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Physicochemical Properties and Trace Metal Contents of Honey in Lowlands of Eastern Hararge, Ethiopia

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Abstract

This study was aimed at characterizing natural hone produced in different lowlands of Eastern Hararge, Ethiopia. Standard methods were employed to study the physicochemical properties and trace metal contents of the selected samples. The results showed that the content of moisture, electrical conductivity, pH, free acid value and total acid value were ranged from 9.07-19.32%, 0.32-0.75 mS/cm, 3.48-4.25, 25.83-64.17 meq/kg and 28.25-66.5 meq/kg respectively. These results showed similarity with national and international reports. The contents of Fe, Zn, Cu, Co, Cd, Pb, Mn, Ni and Cr were determined using Flame Atomic Absorption Spectrophotometry. As to the determination results, the concentrations of the elements were found to be ND - 6.04 mg/kg, ND - 1.01 mg/kg, ND - 0.44 mg/kg, ND - 0.45 mg/kg, ND - 0.28 mg/kg, ND - 0.25 mg/kg, ND - 0.15 mg/kg, ND and ND respectively. These results vividly revealed the amounts of metals in safety baseline levels for human consumption.

Keywords: Atomic absorption, lowland honey, trace metal, natural honey, physicochemical

Introduction

Honey is the natural sweet substance produced by *Apis mellifera,* honeybees. It can be prepared from the nectar of plants, secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants (The Council of the European Union, 2002).

The two common types of honey are *Apinae* honeybees' honey (*Apis mellifera* honey) and stingless honeybees' honey. Both common honeybees (sub family *Apinae*) and stingless bees (sub family *Meliponinae*) are grouped in the family *Apidae* (Culliney, 1983).

Fructose and glucose are the most predominant sugars present and responsible for most of the physical and nutritional characteristics of honey (Alvarez-Saurez *et al.*, 2010). Besides, honey contains volatile compounds such as alcohols, ketones, aldehydes, acids, esters and terpenes

(Manyi-Loh *et al.*, 2011). Phenolic acids (benzoic and cinnamic acids) and flavonoids (flavanones, flavanols) are also found in honey which contributes significantly to the therapeutic capacity of honey which greatly depends on the floral source (Gheldof *et al.*, 2002).

Natural honey contains various minerals with different levels. The level of mineral in honey depends on the natural absorption of the mineral by plants from the soil and the environment. The absorption of minerals can also occur artificially, which is influenced by the composition of artificial sources such as sugar or syrup fed on by the bees (Vanhanen *et al.*, 2011). According to the report of Vanhanen *et al*. (2011), the mineral content of honey is usually in the range of 0.04 - 0.20%, and this contributes to the color of the honey which varies from light to dark. The high nutritional profile of honey with wide range of nutrients encourages its use as food. Consuming honey has an advantage as a source of energy over the commonly used artificial sugar. The major sugar constituents of natural honey are presented in the form of monosaccharides (White and Doner, 1980).

Ethiopia is the largest honey producer in Africa. This country stands tenth in the world. The total honey production in Ethiopia is estimated to be more than 45,300 tons per year (FAO, 2012). The diversified flowering plants in Ethiopia and their blooming seasons, with great variations from place to place, enable the country to sustain a large number of honeybee colonies (Admasu, 1996).

Honey characterization is based on the determination of its chemical, physical or biological properties. Physicochemical parameters such as electrical conductivity, moisture content, free acid and pH have been suggested as criteria for the characterization of honey (Gomes *et al.*, 2010). Even though the physicochemical properties and trace metal composition of various honeybee honeys found in different provinces of Ethiopia were studied, reports regarding the physicochemical properties and trace metal composition of various honeybee honeys produced in the lowlands of Eastern Hararge were very minimal. As a result the present study was intended to determine some selected physicochemical characteristics and the trace metal contents of honey samples found in lowlands of Eastern Hararge, The study could also help to compare and contrast honey composition in the lowlands of Eastern Hararge with national and international levels.

Materials and Methods

Apparatus and glassware

Digital hot air oven (Setwin, India), Refrigerator (LABCOLD), Electronic balance with 0.001 sensitivity (citizon), Borosilicate beakers (100, 150 mL), Erlenmeyer flasks (50, 100, 200 mL), hot plate (Stuart, 300 ^{0}C), volumetric flasks (50, 100 and 1000 mL), Whatman filter paper No. 42, Funnels, Digital pH meter (ME 962p, MAX), Conductivity Meter (4310, JENWAY) and Flame Atomic Absorption Spectrophotometer (FAAS, 210VGP, Buck scientific) and muslin cloth were used in this study.

Chemicals and reagents

HNO_3 (65–68%, Blulux, India), $HClO_4$ (70%, Blulux, India), NaOH (BDH, England), HCl (35-38%, Blulux, India), phenolphthalein (Sigma-Aldrich), prepared stock standard solutions (1000 ppm) of the metals and distilled water were used to undertake this study.

Honey samples and pre-treatment

In the present study, convenience sampling technique was employed to collect hone samples. The samples were collected from the lowlands of Eastern Hararge, Ethiopia, during the months of October and November, 2016 GC. A total of 6 honey samples, 2 kg each, were collected with a labeled sterile screwed bottle from farmers and brought to Central Laboratory of Haramaya University and kept at 4 ^{o}C for further analyses. In the attempt to complete liquefaction and to be sieved with muslin cloth for the analyses of physicochemical compositions, a granular honey was placed in a closed container and heated in water bath for 30 minutes at 60 ^{o}C (AOAC, 1990).

Calibration

The graphical shape of a six point calibration curves (linearity) were established by analyzing the calibration standards that cover the entire desired instrumental measuring range. The regression calculations and a visual inspection of the line were used to establish linearity (AOAC, 1990, INAB, 2012).

Method detection and quantification limits

Method detection limits (MDL) and limit of quantification for trace metals of interest were estimated from the regression of the calibration concentrations and their responses. The limit of detection (LOD) is the concentration of the analyte which gives an instrument signal about 3 units higher than the blank or background signal; and limit of quantification is the lowest limit of quantitative measurements at acceptable accuracy and precision (Miller and Miller, 2010).

Mathematically, LOD is expressed as:

$$LOD = Y_B + 3S_{y/x} \quad (1)$$

Where: Y_B = blank signal, $S_{y/x}$ = standard deviation about the regression, which estimates random error in the Y-direction.

However, the value of the calculated intercept (b) of the regression equation is a more accurate estimate of Y_B than the single measured blank value, it is more appropriate to use intercept (b) in place of Y_B during practical estimation of the LOD. Therefore, Equation 1 becomes:

$LOD = b + 3S_{y/x}$ (2)

Where: b = intercept of the regression line

Likewise, LOQ could be determined as:

$LOQ = b + 10S_{y/x}$ (3)

Physicochemical analysis

Moisture content

Moisture content was determined from the weight lost after taking 10 g of honey samples in to crucible and drying in a dry oven at 105 ^{o}C for 3 hrs and cooling in desiccator for 1hr. Following this, the readings were converted to percent moisture (AOAC, 1990).

Electrical conductivity

The electrical conductivity was measured by means of a conductivity meter after calibrating with a 0.01 N KCl solution; and to each of honey samples (10 g), 75 mL of deionized water was added and mixed thoroughly (IHC, 2000).

pH value

The pH value was measured using a digital pH-meter after calibrating with a buffer solution of pH 4 and 7; and to each of honey samples (10 g), 75 mL of deionized water was added and mixed thoroughly (IHC, 2000).

Free acidity, lactone acidity and total acidity

The contents of free acid, lactone acid and total acid in each honey samples were determined using the standard titrimetric method (AOAC, 1990).

Determination of trace metal contents

The contents of Fe, Zn, Cu, Co, Cr, Mn, Cd, Pb and Ni were determined in each of honey samples using Flame Atomic Absorption Spectrophotometry and standard method (AOAC, 1990).

Statistical analysis

The necessary data were calculated as mean± standard deviation (SD) and analyzed using analysis of variance (ANOVA). Probability of 0.05 or less was considered significant. The statistical package of window 2010 Excel was used for all chemometric calculations.

Results and Discussion

Calibration

The FAAS was calibrated using a blank and five series of standard solutions of each metal. Calibration curves for the various concentration ranges showed good coefficient of determinations ranged between 0.995 and 0.999 (Table 1), which were all within the required limit (≥ 0.995) for trace elements (USEPA, 2007). This showed that there was good correlation between concentration and absorbance indicating good calibration of the instrument and good linearity of the calibration curves.

Table 1. Method detection limit (MDL), method quantification limit (MQL) and coefficients of calibration curves for trace metals determined in honey samples.

Metals	Coefficient of calibration curve (r^2)	Calibration equation	MDL (mg/kg)	MQL (mg/kg)
Fe	0.999	Y=0.008X	0.166	0.552
Zn	0.997	Y=0.044X	0.317	1.056
Cu	0.996	Y=0.010X	0.317	1.057
Mn	0.999	Y=0.017X	0.106	0.354
Pb	0.997	Y=0.005X	0.228	0.761
Cd	0.999	Y=0.014X	0.173	0.576
Cr	0.998	Y=0.014X	0.196	0.653
Co	0.999	Y=0.010X	0.282	0.940
Ni	0.995	Y=0.005X	0.259	0.863

Method detection and quantification limits

The MDL values lied in the range 0.106 (Mn) to 0.317 (Zn and Cu) mg/kg and method quantification limit values 0.354 (Mn) to 1.057 (Cu) mg/kg (Table 1). The values were found to be within the required limit (Temminghoff and Houba, 2004). This indicated that the method was applicable for the determination of the trace metals.

Physicochemical characteristics

The mean values (mean ± SD, n=3) of moisture content, electrical conductivity, pH, free acidity, lactone acidity and total acidity of each honey samples were depicted in Table 2.

Table 2. Mean values (mean ± SD, n=3) of moisture content, electrical conductivity, pH, free acidity, lactone acidity and total acidity of honey samples.

Honey samples	EC (mS/cm)	pH	FA (meq/kg)	LA (meq/kg)	TA (meq/kg)	Moisture content (%)
Fedis-I	0.55±0.007	3.63±0.010	35±0.000	2.33±0.380	37.33±0.380	19.32±0.480
Fedis-II	0.52±0.000	3.85±0.010	30.83±1.440	1.83±0.380	32.67±1.420	9.07±0.420
ErerGuda	0.75±0.004	4.25±0.010	64.17±2.890	2.33±0.380	66.50±2.610	10.71±0.320
Burka Tirtira	0.32±0.001	3.48±0.030	25.83±3.820	2.42±0.520	28.25±3.880	13.41±0.270
Ganda Ifa	0.75±0.022	3.77±0.050	42.50±0.000	4.42±0.290	46.92±0.290	11.56±0.800
Ganda Hasen	0.72±0.007	3.91±0.030	42.50±2.500	3.92±0.520	46.42±2.160	15.00±0.280

Key: EC= electrical conductivity, FA= free acidity, LA= lactone acidity, TA= total acidity

As shown in Table 2, Fedis-I honey had the highest moisture content (19.32%) followed by Genda Hasen(15%), Burka Tirtira (13.41%), Genda Ifa (11.56%), Erer Guda (10.71%) and Fedis-II (9.07%) honey samples. These results were in agreement with the European Community Directive requirements (The Council of European Union, 2002). Similar values were also found in Moroccan honeys (Terrab *et al.*, 2003). Moisture content is highly important for the shelf-life of the honey (Pérez-Arquillué *et al.*, 1994). High moisture content can make honey to be fermented and spoiled. The amount of water in honey is a function of many factors involved in ripening such as weather conditions, original moisture of the nectar, harvest season, the conditions of storage and the degree of maturity (Terrab *et al*., 2003).

The highest EC value (0.75) was recorded in Erer Guda and Ganda Ifa honeys followed by Ganda Hasen (0.72), Fedis-I (0.55), Fedis-II (0.52) and Burka Tirtira (0.32), which were within the international limit,<0.8 mS/cm, (Codex Alimentarius Commission, 2001; Council Directive of the European Union, 2002; Gomes *et al*., 2010). EC can be influenced by the ash, organic acids, proteins, some complex sugars and polyols content. The content of EC also varies with botanical origin in which the value can be changed when the amount of the plant pollen decreases (Kaskoniene *et al.,* 2010**)**. Moreover, the electrical conductivity of honey is closely related to the concentration of mineral salts, organic acids and proteins; and shows great variability according to the floral origin. These characteristic makes EC to be considered as one of the best parameters for differentiating between honeys with different floral origins (Terrab *et al*., 2004).

As shown in Table 2, Burka Tirtira was found to be the most acidic with pH value (3.48) followed by Fedis-I (3.63), Genda Ifa (3.77), Fedis-II (3.85), Genda Hasen (3.91) and Erer Guda (4.25), which were within the limits (3.0-5.6) established by the national and international organizations (The Council of European Union, 2002; Codex Alimentarius Commission, 2001; White, 1978). The pH mean values of honey samples in this study were in agreement with the results reported from other country (Sanz *et al*., 2005). It is important to note that pH of honey is not directly related to free acidity because of the buffering action of the various acids and minerals (White, 1978).

The highest total acid value (meq/kg) was obtained from Erer Guda honey sample (66.5) followed by Ganda Ifa (46.92), Ganda Hasen (46.42), Fedis-I (37.33), Fedis-II (32.67) and Burka Tirtira (28.25). The total acid mean value of Erer Guda exceeded the limit of 50 meq/kg established by the Council of European Union (2002) and Codex Alimentarius Commission (2001). The mean values of total acid in each honey samples found in this study were comparable with those reported in other study (Costa *et al*., 1999). The total acid value reported in this study indicated the absence of undesirable fermentation in all honey samples except in Erer Guda. As to Lorena *et al*. (2012), elevated acidic values of 30.5 - 132.5 were also reported for *Melipona* honey.

The variation in acidity among different honey samples may be attributed to either variation in the contents of organic and inorganic acids due to harvest season (Pérez-Arquillué *et al*., 1994, El-Sherbiny *et al*., 1979) or floral types (El-Sherbiny *et al*., 1979). The free acid values of the honey samples obtained in this study were ranged from 25.83 - 64.17 meq/kg. According to the new limit for free acidity permitted by the Codex (2001) and the European Community Directive (The Council of the European Union, 2002), the free acid value of Erer Guda honey sample was deviated from the legislation limits.

Trace metal analyses

The levels of Fe, Zn, Cu, Mn, Pb, Cd, Cr, Ni and Co were determined in each honey samples and their mean values were depicted in Table 3.

Table 3. Mean values (mean ± SD, mg/kg, n=3) of Fe, Zn, Cu, Mn, Pb, Cd, Cr, Ni and Co in the honey samples

Trace metals	Honey samples					
	Fedis-I	Fedis-II	Erer Guda	Burka Tirtira	Ganda Ifa	Ganda Hasen
Fe	ND**	ND**	0.25±0.03*	2.54±0.44	3.25 ±1.44	6.04±1.88
Zn	ND**	ND**	ND**	0.85±0.06*	0.92±0.09*	1.01±0.03*
Cu	ND**	ND**	ND**	0.35±0.05*	0.44±0.11*	0.19±0.01*
Mn	ND**	ND**	ND**	ND**	ND**	0.15±0.01*
Pb	ND**	ND**	ND**	ND**	ND**	0.25±0.01*
Cd	ND**	0.28±0.07*	0.17±0.02*	ND**	0.28±0.03*	ND**
Cr	ND**	ND**	ND**	ND**	ND**	ND**
Co	ND**	ND**	ND**	0.45±0.06*	ND**	0.32±0.06*
Ni	ND**	ND**	ND**	ND**	ND**	ND**

Key: * = between MDL and MQL; ** = BMDL; ND= not detected; BMDL= below method detection limit; MDL=method detection limit; MQL= method quantification limit

As shown in Table 3, Fe exhibited the highest value with a range of ND - 6.04 mg/kg followed by Zn (ND-1.01 mg/kg), Co (ND-0.45 mg/kg), Cu (ND- 0.44 mg/kg), Cd (ND- 0.28 mg/kg), Pb (ND-0.25 mg/kg), Mn (ND- 0.15 mg/kg), Cr (ND) and Ni (ND). The highest content of Fe (6.04 mg/kg) was recorded in Genda Hasen honey sample followed by Ganda Ifa (3.25 mg/kg), Burka Tirtira (2.54 mg/kg), Erer Guda (0.25 mg/kg); and ND in Fedis-II and Fedis-I. Iron was the most abundant element in the Genda Hasen honey while Cr and Ni were the lowest once. Fe, Zn, Cu, Mn, Pb, Cd, Cr, Ni and Co levels in all the honey samples studied were lower than those found in the Moroccan honeys (Terrab *et al*., 2003).

The level of Zn content was ND in Fedis-I, Fedis-II and Erer Guda; but the researchers were uncertain to report the amount of Zn in Ganda Ifa, Ganda Hasen and Burka Tirtira because it was between MDL and MQL.

The values obtained were below the guideline value (5 mg/kg) established by Codex Alimentarius Commission (2001). Zn and Fe levels in all honey samples reported in this study were also much less than those reported from Kateefash , Saudi Arabian honeys (Hamza *et al*., 1993). Zinc is a nutritionally essential metal; but high intake of it results in gastrointestinal distress and diarrhea (Goyer and Clarkson, 2001).

Copper values were also ND in Fedis-I, Fedis-II and Erer Guda; but between MDL and MQL in Ganda Ifa, Ganda Hasen and Burka Tirtira. As a result, the researchers were uncertain to report for these specified study areas. The concentrations of Cu in all tested samples were below the guideline value (5 mg kg^{-1}) stated in Codex Alimentarius Commission (2001).

The level of Mn was ND in all honey samples, except in Genda Hasen honey sample (0.15 mg/kg), which the researchers could not be certain to report because it was between MDL and MQL. High values of Mn have been reported in other study which can be attributed either to the production stages of honey or the region from where the honey has been taken (Ioannidou *et al*., 2005).

As shown in Table 3, the level of Cd in honey samples collected from Ganda Ifa, Erer Guda and Fedis-II were between MDL and MQL for which the researchers could not be certain to report. However, the level of Cd was ND for honey samples in Fedis-I, Burka Tirtira and Genda Hasen. The apparent levels of Cd detected in honey samples from Ganda Ifa, Erer Guda and Fedis-II were below the standard value (0.5 mg/kg) stated in Codex Alimentarius Commission (2001).

The Pb content was recorded in Genda Hasen honey sample for which the researchers were uncertain to report as it was between MDL and MQL; but ND was obtained in the rest honey samples (Table 3). The content of Pb reported in this study was comparatively less than the one reported from Hungarian honey samples (Ajtony *et al*., 2006).

The level of Co (0.45 mg $kg^{-1}$and 0.32 mg kg^{-1}) recorded in Burka Tirtira and Genda Hasen honey samples respectively; but the researchers were uncertain to report because it was between MDL and MQL; and ND in Ganda Ifa, Erer Guda, Fedis-I and Fedis-II (Table 3). These values were comparable with the report of Kebede *et al*. (2012).

As stated in Table 3, Cr level was ND in all the honey samples and comparable with the finding reported from Kenya (Maiyo *et al*., 2014). The concentration of Cr in this study was also below the report of Fredes and Montenegro (2006) which was 30 - 1900 µg/kg.

The level of Ni was also ND in all honey samples (Table 2). In literatures, Ni content was reported as being 0.01 - 1.04 mg/kg (Fredes and Montenegro, 2006) and 78 - 420 µg/kg (Chakir *et al*., 2011).

Statistical results

One-way ANOVA was performed among the results obtained from the analysis of honey samples. The application of One-way ANOVA was to assess whether the distributed physicochemicals and trace metals significantly in different concentrations or not. The test results showed that there were significant differences ($p < 0.05$, at 95% confidence level) in the concentrations of water content, EC, pH, free acidity, total acidity, Zn, Fe, Mn, Cu, Co, Cd, Cr, Pb and Ni among the analyzed honey samples; whereas there were no significant differences ($p > 0.05$, at 95% confidence level) in the concentrations of Cr and Ni among the analyzed honey samples.

Conclusion

The study revealed that the selected physicochemical properties of the investigated honey samples collected from Eastern Hararge normally fit the nationally as well as internationally set specifications. The levels of trace metals in honey samples were determined and assessed for its quality comparing with permissible limits stipulated by various agencies and organizations. With this regard, the present Fe, Cu, Zn, Mn, Cr, Co, Ni, Pb and Cd levels were in safety base line levels for human consumption. This might be because of the honey samples collected from the rural areas, where there were no effluents released from industries, were in safe condition during their preparation. This study also revealed a high value of total acid in Erer Guda, which might be due to the possibility of fermentation during its production and storage. In the present study, all honey samples used were not contaminated with the investigated toxic heavy metals. The samples showed lower levels than the permissible limits set by European commission and Codex regarding all parameters identified in the present study. However, it is exceptional that high level of total acid was recorded in Erer Guda honey sample.

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