 h burner until the sample is dry and smokeless. After complete ignition to constant weight, the sample was cooled in a desiccator and weighed immediately. The moisture content was determined by measuring 5.0 g of the honey sample and placed into a pre-weighed aluminum drying dish. The sample was dried to constant weight in an oven at 105 °C for 4 h and weighted. Electrical conductivity of honey was determined with a conductometer in a solution containing 133g/L honey solution at 25^{o}C (AOAC, 1999).

Analysis of metals in honey samples

In order to select an optimum procedure for digestion, reagent volume, digestion temperature; and time were optimized on varying one parameter while keeping the other parameters constant until clear and colorless solution was obtained. About 1.0g of homogenized honey samples were weighed into round bottomed flask (Bartha *et al*., 2020). To this, mixture of various volumes of HNO_3 and H_2O_2 were added and digested at different digestion time and temperature. Finally, the optimum procedure which consumed minimum reagent volume at lower temperature and shorter digestion time was chosen (Table 2). Therefore, 4 mL of a mixture of HNO_3 and H_2O_2 (1:1) at a temperature 220 ^{o}C for 1:40 h were chosen as the optimal procedure for determining the concentration of metals in honey samples. After digestion was completed, the solution was cooled and filtered out through Whatman No. 42 filter paper in to 50 mL volumetric flask and finally diluted to the mark with deionized water. Digestion of a reagent blank was also performed with 2 mL of HNO_3 and 2 mL of H_2O_2 at 220 ^{o}C for 1:40 h without honey sample. Finally, all the digests were kept in refrigerator until analyzed with flame atomic absorption spectrometry.

Method Performance Evaluation

Method validation is an analytical method that is used to evaluate the quality, reliability and repeatability of analytical results. The parameters: accuracy, precision, limit of detection, linearity, etc. are used for validating a method. The parameters which were used for validate the analytical procedure are accuracy, precision, limit of detection, linearity, etc (Kilic and Serpil, 2019).

Accuracy of a procedure helps to ensure loss or contamination occurred during preparation steps and matrix interferences (Ayalew *et al*, 2017). Due to the absence of standardized certified reference materials, the accuracy of the method was validated by employing spiking experiments (recovery test). A recovery test was performed by spiking spice samples with various amounts of standard solutions of heavy metals. The spiked samples were digested with the optimized procedures described for the unspiked spice samples (Taghipour and Jalali, 2019). The percentage recovery of Cu, Fe, Mn, Ni and Zn in honey were found be 80.0 %, 102.1 %, 91.2 %, 101.5 % and 99.3% , respectively. The results indicated that the method has good accuracy and applicable for the determination of Cu, Fe, Mn, Ni and Zn in honey samples (Stojsavljevi *et al*., 2019).

Precisions of the results were evaluated by standard deviation of the results of the triplicate digestion and triplicate measurements for each sample (Abraha and Atsbha, 2019). Thus, in this study degree of precision of the data are reported as mean ± SD of triplicate measurements.

Method detection limit (MDL) for analyses of honey samples was calculated by dividing three times the standard deviation of the blank sample with slope of the calibration curve. The blank samples were digested following the same procedures utilized for digesting the spice samples three times the standard deviation of blanks. Three replicate blank samples were digested following the same procedures utilized for digesting the honey samples (Taghipour and Jalali, 2019). The method detection limits (MDL) for the determination of the investigated elements in honey were obtained as 0.009 µg/g for Cu, 0.2 µg/g for Fe, 0.009 µg/g for Mn, 0.02 µg/g for Ni and 0.01 µg/g for Zn.

Data Analysis

Levels of trace metals and physic-chemical properties of honey were expressed as mean ± SD; one-way ANOVA was employed for comparison of the means of honey samples at 95% confident interval. The quantifications and statistical analysis were done with inbuilt IBM SPSS Statistical software Version 20). Pearson correlation coefficients were applied to investigate the correlations among element concentrations and between physicochemical parameters.

Table 2. Optimization of digestion procedure using 1.0 g honey samples

$HClO_4$ (mL)	HNO_3 (mL)	Temperature (^0C)	Time (h)	Observation
1	1	180	2:30	Yellowish solution with residue
2	1	180	2:30	Pale yellow solution
1	2	180	2:30	pale yellow solution
2	**2**	180	2:30	Colorless solution
2	3	180	2:30	Deep yellow solution
2	2	160	2.30	Clear yellow solution
2	2	200	2.30	Deep yellow solution
2	2	220	2:30	Colorless solution
2	2	240	2.30	Pale yellow solution
2	2	300	2.30	Deep yellow solution
2	2	220	1:20	Yellow solution
2	2	220	1:30	Pale yellow solution
2	**2**	**220**	**1:40**	**clear and colorless**
2	2	220	1:45	pale yellow solution
2	2	220	2:30	pale yellow solution

Results

Physicochemical parameters of honey samples

The physicochemical results of the samples are presented in Table 3 as mean ± standard deviation of triplicate analyses. The ash content, pH and electrical conductivity of the honey samples increased in the order of: Beyeda < Jan Amore < Debark < Dabat. However, the moisture content found to be Dabat < Jan Amora < Debark < Beyeda. High moisture content tends to a greater fermentation potential, accelerate crystallization process and makes it more difficult preservation and storage of the honey (Nordin *et al*., 2018). The correlation between the physicochemical parameters were investigated using Pearson correlation (Table 4).

Table 3. Physicochemical properties of honey, n = 3.

District	Moisture content (%, w/w)	Ash content (%, w/w)	pH	EC (mS/cm)
Beyeda	22.9 ± 0.076	0.48±0.046	3.593± 0.012	0.333±0.003
Dabat	16.0 ± 0.015	0.74± 0.162	4.645±0.068	0.401±0.006
Debark	22.3 ± 0.033	0.580± 0.117	4.43±0.032	0.392±0.005
Jan-amora	21.6 ± 0.020	0.502±0.068	3.830± 0.056	0.360±0.035
*Standard	£ 20	£ 0.6	3.2 - 4.5	£ 0.8

* Codex Alimentations, 2001

Table 4. Correlation coefficients between physicochemical parameters in the honey samples

Parameter	Moisture	Ash	pH	Ec
Moisture	1			
Ash	-0.76	1		
pH	-0.56	0.91	1	
EC	-0.58	0.87	0.98	1

Levels of metals in the honey samples

The results found for the total concentration of the mineral elements (Cu, Fe, Mn, Ni and Zn) in honey samples are presented in Table 5. All the analyzed metals (Cu, Zn, Mn, Ni and Fe) were detected in the honey samples. As shown in Table 5, Fe was the most abundant metals analyzed in honey collected at all the study sites compared with other metals. The concentration of this element ranged from 5.80 to *15.30* µg/g. However, the least abundant metal was Mn at Beyeda, Dabat and Debark; and Cu at Jan Amora.

Table 5. The levels (Mean ± SD, µg/g) of metals in honey

Sites	Cu	Fe*	Mn	Ni*	Zn*
Beyeda	0.53 ± 0.004	9.30 ± 0.041	0.45 ± 0.004	4.45±0.015	3.30 ± 0.055
Dabat	*1.62 ± 0.025*	*15.31 ± 0.071*	*0.97±0.003*	*6.75±0.005*	*7.45 ± 0.02*
Debark	0.40 ± 0.004	5.82 ± 0.041	0.20 ± 0.004	1.90 ± 0.013	4.90 ± 0.015
Jan Amora	0.90 ± 0.003	12.90 ± 0.108	1.41 ± 0.029	6.20 ± 0.005	6.80 ± 0.007
p value	>0.05	<0.05	>0.05	<0.05	<0.05

*Significant difference (p<0.05)

Table 6. Pearson correlation coefficients between heavy metal concentrations in honey samples

Metal	Cu	Fe	Mn	Ni	Zn
Cu	1				
Fe	0.91*	1			
Mn	0.62	0.824*	1		
Ni	0.82*	0.98*	0.87*	1	
Zn	0.85	0.75*	0.75*	0.67	1

Correlations marked with *are significant (p < 0.05)

Pearson's correlation analysis and one- way ANOVA were applied to identify the nature and strength of association between metal concentrations and significant variations in the metal contents in honey samples, respectively (Table 6).

Discussion

Physicochemical parameters

Honey is acidic in nature and varies by the nectar and soil types (Nordin *et al.*, 2018). The pH values of the four honey samples were acidic (3.59 to 4.645) and are found within the range of the standard limit (3.40–6.10) (Codex Alimentations, 2001). The pH values of this study agree agreement with values reported from Algeria (Ouchemoukh *et al.*, 2007), Saudi Arabia (Doghairi *et al.*, 2007), Spain (Manzanares *et al.*, 2014), Morroco (Abselami, *et al.*, 2018) and Palestine (Abdulkhaliq & Swaileh, 2017).

Moisture content is the most important physicochemical property which affects storage life of honey. The season of harvesting, degree of maturity that honey reached in the hive, type of hive used, environmental temperature and water content of flora affect the moisture content of the honey. Low moisture content indicates that no microorganisms and can be preserved for longer periods (Awraris *et al.*, 2014).

In this study, the highest value of moisture content was recorded in honey sample collected from Beyeda (22.9 %), followed by Debark (22.3%), Janamora (21.6%), and Dabat (16.0%). The moisture content of honey from Dabat and Janamora are close to those obtained in previous reports (Manzanares *et al*., 2014, Liberato *et al*., 2013) and are also found in the range of values (18–23 %) given by Codex Alimentations (Codex Alimentations, 2001). However, honey samples at Beyeda and Debark are slightly higher than the standards, which may be attributed to inappropriate honey harvesting time, unsuitable storage container and storage places (Meareg and Arega, 2018).

Ash content is reflecting its richness in minerals content and mainly determined by soil and climate characteristics. Honey normally has low ash content and it depends on the composition of source plant nectar (Manzanares *et al*., 2014). The lowest ash content was observed in Beyeda honey (0.475) and the highest ash content was observed in Dabat (0.74) and are found within the acceptable range (0.25 - 1.0 %) (Codex Alimentations, 2001). These values are close to those obtained in Greece (0.75-1.15%) (Karabagias *et al*., 2017), Algeria (0.09-0.54) (Ouchemoukh *et al*., 2007) and Brazil. (0.07-0.71) (Liberato *et al*., 2013).

The electrical conductivity of honey is related with the mineral content, organic acids and proteins. It has been used as a honey quality indicator (Biluca *et al.*, 2016). The electrical conductivity of honey in this study is varied into a range of 0.333 to 0.401. Honey from Beyeda had the lowest Ec, while Dabat had the highest Ec. The EC results are below the limit of electrical conductivity proposed by the Codex Alimentarius Standards ($\leq$0.8 ms/cm) (Codex Alimentations, 2001) and comparable with those reported in previous surveys in, Spain (0.28-0.99) (Manzanares *et al*, 2014), Argentina (0.121-0.684) (Isla *et al*., 2011), Benin (0.53-0.70). If the honey is adulterated with water or saturated sugar solutions, it will display greater conductance than pure honey (Rehman *et al*., 2008).

When the physico-chemical parameters (moisture content, ash content, pH and electrical conductivity) of honey samples investigated under this study were comparable with the reports in Ethiopia. However, the moisture content of Beyeda and Debark honey showed slightly higher results than previous reports (Aregay *et al*., 2018; Mekuanint and Meareg, 2019; Tewodros *et al*., 2013).

Pearson correlation matrix was applied in order to identify correlation between physicochemical parameters and metal concentrations in honey. As can be seen from Table 4, a strong positive correlation was observed between ash content, electrical conductivity and pH. This suggests that the ash content affects the pH and electrical conductivity of honey. The higher the pH and electrical conductivity, the greater the resulting ash content. Similarly, a positive correlation was also observed between pH and electrical conductivity. This agreed with a report from Ethiopia (Belay *et al*, 2017). However, moisture showed a negative correlation with ash content, pH and electrical conductivity, which suggests that high moisture content tends to decrease the pH, ash content and electrical conductivity. For instance, as the moisture content of honey increases, osmotolerant yeasts cause to develop carbon dioxide and ethanol. Ethanol further oxidizes in to acetic acid and as a consequence the pH value decreases (Bako *et al*., 2019).

Distribution of metals in honey samples

Essential trace metals were found to be widely distributed in honey samples. Each honey brand analyzed was found to have different metal contents. Generally, Dabat Honey was found to be richest in its trace mineral content followed by Janamora and Beyeda Honey whereas Debark Honey was found with the least metal contents. The results of this study also indicated that iron has the highest level followed by Zn, Ni, Cu and Mn. They all were found above detection limit.

Copper

Copper is an essential element which plays an important role in variety of biochemical processes and is needed for enzymes in the body. However, at higher levels copper is toxic and affects mainly the blood and kidneys (Guoqiang *et al*., 2011). The copper levels ranged from 0.40 to 1.62 µg/g for Debark and Dabat, respectively (Table 4 and 7). As can be seen in Table 7, the copper contents of honey samples reported from Turkey (Tuzen and Soylak, 2005), Morocco (Belouali *et al*., 2008), Croatia (Bilandzic *et al*., 2019), and Switzerland (Bogdano *et al*., 2007) were generally about the same as our samples. However, the level of Cu in this study was higher than reported from Saudi Arabia (Osman *et al.,* 2007) and Chile (Fredes and Montenegro, 2006).

Iron

Iron is one of the most important minerals for human body and takes part in variety of biochemical processes like synthesis of hemoglobin and myoglobin. It is required to make the protein hemoglobin in red blood cells. The majority of iron in mammals is found in hemoglobin, and minor amount is found in myoglobin. A deficiency of iron in the human body can cause a person feeling tired and listless, and can lead to anemia and cognitive disorders. Conversely, iron is toxic when in excessive amounts (Lachowicz *et al*., 2014). Iron content in honey was ranged from 5.82 µg/g in Debark to 15.31 µg/g in Dabat. The iron contents of honey samples have been reported in the literature in the range of 1.1-5.2 µg/g (Tuzen and Soylak, 2005), 0.88-207.6 µg/g (Belouali *et al*., 2008), 0.1-6.36 µg/g (Fredes and Montenegro, 2006), 4.35-7.54 µg/g (Rehman *et al*., 2008), 0.295-2.34 µg/g (Bilandzic *et al*., 2019) and 0.136-9.85 (Bogdano *et al*., 2007) from Turkey, Morocco, Chile, Pakistan, Croatia and Switzerland, respectively. In general, our values are comparable with values reported above. However, the Fe amount reported from Saudi Arabi (0.31-3.19 µg/g) was slightly lower than the result of this study (Osman *et al.,* 2007).

Manganese

Manganese is an essential micronutrient for development, metabolism, and the antioxidant system. Due to high a cumulative property in the organism, chronic manganese affects the central nervous system with the symptoms Parkinson, poor reproductive performance and growth retardation (Rustamov and Abbasova, 2014). Manganese concentration in honey was found to be highest in honey samples collected from Jan Amora (1.41 µg/g) and the lowest (0.2 µg/g) was in Debark. When compared with literature reports, the concentration of manganese detected in this study were comparable to reports from Turkey (0.18–1.21 µg/g) (Tuzen and Soylak, 2005), Morocco (0.080–9.76) (Belouali *et al*., 2008), and Switzerland (0.125–12.354) (Bogdano *et al*., 2007), while its concentration considerably higher than that reported from Chile (0.06–2.00) (Fredes and Montenegro, 2006) and Saudi Arabia (0.19–0.37) (Osman *et al.,* 2007).

Nickel

Nickel is an essential element for humans, other animals, and plants in small doses. It is moderately toxic element compared to other transition metals that can lead to serious illness including respiratory system cancer and malignant tumors. It also decreases the plants growth and accumulation in animal tissues (Das and Dhundasi, 2008). In this study, nickel concentrations were found to be in the range of 1.90–6.75 µg/g, the lowest was from Debark and the highest from Dabat, which is comparable to a previous report from Turkey (1.1–24.2 µg/g) (Tuzen and Soylak, 2005). However, this concentration is substantially higher than the mean Ni concentration reported in Chile (0.01–0.05 µg/g) (Fredes and Montenegro, 2006), Croatia (0.122–0.523 µg/g) (Bilandzic *et al*., 2019), Pakistan (1.02–1.48 µg/g) (Rehman *et al*., 2008) and Switzerland (0.001–1.97µg/g) (Bogdano *et al*., 2007).

Zinc

Zinc is an essential element important for normal growth and development in human beings. It can enhance the human immune system. High zinc concentrations affect the respiratory, gastrointestinal and blood system causing nauseas, vomiting, diarrhea, skin rashes, anemia and sterility. However, Zn deficiency may lead to result in abnormal physiology disorders such as dwarfism, anemia and dwarfism (Roohani *et al*., 2013). The concentrations of Zn in honey samples were detected in the range of 3.3–7.5 µg/g. The lowest was detected in honey samples collected from Beyeda, while the highest level of zinc was in Dabat. These values are comparable with the values reported from Chile (0.01–4.73 µg/g) (Fredes and Montenegro, 2006) and

Table 7. Comparison of the level of metals in honey of the present study with other reported values

Level of Metals (µg/g)					References
Cu	Fe	Mn	Ni	Zn	
0.25-1.10	1.1-5.2	0.18-1.21	1.1-24.2	NG	Tuzen & Soylak, 2005
0.206-0.389	0.31-3.19	0.19-0.37	NG	0.21-0.75	Osman *et al*., 2007
0.51-4.75	0.88-207.6	0.080-9.76	NG	0.04-2.74	Belouali *et al*., 2008
0.01 -0.05	0.1-6.36	0.06 -2.00	0.01 -0.05	0.01-4.73	Fredes & Montenegro, 2006
NG	4.35 - 7.54	NG	1.02 - 1.48	1.98 - 2.94	Rehman *et al*., 2008
0.051- 3.317	0.136- 9.85	0.125-12.35	0.001-1.97	0.016- 4.13	Bogdano *et al*.,2007
0.103-1.033	0.295-2.34	NG	0.122-0.523	0.44–2.025	Bilandzic *et al*., 2019
0.40-1.62	***5.82- 15.31***	***0.2-1.41***	***1.90-6.75***	***3.30-7.45***	***Present study***

Switzerland (0.016–4.133) (Bogdano *et al*., 2007). However, range of concentration of Zn is slightly higher than reported from Morocco (0.04–2.74 µg/g) (Belouali *et al*., 2008), Croatian (0.442–2.025 µg/g) (Bilandzic *et al*., 2019) and Pakistan (1.98-2.94 µg/g) (Rehman *et al*., 2008).

The results of ANOVA analysis showed that there was no significance difference in in the mean concentrations of Mn and Cu in honey samples at 95% confidence interval ($p \geq 0.05$). However, significance difference ($p < 0.05$) in the mean concentration of Fe, Zn and Ni were observed. These differences could be attributed due to the differences to the floral type, the botanical origin, harvest mechanisms, storage conditions and contamination during transport, season of the year, rain fall (Abraha and Atsbha, 2019).

Regarding to the metallic content, a positive correlation was observed between all the metals (Cu, Fe, Mn, Ni and Zn) in honey samples (Table 6), which suggests these metals may arise from common sources as well as from similarity in chemical properties (Solomon *et al*., 2018). Significant correlations were also found between the concentrations of all metals ($p < 0.05$) except Cu with Mn and Zn; and Ni with Zn ($p \geq 0.05$).

Conclusion

In this study, the physicochemical characteristics and levels of metals in honey from North Gondar were investigated. There was negative correlation of moisture content with ash, pH and electrical conductivity, which indicates that the moisture content may be from different sources compared with aforementioned parameters. Besides, the moisture contents of honey samples from Beyeda and Debark were found to be slightly higher than the value recommended by WHO/FAO. Thus, the local community should take special precautions in the production, storage and transportation of honey to maintain the moisture content of the honey.

The optimized wet digestion method for the analysis of metals in the honey samples provided good recovery, in the range of 80–102.1%. The optimized wet digestion method for the analysis of metals in the honey samples provided good recovery, in the range of 80–102.1%. Among the determined elements, iron was found to be the highest in concentration. There was significant variation, except Cu and Mn, in the level of metals among the honey samples. Pearson correlation indicated that there was strong positive correlation among all the metals, indicating that they are from a common source mainly from flora and anthropogenic.

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