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Evaluation and characterization of physicochemical quality parameters of stingless Bee (*Apidae Meliponini*) honey in Amhara Region

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ABSTRACT

This study aimed to evaluate the physicochemical parameters of honey samples of stingless bees (Meliponinae). Following the honey flow season, the honey samples were collected from 9 potential districts in the Amhara region. About 41 honey samples were evaluated for physicochemical parameters of pH, free acidity, electrical conductivity, hydroxymethylfurfural, moisture content, specific rotation, ash content, color, reducing sugars, and sucrose. The Melissopalynological and sensory analysis had been used as a complement to physicochemical analysis. The composition analysis showed that the mean values of moisture content were 29.69±3.53; electric conductivity, 0.78±0.14; pH, 3.38±0.19; ash, 0.5±0.17; free acidity, 67.47±23.85; HMF, 8.38±4.47; specific rotation, 2.24±4.79; total reducing sugar, 55.27±4.24 and Sucrose, 3.17±1.5. The color of honey ranges from amber (97.6%) to dark amber (2.4%). The values were compared against the standard Apis mellifera honey quality parameters and varied for some parameters based on botanical origin. The parameters pH, electric conductivity, sucrose, ash and HMF in the honey samples comply with the requirements of the previous global reports. The moisture content, reduced sugar, and free acidity do not comply with the limit compared to Apis mellifera honey, implying that this product can undergo fermentation quickly if not properly stored after harvesting. This fact showed the necessity of proper honey harvesting, creating specific legislation for stingless bee honey, and justifies the need for a more harmonized standard of the product. The sensory evaluation result also revealed that on average the judges slightly like the stingless bee honey, which is slightly above the neutral score 5 (neither like nor dislike). The result of the melissopalynological analysis also confirmed the presence of three predominant plant species and important pollen types that confirm the honey botanical and its real geographical origin.

1. INTRODUCTION

Stingless bees are the smallest of the honey producing bees and belong to the family Apidae and subfamily Meliponinae (Danaraddi et al., 2011). They are highly social insects like honey bees living in permanent colonies, nesting in old walls, logs, *crevices and such other concealed places.* Stingless bees are small just few mm in length. The resident species nest among boulders, old walls, dead trees and tree cavities and are widely distributed in tropical and sub temperate region of the world (Suresh Kumar et al., 2012). Stingless bees are locally called as “Tazima Nib” and are uniquely identified in Ethiopia, living by harboring underground nests (Proceedings, 2018). According to Vit et al. (2013), stingless bees construct their nests as spherical pots made of 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 (Gela et al., 2021).

The composition of stingless bee honey differs from other species with some physicochemical parameters (Özbalci et al., 2013). Honey from stingless bees is more valuable, and it has been used for a long time to treat various diseases (Souza et al., 2006). Though the amount of meliponinae honey is smaller than Apis mellifera honey, its medicinal importance makes it attractive and fetches higher market values to it (Santos et al., 2015). Stingless bee honey has high local market demand, attaining better prices than the Apis honey and commercialized in different regions of the country (Gela et al., 2021). 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 (Gela et al., 2021).

Since the aim of the International Honey Commissions is the establishment of quality standards of bee products other than Apis mellifera honey (Codex Alimentarius standard for honey, 2001), stingless bee honey must be considered. The purpose of determining the physical-chemical parameters of honeys is to compare the results obtained with the standards prescribed by international and national institutions to ensure product quality (Damto et al., 2022; Grando et al., 2023; Shamsudin et al., 2019), both for domestic consumption and exports, protecting the consumer from purchasing an adulterated product. Obtaining physical-chemical parameters of honey is important not only for its characterization, but it is also essential to ensure product quality on the market (Damto et al., 2022; Grando et al., 2023; (Nascimento et al., 2015a). Because of the diversity of Meliponinae species and insufficient information regarding the characteristics of the honey produced by this bee species, Even though honey is produced worldwide, its com-position and antimicrobial activity can be variable, and are dependent primarily on their botanical origin geographical and entomological source (Nweze et al., 2017). Other certain external factors, such as harvesting season, environmental factors, processing and storage condition, also play important roles (Souza et al., 2006). Thus, determining the physical-chemical parameters of the stingless bees' honey is crucial for the process of creating specific legislation for stingless bee honey in Ethiopia. This study, therefore, aimed to evaluate and characterize the physicochemical quality of honey produced by the stingless bees (Meliponinae) in the Amhara region.

2. MATERIAL AND METHODS

The study was conducted from November 2018 to February 2019. Nine districts (Sekota, Dehana, Lasta, Tilili, Dangla, Dembecha, Banja, Bure, and Guangua) from the Amhara region were selected based on their potential. Sample kebeles were selected purposely based on their production potential, agro-ecology, and vegetation cover. A total of 41 stingless bee honey (called Tazma-mar in Amharic) samples were collected directly from local beekeepers (stingless bee hunters). A 250g of stingless bee honey was collected from each local beekeeper and kept in the refrigerator (8°C). The physicochemical analysis was performed in the Sekota Dryland Agricultural Research Center Bee Products Laboratory within a month after the samples were collected.

Determination of moisture content

Determination of the refractive index of the honey sample using a refractometer at a constant temperature near 20^0c was adopted by IHC. Conversion of the reading to moisture content (percent by mass) made using a formula or a conversion table. If the determination is made different from a temperature of 20^0c, the reading converted to a standard of 20^0c, according to the temperature corrections quoted.

Determination of pH and acidity

The pH and acidity were determined following the methodology adopted by Moraes and Teixeira (1998) The pH value was determined using a solution containing 10g of honey dissolved in 75ml of distilled water, homogenized and subjected to reading in a pH meter. Acidity was obtained by performing the neutralization of acidic solution of honey (10g of honey dissolved in 75ml of distilled water) using a sodium hydroxide solution 0.1N and 1% of phenolphthalein indicator solution until a pink color was obtained for 10 seconds. The reading of the sodium hydroxide volume 0.1N required in the titration was recorded. The result is expressed in meq/kg (milliequvalent per kilogram) using the equation:

Acidity = V (NAOH) x PA,
Where: V (NAOH) is the volume of NAOH (ml) and PA is the sample weight (g)

Determination of electrical conductivity

The electrical conductivity of a solution of 20 g dry matter of honey in 100 milliliters of distilled water was measured using an electrical conductivity cell. A 0.745 g of potassium chloride (KCl), was dried at 130°C, dissolved in freshly distilled water in a 100 ml flask, and filled to volume with distilled water. Forty milliliters of the potassium chloride solution was transferred to a beaker and the conductivity cell was connected to the conductivity meter, the cell rinsed thoroughly with potassium chloride solution and immersed the cell in the solution, together with a thermometer and reading of the electrical conductance of the solution in millisiemen after the temperature had equilibrated to 20°C was taken, as described in harmonized international commission (Bogdanov, 2009).

Determination of hydroxymethylfurfural (HMF)

The determination of the hydroxymethylfurfural (HMF) content was based on the determination of UV absorbance of HMF at 284nm. The HMF content was calculated after subtraction of the background absorbance at 336nm. The determination of HMF was based on the readings in different absorbance scales (284 and 336nm wavelengths) in a spectrophotometer. The HMF was expressed in mg/kg in the equation:

HMF = (A284 – A336) x 149.7 x 5 x D/W, Where: A284 is the absorbance at 284nm, A336 is the absorbance at 336nm; D is the dilution factor, if necessary and W is the weight of honey sample (g) (Bogdanov, 2009).

Determination of specific rotation

The specific rotation [α] 20 D is the angle of rotation of polarized light at the wavelength of the sodium D line at 20°C of an aqueous solution of 1 dm depth and containing 1g/ml of the substance (Gerginova et al., 2022). The method can be applied to all honey samples. In particular, most of the honeydew samples have positive values of specific rotation whereas nectar honeys have negative values. A clear, filtered solution of honey sample having carrez solutions, were immersed into a 2-dm polarimeter tube and read the angular rotation by Polarimeter.

Determination of ash content

The ash content of honey means the residue which is obtained by a defined procedure and expressed as a percentage by weight. The honey samples were entered in to the muffle furnace and ashed at a temperature no higher than 600°C and the residue weighed and ash content was determined by the equation:

$$Wa = \frac{m3 - m1}{m2} * 100$$

Where: Wa is the ash in g/100g honey, m1 is the weight of dish, m2 is the weight of honey taken and m3 is the weight of dish + ash

Reducing sugar and apparent sucrose determination

This method is a modification of the Lane and Eynon procedure, involving the reduction of Soxhlet's modification of Fehling's solutions. The Layne–Enyon technique as explained in AOAC (1990) was used for the estimation of reducing sugar. Briefly, 5 mL of Fehling's solution A and B were taken in a 250 mL Erlenmeyer flask with 7 mL H_2O and 15 mL of honey. With this solution, 1 mL 0.2% methylene blue indicator was added. Thereafter, titration was continued with heating the solution until decolorization of the indicator. Amount of sucrose was determined using the inversion process. In short, 50 mL of honey was taken in a 100 mL volumetric flask in which 10 mL dilute HCl was added followed by heating in a water bath, and volume made up to the mark. Again, the Layne–Enyon procedure was followed for this solution. Amount of sucrose was calculated using the formula of Saxena et al. (2010) (Kamal et al., 2019).

% Sucrose= [Total Sugar−Total reducing sugar] ×0.95

Pollen analysis

The samples were subjected to qualitative and quantitative pollen analysis following the methodology recommended by the International Commission for Bee Botany (ICBB, 1990). Following Villalpando-Aguilar et al. (2022), the pollen types recovered and identified were placed under four frequency classes as mentioned below.

i. **Predominant pollen type**: More than 45% of the total pollen grains counted.
ii. **Secondary pollen type**: Between 16 and 45% of the total pollen grains counted.
iii. **Important minor pollen type**: Between 3 and 15% of the total pollen grains counted.
iv. **Minor pollen type**: Less than 3% of the pollen grains counted.

Color

The color of the honey samples was characterized using an instrument called

Lovibond comparator 2000 + instrument

Physical characterization using sensory analysis

The sensory testing panel consists of 12 selected and trained people. Testing was performed in a sensory laboratory under sunlight using thirty grams of honey sample per person. Sensory evaluation results of each of the samples were registered on an individual basis. Each term was evaluated using a scale ranging from one to nine (extremely dislike, strongly dislike, moderately dislike, slightly dislike, neutral, slightly like, moderately like, strongly like, extremely like).

Statistical analyses

Results were reported as the mean ± standard deviation of duplicate experiments. Using the SPSS statistical package (version 23), ANOVA and post hoc multi-comparison test were used for comparison of means ($p<0.05$). Several parameters were correlated using Pearson's correlation coefficient (r) ($p<0.01$).

3. RESULTS AND DISCUSSION

3.1. Moisture content

The average moisture content of the stingless bee honey measured for collected honey samples was 29.69±3.53% (Table 1). This result agrees with the moisture content (29%) of stingless bee honey from West Shoa zone of Oromia region (Gela et al., 2021). Global published reports also indicate that moisture content of global stingless bee honey could be between 19.9 and 41.9 (Souza, 2006). The sample of honey analyzed by others also showed the average moisture ranged from 25.99% (M. bicolor) to 36.89% (M. quadrifasciata) in Brazil (Costa dos Santos et al., 2022). The moisture content reported in stingless bees could go from as low as 13.26 g/100 g to as high as 45.8 g/100 g, with a mean of 28.6 g/100 g and a standard deviation of 5.7 g/100 g (Nordin et al., 2018). However, findings showed a slightly higher threshold of 30g/100g in Guatemala, Mexico and Venezuela (Souza et al., 2006). According to this, the mean moisture content value of stingless bee honey of the Amhara Region lies within the reported moisture content range. But the moisture content exceeds the maximum permissible limit for Apis honey (20%) (Alimentarius, 2001, QSAE, 2005). Stingless bee honey has been reported to contain greater water content compared to Apis mellifera honey, this may be due to the high hygro- scopicity nature of stingless bee (Meliponinae) honey (Nascimento et al., 2015b) 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., 2018).

pH and free acidity

Honey is characteristically acidic with pH between 3.2 and 4.5, which is low enough to be inhibitory to several bacterial pathogens (Mandal and Mandal, 2011).The mean pH value of the collected honey samples was 3.38±0.19 for all samples collected from the region (Table 1). This result agrees with the pH value (3.73) of stingless bee honey from West Shoa zone (Gela et al., 2021). 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) (Nascimento et al., 2015b)and pH value for stingless bee honey in Thailand which ranges from 3.10 to3.90 (Chuttong et al., 2016). The lowest pH value 3.15 was detected from the honey of Melipona Scutellaris and the highest pH value was detected from the honey of Melipona quadrifasciata from Brazil (Nordin et al., 2018). Therefore, the mean pH value of stingless bee honey of the study areas lies within the reported pH range. There was a significant difference ($p>0.05$) in pH between honey samples obtained from different sampled locations (Table 1). This variation might be due to the variations in vegetation sources and harvesting practices.

The average acidity of stingless bee's honey samples collected from the study area was 67.47±23.85 (Table 1). The result revealed no significant difference ($p>0.05$) in free acidity between honey samples obtained from different sampled locations. This result is higher than the free acidity value (57.3) of stingless bee honey from the West Shoa zone (Gela et al., 2021). Global published reports indicate that free acidity of global stingless bee honey could vary from 5.9 to 109 (Souza, 2006). 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 (Codex Alimentarius, 2001). The stingless's bee honey analyzed also showed an average acidity ranging from 22.55 to 48.58 meq/kg (milliequivalent per kilogram) in Brazil. Brazil establishes a maximum of 50 milliequivalent per kilogram. The threshold set for Scaptotrigona species, is more tolerant at 85 milliequivalent per kilogram. In addition, a high value of total acidity may imply that at some point the honey began to ferment and that the produced alcohol was transformed into organic acids.

Electrical conductivity

The average electrical conductivity (EC) value was 0.78±0.14 (Table 1). This result is slightly higher than the EC value (0.22) of stingless bee honey from West Shewa zone of Oromia region. Global published reports indicated that EC value of stingless bee honey could vary from 0.49 to 8.77 (Nordin et al., 2018). The IHC recommended that EC should not be more than 0.8mS/cm. According to this, the mean EC value of stingless bee honey of the Amhara Region lies within the reported free acidity range. Honey from the different localities also show significant difference in their EC content ($p>0.05$) (Table 1). This variation might be due to the variations in vegetation sources and mineral content.

Ash content

The average value of ash content was 0.5±0.17 (Table 1). The result is comparable with the ash content (0.41) of stingless bee honey from West Shewa zone (Gela et al., 2021). Past studied reports indicated that ash value of global stingless bee honey could vary from 0.01- 1.18 (Souza et al., 2006). In agreement with this result, (Nordin et al., 2018) reviewed that the ash content of stingless bee honey ranging from 0.01g/100 g (Melipona scutellaris from Brazil) to as high as 3.1g/100 g of honey (Tetrigonamelanoleuca from Thailand) (Chuttong et al., 2016) with the mean value of 0.4g/100g. Both the IHC and Vit et al. (2004) set the ash content threshold at not more than 0.5g/100 g for a good quality honey from both Apis mellifera and Meliponinae species. Therefore, the mean ash content of stingless bee honey lies within the reported ash content range. Honey from the different localities of study area also showed significant differences in its ash content ($p>0.05$). This variation might be due to the variations in vegetation sources and mineral content.

HMF

The average HMF contents of collected honey samples were determined to be 8.38±4.47mg/kg (Table1). This result is much lower than the HMF content (18) of stingless bee honey from West Shewa zone (Gela et al., 2021). Though there is no Ethiopian standard for HMF value of stingless bee honey, published reports indicate that HMF value of global stingless bee honey could vary from 0.4 - 78.4 (Souza et al., 2006). HMF content has been set to be not more than 40 mg/kg (milligram per kilogram) in a good quality Apis mellifera honey (Codex Alimentarius, 2001). As a result, the mean HMF content value of stingless bee honey of the Amhara Region lies within the reported HMF content range.

In reality, though the naturally higher moisture content of Stingless bee honeys directly influences their HMF content, stingless bee honeys have been reported to be more resistant to HMF formation than Apis mellifera honey when subjected to heat treatment. Honey from the different localities also show significant differences in their HMF content ($p>0.05$) (Table 1). This variation might be due to the variations in storage practices, depending on the pH and storage temperature.

Total reducing sugar and sucrose

The mean reducing sugars content of the study areas honey is 55.27±4.24% (Table 1). This indicates that the reducing sugars content of honey of the study area is lower than the minimum limits (58%) reported at (Souza et al., 2006) for global stingless bee honey. According to the standard set by IHC, a good quality honey should have reduced sugar not less than 60g/100g in the case of Apis mellifera honey. Stingless bees have been reported to contain lower sugar content compared to Apis mellifera honey. The low reducing sugars content of the study areas honey may be attributed to its high moisture contents. Honey from the different localities also showed significant differences in its total reducing sugar content ($p>0.05$) (Table 1). This variation might be attributed to the variation in moisture content. Adulteration of honey can be detected by measuring its sucrose content. The mean sucrose content of the study areas honey is 3.17±1.5% (Table 1). Though there is no Ethiopian standard for sucrose content of stingless bee honey, published reports indicate that Sucrose content of global stingless bee honey could vary from 1.1 to 4.8. According to the standards set by the IHC (Bogdanov, 2009). A good quality honey should have a sucrose content that is not more than 5g/100g. Vit et al. (2004) has increased the threshold for sucrose a little higher at 6 g/100 g to accommodate the slightly higher sucrose content reported in stingless bee honey. For Malaysian standards, the maximum threshold of 7.5 g/100 g sucrose was set to regulate honey adulteration. Therefore, the mean sucrose content of stingless bee honey of the Amhara Region lies within the reported range. The amount of sucrose in the honey samples obtained from the different localities also did not show significant difference ($p>0.05$) (Table 1).

Specific rotation

Honey has the property of optical rotation, i.e., its composition includes optically active substances that rotate the polarized light at a certain angle [α]. The specific angle of honey depends on the quantity and ratio between the main sugar constituents the levorotatory fructose (−) and the dextrorotatory glucose (+). However, different low quantity organic components in honey with large positive or negative rotation angles could significantly contribute to its specific rotation. It has been observed that a number of honeydew honeys are dextrorotatory, differing from nectar honeys, which have negative specific angles (Gerginova et al., 2022). The average specific rotation value of the collected samples was 2.24 (Table 1), So it is predominantly dextrorotary showing that it becomes a potential differentiation criterion from Apis mellifera of Ethiopia as these honeys always retain a levorotatory character. From about 41 honey samples analyzed for their specific rotation the majority of the samples 25/41 (about 60.98%) showed a positive value. This indicates that the presence of sugars and other compounds with rotation capacity. Similar with the value (2.3) reported for Vensuelan stingless bee honey. Honey from the different localities also show significant differences in their specific rotation ($p>0.05$) (Table 1). This variation might be attributed to the variations in vegetation sources, sugar and mineral content.

Correlation among some physicochemical parameters

The correlation matrices showed significant correlations between some of the physicochemical parameters. In stingless bee honey samples, a strong negative correlation was found between EC and some parameters (HMF and total reducing sugar: Table 2). The correlation matrices of the honey samples also showed Positive correlations between EC and Ash content. Ash and electrical conductivity values depend on the mineral content of the honey: ash gives a direct measure of inorganic residue after carbonization, while electric conductivity measures all ionizable organic inorganic substances (Rysha et al., 2022). The pH value had negative correlations with total reducing sugar and sucrose content (Table 2). Strong positive correlation was established between HMF and reducing sugar while HMF had negatively correlated with specific rotation (Table 2).

Table 1: Mean values of physicochemical parameters result of sampling areas

Location	pH	Electrical Conductivity	Moisture Content	HMF	Ash	Free acidity	Specific Rotation	Total Reducing Sugar	Sucrose
Sekota	3.25±0.16bc	0.76±0.53bc	25.34±0.96c	6.13±0.67b	0.59±0.11ab	55.52±11.68	0.71±2.56cd	58.58±2.04a	3.69±2.54
Dehana	3.45±0.19abc	0.7±0.27c	27.76±3.5ab	13.3±2.9a	0.62±0.14ab	55.49±15.25	-2.3±2.56d	59.87±0.44a	3.53±1.97
Lalibela	3.23±0.16c	0.67±0.12c	30.85±3.87a	14.48±3.69a	0.35±0.14cd	75.57±47.25	-1.4±5.11d	59.16±1.74a	3.86±1.29
Tillili	3.54±0.19ab	0.84±0.16abc	30.97±1.84a	4.45±1.38b	0.57±0.14abc	72.22±12.85	6.31±1.81ab	52.41±1.98bc	2.35±0.5
Dembecha	3.41±0.21abc	0.97±0.11a	30.0±2.83ab	6.28±0.21b	0.68±0.29a	72.18±7.06	9.73±0.66a	48.68±1.5d	3.23±0.7
Dangla	3.43±0.7abc	0.9±0.1ab	31.43±2.03a	6.11±1.39b	0.41±0.07bcd	69.56±3.26	5.8±2.3ab	49.99±0.73cd	3.65±0.77
Banja	3.34±0.36bc	0.78±0.06abc	32.75±4.31a	7.48±2.53b	0.56±0.21abc	71.75±11.59	4.46±4.37bc	53.8±2.05b	1.97±0.68
Bure	3.67±0.29a	0.95±0.01a	28.65±1.62ab	5.09±0.63b	0.5±0.07abcd	63.58±0.56	6.9±0.18ab	52.23±1.34bc	2.31±0.33
Guangua	3.38±0.06abc	0.69±0.09c	32.7±4.39a	6.71±1.19b	0.3±0.07d	74.9±25.4	-1.97±1.49d	51.56±1.23bc	2.38±0.11
Total	3.38±0.19	0.78±0.14	29.69±3.53	8.38±4.47	0.5±0.17	67.47±23.85	2.24±4.79	55.27±4.24	3.17±1.5
Proposed quality standard stingless bee honey)	3.15-4.5	0.49-8.77	19.9-41.9	0.4-78.4	0.01-1.18	5.9-109	58-75.5	1.1-4.8	
National acceptance (A.mellifera)	3.5-4.5	0.8	Max 21	Max 40	0.6	Max 40	-ve	Min 65	Max 5

Table 2: Correlation analysis of physicochemical parameters

	PH	EC	Moisture content	HMF	Ash	Free acidity	Specific Rotation	Reducing Sugar	Sucrose
PH	**								
EC	.229	**							
Moisture Content	-.142	.077	**						
HMF	-.256	-.503**	.033	**					
ASH	.286	.337*	-.329*	-.299	**				
Free Acidity	-.225	.002	.492**	-.111	-.019	**			
Specific Rotation	.251	.684**	.337*	-.639**	.369*	.333*	**		
Reducing Sugar	-.352*	-.504**	-.429**	.628**	-.015	-.218	-.696**	**	
Sucrose	-.490**	-.119	.003	.227	-.153	.038	-.135	.349*	**

Color analysis

Honey is often judged according to its color. Color and brightness contribute to the aesthetic value of the honey, which is important to the consumer. Besides, color is very important, because this plays an important role to determine its market value. The color of 97.6% of honey samples range from amber to dark amber. All the color of 100% of honey samples from the midland was amber. It Agrees with report that the color of the honey samples ranged from light amber (0.302 nm) to dark amber (1.225 nm) in Brazil (Nascimento et al., 2015b). The honey color depends almost exclusively on floral origin. Generally, dark-colored honey has more minerals than light- colored honey. Studies show that darker honeys may have four to six times more minerals than light-colored honeys, especially manganese, potassium, sodium and iron (Nascimento et al., 2015b).

Table 3: Summary of Honey colors from P-Fund readings

Colour Determined	Pfund Scale	Agroecology and their Percentage		
		Mid land	Highland	Total
Extra white	9-17mm			

White	18-34mm			
Extra light amber	35-48mm			
Light amber	49-83mm			
Amber	84-114mm	100	94.1	97.6
Dark amber	Greater than 114		5.9	2.4

Sensory evaluation

The effects of the main factors on mean acceptance of study honey varieties with respect to color, smell thickness, mouth fill (texture), taste, sweetness, after taste and overall acceptance are presented in Table 3. The score of the color acceptance test ranged from 4.64 (Lalibela location of Eastern Amhara region) to 5.45 (Bure location of Western Amhara Region) on a scale of 9 with a significant difference among different locations. The judges in this sensory evaluation determined the honeys from the Denbecha location as being darker in their color than those from other locations (Table 3). Sensory analysis of honey may be used as a complement to physico-chemical and pollen analyses. It is used to confirm quality, verify the absence of defects, and evaluates the conformity to established sensory profiles of unifloral honeys and also to understand consumer preferences (Araujo et al., 2020). Fructose and glucose are responsible for honey's sweetness and viscosity, but the ratio of fructose to glucose can affect its taste and consistency. Minerals such as calcium, iron, and potassium, as well as enzymes, can contribute to the flavor, color, and aroma of honey (Vîjan et al., 2023)

The floral source has also influenced the aroma of the honey significantly among the honey samples from different locations. In this regard, honey from Banja, Guangua, Bure and Dangla was found to be significantly better preferred honeys in terms of their aroma attributes than honey from Lalibela. Honey from Sekota and Bure were perceived as being more intense regarding the attributes of thickness and mouth fill (texture) when compared to Lalibela honey. The judges determined that the honeys from the majority of locations did not significantly differ in taste, sweetness and after taste attributes, but all are more intense than Lalibela honey (Table 2). The lesser intensity of the taste attributes described in the sensory evaluation for honeys from Lalibela honey compared to those from other locations appeared to be associated with their lower pH and EC related to the higher mineral content (Table 1). The sweet taste of honey is attributed to its higher fructose content and lower pH.

Generally, in all attributes Stingless bee honey produced from Sekota and Dehana from Eastern Amhara Region and Stingless bee honey produced from Bure, Banja, and Dangla from Western Amhara Region perceived as more intense in overall acceptability by the Judges. The results in Table 3 revealed that on average the judges slightly like the stingless bee honey, as the higher acceptance mean was 6.13, which is situated slightly above the neutral score 5 (neither like nor dislike). In agreement with this result reported the neutral acceptance mean score of the stingless honey sample with a maximum of 6.2 score of Melipona favosa honey with the country of origin from Venezuela (Iverson and Dervan, 1993), mean score of 5.1 of Tetragonula carbonaria honey with the country of origin from Australia and mean score of 5.6 scores for Melipona scutellaris honey with the country of origin from Australia given by the Spanish consumers (Vit et al., 2012).

Table 4: Sensory evaluation of stingless bee honey collected from different locations of Amhara region

District	Color	Smell	Thickness	Mouth fill	Taste	Sweetness	After taste	Overall acceptability
Sekota	5.14±2.02a	4.96±2.19abc	5.94±1.73a	5.92±1.48a	5.82±1.71a	5.91±1.8a	5.87±1.6a	6.13±1.49a
Dehana	5.16±2.07a	4.64±2.14abc	5.29±2.29ab	518±2.03ab	5.33±2.13a	5.36±2.14a	5.35±2.0ab	5.73±1.86ab
Lalibela	4.64±2.16a	3.99±1.93c	4.74±1.96b	4.32±1.92c	4.03±2.03b	4.03±2.05b	4.36±2.1c	4.32±2.1c
Tilili	4.77±1.86a	4.61±1.69abc	5.08±1.71ab	4.87±1.46bc	5.1±1.66a	5.03±1.5a	5.03±1.5abc	5.1±1.47bc
Dembecha	3.41±1.87b	4.23±1.72bc	5.32±1.7ab	4.91±1.31bc	5.09±1.54a	5.00±1.41a	4.82±1.14bc	5.05±1.09bc
Dangla	5.09±2.44a	5.00±1.82ab	5.33±1.53ab	5.42±1.68ab	5.73±1.64a	5.73±1.75a	5.58±1.68ab	5.61±1.60ab
Banja	4.73±2.12a	5.32±1.64a	5.14±1.46ab	5.32±1.52ab	5.95±1.46a	5.73±1.28a	5.45±1.68ab	5.73±1.49ab
Bure	5.45±1.65a	5.14±1.52ab	5.82±1.43a	5.82±1.68a	5.82±1.87a	5.73±1.45a	5.73±1.28ab	5.86±1.58ab
Guangua	5.27±1.6a	5.24±1.60a	5.3±1.36ab	5.52±1.37ab	5.15±1.5a	5.03±1.47a	5.21±1.62abc	5.52±1.44ab
Total	4.89±2.04	4.67±1.92	5.28±1.79	5.15±1.74	5.17±1.9	5.15±1.88	5.19±1.79	5.34±1.77

Melissopalynological analysis

Monofloral honey is where the bees have been foraging predominantly on one type of plant, and is named according to that plant (FAO). The result of about 41 studied honey samples in this work shows that from 27 honey samples only 3 pollen types (Eucalyptus camaldulensis, Guizotia abyssinica and Bidens pachyloma) were identified as monofloral honey with a predominant level ranging from 57.71% (Bidens pachyloma) to 77.79% (Eucalyptus camaldulensis) (Table 5).

Among the samples studied, Eucalyptus was the most common dominant pollen types found in the majority of the honey samples (17/27), an indication that the pollen and nectar of these plant species are important bee foods, while Bidens pachyloma was found to be less common pollen types found in the studied samples (4/27) which were found to be dominant. The second major pollen contribution is secondary pollen type of honey which ranges from 16 - 45% of pollen count due to this classification the studied honey samples are listed according to secondary pollen honey in (Table 5). Secondary pollen types (>16 - 44%) identified from the samples in order of decreasing significance were Eucalyptus camaldulensis, Hypericum quartinianum, Gravlliea robusta, Bidens pachyloma, Guizotia abyssinica, Echinops, Viciafaba, Sorghum bicolor, Caesalpinia decapetala and Hypoestes trifolia with their respective percentage shown in the Table 5 below. Among the secondary pollen types identified in the sample, Bidens pachyloma was the most common (15), while Hypoestes trifolia was found to be less common secondary pollen types found in the studied samples (only in 1 sample) (Table 4).

The important minor pollen contributions are the third pollen type of honey which ranges from 3 - 15% of pollen count due to this classification the studied honey samples are listed according to important minor pollen in Table 6. Important minor pollen type (3 - 15% of the count) identified from the samples in order of decreasing significance were Hypericum quartinianum, Vicia faba, Hypoestes trifolia, Caesalpinia decapetala Schinus molle, Bersama abyssinica, Gravillia robusta, Bidens pachyloma, Eucalyptus camaldulensis, Echinops, Zea mays, Sorghum bicolor, and Croton macrostachyus with their respective percentage shown in the Table 5 below. Among these secondary pollen types identified in the sample, Bidens pachyloma was the most common Important minor pollen type found in the honey samples (9), while Hypericum quartinianum was found to be less common Important minor pollen type found in the studied samples (only in 1 sample) (Table 4).

The presence of these prominent and important pollen types in these samples has confirmed that the honey samples were of botanical origin and also a true indication of their geographical origin, while the rest pollen types were categorized as important minor and minor pollen types. Minor pollen type (less than 3% of the count) identified from the samples were Acacia brevispica, Acacia Senegal, Acacia seyal, Becium grandiflorum, Bersama abyssinica, Caesalpinia decapetala, Echinops spps, Zea mays, Ocimum bacilicum, Sorghum bicolor and Vernonia spps with their respective percentage shown in Table 7 below.

Table 5: Identified predominant pollen types (more than 45% of the count)

Botanical sources	Family name	No of samples	Mean pollen percentage	Range	Agroecology
Guizotia abyssinica	*Asteraceae*	6	67.05%	45.53 - 91.77	Mid & highland
Bidens pachyloma	*Asteraceae*	4	57.71%	53.2 - 61.15	Midland
Eucalyptus camaldulensis	*Myrtaceae*	17	77.79%	59.15 - 92.98	Mid & highland
Total	27/41 (65.58%)				

Table 6: Identified secondary pollen types (16 - 45% of the count)

Botanical sources	Family Name	No of samples	Mean Pollen percentage	Range	Agroecology
Bidens pachyloma	*Asteraceae*	15	29.03	16.4 - 44.83	Midland
Caesalpinia decapetala	*Fabaceae*	2	22.93	22.8 - 23.06	Midland
Echinops	*Asteraceae*	2	27.55	17.62 - 32	Highland

Eucalyptus camaldulensis	*Myrtaceae*	2	31.01	30.79 - 44.83	Highland
Gravillea robusta	*Proteaceae*	3	29.77	22.86 - 27.36	Midland
Guizotia abyssinica	*Asteraceae*	7	28.5	16.4 - 35.93	Mid & highland
Hypericum quartinianum	*Guttiferae*	6	30.52	17.64 - 42.1	Mid & highland
Hypoestes trifolia	*Acanthaceae*	1	20		Highland
Sorghum bicolor	*Poaceae*	2	27.26	16.7 - 21.21	Midland
Vicia faba	*Fabaceae*	4	27.39	16.4 - 41.17	Midland

Table 7: Identified minor pollen type (3 - 15% of the count)

Botanical sources	Family Name	No of samples	Mean pollen percentage	Range	Agroecology
Gravillea robusta	*Proteaceae*	5	9.15	4.13 - 157.17	Midland
Bersama abyssinica	*Francoaceae*	6	10.07	6.8 - 13.6	Midland
Bidens pachyloma	*Asteraceae*	9	8.72	3.76 - 15.5	Midland
Caesalpinia decapetala	*Fabaceae*	3	11.25	8.3 - 12.85	Midland
Croton macrostachyus	*Euphorbiaceae*	2	4.15	3.6 - 4.7	Midland
Echinops spps	*Asteraceae*	7	7.3	3.15 - 14.65	Midland
Eucalyptus camaldulensis	*Myrtaceae*	7	7.67	3.58 - 13.1	Mid & highland
Hypericum quartinianum	*Guttiferae*	1	13.3		Midland
Hypoestes trifolia	*Acanthaceae*	3	12.16	5.3	Midland
Zea mays	*Poaceae*	6	6.8	3.3	Highland
Schinus molle	*Anacardiaceae*	5	10.71	7 - 15.47	Midland
Sorghum bicolor	*Poaceae*	2	5.21	3.72 - 6.7	Midland
Viciafaba	*Papilionaceae*	5	12.7	10.12 - 14.4	Mid & highland

Table 8: Identified minor pollen type (less than 3% of the count)

Botanical sources	Family Name	No of samples	Mean Pollen percentage	Range	Agroecology
Acacia brevispica	*Fabaceae*	1	0.8		Highland
Acacia senegal	*Fabaceae*	1	0.5		Highland
Acacia seyal	*Fabaceae*	3	2.3	2.3 - 2.7	Midland
Becium grandiflorum	*Lamiaceae*	3	2	1 - 2.7	Highland
Bersama abyssinica	*Francoaceae*	1	2.3		Midland
Caesalpinia decapetala	*Fabaceae*	1	2.8		Highland
Echinops spps	*Asteraceae*	1	1.3		Midland
Zea mays	*Poaceae*	3	1.42	1.3 - 1.65	Midland
Ocimum bacilicum	*Lamiaceae*	2	1.58	1.25 - 1.9	Midland
Sorghum bicolor	*Poaceae*	1	2.06		Midland
Vernonia spps	*Asteraceae*	1	2.18		Midland

4. CONCLUSION AND RECOMMENDATION

The parameters pH, electric conductivity, sucrose, ash and HMF in the honey samples comply with the requirements of the previous global reports. The parameters of moisture content, reducing sugar and free acidity do not comply with the limit compared to Apis mellifera honey. This fact points to the necessity of proper honey harvesting, creating specific legislation for stingless bee honey and justifies the need for a more harmonized standard of this food product, which will include stingless bee honey from around the country. The study has confirmed that the stingless bee honey produced in the Amhara Region is with in acceptance score supported by the sensory panel evaluation. This study has also led to the identification of major plants visited by stingless bees in the study area. In this research, the possibility of producing three monofloral honey (Gizotia, Bidens and Eucalyptus honey) from stingless bee honey in Amhara region could also be explored. Furthermore, on the trehalulose sugar content and other nutritional quality of stingless bee honey, scientific studies need to be conducted.

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