International Scientific Organization
http://iscientific.org/
Chemistry International
www.bosaljournals.com/chemint/

Assessment of trace metals and physicochemical parameters of commercially available honey in Ethiopia

Weldegebriel Yohannes, Bhagwan Singh Chandravanshi* and Ghirma Moges

Department of Chemistry, College of Natural Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
*Corresponding author's E. mail: bscv2006@yahoo.com

A R T I C L E   I N F O

Article type:
Research article
Article history:
Received November 2017
Accepted March 2018
April 2018 Issue
Keywords:
Honey
Trace metals
Physicochemical properties
Ethiopia

A B S T R A C T

Trace metal contents and quality parameters (physicochemical properties) such as pH, electrical conductivity and ash content of four Ethiopian honeys were determined using flame atomic absorption spectrometry, pH-meter, conductivity meter and ashing, respectively. The optimized wet digestion method for honey analysis was found efficient for the metals determined and it was validated through the recovery experiment and a good percentage recovery was obtained (93-104%). Fe was found in highest amount with mean concentration ranging from 5.37 to 12.4 μg/g followed by Ni with mean concentration range of 0.80 to 4.46 μg/g, Cr (1.20-4.33 μg/g), Zn (1.92-4.22 μg/g), Co (0.60-1.17 μg/g), Mn (0.16-0.89 μg/g), Cd (ND-0.69 μg/g) and Cu (0.09-0.47 μg/g). The toxic metal Pb was not detected. The ranges of physicochemical properties determined were summarized as: pH 4.11-4.33, electrical conductivity of 0.10-0.29 $mscm^{-1}$ and ash content 0.17-0.46%. The metals content and the physicochemical properties investigated in honey samples were found within the ranges established by national and international standards.

Capsule Summary: Trace mineral contents and physicochemical properties of four Ethiopian honeys were determined using flame atomic absorption spectrophotometry, pH-meter, conductivity meter and ashing, respectively. The metals content and the physicochemical properties investigated in honey samples were found within the ranges established by national and international standards.

Cite This Article As: W. Yohannes, B. S. Chandravanshi and G. Moges. Assessment of trace metals and physicochemical parameters of commercially available honey in Ethiopia. Chemistry International 4(2) (2018) 91-101.

INTRODUCTION

Ethiopia is home to some of the most diverse flora and fauna in Africa due to its varied ecological and climatic conditions. As a result, the country is the leading honey and wax producer in Africa (Demewez et al., 2012). Ethiopian honey production accounts for approximately 2.5% of world production and 21.7% of African honey production (Sebeho, 2015). Honey is a natural sweet viscous liquid that honeybees *Apis mellifera* produce from nectars of flowers, from plant secretions, as well as from excretions of plant sucking insects on the living parts of plants (Tuzen et al., 2007; Saif-ur-Rehman et al., 2008; Nigussie et al., 2012; Nwankwo et al., 2014; Sime et al., 2015; Gul et al., 2015). Honeybees collect these materials, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature (Codex Alimentarius Commission, 2001; Osman et al., 2007; Bibi et al., 2008; Flores et al., 2015). Freshly extracted honey is a viscous liquid with a higher density (1.5 g/cm^3) than water (1 g/cm^3 at 4 °C); having a strong hygroscopic character, relatively low heat of conductivity, low surface tension and various colors (Atrouse et al., 2004). It is important to note that honey produced by insects other than honeybees has very different properties (Dhahir et al., 2015). The composition of nectar and honeydew honeys is different. Honeydew honey is a type of honey that honeybees produce from excretions of plant-sucking insects (*Hemiptera*) on the living parts of plants or secretions from the living parts of plants (Flores et al., 2015). Light nectar honeys have a lower content of components than darker honeys (Bogdanov et al., 2007; Noori et al., 2013; Pathare et al., 2015). In general, higher antioxidant content was found in darker honeys and in honeys with higher water content (Atrouse et al., 2004). The most beneficial and healthy type of honey does not come from nectar, but rather from honeydew (Sanz et al., 2005).

Honey consists of a range of nutritionally important compounds and several health-promoting and curative properties. It is considered as an important part of traditional medicine throughout the world today (Kaakeh and Gadelhak, 2005; Kahraman et al., 2010). There are many reports on the medicinal importance of honey (Aljadi et al., 2003; Lachman et al., 2010; Nwankw et al., 2014; Pathare et al., 2015; Gul et al., 2015). The major components of honey include various saccharides, water, proteins, vitamins, organic acids, flavonoids, phenolic compounds, inorganic substances, including trace elements and unstable compounds such as enzymes (Bogdanov et al., 2008; Sime et al., 2015; Pathare et al., 2015). If honey is consumed at higher doses of 50 to 80 g per intake, it has a variety of positive nutritional, healing, and prophylactic properties (Bogdanov et al., 2008; Pathare et al., 2015). Bee honey can be a good source of major and trace elements needed by humans. The mean content of mineral substances in honey has been reported to be 0.17%, although this can vary within a wide range (Matei et al., 2004; Osman et al., 2007). Trace elements are the constitutive parts of the structures of different active bio-compounds: zinc, copper and manganese are found in enzymes, cobalt in vitamins and hormones, copper and iron in respiratory enzymes and so on (Tuzen et al., 2005; Staniškienė et al., 2006; Osman et al., 2007).

However, in order to have a beneficial effect, honey must be free of any contaminating agents and excessive trace metals. Any heavy metal present in honey above the admitted level by pollution standards, is threats to human body through the possible negative effect of the contaminants (Aljadi et al., 2003). For example, lead, cadmium, arsenic and mercury are potentially toxic within specific limiting values. Excessive content of these metals in food is associated with serious health problems, especially of the cardiovascular, renal, nervous and skeletal systems (Tuzen et al., 2005; Staniškienė et al., 2006; Maleki et al., 2008). Therefore, the contents of these metals in honey samples should be determined for human health. In general, the chemical composition of honey depends on several factors such as plant source (botanical origin of the nectar) visited by bees, season, environmental and climatic conditions, production methods, storage conditions, etc. (Naab et al., 2008; Yang et al., 2013; Noori et al., 2013; Nicolson and Human, 2013; Popova et al., 2014), but the main constituents are more or less identical in all honey types.

Honey specifications such as Codex Alimentarius Commission (2001) and Ethiopian Standard (2005) require physicochemical criteria for honey characterization: namely, water content, ash, pH, electrical conductivity, hydroxymethylfurfuraldehyde (HMF), glucose, fructose, sucrose as well as diastase activity. Reported works (Sanz et al., 2004; Fredes and Montenegro, 2006; Osman et al., 2007; Naab et al., 2008; Chua et al., 2012; Almeida-Muradian et al., 2013; Kamboj et al., 2013) in the published scientific journals also reflect the importance of these honey specifications. Liquid honey does not spoil because of its high sugar concentration and low moisture content. Honey has moisture content with a range of between 15.1 and 21.0%. As long as the moisture content remains below 18%, virtually no microorganisms can successfully multiply in honey. Anomalous values may be an index of adulterations (Conti et al., 2007; Saif-ur-Rehman et al., 2008). Another important physicochemical characteristic of honey is pH. All honeys are mildly acidic with pH-value generally lying between 3.5 and 5.5. This is because gluconic acid is formed in honey when bees secrete the enzyme glucose oxidase, which catalyzes the oxidation of glucose to gluconic acid. The low pH alone is inhibitory to many pathogenic bacteria and, in topical applications at least, could be sufficient to exert an inhibitory effect (Tuzen et. al., 2007; Conti et. al., 2007; Pathare et al., 2015). Moreover, the presence of organic acids in honey contributes to honey flavor and stability against microbial spoilage (Amri and Ladjama, 2013). The pH is indeed a useful index of possible microbial contamination and adulteration. The pH of adulterated honey samples is higher than that of pure samples (Saif-ur-Rehman et al., 2008). Moreover, electrical conductivity is another physicochemical property of honey, which is predominantly dependent on the mineral content of honey (Garcia-Alvarez et al., 2000). Olga et al. (2012) have reported that honeydew honeys generally contain higher amounts of minerals compared to blossom honeys. Another parameter associated to the mineral content of honey is ash content. The ash content in honey is generally low. It can vary between 0.02 and 1.0% and the maximum limit allowed by legislation for honey from floral sources is 0.6% according to Council Directive of the European Union (2002). Very high mineral contents about 1.0% are actually encountered only in honeydew honey and ash content is often used to identify this kind of honey (Felsner et al., 2004, Hernandez et al., 2005). Research concerning the determination of mineral content of honey and other physicochemical parameters is increasing during the last years. Since honey offer a potential dietary supplement and shows therapeutic features, it is important to know the quality of honey from the point of view of physicochemical properties and the content of trace metals levels that are essential to health.

Recently some studies have been carried out on the mineral contents of beverages and mineral waters in

Ethiopia. These are mineral contents in tea (Gebretsadik and Chandravanshi, 2010), coffee (Ashu and Chandravanshi, 2011), mineral waters (Seda et al., 2013), wines (Woldemariam and Chandravanshi, 2011), ouzo (Bekele and Chandravanshi, 2012), traditional fermented alcoholic beverages (Debebe et al., 2017a). Phenolic contents (Debebe et al., 2016) and level of alcohol in fermented (Debebe et al., 2017b) and distilled (Debebe et al., 2017c) alcoholic beverages have also been reported.

However, to the best of our knowledge, no systematic study on the levels of trace heavy metals and physicochemical parameters in Ethiopian honey has been made. Trace heavy metals are important from an economic standpoint as they have to not exceed maximum limits regulated by importing countries such as the EU countries. Therefore, the main objectives the present study are: (i) to determine the trace heavy metals: Cd, Pb, Zn, Co, Cu, Ni, Mn, Fe and Cr in Ethiopian honey by flame atomic absorption spectrometry, (ii) to determine some physicochemical parameters such as pH, electrical conductivity, ash content of the honey samples collected from Addis Ababa supermarkets and Beza-Agro Honey Processor (Adama) and compare with national and international standards and (iii) to evaluate the quality of the samples of honey from the market from the point of view of physicochemical properties and content of trace metals and comparing these samples with honey from other part of the world.

MATERIAL AND METHODS

Reagents, chemicals and equipment(s)

A refrigerator (Hitachi, Tokyo, Japan) was used to keep the collected honey samples and digested samples until analysis. A digital analytical balance (Mettler Toledo, Model AG204, Switzerland) with ± 0.0001 g precision was used to weigh honey samples. The pH and electrical conductivity were measured by a pH-meter (pH/Ion Level 2, Germany) and conductivity meter (HANNA Instruments, Portugal), respectively. Round bottom flasks (250 mL) fitted with reflux condenser were used in Kjeldahl digestion block (Gallenkamp, England) apparatus to digest honey samples, spiked honey samples and blank solutions. Buck Scientific Model 210 VGP (East Norwalk, USA) atomic absorption spectrophotometer equipped with deuterium ark background correctors using air-acetylene flame was used for analysis of the digested honey samples for the trace heavy metals Mn, Ni, Pb, Fe, Zn, Cu, Co, Cr and Cd.

All the reagents used were of analytical grade. 30% hydrogen peroxide (H_2O_2), 69-72% nitric acid (HNO_3) and 70% perchloric acid ($HClO_4$) (Fine Chemical, Mumbai, India) were used for the digestion of honey samples. Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Mn, Ni, Pb, Fe, Zn, Cu, Co, Cr, Cd (Buck Scientific Puro-Graphic^{tm}, USA) were used for preparation of calibration standards and in the spiking experiments. Deionized water (chemically pure < 1.5 μscm^{-1}) was used throughout the experiment for sample preparation and dilution.

Sample collection

Four different types of honey samples were collected from different supermarkets in Addis Ababa (Ethiopia) and from the processor Beza Agro Industry in Adama (Ethiopia), randomly. The honey samples were stored in glass jars. The samples were kept in the refrigerator, until analysis. All the examined natural samples were processed honeys of random (mixed) floral type.

Sample preparation

In accordance with AOAC (1999), the honey samples were heated to 65 ^oC in a water bath until liquefied to permit easier handling and to decrease viscosity for more uniform distribution. The samples were then cooled and weighed for subsequent analysis.

Physicochemical property determinations

To determine the physicochemical parameters, the honey samples were analyzed according to AOAC (1999) and Ethiopian Standard (2005) in order to determine pH, electrical conductivity and ash content. Three replicates were used for each honey sample.

pH and electrical conductivity

A 70 mL aliquot of deionized water (pH 7.0) was added to 10 g of honey and mixed thoroughly. Then the pH and EC were measured directly by a pH-meter and conductivity meter, respectively.

Determination of ash content

Samples were prepared according to method of the AOAC (1999) and Ethiopian Standard (2005). Honey sample of 5 g was weighed accurately in a pre-weighed porcelain crucible and gently heated on a hot plate until the sample was turned to black and dry so that there was no danger of loss by foaming and overflowing. The sample was then ignited at 600 ^oC in a furnace (overnight) to constant weight. Then the samples were cooled in desiccators and weighed.

Optimization of digestion procedure

Basically, the extraction should be performed in such a way that the analyte is separated from the interfering matrix without loss, contamination, or change of speciation and with minimum interference (Soares et al., 2008). Series of digestion procedures were involved to optimize digestion of honey at different conditions by varying digestion time, reagent volume, volume ratio of reagents and digestion temperature. Accordingly, fifteen digestion procedures were tested for the digestion of honey sample. The optimum procedure of honey digestion was selected depending on the minimum reagent volume consumption, digestion time, obtaining clear solution, complexity and simplicity. Finally the optimal procedure was chosen on the basis of these criteria requiring three hours for complete digestion of 0.5 g honey sample with 2.0 mL of 69-72% nitric acid (HNO_3) and 2.0 mL 70% perchloric acid ($HClO_4$).

Table 1: Recovery values of metals for the analyzed honey samples

Metal	Conc. in sample (µg/g)	Amount added (µg/g)	Conc. in spiked sample (µg/g)	% Recovery
Zn	4.22 ± 1.02	1.69	5.89 ± 0.55	98.9 ± 1.5
Co	0.83 ± 0.02	0.332	1.12 ± 1.6	93.0 ± 5.5
Cd	0.67 ± 0.06	0.268	0.93 ± 0.8	97.0 ± 8.1
Cr	3.32 ± 0 .09	1.33	4.60 ± 1.3	95.7 ± 2.2
Fe	8.53 ± 4.12	3.412	12.1 ±1.0	104 ± 1.3
Ni	0.8 ± 0.05	0.32	1.1 ± 0.01	101 ± 5.4
Pb	ND	-	-	-
Mn	0.18 ± 0.011	0.072	0.25 ± 0.6	97.2 ± 4.1
Cu	0.091 ± 0.08	0.036	0.125 ± 0.7	93.4 ± 2.2

Values are mean ± SD of triplicate analysis. ND = Not detected, concentration was below detection limit

Table 2: pH, electrical conductivity and ash content of the of four brands of honey samples

Honey type	pH	EC (mscm^{-1})	Ash content (% w/w)
Tutu	4.11± 0.03	0.12 ± 0.02	0.31 ± 0.01
Yeshi	4.31 ±0.01	0.10 ± 0.01	0.17 ± 0.01
Beza	4.33 ± 0.11	0.29 ± 0.01	0.46 ± 0.01
Dima	4.33 ± 0.01	0.11 ± 0.01	0.20 ± 0.01

Values are mean ± SD of triplicate analysis

Table 3: Concentration (µg/g) of trace metals in four types of honey samples

Metal	Concentration of metal (mean ± SD) (µg/g) (n = 3)			
	Beza	Dima	Tutu	Yeshi
Zn	1.92 ± 0.18	4.22 ±0.31	4.17 ± 0.41	1.10 ± 0.09
Co	1.17 ± 0.08	0.83 ± 0.02	0.98 ± 0.08	0.60 ± 0.05
Cd	0.19 ± 0.01	0.67 ± 0.06	ND	0.54 ± 0.05
Fe	12.43 ± 1.0	8.53 ± 0.7	12.3 ± 0.97	5.37 ± 0.36
Ni	4.46 ± 0. 30	0.8 ± 0.01	1.24 ± 0.1	3.96 ± 0.25
Cr	4.33 ± 0.33	3.32 ± 0.22	3.828 ±0.36	1.20 ± 0.10
Pb	ND	ND	ND	ND
Mn	0.88 ± 0.07	0.18 ± 0.011	0.16 ± 0.01	0.64 ± 0.06
Cu	0.46 ± 0.04	0.091 ± 0.08	0.362 ± 0.02	0.09 ± 0.006

ND = Not detected

Digestion of honey samples

Exactly 0.5 g of honey sample was accurately weighed and transferred into a 250 mL round bottom digestion flask. 4 mL of freshly prepared 1:1 mixture of conc. HNO_3 and conc. $HClO_4$ was added to the sample. The sample was swirled gently to homogenize the mixture then the flask was fitted to a reflux condenser and the sample was digested continuously for three hours on a Kjeldahl digestion block by setting the temperature at 240 °C. Each honey sample was digested in triplicate and hence a total of twelve digests were made for the four types of honey samples. Then it was cooled to room temperature for 10 min without removing the condenser from the flask and for 10 min after removing the condenser. To the cooled solution, deionized water was added to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digest residue while filtering with Whatman®, (110 mm, diam), filter paper. The round bottom flasks were rinsed subsequently with deionized water in to 50 mL volumetric flasks and finally the volumetric flasks were made up to the mark with deionized water. The digestion gave a clear colorless solution. Digestion of six reagent blank were also performed in parallel with the honey samples keeping all the digestion parameters the same. All the digested samples were stored in refrigerator until their analysis using FAAS.

Determination of the metals

Working standard solutions (10 mg/L) of metals of interest

Table 4: Comparison of pH, electrical conductivity and ash content of honey from different countries

Country	pH	EC (mscm^{-1})	Ash content (% w/w)	Reference
Saudi Arabia	3.88–4.25	0.139–0.398	0.11–3.398	(Osman et al., 2007)
Czech Republic	3.70–4.40	0.239–0.613	0.14– 0.58	(Čelechovská and Vorlova, 2001)
Chile	3.79–5.08	0.11–0.97	-	(Fredes and Montenegro, 2006)
Croatia	4.21–5.55	0.45–0.89	-	(Daniela et al., 2008)
Argentina	3.19–4.06	0.113–0.278	-	(Naab et al., 2008)
Brazil	-	0.25–1.07	0.04–0.76	(Soares et al., 2008)
Venezuela	-	-	0.352–0.643	(Ferrer et al., 2004)
Ethiopia	4.11–4.45	0.1–0.29	0.17–0.46	Present study

Table 5: Comparison of the concentration of metals in honey of the present study with other reported values

Country	Concentration of metals (μg/g)					Reference
	Fe	Ni	Zn	Cr	Co	
Iran	-	-	0.123-0.227	0.17- 2.27	-	(Aghamirlou et al., 2015)
Chile	0.1-6.36	0.01-1.04	0.01-4.73	0.03-1.92	0.03-0.60	(Fredes and Montenegro, 2006)
Pakistan	4.35-7.54	1.02-1.48	1.98-2.94	-	0.84-1.12	(Saif-ur-Rehman et al., 2008)
Brazil	1.50-6.24	-	ND-0.94	-	-	(Soares et al., 2008)
Turkey	1.1-5.2	-	1.1-24.2	-	-	(Tuzen and Soylak, 2005)
Venezuela	3.5-39	-	-	-	-	(Ferrer et al., 2004)
Morocco	0.88-207.6	-	0.04-2.74	-	-	(Belouali et al., 2008)
Ethiopia	5.37-12.4	0.8- 4.46	1.92-4.22	1.20-4.33	0.60-1.17	Present study
Country	**Cd**	**Cu**	**Mn**	**Pb**		**Reference**
Iran	0.0014-0.0 83	0.028-0.150	-	0.145-0.961		(Aghamirlou, 2015)
SaudiArabia	0.038-0.080	0.206-0.389	0.188-0.373	0.002-0.037		(Osman et al., 2007)
Chile	0.01-0.05	0.06-2.00	0.01-3.14	0.01-0.11		(Fredes and Montenegro, 2006)
Brazil	0.019-0.059	0.008.3-0.08	ND-3.0	0.001-0.10		(Honório et al., 2014)
Turkey	0.010-0.02	0.25-1.10	0.18-1.21	0.017- 0.03		(Tuzen and oylak, 2005)
Venezuela		0.3-1.67	0.4-1.67	-		(Ferrer et al., 2004)
Morocco	0.0013-0.0249	0.51-4.75	0.080-9.76	0.036-1.88		(Belouali et al., 2008)
Ethiopia	ND-0.69	ND-0.468	ND-0.885	ND		Present study

ND = Not detected

were prepared from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L. The working standards were diluted with deionized water to obtain four standards of each metal for the preparation of calibration curves for the determination of Mn, Cd, Co, Cr, Zn, Ni, Pb, Fe and Cu by FAAS. Triplicate determinations were carried out on each sample. The same analytical procedure was employed for the determination of elements in the digested blank solutions.

Calibration of the instrument

Atomic absorption spectrophotometer was used to determine trace metals concentration. Calibration of the instrument was done by the standards prepared before the determinations were done. The standards were prepared from the intermediate 10 mg/L of the metals which were prepared prior by taking 1 mL from the stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals. After making sure that the instrument was properly calibrated, concentration of metals in each sample was measured. The correlation coefficient (R^2) of the calibration curves of each element was determined by plotting prepared standards concentration versus their corresponding absorbance. The correlation coefficients obtained were ranged between 0.9994-0.9999, which indicated that there was a good correlation between concentration and absorbance.

Recovery

To check the efficiency of the developed optimized digestion procedure spiking experiments were carried out. Known amount of each metal was added from the 1000 mg/L of stock solution into flasks containing 0.5 g honey sample. The procedure was as the following: in the first spiking the standards of four of the metals (7 µL of Cr, 16 µL of Ni, 85 µL of Fe and 13 µL of Cd) which were prepared from the working standard solution 10 mg/L of the elements prior by taking 1 mL from the stock standard solutions containing 1000 mg/L of the metals were spiked at once in round bottomed flask containing 0.5 g of honey sample and similarly, the remaining metals (84 µL of Zn, 20 µL of Cu, 17 µL of Co and 4 µL of Mn) were spiked at once into other round bottomed flask containing 0.5 g of honey sample. Then, the spiked samples were digested simultaneously with the unspiked samples based on the optimized (developed) digestion method for honey. Each sample was then analyzed for their respective spiked metals by FAAS. The results are given in Table 1. As can be seen from Table 1, the percentage recoveries are between 93 to 104%, which are within the acceptable range for all metals.

Method detection limit

Method detection limit (MDL) is defined as the minimum concentration of analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero, but it may not necessarily be quantified as an exact value (Harris, 2006). It is the amount of analyte that gives a signal equal to three times the standard deviation of the blank (Butcher et al., 1998). In the present study, six reagent blank solution were digested and each of the blank samples were analyzed for metal concentrations of Mn, Fe, Cu, Zn, Mn, Co, Ni, Pb and Cd by AAS. The detection limits were obtained by multiplying the standard deviation of the reagent blank by three. The detection limits were found in the range 0.03-09 µg/g which clearly showed that the method developed is applicable to determine the metal concentration in the honey samples at trace levels (µg/g).

RESULTS AND DISCUSSION

pH and electrical conductivity

pH and electrical conductivity of honey samples are given in Table 2. The pH values of all honey brands were found to be acidic and the values were also comparable with each other (Table 2), i.e. there was only slight difference in the pH among the samples analyzed. The mean pH values recorded were: 4.10, 4.31, 4.32, and 4.33 for Tutu, Yeshi, Dima and Beza honey, respectively. The pH was also within the accepted range (3.5-5.5) according to the Codex Alimentarius (Codex Alimentarius Commission, 2001). Pure honey is characterized by a conductance near zero. It was reported that if honey is adulterated with water or saturated sugar solutions, it will display greater conductance than pure honey (Saif-ur-Rehman et al., 2008). The electrical conductance of the four honey samples were found considerably lower than the maximum electrical conductivity given by the Codex Alimentarius (0.8 $mscm^{-1}$) (Codex Alimentarius Commission, 2001). As can be seen from Table 2, the highest electrical conductivity was recorded for Beza (0.29 ± 0.01) honey followed by Tutu (0.12 ± 0.021), Dima (0.11 ± 0.01) and Yeshi (0.1 ± 0.01). From Tables 2, the ranges of physicochemical properties analyzed could be summarized as: pH 4.11-4.45, and electrical conductivity of 0.10-0.29 $mscm^{-1}$, which are all within the limits established by national and international standards (Ethiopian Standard (2005) and Codex Alimentarius Commission (2001).

Ash content

Honey normally has low ash content and it depends on the materials collected by the bees for aging on the flora (Terrab et al., 2003; Belouali et al., 2008). This variable was calculated adapting method of AOAC (1999) and the Ethiopian Standard (2005).

The ash contents (Table 2) in the analyzed honey brands were below the limit (0.6%) allowed by the, Codex Alimnetarius Standards (Codex Alimentarius Commission, 2001) and Ethiopian Standard (2005). The highest value of ash content was recorded for Beza honey samples (0.46%), followed by Tutu (0.31%), Dima (0.20%), and Yeshi (0.17%). Beza honey exhibited almost twice as much element as light honey samples Dima and Yeshi. It is known that the mineral content is closely related to the electrical conductivity, i.e. the higher the mineral content, the higher the electrical conductivity. In this study it was found that samples that had high ash percentages manifested the highest electrical conductivity. The ash content and electrical conductivity are important as these parameters can permit an indirect estimate of mineral content.

Concentration of metals in different honey brands

The concentrations of nine trace metals in four commercially available Ethiopian honey brands were determined by using FAAS. The result showed that the metal content of each honey brand is different from one to the other. Of the nine elements determined seven were found above the detection limit in all of the four honey brands. Cd was not detected in one honey type (Tutu honey) as it was found below the detection limit, i.e. < 0.06 µg/g whereas Pb was not detected in all of the honey types (< 0.05 µg/g). Therefore, the commercially available honeys are free from the non-essential toxic metal Pb whereas the other non-essential toxic metal Cd was found at very trace level in the three honey types and none in one honey type. The concentrations of the metals in the four honey brands are summarized in Table 3.

Distribution of metals in honey samples

Essential trace metals are widely distributed in honey samples. Each honey brand analyzed was found to have different metal contents. Generally, Beza Honey was found to be richest in its trace mineral content followed by Dima and Tutu Honey whereas, Yeshi Honey was found with the least mineral content. The results of this study also indicated that Fe has the highest concentration in all honey samples studied followed by Ni, Cr, Zn, Co, Mn, Cd and Cu whereas Pb was found below the detection limit.

Concentration of metals in Beza Honey

Beza Honey contains Fe in the highest amount with concentration of 12.43 ± 1.01 µg/g followed by Ni (4.46 ± 0.31 µg/g), Cr (4.33 ± 0.37 µg/g), Zn (1.92 ± 0.18 µg/g), Co (1.17 ± 0.08 µg/g), Cu (0.885 ± 0.07 µg/g) and Mn (0.467 ± 0.36 µg/g) whereas Cd (0.19 ± 0.51 µg/g) was found to have the least concentration. Lead was observed to be below the detection limit, i.e. < 0.05 µg/g. These results showed that the essential trace metals are found at higher amount whereas the non-essential in a lower amount or none.

Concentration of metals in Dima Honey

Dima Honey was another honey type analyzed and as it can be seen in Table 3, generally, the concentration of the metals in this brand was found to have the same trend as the previous brand. It included the following average concentrations (µg/g) in the decreasing order: Fe (8.53 ± 0.7), Zn (4.22 ± 0.31), Cr (3.32 ± 0 .09), Co (0.83 ± 0.02), Ni (0.8 ± 0.01), Cd (0.67 ± 0.06), Mn (0.18), Cu (0.091), but similarly, Pb was not detected as it was found below the detection limit.

Concentration of metals in Tutu Honey

The third type of honey sample whose trace metals analyzed was Tutu Honey. The general trend of the content of the metals was not much different from the previous two brands. This honey is also very rich in its content of Fe (12.3 ± 0.97± 4.6 µg/g), followed by Zn (4.17 ± 0.41), Cr (3.82 ± 0.36 µg/g), Ni (1.24 ± 0.01 µg/g), Co (0.98 ± 0.08 µg/g), Cu (0.362 ± 0.02 µg/g) and Mn with the least concentration (0.16 ± 0.01 µg/g). In contrast to the three honey brands, not only Pb but also Cd was found below detection limit, i.e. < 0.06 µg/g.

Concentration of metals in Yeshi Honey

The fourth honey sample studied was Yeshi. Compared to other honey brands, Yeshi Honey was found with the least amount of the corresponding metals except for the metals Ni, Mn and Cd. However, the level of metals followed generally the same trend as the previous three honey brands. The concentrations of the metals for this honey were found in the following decreasing order: Fe (5.37 ± 0.36 µg/g), Ni (3.96 ± 0.25µg/g), Zn (1.1 ± 0.09 µg/g), Cr (1.20 ± 0.10 µg/g), Co (1.17 ± 0.08 µg/g), and Mn (0.64 ± 0.06), Cd (0.19 ± 0.51 µg/g) and Cu (0.09 ±0.006). Likewise, the non-essential trace heavy metal Pb was not detected in Yeshi Honey samples.

Comparison of the concentration of metals in four different honey brands

Even though the trend of levels of metals concentration in each brand is similar, the metal content of each brand is found to be quite different (Table 3). These show that there is considerable variation in mineral composition among honey samples of different origins. The data variation is probably due to the floral type, the botanical origin, storage conditions anthropogenic factor, season of the year, rainfall and so on. From the data in Table 3, one can summarize that in general Beza Honey has the highest amount of essential trace metals followed by Tutu, Dima and finally by Yeshi. Unlike to the three honey brands (Beza, Dima and Yeshi), the non-essential toxic metal Cd was too low to be detected in one honey brand (Tutu Honey) where as in the rest honey brands, it was found in very small amount below the maximum values allowed according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO 1993). The other non-essential toxic heavy metal Pb was found too low to be detected in all honey brands.

As can be seen from Table 3, Fe was found in highest amount with mean concentration ranging from 5.37 to 12.4 µg/g followed by Ni with mean concentration range of 0.8 to 4.46 µg/g, Cr (1.20-4.33 µg/g), Zn (1.92-4.22 µg/g), Co (0.60-1.17 µg/g), Cd (ND-0.69 µg/g), Mn (0.16- 0.885 µg/g) and Cu (0.09-0.4676 µg/g). The non-essential metal Pb was not detected.

Comparison of metal content and physicochemical properties of Ethiopian honey with other reported values

Although various chemical analysis target to a similar objective, there may be a difference in sampling, sample preparation and analysis techniques. Considering all these, the results of the present study can be compared to the findings of other authors. Comparisons of pH, electrical conductivity and ash content and metal contents of honeys from different countries are given in Table 4 and 5.

As can be seen in Table 4, the present studied physicochemical properties: pH, EC and ash content are remarkably in a good agreement with the reported results from different countries in the world.

In the present study Fe, Ni and Cd are at slightly higher concentration than those found in Chilean and Turkish honeys. However, Fe is within the concentration range found in Moroccan and Venezuelan honeys. The levels of Cu, Zn and Mn are in a very good agreement within the values found in the countries: Chile, Turkey, Venezuela, Brazil, honeys whereas Co and Ni are comparable with the Pakistani honey. The non-essential metal Pb in this study was found below the detection limit, i.e. < 0.05 µg/g and this is in a very good agreement with most of the results reported from different countries (Table 5). Except honey from Tutu, the other non-essential metal Cd was found slightly higher than most of the reported results. This might need further studies on the geographical origin to help in finding out the possible sources of heavy metal pollution and vegetation of the area from where the honey was originated. In general, the results obtained in this study are remarkably in a good agreement with those reported from other parts of the world implying acceptability and validity of this work regardless of some factors contributing deviation in some ways.

Statistical analysis

Analysis of variance (ANOVA) is a powerful statistical method which is used to identify the source of variation of more than one means obtained from different experiments. Since the sample means vary from one sample to another analysis of variance is used to test whether there is significance difference or not between samples mean thus enabling the source of errors. For the present study, the significance of variation within sample and between samples was studied using ANOVA. For the present study SPSS 20.0 for window software was used to calculate the presence or absence of significant difference in mean concentration of each metal among the four brands of Ethiopian honey namely, Beza, Dima, Tutu and Yeshi Honey. For Co, no significance difference at 95% confidence level ($p \geq 0.05$) was observed in the mean concentrations between all the four honey samples. The mean concentrations of Zn do not differ significantly ($p \geq 0.05$) for Dima and Tutu while it differs significantly for Dima and Tutu compared to Yeshi and Beza. Fe mean concentrations do not also vary significantly ($p \geq 0.05$) for Beza and Tutu while it differs significantly for Beza and Tutu compared to Dima and Yeshi. Similarly, the mean concentrations of Cu do not vary significantly for Dima and Yeshi but it differ significantly ($p \geq 0.05$) for Dima and Yeshi compared to Beza and Tutu. The mean concentrations of Zn do not vary significantly ($p < 0.05$) for Beza and Yeshi and also for Dima and Tutu. In the case of Cd there is no significant difference between three honey brands Dima, Yeshi and Beza. In a similar manner, in the case of Cr significant difference ($p < 0.05$) occurs between each honey brand except between Dima and Tutu, and Beza and Tutu. For Zn, Ni and Mn there was no significant ($p < 0.05$) difference in means between Beza and Yeshi and between Dima and Tutu where as there is significant difference ($p < 0.05$) when Beza and Yeshi are compared to Dima and Tutu.

The analysis of variance showed that there was significant variation in levels of elements between each brand of honey. The difference may be due to the floral type, the botanical origin, storage conditions anthropogenic factor, season of the year, rainfall and so on.

CONCLUSIONS

In this work, physicochemical properties (ash content, electrical conductivity and pH values) and nine trace metal contents in the four different brands of commercially available Ethiopian honey were determined. The optimized wet digestion method for honey analysis was found efficient for the metals determined with a good percentage recovery. The non-essential toxic metal Pb was found below the detection limit, i.e. < 0.05 µg/g and this is in a very good agreement with most of the results reported from different countries. Although the amount of Cd detected was in a safe amount for human consumption, it was found slightly higher than most of the reported results. This might need further studies on the geographical origin to help in finding out the possible sources of heavy metal pollution and vegetation of the area from where the honey was originated. ANOVA suggested that there were significant variation in the levels of some elements between different brands of the honey, which could be attributed to different factors such as geographical origin of the honey, different precautions taken during processing, storage conditions and floral source. For some elements the variations were insignificant, which could be attributed to having similar factors mentioned above. The metals content and the physicochemical properties investigated in honey samples were found within the ranges established by national and international standards. Thus the Ethiopian honey is in safety baseline levels for human consumption.

ACKNOWLEDGEMENTS

The authors acknowledge Department of Chemistry, Addis Ababa University, Addis Ababa, Ethiopia for providing the laboratory facilities. Yohannes, W. is grateful to the Department of Chemistry, Addis Ababa University for sponsoring his study.

REFERENCES

Aghamirlou, H.M., Khadem, M., Rahmani, A., Sadeghian, M., Mahvi, A.H., Akbarzadeh, A., Nazmara, S., 2015. Heavy metals determination in honey samples using inductively coupled plasma-optical emission spectrometry. Journal of Environmental Health Science and Engineering 13(39), 1-8. DOI: 10.1186/s40201-015-0189-8.

Aljadi, A.M., Yusoff, K.M., 2003. Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. Turkish Journal of Medical Sciences 33(4), 229-236.

Almeida-Muradian, L.B., Stramm, K.M., Horita, A., Barth, O.M., Silva de Freitas, A., Estevinho, L.M., 2013. Comparative study of the physicochemical and palynological characteristics of honey from *Melipona subnitida* and *Apis mellifera*. International Journal of Food Science and Technology 48(8), 1698-1706.

Amri, A., Ladjama, A., 2013. Physicochemical characterization of some multifloral honeys from honeybees *Apis mellifera* collected in the Algerian northeast. African Journal of Food Science 7(7), 168-173.

AOAC, 1999. Official Methods of Analysis of AOAC International. 16th ed. 5th rev. AOAC International: Arlington.

Ashu, R., Chandravanshi, B.S., 2011. Concentration levels of metals in commercially available Ethiopian roasted coffee powder and their infusions. Bulletin of the Chemical Society Ethiopia 25, 11-24.

Atrouse, O.M., Oran, S.A., Al-Abbadi, S.Y., 2004. Chemical analysis and identification of pollen grains from different Jordanian honey samples. International Journal of Food Science and Technology 39(4), 413-417.

Bekele, D., Chandravanshi, B.S., 2012. Levels of essential and non-essential metals in Ethiopian ouzo. SINET: Ethiopian Journal of Science 35, 19-28.

Belouali, H., Bouaka, M., Hakkou, A., 2008. Determination of some major and minor elements in the east of Morocco honeys through inductively coupled plasma optical emission spectrometry. Apiacta 43, 17-24.

Bibi, S., Husain, S.Z., Malik, R.N., 2008. Pollen analysis and heavy metals detection in honey samples from seven selected countries. Pakistan Journal of Botany 40(2), 507-516.

Bogdanov, S., Haldimann, M., Luginbühl, W., Gallmann, P., 2007. Minerals in honey: Environmental, geographical and botanical aspects. Journal of Apicultural Research 46(4), 269-275.

Bogdanov, S., Jurendic, T., Sieber, R., Gallmann, P., 2008. Honey for nutrition and health: A review. Journal of the American College of Nutrition 27(6), 677-689.

Butcher, D.J., Sneddon, J., 1998. A Practical Guide to Graphite Furnace Atomic Absorption Spectrometry. John Wiley and Sons: New York.

Čelechovská, O., Vorlova, L., 2001. Groups of honey-physicochemical properties and heavy metals. Acta Veterinaria Brno 70(1), 91-95.

Chua, L.S., Abdul-Rahaman, N.L., Sarmidi, M.R., Aziz, R., 2012. Multi-elemental composition and physical properties of honey samples from Malaysia. Food Chemistry 135(3), 880-887.

Codex Alimentarius Commission, 2001. Revised Codex Standard for Honey. Food and Agriculture Organization of the United Nations: Rome.

Conti, M.E., Stripeikis, J., Campanella, L., Cucina, D., Tudino, M.B., 2007. Characterization of Italian honeys (Marche Region) on the basis of their mineral content and some typical quality parameters. Chemistry Central Journal 1(1), 1-10.

Council Directive of the European Union, 2002. Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities L10, 47-52.

Daniela, K., Ljiljana, P., Dragan, B., Frane, C., Ivan, C., 2008. Palynological and physicochemical characterisation of Croatian honeys – Christ's thorn (*Paliurus Spina* Christ Mill) honey. Journal of Central European Agriculture 9(4), 689-696.

Debebe, A., Redi-Abshiro, M., Chandravanshi, B.S., 2016. Total contents of phenolics, flavonoids, tannins and antioxidant capacity of selected traditional Ethiopian alcoholic beverages. Bulletin of the Chemical Society Ethiopia 30, 27-37.

Debebe, A., Redi-Abshiro, M., Chandravanshi, B.S., 2017a. Assessment of essential and non-essential metals in Ethiopian traditional fermented alcoholic beverages. Bulletin of the Chemical Society of Ethiopia 31, 17-30.

Debebe, A., Redi-Abshiro, M., Chandravanshi, B.S., 2017b. Non-destructive determination of ethanol levels in fermented alcoholic beverages using Fourier transform mid-infrared spectrometry. Chemistry Central Journal 11, 27. DOI: 10.1186/s13065-017-0257-5.

Debebe, A., Anberbir, A., Redi-Abshiro, M., Chandravanshi, B.S., Asfaw, A., Asfaw, N., Retta, N., 2017c. Alcohol determination in distilled alcoholic beverages by liquid phase Fourier transform mid-infrared and near-infrared spectrophotometers. Food Analytical Methods, 10, 172-179.

Demewez, M.H., Hulugeze G.S., Getenet, B.G., 2012. Effect of improved preparation methods on physicochemical characteristics and consumer acceptability of honey wine (mead). African Journal of Food Science and Technology 3(9), 227-235.

Dhahir, S.A., Hemed, A.H., 2015. Determination of heavy metals and trace element levels in honey samples from different regions of Iraq and compared with other kind. American Journal of Applied Chemistry 3(3), 83-92.

Ethiopian Standard, 2005. Honey - Specification ES 1202:2005. Quality and Standards Authority of Ethiopia: Addis Ababa.

Felsner, M.L., Cano, C.B., Matos, J.R., Almeida-Muradian, L.B.D., Bruns, R.E., 2004. Optimization of thermogravimetric analysis of ash content in honey. Journal of the Brazilian Chemical Society 15(6), 797-802.

Ferrer, B.S., Rodrguez, G.O., Martinez, J., Mordan, M., 2004. Mineral content of the honey produced in Zulia state, Venezuela. Archivos Latinoamericanos de Nutricion 54, 346-348.

Flores, M.S.R., Escuredo, O., Seijo, M.C., 2015. Assessment of physicochemical and antioxidant characteristics of *Quercus pyrenaica* honeydew honeys. Food Chemistry 166, 101-106.

Fredes, C., Montenegro, G., 2006. Heavy metal and other trace elements contents in honey bee in Chile. Ciencia E Investigacion Agraria 33(1), 50-58.

Garcia-Alvarez, M., Huidobro, J.F., Hermida, M., Rodriguez-Otero, J.L., 2000. Major components of honey analysis by near-infrared transflectance spectroscopy. Journal of Agricultural and Food Chemistry 48(11), 5154-5158.

Gebretsadik, D.W., Chandravanshi, B.S., 2010. Levels of metals in commercially available Ethiopian black teas and their infusions. Bulletin of the Chemical Society Ethiopia 24, 339-349.

Gul, W., Farooq, N., Khan, U., Rehan, F., Anees, D., 2015. Honey: A nectarous anti-infective agent. World Journal of Pharmacy and Pharmaceutical Sciences. 4(4), 208-215.

Harris, D.C., 2006. Quantitative Chemical Analysis, 7th ed. W.H. Freeman and Company: New York.

Hernández, O.M., Fraga, J.M.G., Jiménez, A.I., Jimenez, F., Arias, J.J., 2005. Characterization of honey from the Canary Islands: Determination of the mineral content by atomic absorption spectrophotometry. Food Chemistry 93(3), 449-458.

Honório, G.G., Azevedo, G.C., Matos, M.A., de Oliveira, M.A., Matos, R.C., 2014. Use of boron-doped diamond electrode pre-treated cathodically for the determination of trace metals in honey by differential pulse voltammetry. Food Control 36(1), 42-48.

Kaakeh, W., Gadelhak, G.G., 2005. Sensory evaluation and chemical analysis of *Apis mellifera* honey from the Arab Gulf Region. Journal of Food and Drug Analysis 13(4), 331-337.

Kahraman, T., Buyukunal, S.K., Vural, A., Altunatmaz, S.S., 2010. Physico-chemical properties in honey from different regions of Turkey. Food Chemistry 123(1), 41-44.

Kamboj, R., Bera, M.B., Nanda, V., 2013. Evaluation of physico-chemical properties, trace metal content and antioxidant activity of Indian honeys. International Journal of Food Science and Technology 48(3), 578-587.

Lachman, J., Orsák, M., Hejtmánková, A., Kovárová, E., 2010. Evaluation of antioxidant activity and total phenolics of selected Czech honeys. LWT - Food Science and Technology 43(1), 52-58.

Maleki, A., Zarasvand, M.A., 2008. Heavy metals in selected edible vegetables and estimation of their daily intake in Sanandaj, Iran. Southeast Asian Journal of Tropical Medicine and Public Health 39, 335-340.

Matei, N., Birghila, S., Dobrinas, S., Capota, P., 2004. Determination of C vitamin and some essential trace elements (Ni, Mn, Fe, Cr) in bee products. Acta Chimica Slovenica 51, 169-175.

Naab, O.A., Tamame, M.A., Caccavari, M.A., 2008. Palynological and physicochemical characteristics of three unifloral honey types from central Argentina. Spanish Journal of Agricultural Research 6(4), 566-575.

Nicolson, S.W., Human, H., 2013. Chemical composition of the low quality pollen of sunflower (*Helianthus annuus*, Asteraceae). Apidologie 44(2), 144-152.

Nigussie, K., Subramanian, P.A., Mebrahtu, G., 2012. Physicochemical analysis of Tigray honey: An attempt to determine major quality markers of honey. Bulletin of the Chemical Society of Ethiopia 26(1), 127-133.

Noori, A.L., Al Ghamdi, A., Ansari, M.J., Al-Attal, Y., Al-Mubarak, A., Salom, K., 2013. Differences in composition of honey samples and their impact on the antimicrobial activities against drug multiresistant bacteria and pathogenic fungi. Archives of Medical Research 44(4), 307-316.

Nwankwo, C.M., Ezekoye, C.C., Igbokwe, S.O., 2014. Phytochemical screening and antimicrobial activity of apiary honey produced by honey bee (*Apis mellifera*) on clinical strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. African Journal of Biotechnology 13(23), 2367-2372.

Olga, E., María, F.G., Carmen, S.M., 2012. Differentiation of blossom honey and honeydew honey from Northwest Spain. Agriculture 2(1), 25-37.

Osman, K.A., Al-Doghairi, M.A., Al-Rehiayani, S., Helal, M.I., 2007. Mineral contents and physicochemical properties of natural honey produced in Al-Qassim region, Saudi

Arabia. Journal of Food, Agriculture and Environment 5(3/4), 142.

Pathare, S., Ravikumar, P., Mistry, A., 2015. Promising pharmaceutical applications of honey. Journal of Pharmacy and Pharmaceutical Sciences 4, 377-392.

Popova, M., Reyes, M., Le Conte, Y., Bankova, V., 2014. Propolis chemical composition and honeybee resistance against Varroa destructor. Natural Product Research 28(11), 788-94.

Quality and Standard Authority of Ethiopia, 2005. Ethiopian Standard Specifications for Bees Wax (ES 1203:2005), Honey (ES 1202:2005) and Bee Hives (ES 1204:2005). Quality and Standard Authority of Ethiopia: Addis Ababa.

Saif-ur-Rehman, Z.F., Maqbool, T., 2008. Physical and spectroscopic characterization of Pakistani honey. Ciencia E Investigacion Agraria 35(2), 199-204.

Sanz, M., Gonza´ lez, M., de Lorenzo, C., Sanz M., Inez-Castro, J., 2004. Carbohydrate composition and physicochemical properties of artisanal honeys from Madrid (Spain): Occurrence of *Echium* sp honey. Journal of Science of Food and Agriculture 84, 1577-1584

Sebeho, H.K., 2015. Production and quality characteristics of Ethiopian honey. Academic Journal of Entomology 8(4), 168-173.

Seda, T., Assefa, M., Chandravanshi, B.S., Redi, M., 2013. Levels of common ions in bottled mineral waters consumed in Addis Ababa, Ethiopia. SINET: Ethiopian Journal of Science 36, 27-40.

Sime, D., Atlabachew, M., Redi-Abshiro, M., Zewde, T., 2015. Total phenols and antioxidant activities of natural honeys and propolis collected from different geographical regions of Ethiopia. Bulletin of the Chemical Society of Ethiopia 29(2), 163-172.

Soares, J., Soares, N., Pires, M.L., Novaes, S., Lacerda, J.J., 2008. Honey classification from semi-arid, Atlantic and transitional forest zones in Bahia, Brazil. Journal of the Brazilian Chemical Society 19, 502-508.

Staniškienė, B., Matusevičius, P., Būdreckienė, R., 2006. Honey as an indicator of environmental pollution. Environmental Research, Engineering and Management 36(2), 53-58.

Terrab, A., Gonzalez, A.G., Díez, M.J., Heredia, F.J., 2003. Mineral content and electrical conductivity of the honeys produced in Northwest Morocco and their contribution to the characterisation of unifloral honeys. Journal of the Science of Food and Agriculture 83(7), 637-643.

Tuzen, M., Soylak, M., 2005. Trace heavy metal levels in microwave digested honey samples from Middle Anatolia, Turkey. Journal of Food and Drug Analysis 13(4), 343-347.

Tuzen, M., Silici, S., Mendil, D., Soylak, M., 2007. Trace element levels in honeys from different regions of Turkey. Food Chemistry 103(2), 325-330.

Woldemariam, D.M, Chandravanshi, B.S., 2011. Concentration levels of essential and non-essential elements in selected Ethiopian wines. Bulletin of the Chemical Society Ethiopia 25, 169-180.

World Health Organization, 1993. Evaluation of certain food additives and contaminants. WHO Technical Report Series, Number 837. World Health Organization: Geneva.

Yang, K., Wu, D., Ye, X., Liu, D., Chen, J., Sun, P., 2013. Characterization of chemical composition of bee pollen in China. Journal of Agricultural and Food Chemistry 61(3), 708-718.

Visit us at: http://bosaljournals.com/chemint/
Submissions are accepted at: editorci@bosaljournals.com