



Research article

Physico-chemical characteristics of honey produced by stingless bees (*Meliponula beccarii*) from West Showa zone of Oromia Region, EthiopiaAlemayehu Gela^{a,b,*}, Zewdu Ararso Hora^a, Deresa Kebebe^a, Araya Gebresilassie^b^a Oromia Agricultural Research Institute, Holeta Bee Research Centre, Holeta, Ethiopia^b Department of Zoological Sciences, Addis Ababa University, Addis Ababa, Ethiopia

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ABSTRACT

The study was investigated to characterize the physico-chemical properties of honey produced from stingless bees (*Meliponula beccarii*) in Oromia Region of Ethiopia. Honey samples were collected from underground soils through an excavation of natural nests from four representative districts (Wolmera, Jeldu, Toke Kutaye and Chalya). About 120 honey samples (0.35–1.5 lit volumes) were collected and pooled for each respective area (district) for analysis. Principal component analysis (PCA) of pollen data and GPS were used to categorize the botanical origin and geographical location of honey sample sources, respectively. Consecutively, the physico-chemical characters were evaluated for the moisture content (MC), electrical conductivity (EC), pH, ash, total acidity (TA), hydroxyl methyl furfuraldehyde (HMF), insoluble materials (IM), and proline. The study results showed that the mean values of parameters for honey samples were: MC (29 ± 1.45), EC (0.22 ± 0.03), pH (3.73 ± 0.13), Ash (0.41 ± 1.1), TA (7.3 ± 0.36), HMF (18 ± 3.45), IM (0.70 ± 0.3) and proline (214.5 ± 15). There was no mean significance differences ($P > 0.05$) observed among the sampled districts for the analyzed parameters, except for proline. The values were compared against the standard physico-chemical parameters of *Apis mellifera* honey of Ethiopia and these values significantly varied for moisture content, total acidity and HMF. This is the first study investigated on composition property of stingless bee honey in Ethiopia and recall for further study on its nutritional and medicinal values so as to create a specific standardization.

1. Introduction

Honey is a natural substance that is sweet sticky and viscous solution produced by different bee species either from the juice of blossoms or from exudations of living parts of plants or excretions of insects those sucking plant juices from the living parts of plants (Alimentarius, 2001). With its composition and constituents, honey is known globally to have a wide variety of uses and applications, such as for nutritional, medicinal and industrial purposes. Honey is composed of sugars, mainly monosaccharide, with constituting about 95–97% of carbohydrates of its dry weight (Buba et al., 2013; Cantarelli et al., 2008). Fructose (38%) and glucose (31%) are the foremost transcendent sugars present and contribute in nutritional characteristics of honey (Alvarez-Suarez et al., 2010; Sato and Miyata, 2000). Bastos and Alves (2003) stated that honey flavor and aroma is determined by volatile compounds includes alcohols, aldehydes, ketones, esters and acids. Nevertheless, depending on the source(s) of nectar, geographical origin, seasonal and environmental factors and handling techniques, honey can have a variable chemical

composition (Alvarez-Suarez et al., 2010; Silva et al., 2013). Moreover, a research finding indicate that honey-making processes are profoundly related to enzymes included by the bees so that composition of honey is additionally influenced by the sorts of bee species (Kek et al., 2017).

Based on its entomological derivation, honey may be from the species *Apis mellifera*, which is the most broadly known and commercially accessible around the world, or from meliponines, which are commonly known as stingless bees without sting, or native bees (Temaru et al., 2007). In Ethiopia, both the honey types are produced all over the country. Several studies have been conducted to characterize Ethiopian *Apis mellifera* honey depending on its botanical and topographical origins (Belay et al., 2013, 2017; Tesfaye et al., 2016), so that its quality parameters have been standardized both at national and international levels (QSAE, 2005; Alimentarius, 2001). Exclusively, the stingless bee, *Meliponula beccarii* (Gribodo, 1879) honey commonly known as “Damuu” or “Tazma” honey is a valuable bee product with long consumption tradition, to which several medicinal uses are attributed. According to Vit et al. (2013) stingless bees construct their nests as spherical pots made of

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cerumen, that is a mixture of propolis and wax to store their honey and the honey produced in such a way is called pot honey. In Ethiopia, the way stingless bee honey (“*damma damuu*”) harvested from feral colonies is absolutely traditional and destructive. The process is not only reducing quality of honey but also endangered the existing local species because of total nest destruction.

Certainly with its specific delicate taste and its importance in traditional medicine in rural communities of Ethiopia, stingless bee honey has greater value than *A. mellifera* honey (Andualem, 2013; Ewnetu et al., 2013; Pimentel et al., 2013; Vit et al., 1998). As a result, stingless bee honey has high local market demand, attaining better prices than the Apis honey and commercialized in different regions of the country. Despite its high demand and medicinal value, the issue of its quality and authenticity remain as important factors in its consumption and marketing due to the scant knowledge about its production system and composition. As a result, the proximate composition property of stingless bee honey is not yet characterized and documented even to set its quality standard both for nutritional and medicinal value. Eventually, the result is helpful for setting stingless bee honey quality standard and its characterization particularly in the identification of Ethiopian stingless bee honey. Therefore, the aim of the current study was to evaluate the proper physico-chemical characteristics of honey from native stingless bees (*Meliponula beccarii*) of Ethiopia and determine some of its quality level in comparison with *A. mellifera* honey.

2. Material and methods

2.1. Description of study area

This study was conducted in four representative districts of West Shoa Zone of Oromia Regional State in western part of Ethiopia. The study focused on high land areas where stingless bees potentially exist which includes Wolmera, Jeldu, Toke Kutaye and Chaliya districts of West Shoa Zone. Wolmera is located at 09°03′.24 N and 038°30′.72 E latitude and longitude, respectively with 2498 m altitudes, Jeldu at 09°04′.01 N and 039°06′.45 E latitude and longitude, respectively with 2400 m altitudes, Toke Kutaye at 038°51′.60 N, 037°43′.92 E and 2379 m of latitude, longitude and elevation, respectively, and Chaliya at 09°20′.11N of latitude and 037°25′.35 E of longitude with 2269 m elevation. The locations were purposively chosen for the study representing the high potential areas of stingless bee, *M. beccarii* and with diverse floral composition.

2.2. Honey samples collection

Following the honey flow season (October–November, 2018), the samples of were collected from various spot areas of each district. The feral stingless bee colony nests were first located by the help of experienced local traditional hunters (Figure 1A). To collect the samples, the underground nests were then carefully excavated without damaging the nest chambers containing both honey and pollen stores. Directly from the honey chamber, honey samples were harvested from sealed honey pots using disposable syringes (10 ml) and pooled in to air tighten glass jars for each sampling area (Figure 1B–F). Accordingly, about 120 honey samples pooled in to 20 groups were collected and stored in refrigerator (4 °C) until laboratory analysis.

2.3. Botanical origin identification

The botanical origin of stingless bee honey was determined using harmonized methods of melissopalynology (Von Der Ohe et al., 2004), with little modification. Dominant stingless bee honey plants were determined through pollen analysis. Each 10 g sample of honey was weighed in a glass centrifuge tube with 50 mL capacity and dissolved in distilled water (20 mL). The solution was then centrifuged for 10 min at temperature of 20–40 °C and the supernatant was descanted. To fully dissolve the remaining sugar crystals in a solution, another 20 mL distilled water was added and centrifuged for 5 min at the same temperature, then the supernatant was again descanted. The remaining residue was uniformly spread on a microscopic slide using a micro spatula and allowed to dry and fix. Finally, one drop of glycerin jelly was added to the cover slip and the sample was observed under Zeiss Axio-Vert A.1 light microscope (Mg. Power 40X). Selected pictures of pollen finger prints were taken from each slide (Figure 2) and the source of dominant pollen plants were identified using reference slides and pollen atlas (Adgaba, 2007).

Furthermore, PCA was employed to categorize the botanical origin and geographical locations of stingless bee honey samples.

2.4. Analysis of physico-chemical properties

Chemical compositions (pH, total acidity, ash or mineral content, HMF, insoluble materials and proline) and physical compositions including: moisture content, electrical conductivity of the stingless bee honey samples were determined according to according to the standards protocols for each respective parameters at Holeta Bee Research Center



Figure 1. Stingsless bee honey harvesting and sample collection process. A shows undisturbed nest entrance with guard bees, B and C show nest excavation process, D shows sealed honey pots, E shows honey harvesting and F shows strained stingsless bee honey samples.

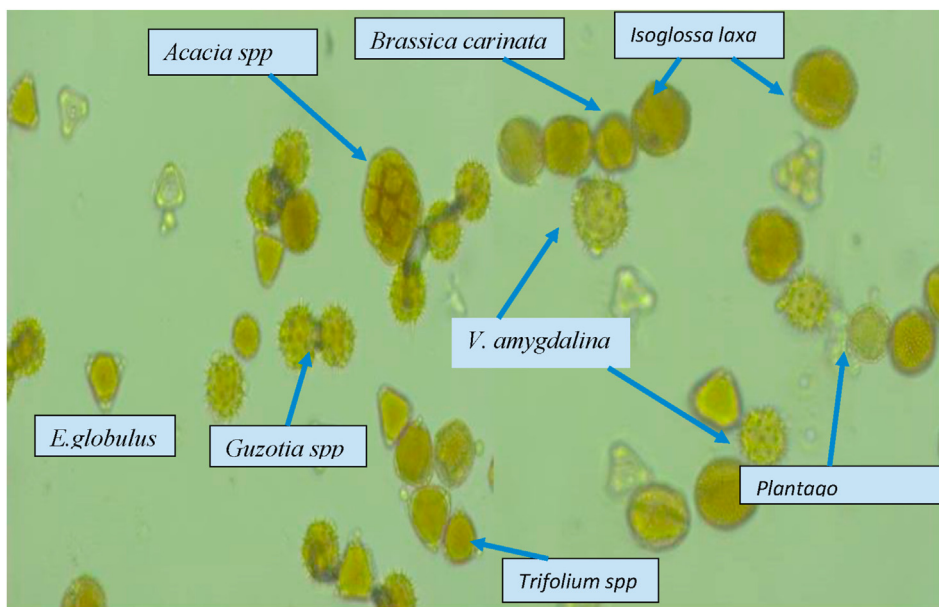


Figure 2. Pollen morphology of dominant stingless bee honey plants (under Zeiss AxioVert A.1 light microscope, MP 40 X).

Laboratory. Each physico-chemical property parameter was analyzed with three replications under each respective procedure.

2.5. Moisture content

The honey sample moisture content was analyzed using a portable digital, professional hand held refractometer (Bellingham RFM 330, SER. No.016468, made of UK) with the range expressed in percentage (%) and with the refractive index for water (n_D) at 20 °C waiting for 6 min to reach at equilibration point. This technique was assumed according to the rules that the honey refractive index rises with the amount of solid substances contained in the honey sample. Then the moisture contents of each sample were recorded three times, and the mean value was recorded as described elsewhere in (Alimentarius, 2001).

2.6. pH and total acidity

The honey samples pH and total acidity were considered according to the procedure of Bogdanov (2009). For the measure of pH value, a solution of 10 g stingless bee honey was dissolved in 75 mL of distilled water, then homogenized and subjected to reading in a pH meter (3100 Janeway, England) that was calibrated at pH 4.0 and 7.0. Furthermore, the honey solution was titrated with 0.1 M sodium hydroxide (NaOH) to the value of pH 8.30 at a steady reading after 2 min of the starting of titration. The readings were then recorded to the closest 0.2 mL employing a 10 mL burette to obtain precision value.

The total acidity of the honey was expressed in milli equivalents or mill moles of acid per kg of honey sample, which was equivalent to ml of 0.1M NaOH x 10 Eq. (1). Then, the value was expressed to the nearest one decimal point according to the standard procedure the Harmonized Methods of International Honey Commission (Bogdanov, 2009).

$$\text{Acidity} = 10V \quad (1)$$

where; V = the volume of 0.1 N in a 10 g of honey sample.

2.7. Determination of the ash content

The contents of ashes in the stingless bee honey samples were determined based on the standard procedure of (Marchini et al., 2007). First, the selected crucibles were heated in a furnace for about 25 min at a temperature of 300 °C. Then they were transferred to the desiccators for

20 min to cool down and were weighed separately to 0.001g (M_1). Then, 10 g of stingless bee honey sample was accurately measured and put into an ignited and pre-weighed crucible. In each sample, two droplets of olive oil were added to prevent frothing, and then the samples were gently heated on an electric hot plate until the samples were completely carbonized. The honey samples were incinerated in an electric muffle furnace (CFS 11/B, England) (600 °C) for about 5 h until complete incineration (white to light gray color) to reach the constant weight. The crucibles were then taken out and cooled in the desiccators and weighted for constant weight (M_2). Finally, the ash content was calculated in percent in g/100g mass using the formula Eq. (2), as described in (Alimentarius, 2001).

$$\text{Ash, \% by mass} = \frac{(M_2 - M_1)}{M} \times 100 \quad (2)$$

where;

- M_1 = Weight of empty crucible
- M_2 = Weight of the ash and crucible
- M = Mass of the sample taken for the test

2.8. Determination of HMF

The honey sample HMF content was determined based on the ability of UV absorbance of HMF at 284 nm according to the harmonized Methods of International Honey Commission (Bogdanov, 2009). For the purpose, the difference between the absorbance of aqueous honey solution and the solution after the addition of bisulphate was determined to eliminate the intrusion of other components at the same wavelength. Then, HMF was calculated after subtracting the background absorbance at 336 nm. Subsequently, a spectrophotometer working in a wavelength range of 284 nm–336 nm was employed. At the end, HMF was expressed in mg kg⁻¹ Eq. (3):

$$\text{HMF} = (A_{284} - A_{336}) \times 149.7 \times 5 \times D/W \quad (3)$$

where;

- A₂₈₄ = absorbance at 284 nm
- A₃₃₆ = absorbance at 336 nm
- D = dilution factor, when necessary

W = weight of the honey sample (g)

2.9. Electrical conductivity

The electrical conductivity of stingless bee honey samples was measured using a conductivity meter (LF 90, Astute Tech. Werkstätten D821 Weilheim, Germany). 20 g of an anhydrous honey sample was diluted in 100 mL distilled water and from the dissolved solution, 40 mL was poured into a measuring beaker and placed in a thermostat-controlled water bath (GFL Labortechnik mbh D3006 Burgwedel) at 20 °C. Then, the measurements of electrical conductivity were acquired by conductivity meter (low range, 4310 Wagtech, England) with 1.03 cell constant. After the conductivity cell was immersed in to the honey solution, the conductance read was recorded in mS at an equilibrium temperature. Finally, the value of electrical conductivity was calculated by the following equation Eq. (4) as described in International Honey Commission (Bogdanov, 2009).

$$SH = K.G \quad (4)$$

where:

SH = electrical conductivity in mScm^{-1}
 K = cell constant in cm^{-1}
 G = conductance in mS

2.10. Determination of proline

For the determination of proline content, 5g of homogenized honey sample was balanced and diluted in 50 mL H₂O, then transferred to a 100 ml volumetric flask and again dissolved with distilled water. Then a Lambda 25 double-beam spectrophotometer UV/Vis (Perkin Elmer, Waltham, Massachusetts, USA) was used to determine the absorbance. When reach at a maximum absorbance (510 nm), a spectrum of proline from 440 to 560 was determined to evaluate the wavelength at the point of maximum absorbance. Then, the amount of proline in mg/kg of honey at one decimal point was calculated using the equation Eq. (5) developed by International Honey Commission (Bogdanov, 2009).

$$\text{Proline} \left(\frac{\text{mg}}{\text{kg}} \right) = \frac{E_s}{E_a} + \frac{E_1}{E_2} \times 80 \quad (5)$$

where;

Es = absorbance of the honey solution
 Ea = absorbance of the average proline standard solution
 E1 = mg proline taken for standard solution
 E2 = weight of honey in grams
 80 = dilution factor

2.11. Statistical analysis

One-way ANOVA was employed to compute the variations between the means of each variable in every parameters of the analysed honey samples. For all the computations, SPSS version-20 statistical software was employed and tests were made at 5% level of significance. Moreover, principal component analysis (PCA) was employed to categorize the botanical origins of the stingless bee honey samples based on pollen finger print data (melissopalynology) Figure 3.

3. Results and discussion

The study result was revealed that average volume of stingless bee honey collected from each nest ranged from 350 ml-1.5 l, which varied among districts (Table 1). The highest volume of honey (2.3 l) was harvested from Chaliya district, while the lowest honey volume (250 ml)

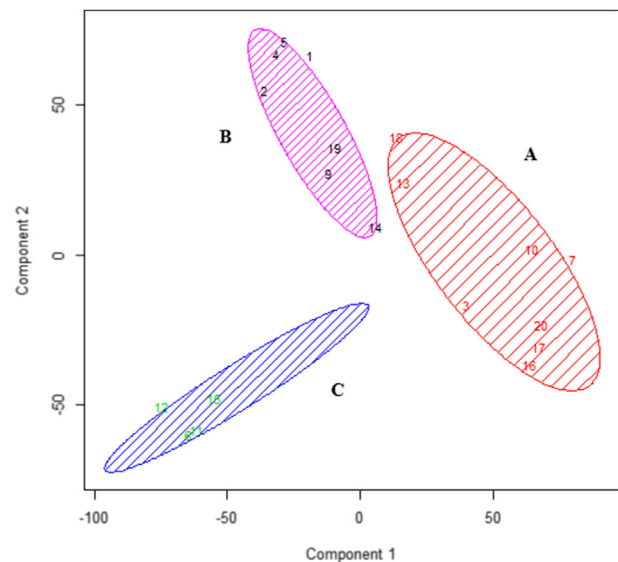


Figure 3. Clusters of the study sites based on the species diversity from honey pollen analysis of stingless bee honey (*M. beccarii*) using PCA plot analysis. Component 1 and 2 are PCA axis, a number in the clusters represent the sampling site, letters represent number of clusters.

was recorded from Toke Kutaye district. The variations in volumes of honey yield among districts might be affected by several factors such as the nest size, colony population size, vegetation type and longevity of established colony harbored in the specific nest. Traditionally, the year of colony established is estimated by counting the number of guard bees on their nest entrance, which can be related to amount of honey production.

Interestingly, melissopalynology finding of this study indicated the botanical origin of stingless bee honey. This showed that the pollen dominance and morphology of diverse plant species from which the honey was collected. Major honey plants identified from pollen morphology analysis included *Guzotia* spp, *Acacia* spp, *Eucalyptus globulus*, *Vernonia amygdalina*, *Trifolium* spp, *Plantago lanceolata*, *Brassica carinata* and *Isoglossa laxa* (Figure 2). These plant species are also known as major honey plants of *A. mellifera* bees (Admassu et al., 2014).

Moreover, PCA of pollen data was categorized briefly the botanical and geographical origin of stingless bee honey samples. From the dominant pollen analysis in the honey samples, it was found that stingless bee honeys are produced from diverse forage plant species and the study sites were classified accordingly into three clusters. Cluster A includes Wolmera site, Toke Kutaye site and Jeldu site which is dominated by *Guizotia scabra* honey due to its abundance and major source of pollen and nectar. Cluster B include Chaliya and Jeldu sites are dominated by *Vernonia amygdalina* which is adapted to grow in the mid and highlands. Cluster C includes Wolmera and Jeldu sites which were dominated by the *Eucalyptus globulus* honey since it is multipurpose tree widely grown by the respective community and the major source of nectar for honeybees (Figure 3).

Subsequently, physico-chemical analysis of twenty pooled honey samples revealed no significant differences ($P > 0.05$) among the districts for all the measured values (moisture content, electrical conductivity, pH, ash, total acidity, HMF, and insoluble materials) except for the proline (Table 2). Proline content was higher in the honey sampled from Jeldu district (293 ± 14), while the lower value was recorded from Wolmera district (171 ± 13). The value of moisture content, electrical conductivity, pH, FA, and HMF were compared against standard values of *A. mellifera* honeys, and parameters like moisture content, HMF and TA were significantly varied (Table 2).

Water (moisture) content is one of the most pertinent characteristics of honey, since it impacts the thickness, specific weight, ripeness, crystallization and taste of the honey which enhances the shelf life of the

Table 1. Area description and stingless bee honey volumes collected from underground nest.

No.	Zone	District	Bee species	Agro ecology	Average honey volume/nest
1	West Shoa	Chaliya	<i>M. beccarii</i>	High land	1.5 lit
2	West Shoa	Jaldu	<i>M. beccarii</i>	High land	500ml
3	West Shoa	Wolmera	<i>M. beccarii</i>	Mid high land	680 ml
4	West Shoa	TokeKutaye	<i>M. beccarii</i>	Mid high land	350ml

Table 2. Mean comparison of physico-chemical properties of stingless bee honey samples collected from four districts of West Shewa zone, Oromia and compared to *Apis mellifera* honey standards (N = 20).

Districts	N = 20	Parameters (Mean ±SE)								
		MC (%)	EC (mScm ⁻¹)	pH	Ash (%)	TA (meqkg ⁻¹)	HMF (mgkg ⁻¹)	IM (%)	Proline (mgkg ⁻¹)	
Wolmera	5	28.2 ± 1.5 ^a	0.21 ± 0.1 ^a	3.8 ± 0.3 ^a	0.41 ± 3.2 ^a	16.9 ± 0.5 ^a	18.6 ± 4.3 ^a	0.71 ± 0.06 ^a	171 ± 13 ^a	
Jeldu	5	32.5 ± 2.5 ^a	0.20 ± 0.1 ^a	3.7 ± 0.1 ^a	0.38 ± 2.5 ^a	17.1 ± 0.1 ^a	15.9 ± 2.6 ^a	0.68 ± 0.00 ^a	293 ± 14 ^b	
T/Kutaye	5	29.0 ± 1.0 ^a	0.22 ± .01 ^a	3.8 ± 0.1 ^a	0.56 ± 4.0 ^a	16.8 ± 0.5 ^a	22.4 ± 0.0 ^a	0.66 ± 00 ^a	213 ± 21 ^a	
Chaliya	5	28.7 ± 0.8 ^a	0.24 ± 0.6 ^a	3.6 ± 0.1 ^a	0.23 ± 0.2 ^a	18.4 ± 1.3 ^a	15.1 ± 0.0 ^a	0.73 ± 0.70 ^a	181 ± 14 ^a	
Overall mean		29.6 ± 1.4	0.21 ± 0.16	3.7 ± 0.15	0.41 ± 1.1	17.3 ± 0.7	18 ± 1.7	0.69 ± 0.06	214 ± 15	
Overall range		25–35	0.16–0.34	3.4–3.9	0.21–0.57	16.7–21	11.2–22.4	0.56–0.87	124–307	
Standards <i>Apis</i> honey (Ethiopia)		18–23	0.22–1.52	3.2–4.5	0.14–0.30	<40	<40	0.12–0.5	>180	

Means followed by different superscripts within column are significantly different ($P < 0.05$), N = Number of Sample, SE = Standard Error. Notice: MC = moisture content, EC = Electric conductivity, PH = PH value, TA = Total acidity, HMF = hydroxyl methyl furfural, IM = insoluble matter, P = Proline.

product at certain value (do Nascimento et al., 2015). From this study, the value of moisture contents in stingless bee honey samples were recorded from 25.1 - 35.0 % with mean value of $29.6 \pm 1.4\%$ (Table 2). It was observed that there was no statistical difference ($P > 0.05$) in the value of moisture contents of stingless bee honey samples collected among the four districts. Nevertheless, the overall mean value of moisture content of the analyzed stingless bee honey sample ($29.6 \pm 1.4\%$) was higher than the threshold value of Ethiopian (QSAE, 2005) and international standard (Alimentarius, 2001) moisture content for *A. mellifera* honey which permits a maximum moisture content of 20 % in honey. The moisture contents of honey samples in the present study (25.1–35.0%) are in the range of previously reported moisture content values of stingless bee honeys produced in Venezuela, and Parana State (Brazil) to be 26–42%, and 25–36%, respectively (do Nascimento et al., 2015; Souza et al., 2006; Moo-Huchin et al., 2015). From this it is demonstrated that stingless bee honey in general has higher moisture content than *A. mellifera* honey. This may be due to the high hygroscopicity nature of stingless bee (Meliponinae) honey (Alves et al., 2005; do Nascimento et al., 2015) indicating that the moisture content in honey in turn can be influenced by intrinsic characteristic of bee species and the material they used to construct for their honey storage. For instance, stingless bees use unique cerumen made up of wax combined with propolis and plant resins to construct their honey pots for honey storage which may contribute to high moisture content as compared to honey combs of *Apis* spp. built from only beeswax content (Kek et al., 2017). The higher moisture in stingless bee honey is therefore; emphasize as precaution to store stingless bee honey in the refrigerator chambers in order to avoid its degradation or fermentation, thereby to ensure a quality product for the consumers.

The pH and total acidity parameters showed statistically no significant differences among stingless bee honey sampled from the four districts. The overall pH value ranged between 3.4–3.9 with mean value of 3.7 ± 0.15 , which is lower than commonly known standard pH value of 3.42–6.10 for honey from *A. mellifera* (Tesfaye et al., 2016), but not significantly different. These pH values are comparatively in line with past study reports for stinging bee honey pH value (3.75 and 4.21) in Nigerian (Nweze et al., 2017), Brazilian stingless bee honeys (2.93–4.08) (do Nascimento et al., 2015), and pH value for stingless bee honey in Thai which ranges from 3.10 to 3.90 (Chutong et al., 2016). As a result, the acidic and low pH value of Melipona honey is highly contribute to inhibit

the presence and growth of microorganisms (Silva et al., 2013). In addition, this low pH parameter of stingless bee honey has great importance in the process of extraction and storage of the honey so as to enhance its shelf life, texture and stability (Alves et al., 2013). Whether it is produced from *Melipona* or *Apis* bees, honey has usually low pH value, which acts effectively as antimicrobial agents against microbes used as potential substitute in reducing some infectious diseases such as coughs and wounds (Eteraf-Oskouei and Najafi, 2013; Meo et al., 2017). To this fact, Meliponan honey has higher market demand and traditional medicinal value than *Apis mellifera* honey in Ethiopia.

Total acidity is also a quality parameter corresponds to the presence of organic acids in honey that can influence honey fermentation feature (da Silva et al., 2016). In this study, there was no total acidity significant difference ($P > 0.05$) seen between the stingless bee honey samples from the four districts, in which it ranged from 16.7–21 meqkg⁻¹ with mean value of 17.3 ± 0.7 meqkg⁻¹ value (Table 2). This average values of acidity for the stingless bee honey samples are laid within the acceptable limit of international standard values (<50 meqkg⁻¹) for *Apis* honey (Alimentarius, 2001). But, the variation in acidity value amongst different honey samples can generally be attributed to the floral origin, location, harvest season, management and particularly with the bee species that produced the honey (Vit et al., 1998). Stramm (2011) also stated that the higher the acidity value indicates the presence of abundant organic acids in the stingless bee honey which can be related to the enzymatic activity of glucose-oxidase on glucose, a content of low pH that contributes for acidic characteristics. Such organic acids are an important component and indicator of good flavor and aroma in most honeys. In other case, the total acidity of honey can also be influenced by the nectar pH and mineral (ash) content derived from the soil composition or the plant species from which the final honey composition made (Périco et al., 2011).

The ash content demonstrates the abundance of mineral content in honey sources, which is mainly influenced by the nectar botanical origin, location, species of the bee and processing and handling. With this respect, Biluca et al. (2016) also indicated that the mineral content in honey is depends on nectar composition of major bee forages during the honey. In this study, the honey samples assessed for ash content exhibited no statistical difference among the locations (Table 2). The stingless bee honey samples had the ash content reaching from 0.21 to 0.57%, that is consistent with allowable range. The values were almost similar to

previous study in Brazil for which the stingless bee honey ash content ranged from 0.19 to 0.33% (do Nascimento et al., 2015). However, the average ash content of stingless bee is relatively higher ($0.41 \pm 1.1\%$) than the *Apis* honey (0.14–0.3%). This might be associated to the origin of *Meliponula beccarii* honey which is uniquely harvested from the ground in the soil where the mineral content is expected richer than in the beehives. Hence, the results of present study suggest that honey produced from native stingless bees (*M. beccarii*) in Ethiopia is richer in mineral content and good if standardized for both medicinal and nutritional consumption.

Electrical conductivity is a parameter of honey which is related to the concentration of mineral salts, organic acids, and protein in honey samples and measures all insoluble organic and inorganic substances (da Silva et al., 2016). The calculated value of electrical conductivity in the twenty pooled honey samples of stingless bees from the four study locations found to be 0.16 mScm^{-1} minimum value and 0.34 mScm^{-1} of maximum value, with overall average value of $0.21 \pm 0.16 \text{ mScm}^{-1}$, while values for *Apis* honey ranged from $0.22\text{--}1.52 \text{ mScm}^{-1}$ (Table 2). The electrical conductivity values of the explored honey samples are within the range of allowable value of international standard value (i.e., not greater than 0.8 mScm^{-1}) (Alimentarius, 2001). Similar values have been reported by Biluca et al. (2016) ranging from 0.15 to 1.34 mScm^{-1} , in the stingless bee honeys of Brazil. The non-significance differences in electric conductivity of honey samples between the four locations suggested the similarity of flora composition in similar ecological condition of West Shewa zone.

The Hydroxymethylfurfural (HMF) is a compound made from chemical reaction of some sugars and acids, and it is used as an indicator of honey freshness and good quality (Marchini et al., 2007) regarding product adulteration or improper storage conditions. The highest HMF value was recorded in honey samples collected from Toke Kutaye district (22.4 mgkg^{-1}), while the lowest record was for honey samples collected Chelyia district (15.1 mgkg^{-1}) with the overall mean of $18 \pm 1.7 \text{ mgkg}^{-1}$ (Table 2). Even though the honey samples were freshly harvested and processed, the higher HMF recorded in honey from Toke Kutaye district might be due to its long storage or aging in its natural nest. Previous study indicated that aged honey even in its natural nest has high HMF concentration (Shapla et al., 2018). However, this parameter showed values within the range of established national honey standard (Alimentarius, 2001) that permits a maximum of 40 mg kg^{-1} , which is in agreement with previous studies conducted on Ethiopian *Apis* honey HMF value (Adgaba, 1999; Belie, 2009; Tesfaye et al., 2016).

Insoluble matter is an estimation for the presence of impurities in the product (in %), and therefore, it indicates the cleanliness of honey as described by the international honey commission (Bogdanov, 2009). The honey samples analyzed in the present study showed a water-insoluble solids content ranging from 0.56% to 0.87% with a mean value of $0.69 \pm 0.06\%$. The results show that the honey samples used in the present study were clean suggesting good harvesting techniques. In overall, good manufacturing practices in production and processing are generally effective to ensure the low limits of insoluble matters in honey.

The proline content in honey is measured as a criterion for estimating the quality (Bogdanov et al., 2002) and the antioxidant activity of the honey (Meda et al., 2005; Saxena et al., 2010). It is the principal free amino acid of honey and hence measured as the level of total amino acids in honey (Iglesias et al., 2004). It may also be used for characterization of honey based on its botanical origin. The value of proline in the present study significantly different between the localities, where the highest mean value was recorded in the honey sample from Jeldu district ($293 \pm 14 \text{ mgkg}^{-1}$), while the lowest mean was recorded in the honey sample from Wolmera district ($171 \pm 13 \text{ mgkg}^{-1}$). These differences could be correlated with the degree of nectar processing by the bees themselves making the honey proline amount which is a criterion for ripeness of honey in combination with other factors such as saccharide and glucose oxidase activities (Truzzi et al., 2014). Moreover, the difference between origin of honey (nectar source) among the two locations (Wolemera and

Jeldu districts) might have revealed the difference in the proline value in the honey samples. Similar investigation by Kečkeš et al. (2013) also demonstrated that proline content of honey was significantly associated with its floral and geographical origins.

4. Conclusions

The physico-chemical parameters assessed in twenty pooled samples of stingless bee honey, principally moisture, pH, total acidity, ash content, electrical conductivity, HMF and insoluble matter are demonstrated no significance differences among the four study districts except for the value of proline. The majority of physico-chemical parameters comply with the quality standard limits of Ethiopian honey from that of *A. mellifera* as they share common characteristics of their botanical origin. However, the values of the moisture content and total acidity in this specific honey are higher when compared with the standard limits of *A. mellifera* honey emphasizing product differentiation and the need for specific regulations for the native species (*Melipona beccarii*) honey. The distinctive low pH and high acidity value of the stingless bee honey may represent its potential medicinal value because these conditions inhibit the microbial growing and favor its shelf life. As a result, stingless bee honey has greater market demand and higher price than the *Apis* honey in Ethiopia. Although the study demonstrated the first partial characteristics of honey from native stingless bee species in the country, and further study on comprehensive properties including sugar and enzyme contents in the specific honey originate from wider ecological zones is much more recommended to suggest establishing a specific quality standard for stingless bee honey.

Apart from this, the current traditional way of honey harvesting from feral colonies is destructive which reduces the volume and quality of stingless bee honey because of total nest damaging. As a result, conserving the feral colonies under suitable management is well necessary that enables sustainable species conservation for pollination services and enhance honey quality production as source of income generation for small scale farmers, particularly for traditional stingless bee hunters.

Declarations

Author contribution statement

Alemayehu Gela: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zewdu Ararso Hora: Conceived and designed the experiments.

Deresa Kebebe: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Araya Gebresilassie: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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