

Proximate and Mineral Composition of *Meliponula beccarii* Bees Honey from Potential Areas of Oromia

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Abstract

Stingless bee honey from various areas has its own unique components because of difference in flora, climate condition, and different methods of purifying and storage condition of honey. The objective of this study was to investigate the proximate and mineral composition of *M. beccarii* honey from different areas including Tokke kuttaye, Chalia, Gera, Bacho, Alle and Didu districts of Oromia region in Ethiopia. Moisture, ash, protein and fat contents were determined using oven drying, muffle furnace, Kjeldahl and Soxhlet extractor methods, respectively. Mineral composition of honey was analyzed using Atomic Absorption Spectrometry method after digestion. The proximate results of moisture, ash, protein, fat, and carbohydrate were 23.20-34.88, 0.14-0.34, 0.11-1.42, 0.11-0.16 and 63.02-75.23 g/100g, respectively. Mineral composition of stingless bee honey for Na, K, Ca, Mg and P range was 59.46-490.60, 1351.60-5892.11, 67.58-426.63, 20.16- 96.57 and 0.1-0.18 mg/kg, respectively. The results have shown that this honey is a good source of carbohydrate and potassium because the contents of these macronutrients were found to be high in the honey when compared to others. This could show the honey can be branded and supplied in honey supply chains especially at supermarkets. The results of this study also indicated that the mineral contents of honey varied among the locations which might be caused by the natural absorption of minerals by plants from the soil and the environment.

Key word: Honey; *M. beccarii* bees; Mineral; Proximate; Underground soil honey.

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1. Introduction

The honey of stingless bee is produced from the nectar of flowers that bees collect, transform, combine with specific substances of their own, store and let it mature inside pots in the colonies [1]. Honey from different locations possesses a unique combination of different ingredient and properties due to the variety of floral origin, climatic conditions, and application of different processing technologies and storage conditions of honey [2]. In Ethiopia, honey produced by stingless bees is considered to be important in traditional medicine for the treatments of wound, respiratory ailments, surface infection, diarrheal and various diseases in line with treatments with other honey [3]. These attributes made the consumers to afford to buy stingless bee honey in comparison with *A. mellifera* honey. As the result of medicinal value of stingless bee honey, it has high market demand, achieving higher prices than the honey produced by bees of the genus *Apis*, and commercialized in different regions of Ethiopia. The antibiotic activity of stingless bee honey in medicinal products is depended on its physical and chemical properties that determine its quality standard [4; 5].

Limited studies are available on characterization of stingless bees honey as compared to the extensive literature available on *A. mellifera* honeys. It has valuable nutritional and medicinal qualities in Ethiopia. Although the use of honey has been of great importance in Ethiopia, there is little or no information on the proximate and mineral contents of stingless bee honey produced in artificial pots hives and underground nests of stingless bee. The stingless bee honey is well known for its valuable nutritional and medicinal qualities. These properties, among other benefits, are determined by the mineral components while the content of honey depends on its type and botanical origin. The sources of the mineral components of honey are primarily comes from nectar, honeydew and the pollen grains but not the bee, as previously suggested by [6]. The mineral content in stingless bee honey is dependent on the natural absorption of minerals by plants from the soil and the environment. The absorption of minerals can also occur artificially, influenced by the composition of artificial sources such as sugar or syrup fed on by the bees. The mineral content of honey is usually between 0.04 and 0.20%, and it contributes to the color of the honey, which may vary from light to dark [7] in honey collected from Malesia. Recently, there is growing interest in use of stingless bee honeys for various purposes and therefore it is important to find quality standard parameters, showing the high compositional and functional variability which is attributed by ethnological and botanical sources, geographical origin and harvesting season [8].

However, there is little information on the proximate and mineral contents of stingless bee honey from Ethiopia. Thus, it is crucial to have information on composition of stingless bee honey for investors, consumers, traders and researchers in order to plan future work. Therefore, the composition of the honey is important not only for its characterization [9], but it is also essential to ensure product quality on the market and to generate information on the bee products. Thus, the aim of this study is to investigate the mineral contents and proximate composition in honey produced by stingless bees from potential areas of Oromia region, Ethiopia.

2. Materials and Methods

2.1. Study area

This study was conducted in potential districts of West Shoa, Jimma and Ilu Abba bora zones of Oromia region.

These districts were Chalia and Tokke Kuttaye from West Shoa, Gera from Jimma and Alle, Bacho and Didu from Ilu Abba Bora zones (Fig. 1).

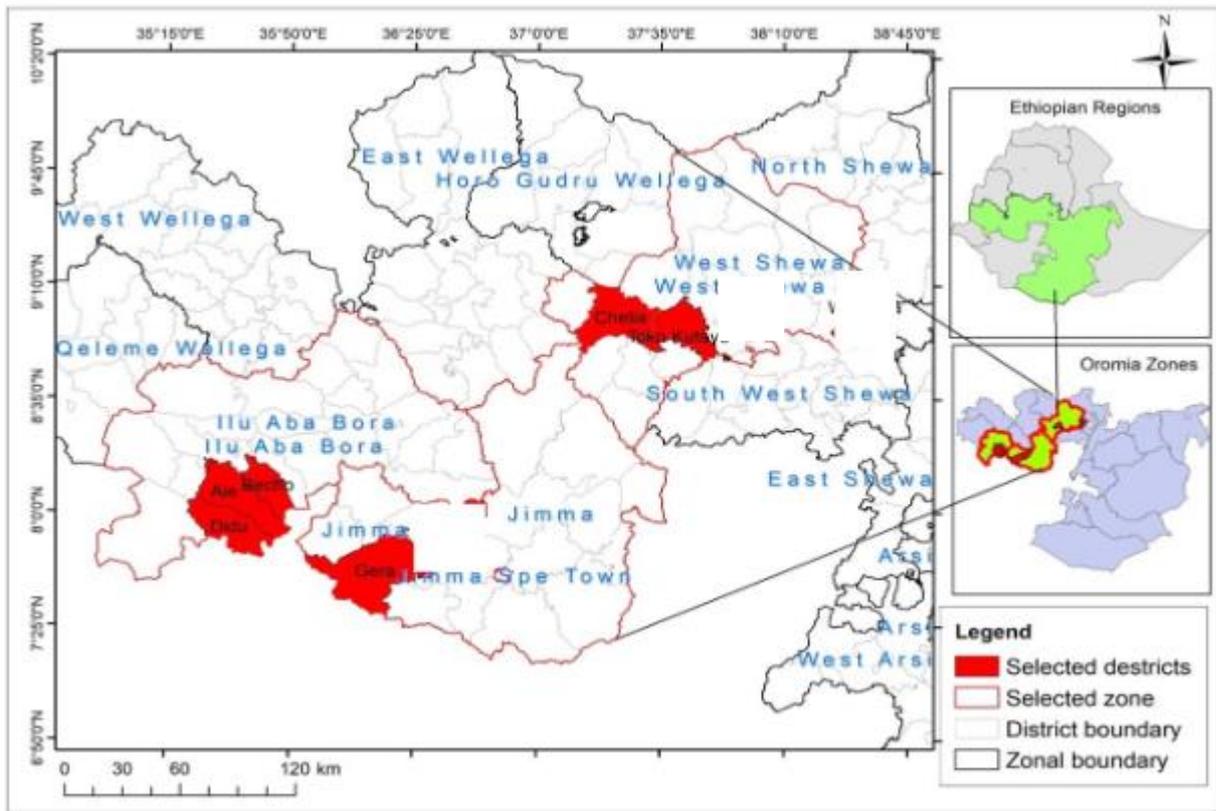


Figure 1: Map of study areas

2.2. Stingless Bee Honey (*M. Beccarii*) Sample Collection

The stingless bee honey samples were collected following the honey flow season and, thirty (30) stingless bee honey samples were collected from each potential district of the study areas. Fresh and pure honey samples (Fig 2.) were collected directly from sealed honey pots with disposable syringes and it was sieved to remove impurities and placed in uncontaminated plastic containers. Finally, it was properly packed in boxes and brought to the Holeta Bee Research Laboratory, where it was stored under refrigeration at 4°C until analyses.

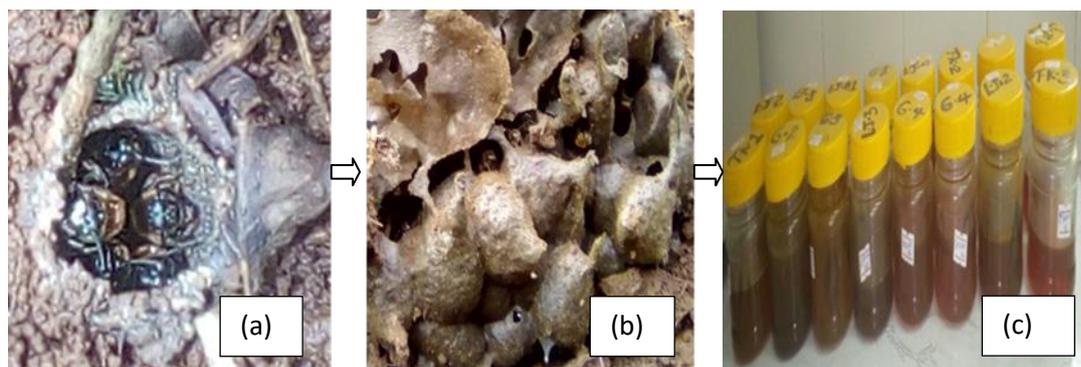


Figure 2 a): Stingless bees on their entrance on nest b) Stingless bee's nest) (c) Harvested stingless bee honey

2.3. Proximate Analysis of honey

2.3.1. Crude protein Analysis

The total nitrogen content was determined by the Kjeldahl method [10]. About 1 gm of honey sample was measured in Tecator tubes and they were placed in the Tecator rack. Three blanks were used. About 6 ml of conc. H_2SO_4 was added and 3.5 ml of H_2O_2 was added step by step. The tubes were shaking a few times after the violent reaction has ceased and put back into the rack. Three grams of $CuSO_4$ and K_2SO_4 catalytic mixture were added and the tubes were let to stand for 15 minutes before digestion. The sample tubes were placed in a digester after the working temperature ($370\text{ }^\circ\text{C}$) has reached and the digestion process has continued until clear solution was observed. The sample tubes were allowed to cool in fume hood. About 20 ml of distilled water was added to avoid precipitation of sulphate and then 40 ml of NaOH solution was added. A conical flask of 250 ml containing 25 ml of boric acid, 25 ml of distilled water and an indicator solution was placed under the condenser of the distiller with its tip immersed into the solution. The solution was titrated using 0.1 N HCl till the color of the solution changes to reddish and the total volume of the HCl was recorded for blank and each sample.

$$\%N \text{ in stingless bee honey} = (V_{HCl} \times N_{HCl} \times 14.0 \times 100) / (1000 \times W_o) \quad (1)$$

Where; V=volume of HCl consumed (ml), N=the normality of the HCl used, W_o =Sample weight on dry matter basis, 14.0=the molecular weight of atomic nitrogen

$$(\%) \text{ Crude protein} = \%N \text{ in honey} \times 6.25 \quad (2)$$

2.3.2. Fat content

The fat content was determined by using acid hydrolysis method based on the AOAC Method [11]. About 5 gm of honey sample of stingless bee was measured in the thimbles and covered with a layer of fat free cotton. The thimbles were put in the extraction chamber. The extraction cylinders were taken out of the desiccators and put on the bracket. About 50 ml of ether was added into the extraction cylinders and moved into the heating plank and the extraction can take for about 4 hours. The extraction cylinders were disconnected and put in a drying oven at 70°C for about 30 minutes. The cylinders was taken out of the oven and cooled in desiccators for 30

minutes. The weight of the cylinders was measured immediately after they have taken out of the desiccators. The percentage of fat in the sample was calculated using the following formula:

$$(\%) \text{ Crude Fat content} = ((W_2 - W_1) / W) \times 100 \quad (3)$$

Where; W_1 = Weight of the extraction cylinder, W_2 = Weight of the extraction cylinder plus the dried crude fat, W = Weight of the sample

2.3.3. Carbohydrate content of the honey

Carbohydrate value of the honey samples was estimated using the method [12].

$$\% \text{ Carbohydrate in honey} = 100 \% - (\% \text{ MC} + \text{Crude Fat} + \text{Protein} + \% \text{ Ash}) \quad (4)$$

The energy values of the samples were determined using the following calculation as follows:

$$\text{Energy (KJ/100g)} = 4.186 [(\% \text{ Cru. Pr.} \times 4) + (\% \text{ Crude Fat} \times 9) + (\% \text{ Carbo} \times 4)] \quad (5)$$

2.3.4. Ash content in stingless bee honey sample

About 5 gm stingless bee honey sample (W) was measured in each crucible (W_2) based on method [13]. The sample was charred on a hot plate under a fume hood until the smoke stopped and the samples were asked in a muffle furnace at 550 °C for 5 hours. A crucible was cooled in desiccators and their weight was measured (W_1) and the ash content was determined by using the following equation.

$$\% \text{ Ash content in stingless bee honey} = 100 \times (w_1 - w_2) / w \quad (6)$$

Where; W_1 = Mass of the ash and crucible, W_2 = Mass of empty crucible, and W = Mass of the sample taken for the test

2.4. Mineral analyses in stingless bee honey

Determination of Na, K, Ca, and Mg were undertaken by [10] quantization with some modification. Briefly, about 1.5 g of the stingless bee honey sample was digested with 4 ml HNO_3 (65% v/v) and 2 ml H_2O_2 (30% v/v). A blank control was digested in a similar manner. The digestion program began initially at 500 W, ramped for 1 min, and was held for 4 min. The second step began at 1000 W, ramped for 5 minutes, and was followed by a hold period for 5 min. The third step began at a power of 1400 W and then ramped for 5 minutes with a hold period of 10 minutes. The digested samples were diluted to a final volume of 50 mL with double deionized water for the analysis of all mineral types. The standard solutions of the elements used for calibration were prepared by diluting stock solutions (1000 mg/L) of each element. The serial dilutions for each of the elements analyzed were freshly prepared on the day of analysis. Atomic absorption spectrometry was used to read the absorbance at selected wave length. Mineral content of the sample was read from relevant calibration curve.

$$\text{Mineral content (ppm)} = [(cs-cb) \times \text{Vol.}] / (10 \times m \times 1000) \quad (7)$$

Where m= weight (gm) of sample, Vol. =50ml= volume of extract, cs = concentration (ppm) of sample solution, cb = Concentration (ppm) of blank solution.

2.5. Phosphorus determination in stingless bee honey

Phosphorus was determined using the molybdovanadate method [14]. About, 5 ml of honey were measured from the sample digested for protein determination and placed in a 100 ml volumetric flask. Ten (10) ml of the molybdate and vanadate solution were added to the samples. After 30 minutes the color was measured at 460nm wavelength using spectrophotometer. Data from the blank absorbance, sample and standard were used to calculate phosphorus content using the following formula:

$$P \text{ (ppm)} = (\text{conc}_1 * \text{vol}_1 * \text{vol}_2 * \text{mcf}) / (S * A) \quad (8)$$

Where: conc_1 = P concentration in sample digest read from the Curve, ppm, Vol_1 = volume of the digest, Vol_2 = volume of the dilution, S = weight of the stingless bee honey in gm and A = Aliquot

2.6. Statistical Analysis

The statistical analysis was performed by using statistical package of social science (SPSS) version 20. One-way analysis of variance (ANOVA) and Tukey were used to compare the mean difference between various parameters of each honey group at $p < 0.05$.

3. Result and discussion

3.1. Moisture content

There are significant differences between the honey from Didu and other districts. The moisture content of the honey samples investigated fall in between $23.70 \pm 0.50\%$ to $33.14 \pm 1.14\%$. Stingless bee honey samples collected from Alle district has shown that the highest moisture content followed by samples from Gera ($31.90 \pm 0.50\%$) and the lowest from Didu district (24.20% w/v) origin (Table 1). A report by [15] stated that the moisture value of stingless bee honey collected from different botanical origins ranged from 27.00% to 31.00%. A similar study conducted stated that the moisture content of stingless bee honey ranged from 28.30%-33.70% [16]. The difference can be occurred due to variation in botanical and geographical origin. According to East African stingless bee honey standard, moisture content should not exceed 32 % (m/m). Meanwhile, the moisture content of all the samples tested fall within the limit [17] but that of Alle district is out of the standard which may be due to the botanical and geographical origin of the area. This high moisture content can affect the age of honey. The shelf-life of honey was greatly affected by the moisture value as it will promote fermentation [18, 19].

3.2. Ash content

There was no statistical significant difference between the results of ash contents in the study locations but numerical difference was observed among the study areas. The stingless bee honey samples obtained from Bacho district ($0.30\pm 0.05\%$) has the highest ash content while that of Alle district ($0.14\pm 0.045\%$) had the lowest as compared to each other (Table 1). The ash content of the honey samples varied from 0.14 ± 0.045 to 0.30 ± 0.05 % (m/m). The ash contents obtained from these study areas were all within the limits of maximum 0.50 % (m/m) as stated by the East African Standards [19]. Study results reported the ash value of stingless bee's honey was recorded a low amount of 0.08 % and ranged from 0.15 ± 0.01 to 0.67 ± 0.00 % [20, 21]. However, the results were significantly different from reports stated the ash value of all the stingless bee honey obtained in their study were < 1.8 % [22]. This is due to difference in botanical and geographical origins of honey samples. These could lead to the numerical differences in the ash composition determined. A study report by [20, 23] stated that botanical origins affect the ash content of the honey and lighter color indicated that honey deemed to have lower ash content than that of darker color honey.

Table 1: Proximate analysis of *M. beccarii* bee honey (mean \pm SD) from different study districts

Study district	MC (%)	AC (%)	Protein (%)	Fat (%)	Carb (%)	Energy (KJ/100 g)
Chalia	29.96 ± 2.85^a	0.23 ± 0.11^a	0.75 ± 0.18^a	0.24 ± 0.17^a	68.88 ± 2.76^b	1174.89 ± 49.14^b
Tokke Kuttaye	31.15 ± 1.63^a	0.24 ± 0.12^a	0.70 ± 0.15^a	0.31 ± 0.02^a	67.60 ± 1.84^b	1155.33 ± 29.10^b
Alle	33.14 ± 1.14^a	0.14 ± 0.045^a	0.51 ± 0.37^a	0.31 ± 0.10^a	65.28 ± 1.82^b	1118.79 ± 25.72^b
Didu	23.70 ± 0.50^b	0.28 ± 0.06^a	0.85 ± 0.26^a	0.27 ± 0.10^a	74.86 ± 0.62^a	1279.19 ± 8.70^a
Bacho	30.11 ± 0.40^a	0.30 ± 0.05^a	0.93 ± 0.71^a	0.31 ± 0.02^a	63.16 ± 0.13^c	1089.58 ± 0.49^b
Gera	31.90 ± 0.50^a	0.22 ± 0.04^a	0.43 ± 0.09^a	0.13 ± 0.03^a	67.25 ± 0.33^b	1141.99 ± 11.40^b
Range	23.20-34.88	0.14-0.34	0.11-1.42	0.11-0.16	63.02-75.23	1089.00-1286.8
P value	0.003	0.54	0.11	0.30	0.00	0.00

Different letters down the column indicated significant differences ($p < 0.05$). Where, SD=Standard Deviation, MC =Moisture Content, AC= Ash Content, Carb= Carbohydrate

3.3. Protein content

Protein in *M. beccarii* honey samples from study areas were in the ranged of 0.11 to 1.42%. The highest value of ($0.93\pm 0.71\%$) was recorded in the samples retrieved from Bacho district of Ilu Aba Bora zone while the lowest (0.43 ± 0.09 %) was obtained from Gera district of Jimma zone. A study result reported by [20] has shown that the highest value of protein content in stingless bee honey was 0.85%. A similar report by stated that protein in honey was mostly made up of enzymes and free amino acids [23, 24]. The main specialized protein act as biological catalysts (enzymes) in honey that has function in promoting health benefits in human are enzyme amylase, invertase and glucose oxidase. Stingless bee honey protein content has many uses such as moisturizing, antioxidant protection and wound healing but its content can be affected by plant species that bees consume nectar from and the bee species that produce it [25]. Thus, the stingless bee potentiality of the study areas in the Oromia region can get this benefit if the resources are used properly.

3.4. Fat content in honey samples

The *M. beccarii* bee honey samples tested in this study indicated too little amount of fat which ranged from 0.13 ± 0.03 to 0.31 ± 0.10 . The highest amount of fat was found in honey samples taken from Tokke kuttaye of West Shoa zone and the lowest from Gera district of Jimma zone. Some study results stated that as there was no significant value in honey for fat content [20].

3.5. Carbohydrate content in honey samples

The total carbohydrate contents of samples investigated in this study ranged between 63.16 ± 0.13 percent to 74.86 ± 0.62 % (m/m). It was stated that stingless bee honey with lowest moisture and protein contents have the highest carbohydrate content [20]. The previous study stated that composition of stingless bee honey depends on the source(s) of nectar, geographical origin, seasonal and environmental factors and handling techniques [18, 26]. The honey from Didu district had the highest carbohydrate content of 74.86 ± 0.62 % while honey sample from Bacho district had the lowest carbohydrate content of 63.16 ± 0.13 %, and the lower carbohydrate content making honey less sweet. The high carbohydrate content of stingless bee honey can be recommended for athletes since it provides a good energy supplier [27] since it contains high concentration of sugars, with low levels of amino acids, phenolic acids, organic acids, vitamins, minerals, enzyme and other phytochemicals [18, 28]. The result obtained from this study also revealed that the *M. beccarii* honey has high carbohydrate contents which may be useful for athletes as source of energy.

Generally, the results of proximate contents comparison of honey samples collected from different areas and botanical origins such as moisture, ash, carbohydrate, and protein and fat contents varied from places to places, country to country (Table 1). For stingless bee honey from Malesia it was stated that the ash, moisture, protein and carbohydrate contents of honey samples ranged from 0.15 to 0.90 g/100 g, 27.00 to 31.00 g/100 g, 0.20 to 0.80 g/100 g, and 67.58 to 72.25 g/100 g, respectively. The ash content is somewhat higher than the current results but other proximate content results had similarity. It was also reported that the mean values of stingless bee honey were found to be 29 ± 1.45 and 0.41 ± 1.1 for moisture and ash contents in West Shoa zone of Oromia region in Ethiopia, respectively [29] which similar to the present findings. In this study, the average moisture content of the honey samples was determined to be 29.04 % and the other report was $27.6\% \pm 1.26$ which had similarity to the current findings [30].

3.6. Mineral Contents in honey samples

The highest potassium and magnesium content of stingless honey were found in Didu district of Ilu Aba Bora zone (5569.75 ± 64.47) and (81.64 ± 2.40) while the highest phosphorous, sodium and calcium found in Chalia district (0.09 ± 0.04 , 207.73 ± 106.05 , and 255.34 ± 104.61 ppm), respectively. The composition of honey depends, on the plant sources from which honey is originated, the species of the bee, the physiological state of the colony, the state of maturity of the honey and the weather conditions during the honey harvest [31].

Table 2: The mineral content analysis of *M. beccarii* (mean \pm SD) from different study districts

Study districts	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	P (ppm)
Chalia	207.73 \pm 106.05 ^a	4029.05 \pm 1159.7 ^a	255.34 \pm 104.6 ^a	66.70 \pm 22.3 ^{ab}	0.09 \pm 0.04 ^a
Toke Kuttaye	151.68 \pm 22.71 ^a	3391.04 \pm 1050.09 ^a	235.99 \pm 58.79 ^a	56.85 \pm 21.1 ^{ab}	0.08 \pm 0.01 ^{ab}
Alle	193.90 \pm 30.72 ^a	5376.33 \pm 515.78 ^a	167.20 \pm 19.40 ^a	74.45 \pm 19.2 ^{ab}	0.05 \pm 0.06 ^{ab}
Didu	156.83 \pm 15.63 ^a	5569.75 \pm 64.47 ^a	159.93 \pm 2.43 ^a	81.64 \pm 2.40 ^a	0.01 \pm 0.01 ^b
Bacho	96.33 \pm 36.87 ^a	5545.57 \pm 8.06 ^a	160.83 \pm 0.31 ^a	80.74 \pm 0.30 ^{ab}	0.01 \pm 0.01 ^b
Gera	80.76 \pm 11.64 ^a	1615.20 \pm 232.75 ^c	80.76 \pm 11.64 ^a	40.38 \pm 5.82 ^b	0.04 \pm 0.01 ^{ab}
Range	59.46-490.60	1351.60-5892.11	67.58-426.63	20.16- 96.57	0.1-0.18
P value	0.008	0.05	0.003	0.028	0.008

Different letters down the column indicated significant difference ($p < 0.05$). Where, SD=Standard deviation.

A study result was reported that the total mineral contents founded in all stingless bee honey samples ranging from 545.76 to 570.66 mg/kg, with potassium, sodium, and calcium being the most abundant elements. The study results confirmed that *M. beccarii* bees honey is a good source of potassium and sodium [32]. The most abundant mineral element determined in all the honey samples analyzed was K, followed by Na and Ca. This finding is similar with results from the literature [20, 33]. The results of mineral contents comparison of *M. beccarii* honey samples collected from different areas and botanical origins such as K, Na, Ca, Mg and P varied from places to places, country to country. According to study results report potassium and sodium were major minerals in all stingless bee honey with an average of 622.36 and 496.01 ppm, respectively [34]. Potassium content in forest stingless bee honey was 2669.23 mg/kg which is lower compared to the current finding which ranged from 1351.60 to 5892.11 mg/kg (Table 2) and it was ranged from 263 to 4980 mg/kg [35]. Magnesium content in the current SBH was higher compared to 137.65 ppm that reported [34]. The variation of mineral contents in honey can be caused by the material for nest or beehive construction such as resin and wax which differs depending on the natural sources available [36]. The honey of stingless bees contains mineral elements, predominantly potassium, calcium, sodium and magnesium, and this honey demonstrates a high bio accessibility of minerals. However, the research shows a variation in the mineral compositions of the stingless bee honeys from sample to sample.

4. Conclusion and recommendation

The stingless bee honey from the study areas was highest in carbohydrate content but it has low fat and protein contents. It was also a good source of potassium and sodium. The proximate and mineral contents of *M. beccarii* bees honey varied with its botanical and geographical origins it can be influenced by environmental, storage and processing factors. The stingless bee honey obtained from Chalia district is the highest in P content while that of Bacho and Didu district is the lowest as compared to honey from other districts. Honey samples with higher moisture content noted lower carbohydrates content and the moisture content of *M. beccarii* bees honey dehydration to discourage its fermentation which is important to improve its shelf life. Stingless bee

honey can contribute to food security and income generation if it is used properly. Stingless bee honey and the bees safety should also be studied and got focus in the future studies by researchers.

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5. Conflict of interest

The authors of this paper state that there is no conflict of interest.

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