

Influence of spatial variation on the physicochemical properties and mineral content of stingless bee honey (*Heterotrigona itama*) in Terengganu, Malaysia

Izzati Shahira Rosidi Sujanto, Nur Syahidah Ramly, John Yew Huat Tang, Asmaliza Abd Ghani, Nadiawati Alias, Salmah Mohamed, Norhayati Ngah*

Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200, Besut, Terengganu, Malaysia

Received:
April 27, 2021

Accepted:
December 28, 2021

Online First:
March 09, 2022

Published:
September 4, 2022

Abstract

The growing interest in the usage of stingless bee honey as a functional food proceeds from its composition, which has been associated with bio-medicinal properties. However, the composition of honey is dependent on the types and origin of bees' foods, which are flower nectar and plant honeydew. Thus, this study was done to investigate the influence of spatial variation on the physicochemical and mineral content of stingless bee honey in Terengganu, Malaysia. Honeys produced by *Heterotrigona itama* were collected from five different locations around Terengganu, Malaysia that are surrounded by different plant diversity. The physicochemical properties, antioxidant activity, total phenolic compounds, total flavonoid compounds, and minerals of stingless bee honey were evaluated. Results obtained show that the pH of honey at all locations differed significantly, ranging from 3.30 to 3.54. The sweetness of honey varied between locations, from 69.30 to 75.76 °Brix. The moisture content of honey ranged from 27.28 to 33.13% and the electrical conductivity from 0.65 to 1.46 mScm⁻¹, which differed significantly among the five selected locations. The parameters for colour, *L** (lightness of the sample), *a** (the colour scale from red to green) and *b** (the colour scale from yellow to blue) values ranged from 25.02 to 52.55, -0.12 to 7.42, and 10.36 to 14.88, respectively. The total flavonoid content of honey ranged from 1.41 to 6.64 mg CE/g, while total phenolic content gave a value of 3.94 to 10.54 mg GAE/g. The antioxidant activity of honey gave a value ranging from 71.14 to 85.27%. The location of hives had a significant influence on the amount of minerals in stingless bee honey. It can be concluded that the physicochemical properties and mineral content of honey produced by *H. itama* were different between locations due to the variation of plant diversity at respective locations. The data obtained provides information on the effect of spatial biodiversity variation on the quality of stingless bee honey in selected locations in Terengganu, Malaysia.

Keywords: Stingless bee honey, Physicochemical properties, Mineral content, Spatial variation

How to cite this:

Sujanto ISR, Ramly NS, Tang JYH, Ghani AA, Alias N, Mohamed S and Ngah N. Influence of spatial variation on the physicochemical properties and mineral content of stingless bee honey (*Heterotrigona itama*) in Terengganu, Malaysia. Asian J Agric & Biol. 2022(4): 202104176. DOI: <https://doi.org/10.35495/ajab.2021.04.176>

*Corresponding author email:
norhayatingah@unisza.edu.my

This is an Open Access article distributed under the terms of the Creative Commons Attribution 3.0 License. (<https://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Introduction

Stingless bees are the major group of eusocial bees in the world with more than 600 species reported worldwide (Lavinias et al., 2019). In Malaysia, *Heterotrigona itama* is the most popular stingless bee species bred by meliponarists since their hives are easily found in Malaysian forests (Kelly et al., 2014; Shamsudin et al., 2019) and they are used as crop pollinators (Ibrahim et al., 2016). In addition, the high demand due to the nutritional value of *H. itama* honey makes them the most common meliponiculture species in Malaysia (Wong et al., 2019).

Stingless bee honey has been gaining attention as a source of curing and healing properties. Its therapeutic abilities have been well documented by many researchers (Abd Jalil et al., 2017; Cianciosi et al., 2018; Rosli et al., 2020). The proven efficacy of stingless bee honey for medicinal purposes has made this stingless bee product claimed as the most natural superfood in the world (Rosidi Sujanto et al., 2021a). Stingless bees produce honey that has higher phenolic content compared to the honey produced by *Apis* sp. (Ranneh et al., 2018), thanks to their smaller body size that allows them to collect nectar from diverse species of flowers (Omar et al., 2019). This stingless bee's ability, as well as their flower constancy behaviours, influences the value of the bioactive compound in their honey (Aljadi and Kamaruddin, 2004).

However, the inconsistency in the quality of honey is always an issue (Ramly et al., 2021a). The differences in the colour and taste of honey make it arguable that either it is pure or fake. The characteristics of stingless bee honey vary depending on the bee species and location of the beehives (Ramly et al., 2021b), which led to a problem in determining the clarity and originality of stingless bee honey. According to Fatima et al. (2018) and Karabagias et al. (2014), stingless bees exhibit different honey compositions due to their foraging activity on wide groups of flowers in different types of habitats. Razali et al. (2018) stated that the pureness of honey can only be determined by evaluating its physicochemical properties such as moisture, pH, total soluble solids, colour attributes and intensity, antioxidant content, and minerals, while the originality of honey can be identified by its origin.

To date, the market for high-quality stingless bee honey is expanding. On top of that, interest in farming the stingless bee is increasing among people in the countryside, especially in the east coast region

(Kelantan, Terengganu), west coast region (Perak), and northern region (Kedah) of Malaysia to fulfil market demand (Fatima et al., 2018). However, information on its valuable effects is still scarce, especially on the physicochemical characteristics and minerals compounds. Indeed, current international legislation is only concerned with *Apis mellifera* honey. Research that is more systematic is needed to provide a piece of complete scientific evidence as well as to make clear any fraud, false claims, and to establish a standard for the quality of stingless bee honey. Thus, this study aims to explore the influence of spatial variation on the physicochemical, phytochemical characteristics, and mineral content of stingless bee honey in a selected location in Terengganu, Malaysia.

Material and Methods

Sample collection

H. itama honey was collected at five different locations in Terengganu, Malaysia. All of those locations are surrounded by different plant diversity; Banggol Peradong (5.3065501, 103.0450277) is dominant with fruit orchards (*Durio zibethinus*, *Nephelium lappaceum*, *Lansium parasiticum*, *Garcinia mangostana*), Kubang Jela (5.3161074, 103.0799022) is dominant with ornamental plants, Benting Lintang (5.7318803, 102.6553897) is dominant with coconut trees, Tembila (5.760968, 102.636176) is dominant with acacia trees, and Telaga Papan (5.534998, 102.889181) is dominant with gelam (*Melaleuca cajuputi*) trees. Three hives for each location that are in good condition, free from any disease, and have an active colony were selected for this experiment. The honey pots in selected hives were emptied on June 1st, 2020. Stingless bees were then allowed to collect honeydew from their natural surroundings to produce honey. The honey was then collected on October 30th, 2020. The samples were obtained from sealed honey pots, transferred into a sterilised bottle, and kept at a 4°C chiller (Sun-tech LC-213LD, Taiwan) prior to further analysis.

Physicochemical analysis

Determination of pH

The pH value of the honey samples was calculated by using the pH meter (Thermo Scientific Orion 2-Star Benchtop). The electrode of the pH meter was immersed in 6 g of the honey sample that had been diluted with 45 mL of distilled water, and the pH meter



reading was recorded. For each sample, the measurement was repeated quintuplicate to get the mean value (Omar et al., 2019).

Determination of total soluble solids

A handheld refractometer (HANNA instrument-96801) was used to evaluate the total soluble solids of stingless bee honey. Two drops of concentrated honey were placed and spread on the entire surface of the prism of the refractometer, and the readings were recorded as °Brix. On average, the reading was recorded in quintuplicate to get the mean value (Moniruzzaman et al., 2013).

Determination of electrical conductivity

The electrical conductivity was determined at 20% of the honey solution (w/v) in distilled water. The electrical conductivity of honey was then measured using an electrical conductivity meter (Horiba LAQUA twin). The results obtained were then expressed in mScm^{-1} (Omar et al., 2019).

Determination of moisture

Moisture content was analysed according to the AOAC official method (Nielsen, 1998). The empty crucible was dried in an oven (Mettler) at 105°C for 4 hours prior to being used to remove all the excess moisture. Then the crucible was cooled in the desiccator and weighed as W1. Next, the honey samples were weighed for approximately 2 g into the crucible and weighed as W2. After that, the crucible was placed in an oven at 105°C for 24 hours. After 24 hours, the crucible containing the dried sample was transferred into the desiccator to let it cool down and then weighed as W3. For each sample, the measurement was repeated quintuplicate to get the mean value. Finally, the percentage of moisture content was calculated using the formula:

$$\% \text{ Moisture} = (W2-W3)/(W2-W1) \times 100$$

Where:

W1 = Weight of the crucible;

W2 = Weight of the sample plus crucible before drying;

W3 = Weight of the sample plus crucible after drying.

Determination of colour

The Chroma meter (Konica Minolta CR-400) device was used to determine the colour of the honey samples. Approximately 2 g of honey was poured into the reading plate. The sample was then analysed, and

the results were shown in terms of colour parameters, which are L^* , a^* , and b^* , where L^* represented the lightness of the sample, a^* represented the change in hue from red to green, and b^* represented the change in hue from yellow to blue. The measurement was repeated quintuplicate for each sample to get the mean value (Pathare et al., 2013).

Phytochemical and antioxidants analysis

Determination of total phenolic content

Total phenolic content was analysed using the Folin-Ciocalteu assay described by (Moniruzzaman et al., 2013) with some modification. A 10% concentration of honey solution was prepared by diluting 1 g of honey with 9 mL of methanol (99.99%, HmbG). After that, 0.5 mL of that solution was mixed with 2.5 mL of 0.2 N Folin & Ciocalteu's phenol reagent (Himedia) and left for 5 minutes. Then, 2 mL of 7.5% Na_2CO_3 (Bendosen) solution was added to the mixture and incubated at room temperature in the dark for 2 hours. After that, the samples were measured at the absorbance of 760 nm by using a UV-Visible Spectrophotometer (Shimadzu UV mini-1240) against a mixture of methanol, 0.2 N Folin & Ciocalteu's phenol reagent, and 7.5% Na_2CO_3 as a blank. A calibration curve was created by using a standard solution of Gallic acid (Merck) (0, 25, 50, 125, 250, 377, 500, 1000, and 1500 mg/L). The results were expressed as the total phenolic content in mg of Gallic acid equivalents (GAE) per g of honey.

Determination of total flavonoid content

The total flavonoid content in the honey sample was determined using the method described by Moniruzzaman et al. (2013) with some modification. One millilitre of honey was diluted with 4 mL of methanol (99.99%, HmbG). Then, 0.3 mL of 5% NaNO_2 (Merck) was added. After 5 minutes, 0.3 mL of 10% AlCl_3 (HmbG) was added, followed by the addition of 2 mL of 1M NaOH (Bendosen) after 6 minutes. Next, 2.4 mL of distilled water was added to the mixture to reach a 10 mL solution. Results obtained were analysed at the absorbance of 510 nm by using a UV-Visible spectrophotometer (Shimadzu UV mini-1240) against a blank sample consisting of a solution consisting of methanol, 5% NaNO_2 , 10% AlCl_3 , and NaOH (1 M) without the honey sample. A calibration curve was created by using a standard solution of catechin (Santa Cruz) (0, 0.04, 0.08, 0.12, 0.16, 0.20, 0.24, and 0.28 mg/mL) and the results obtained were expressed as mg of catechin equivalents



(CE) per g of honey.

DPPH radical scavenging activity (% RSA)

The scavenging activity against 1,1-diphenyl-2-picrylhydrazil (DPPH) (Sigma Aldrich) radical was performed according to the methodology described by Chua et al. (2013) and Shamsudin et al. (2019) with minor modification. In brief, 0.75 mL of the honey solution in methanol at different concentrations, ranging from 20 to 40 mg/mL, was mixed with 1.5 mL of 0.02 mg/mL of DPPH in methanol. The mixture was then incubated at room temperature in the dark for 15 mins. After that, the absorbance was measured at 517 nm against a blank sample consisting of methanol. The absorbance of the control was also measured using 0.75 mL of methanol mixed with 1.5 mL of DPPH solution. The DPPH inhibition of honey was expressed in terms of the percentage inhibition of the DPPH radical and was calculated as below:

$$I = [1 - (A_A/A_B)] \times 100$$

Where:

I = DPPH inhibition (%);

A_B = absorbance of the control;

A_A = absorbance of the honey sample.

Minerals analysis

Inductively coupled plasma atomic emission spectroscopy (ICP-OES) (Thermo Scientific iCAP 7000 Series, UK) was used to determine the concentrations of calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), copper (Cu), aluminium (Al), phosphorus (P), manganese (Mn), and sulphur (S) in honey samples. The sample preparation was done by following the methodology of Yücel and Sultanoğlu (2013) with slight modification. First, the sample needs to be digested before being used as the final sample for the ICP-OES. A microwave digestion procedure was applied to honey samples. Five milligrams of each honey sample were digested with 9 mL of 65% HNO₃ (65%, Merck) using a microwave digestion system (Anton Paar-Multiwave GO). Then, the digested sample was marked up to 25 mL with ultra-pure water to be diluted. A blank digest was carried out in the same way. After that, approximately 10 mL of the digested sample was poured into the test tube and was ready to be used as the sample for the ICP-OES. The result obtained then was expressed as mineral content in mg per kg of honey.

Statistical analysis

All the statistical analysis was performed using R statistical software Version 4.0.3 (R Core Team, 2020). The data obtained were analysed using one-way ANOVA (Analysis of Variance) for a significant difference at a 95% confidence level ($p \leq 0.05$). Significant differences between mean values were analysed by using Lsmeans, where the location of stingless beehives was set as an explanatory variable by using post-hoc Tukey tests.

Results

Table 1 shows the mean value of the physicochemical characteristics of stingless bee honey collected at five different locations in Terengganu, Malaysia. The results obtained showed that the physicochemical characteristics of honey differed significantly between locations. All the honey from all locations was found to be acidic, with a pH value range of 3.30 to 3.54. Amongst all, stingless bee honey collected from the hives at Telaga Papan was the most acidic compared to other places. The °Brix value of stingless bee honey recorded in this study also differed significantly between locations. It was between 69.34 and 75.76 °Brix. Stingless bee honey collected from Banggol Peradong had the lowest Brix value, while the highest Brix value was recorded in the stingless bee honey from Telaga Papan. The electrical conductivity (EC) was related to the ash and acidity value, indicating the existence of ions, natural acids, and proteins in honey (Majid et al., 2019). Our results showed significantly different EC values between honey samples, which ranged from 0.65 to 1.46 mScm⁻¹. Stingless bee honey collected from Benting Lintang had the highest value of EC, and the lowest was Banggol Peradong. The result of this research shows that there was no significant difference in moisture content in stingless bee honey collected from all locations. However, honey collected from Banggol Peradong had the highest moisture content (33.13%), followed by Kubang Jela, Tembila, Benting Lintang, and Tembila. The smaller the *L** value, the darker the colour of honey. The *L** value in this study had a range from 25.02 to 52.55, where stingless bee honey collected from Telaga Papan had the lowest *L** value, which exhibits a darker colour, followed by Kubang Jela, Benting Lintang, Banggol Peradong, and Tembila. Our findings show that there was no significant difference in terms of colour between the honeys



collected at Banggol Peradong and Tembila (Figure 3). Meanwhile, for the a^* value, Banggol Peradong and Tembila showed a negative value of -0.12, which indicates the presence of more green components in the samples. The b^* value ranged from 10.36 to 14.88, meaning that the honey samples contained higher yellow components.

Table-1: The physicochemical characteristics of stingless bee honey from five different locations in Terengganu, Malaysia

Parameters	Kubang Jela	Banggol Peradong	Benting Lintang	Tembila	Telaga papan
pH	3.54 ± 0.01 ^a	3.52 ± 0.01 ^a	3.46 ± 0.01 ^b	3.31 ± 0.01 ^c	3.30 ± 0.01 ^c
Brix (°Brix)	69.68 ± 0.10 ^a	69.40 ± 0.05 ^a	71.74 ± 0.12 ^b	70.51 ± 0.03 ^c	75.76 ± 0.08 ^d
Energy (Kcal)	278.72 ± 0.41 ^a	277.36 ± 0.37 ^a	286.96 ± 0.53 ^b	282.00 ± 0.22 ^c	302.96 ± 0.27 ^d
EC (mScm ⁻¹)	1.06 ± 0.01 ^a	0.65 ± 0.01 ^b	1.46 ± 0.01 ^c	0.96 ± 0.01 ^a	1.04 ± 0.01 ^d
Moisture (%)	32.65 ± 0.08 ^a	33.13 ± 0.09 ^b	32.11 ± 0.12 ^c	32.52 ± 0.03 ^d	27.28 ± 0.10 ^e
Colour L^*	44.35 ± 0.12 ^a	51.95 ± 0.35 ^b	48.59 ± 0.38 ^c	52.55 ± 0.52 ^b	25.02 ± 0.21 ^d
Colour a^*	2.14 ± 0.05 ^a	-0.12 ± 0.03 ^{bcd}	0.04 ± 0.02 ^{bcd}	-0.12 ± 0.03 ^{bcd}	7.42 ± 0.12 ^e
Colour b^*	14.88 ± 0.14 ^{abc}	13.34 ± 0.60 ^{abce}	14.82 ± 0.19 ^{abc}	10.36 ± 0.80 ^{de}	12.16 ± 0.40 ^{bed}

*Values expressed as mean ± standard error. Different superscript letters in the same row show the significant differences at $p < 0.05$.

Figure 1 represents the total phenolic (TPC) and total flavonoid (TFC) content of stingless bee honey. The results obtained showed that the stingless bee honey collected at Telaga Papan had a significantly higher TPC than Benting Lintang, Kubang Jela, Tembila, and Banggol Peradong. Stingless bee honey collected from Benting Lintang and Kubang Jela showed non-significant differences on TPC, but both had significant differences compared to Telaga Papan, Banggol Peradong and Tembila. The TPC of stingless bee honey collected from Telaga Papan was 10.54 ± 0.53 mg GAE/g, Benting Lintang was 7.87 ± 0.09 mg GAE/g, Kubang Jela was 7.74 ± 0.16 mg GAE/g, Tembila was 5.70 ± 0.34 mg GAE/g, and Banggol Peradong was 3.93 ± 0.12 mg GAE/g. Meanwhile, for TFC value recorded for stingless honey collected from all locations ranged from 1.41 to 6.64 mg CE/g. Stingless bee honey collected from Telaga Papan (6.64 ± 0.08 mg CE/g) has the highest TFC value. Meanwhile, the TFC values of stingless bee honey at

Kubang Jela (2.13 ± 0.05 mg CE/g) and Benting Lintang (2.00 ± 0.01 mg CE/g) show no significant differences between these two locations. Stingless bee honey collected from Banggol Peradong recorded the lowest TFC value (1.41 ± 0.01 mg CE/g), and it did not significantly differ from the honey collected from Tembila (1.51 ± 0.05 mg CE/g).

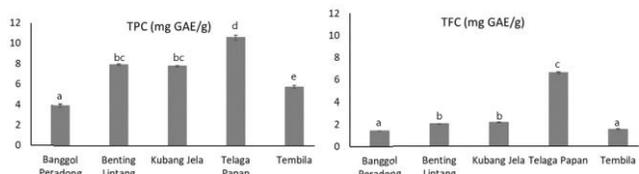


Figure-1: The total phenolic content (TPC) and total flavonoid content (TFC) of stingless bee honey. Values are expressed as mean ± standard error. Different superscript letters show the significant differences at $p < 0.05$.

Figure 2 shows that ascorbic acid inhibited nearly 100% of the DPPH in the solution, while 40 mg/mL inhibited 99.50% of the DPPH in the solution. When compared, the results obtained indicate that stingless bee honey collected from Benting Lintang (85.27%) showed the highest percentage of DPPH inhibition, while Telaga Papan (71.14%) had the lowest at a concentration of 40 mg/mL (Figure 2).

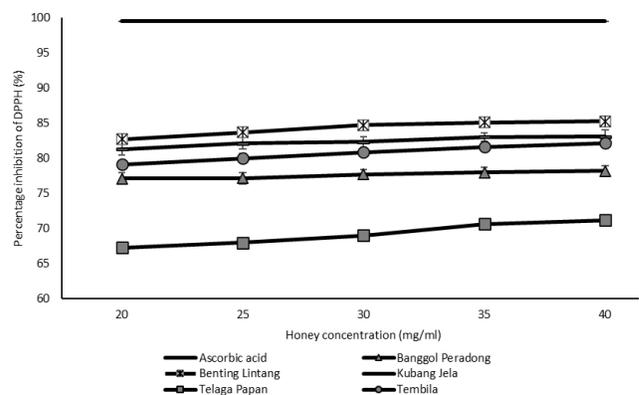


Figure-2: The percentage of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of stingless bee honey at different concentrations of honey samples.

Stingless bee honey collected from Benting Lintang displays the greatest scavenging activity against DPPH with a scavenging rate of 85.27% while Telaga Papan shows the weakest scavenging

activity with a scavenging percentage of 67.24 to 71.14%. Stingless bee honey collected from Kubang Jela and Benting Lintang had no significant difference in DPPH at all concentrations. In addition, stingless bee honey collected from Kubang Jela did not significantly differ compared to the honey collected from Tembila at all five concentrations. Meanwhile, stingless bee honey collected from Telaga Papan had a significant difference with the honey collected from all four other locations at the concentrations. Overall, the results indicate that the stingless bee honey collected at all five different locations in Terengganu had high antioxidant capacity, even at a low concentration of 20 mg/mL.

Table-2: The mineral content in stingless bee honey from five different locations in Terengganu, Malaysia

Minerals (mg/L)	Kubang Jela	Banggol Peradong	Benting Lintang	Tembila	Telaga papan
Potassium (K)	3651.65 ± 21.16 ^a	3633.98 ± 11.62 ^a	3688.35 ± 6.37 ^a	3591.88 ± 45.77 ^a	2551.77 ± 89.54 ^b
Sodium (Na)	142.45 ± 0.23 ^a	142.25 ± 5.08 ^a	346.35 ± 30.54 ^b	307.45 ± 20.06 ^b	97.18 ± 26.49 ^a
Calcium (Ca)	181.73 ± 29.08 ^{abc}	204.55 ± 10.57 ^{abc}	128.40 ± 22.86 ^{abcde}	61.53 ± 2.01 ^{cde}	37.06 ± 22.21 ^{cde}
Magnesium (Mg)	123.88 ± 14.04 ^a	153.30 ± 13.14 ^a	143.85 ± 24.65 ^a	23.63 ± 0.10 ^b	6.75 ± 3.89 ^b
Phosphorus (P)	59.45 ± 6.80 ^{abc}	46.78 ± 5.07 ^{abcd}	63.45 ± 0.75 ^{abc}	31.50 ± 3.61 ^{bd}	7.83 ± 1.79 ^e
Iron (Fe)	13.13 ± 1.66 ^{abcde}	16.03 ± 0.22 ^{abc}	12.38 ± 1.98 ^{abcde}	8.40 ± 0.54 ^{cde}	5.80 ± 2.24 ^{acde}
Manganese (Mn)	3.80 ± 0.49 ^{ac}	9.65 ± 0.03 ^b	2.75 ± 0.55 ^{aced}	0.75 ± 0.36 ^{cde}	0.82 ± 0.74 ^{cde}
Copper (Cu)	1.47 ± 0.17 ^a	1.23 ± 0.04 ^a	1.30 ± 0.08 ^a	1.42 ± 0.07 ^a	0.85 ± 0.25 ^a
Zinc (Zn)	4.93 ± 0.52 ^{abc}	2.98 ± 0.43 ^{abcde}	2.88 ± 0.02 ^{abcde}	1.95 ± 0.20 ^{bcde}	1.62 ± 0.80 ^{bcde}

*Values are expressed as mean ± standard error. Different superscript letters in the same row show the significant differences at $p < 0.05$.

Table 2 shows the mean value of minerals detected in the honey collected from all five locations in Terengganu. There are four major minerals detected, which are potassium (K), sodium (Na), calcium (Ca), and magnesium (Mg) whose values ranged from 2551.77 to 3688.35 mg/kg, 97.18 to 346.35 mg/kg, 37.06 to 204.55 mg/kg and 6.75 to 153.30 mg/kg, respectively. The results obtained show that stingless bee honey collected at all locations has significantly different mineral content except for potassium and copper.

Discussion

The pH value of stingless bee honey recorded at these five selected locations in Terengganu, Malaysia is still in the normal range as suggested by the Malaysian Standard of Stingless Bee Honey (Nordin et al., 2018). Previous research done by Julika et al. (2019) on six random samples obtained from the local market in Malaysia found that the lowest pH values for stingless bee honey are between 2.51 and 3.26. In other research by Chan et al. (2017), the pH value of stingless bee honey from three different regions (northern, east, and central) in Peninsular Malaysia ranged from 3.29 to 3.71. In addition, the pH value of *H. itama* honey that was collected from the east coast parts (Terengganu and Pahang) and the northern part (Johor) of Malaysia ranged from 3.19 to 3.38 (Shamsudin et al., 2019).

The Brix value is the total soluble solid that represents the value of sugars, organic acids, and minerals in the solution (Biluca et al., 2016). Thus, the higher the value of Brix, the sweeter the honey. Our findings were in agreement with many other studies on stingless bee honey collected from variable locations where the values of total soluble solid were recorded between 66.23 and 75.2 °Brix (Chan et al., 2017; Tuksitha et al., 2018; Shamsudin et al., 2019; Rosidi Sujanto et al., 2021b). Stingless bee honey has lower total soluble solids than the honey produced by honey bees due to its high moisture content and low sugar (Nordin et al., 2018).

A study by Souza et al. (2006) shows that the EC value of stingless bee honey collected from 152 hives ranged from 0.49 to 8.77 mScm⁻¹. The differences in EC value are influenced by the various types of protein content, organic acids, and mineral concentration of nectar obtained from the botanical sources collected by the bees (Omar et al., 2019). As an example, previous research by Majid et al. (2019) shows that multiflora, multifruit, and uniflora of *H. itama* honey have an EC value ranging from 0.04 to 0.22 mScm⁻¹.

Moisture content is an essential criterion in establishing the grade of honey, as higher moisture content may lower the storage life and microbial activity of honey. Lim et al. (2019) proposed moisture content values for honey from various floral origins ranging from 27 to 31%. In the other study by Pontara et al. (2012), the moisture content of honey might be linked to several factors such as the floral and spatial origin of nectar, soil and climate conditions, harvesting time, and the right handling procedure by beekeepers during harvest. Stingless bee honey has



been reported to contain high moisture content as opposed to *A. mellifera* honey (De Almeida-Muradian et al., 2013). Generally, Malaysian stingless bee honey was reported to have a moisture content that ranged from 21.32 to 31.67%.

The colour of stingless bee honey was examined in terms of three-point values, which are L^* , a^* , and b^* . The L^* value expresses the lightness, the a^* points out the redness or greenness component, and the b^* shows the yellowness or blueness component. Shamsudin et al. (2019) reported that *H. itama* honey showed a broad range of L^* , a^* , and b^* values, ranging from 73.98 to 92.49 for L^* value, -0.47 to 16.65 for a^* value, and 29.42 to 88.66 for b^* value. Shamsudin et al. (2019) also conclude that the variation of honey colour might be reflected by the several kinds of floral, type of bee, and location of honey's origin. According to Can et al. (2015), the origin of nectar and pollen collected by stingless bees influences the honey colour; as various colour pigments deriving from various phytochemicals such as anthocyanins, phenolic acids, proanthocyanins, flavonoids, and minerals make up the primary colour of honey. In addition to that, Ramly et al. (2021c) claimed that the colour and sweetness of stingless bee honey can only be used to estimate the nutrition and mineral content of honey.

In our study, the TPC in stingless bee honey varied according to the locations of beehives. The investigation by Ya'akob et al. (2019) shows that the stingless bee honey from multiple locations in Johor has a broad range of TPC, valued from 414.53 to 778.23 mg GAE/100g, which is in agreement with this study. In another research by Wong et al. (2019), the detected value for TPC of *H. itama* honey originated from Sarawak was 477.40 mg GAE/kg. Contrary to that, Biluca et al. (2016) stated a far smaller value of TPC of thirty-three samples in the range of 10.3-98.0 mg GAE/100g compared to others. Phenolic content is the major group of phytochemicals that are derived from plants and accumulate in honey through nectar or pollen from plants visited by bees. Da Silva et al. (2013) stated that the total phenolic content (TPC) differed among honeys as it was related to the type of bee species, location of beehives, flowering season, and floral source. Findings by Kamboj et al. (2013) come together on the same page as Iglesias et al. (2012) and Khalil et al. (2011) that the differences in polyphenol compounds in honey were largely correlated with the floral sources of the nectar and also the type of bee species. Besides, these compounds are

also important in contributing to the appearance and useful properties of honey (Abu Bakar et al., 2017).

In a study by Chan et al. (2017), it was indicated that the TFC value of stingless bee honey ranged between 44.60 and 79.13 mg QE/kg. On the other hand, Abu Bakar et al. (2017) reported that honey of *H. itama* collected from Jasin Melaka had a TFC of 549.05 mg Rutin/kg. Similar to total phenolic content, the differences in the reported value of TFC may be due to the floral sources of the honey nectar and also the type of bee species (Kamboj et al., 2013). Muruke (2014) also stated that the flavonoid content in honey has been reported to be linked with the environmental conditions and geographic area of the beehives.

Ascorbic acid, a natural water-soluble vitamin C, is used as a standard in comparing the antioxidant capacity for this experiment. Chan et al. (2017) found that the stingless bee honey collected from Pahang demonstrated the greatest percentage of inhibition compared to the stingless bee honey collected from Kedah and Selangor, with a percentage of 44.12 to 79.99% at a concentration of 10 to 60 mg/mL. In the other research conducted by Abu Bakar et al. (2017), the honey of *H. itama* collected from Jasin Melaka tended to scavenge 52.33–97.30% of the DPPH radicals. The differences in antioxidant capacity of honey were closely related to the floral origin of nectar due to the pollen, which was the main food source for the bees, containing different kinds of amino acid content (Weston et al., 2000).

Our findings show that the amount of potassium and magnesium in stingless bee honey collected from all locations in Terengganu was much higher as compared to the *H. itama* honey collected from Johor and Melaka as declared by Abu Bakar et al. (2017). However, the calcium content of *H. itama* honey samples from Melaka was a bit higher compared to *H. itama* honey collected from Terengganu, with a value ranging from 51.83–292.67 mg/kg (Abu Bakar et al., 2017). Cheng et al. (2019) also indicated lower potassium content ranging from 484.11 to 761.22 mg/kg but higher sodium and calcium of *H. itama* honey that originated from six different locations around Malaysia, one of which was Kuala Terengganu. Meanwhile, from previous studies conducted by Biluca et al. (2016), Kek et al. (2018), Cheng et al. (2019) and Abu Bakar et al. (2017), it was found that micro minerals show only small differences from each other. In conclusion, the differences in mineral content of honey samples originating from various locations could be linked to the variation of



soil in which the original nectar was found and also usually reflect the standards that signify the probable floral sources of honey (Alvarez-Suarez et al., 2010).

Conclusion

The physicochemical characteristics, antioxidant properties, and mineral content of *H. itama* honey collected from different locations in Terengganu were discovered. The research outcome showed that the physicochemical characteristics, antioxidant properties, and mineral content of those honeys are significantly influenced by the location of beehives as it was related to the floral origin of that area. It was predictable since the honey, even though produced by the same species of stingless bee, was harvested from different locations that were surrounded by different plant diversity. Our result clearly shows the variability in the value of Brix, moisture content, electrical conductivity, total phenolic content, total flavonoid content, and percentage of DPPH inhibition for all honey samples from five locations in Terengganu. In this study, honey with a darker colour (collected from Telaga Papan, Kubang Jela, and Benting Lintang) exhibited a better potential source of TPC, TFC, and antioxidant properties in comparison with the light-coloured honey. Meanwhile, for mineral content, the highest concentration was shown for potassium (K) among all the other minerals, and it also had a far higher value when compared to literature. This data can be additional knowledge for future studies related to the correlation between physicochemical and antioxidant capacity of the Malaysian stingless bee honey from different botanical origins. Further research should be done to investigate the source of plant nectar collected by the stingless bee and its influence on the quality of honey.

Acknowledgement

This work was funded by the Fundamental Research Grant Scheme (FRGS/1/2019/WAB13/UNISZA/02/1 RR311), Ministry of Higher Education Malaysia, and supported by the Faculty of Bioresources and Food Industry, University Sultan Zainal Abidin, Terengganu, Malaysia.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: This research was supported by the Fundamental Research Grant Scheme

(FRGS/1/2019/WAB13/UNISZA/02/1 RR311).

References

- Abd Jalil MA, Kasmuri AR and Hadi H, 2017. Stingless bee honey, the natural wound healer: A review. *Skin Pharmacol. Physiol.* 30(2):66-75.
- Abu Bakar MF, Sanusi SB, Abu Bakar FI, Cong OJ and Mian Z, 2017. Physicochemical and antioxidant potential of raw unprocessed honey from Malaysian stingless bees. *Pak. J. Nutrition.* 16(11):888-894.
- Aljadi AM and Kamaruddin MY, 2004. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chem.* 85(4):513-518.
- Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E and Battino M, 2010. Contribution of honey in nutrition and human health: A review. *Mediterranean J. Nutr. Metab.* 3:15-23.
- Biluca FC, Braghini F, Gonzaga LV, Costa ACO and Fett R, 2016. Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (Meliponinae). *J. Food Compost. Anal.* 50:61-69.
- Can Z, Yildiz O, Sahin H, Akyuz E, Silici S and Kolayli S, 2015. An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chem.* 180:133-141.
- Cianciosi D, Forbes-Hernández TY, Afrin S, Gasparrini M, Reboredo-Rodriguez P, Manna PP, Zhang J, Bravo Lamas L, Martínez Flórez S, Agudo Toyos P, Quiles JL, Giampieri F and Battino M, 2018. Phenolic compounds in honey and their associated health benefits: A review. *Molecules.* 23(9):2322.
- Chan BK, Haron H, Abdul Talib Ra nd Subramaniam P, 2017. Physical properties, antioxidant content and anti-oxidative activities of Malaysian Stingless Kelulut (*Trigona spp.*) honey. *J. Agric. Sc.* 9(13):1-9.
- Cheng MZSZ, Ismail M, Chan KM, Ooi DJ, Ismail N, Zawawi N and Mohd Esa N, 2019. Comparison of sugar content, mineral elements and antioxidant properties of *Heterotrigona itama* honey from suburban and forest in Malaysia. *Malays. J. Med. Health Sc.* 15(1):104-112.
- Chua LS, Rahaman NLA, Adnan NA, Tjih T and Tan E, 2013. Antioxidant activity of three honey samples in relation with their biochemical components. *J. Anal. Methods Chem.* Article ID 313798.



- Da Silva IAA, da Silva TMS, Camara CA, Queiroz N, Magnani M, de Novais JS, Soledade LEB, de Oliveira Lima E, de Souza AL and de Souza AG, 2013. Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. *Food Chem.* 141:3552-3558.
- De Almeida-Muradian LB, Stramm KM, Horita A, Barth OM, Da Silva de Freitas A and Estevinho LM, 2013. Comparative study of the physicochemical and palynological characteristics of honey from *Melipona subnitida* and *Apis mellifera*. *Int. J. Food Sci. Tech.* 48:1698-1706.
- Fatima IJ, Mohd Hilmi AB, Salwani I and Lavaniya M, 2018. Physicochemical characteristics of Malaysian stingless bee honey from *Trigona* species. *Int. Med. J. Malays.* 17(1):187-191.
- Ibrahim N, Zakaria AJ, Ismail Z and Mohd KS, 2016. Antibacterial and phenolic content of propolis produced by two Malaysian stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica*. *Int. J. Pharmacog. Phytochem Res.* 8(1):156-161.
- Iglesias A, Feás X, Rodrigues S, Seijas JA, Vázquez-tato MP, Dias LG and Estevinho LM, 2012. Comprehensive study of honey with protected denomination of origin and contribution to the enhancement of legal specifications. *Molecules.* 17:8561-8577.
- Julika WN, Ajit A, Sulaiman AZ and Naila A, 2019. Physicochemical and microbiological analysis of stingless bees honey collected from local market in Malaysia. *Indo. J. Chem.* 19(2): 522-530.
- Kamboj R, Bera MB and Nanda V, 2013. Evaluation of physico-chemical properties, trace metal content and antioxidant activity of Indian honeys. *Int. J. Food Sc.Tech.* 48:578-587.
- Karabagias IK, Badeka AV, Kontakos S, Karabournioti S and Kontominas MG, 2014. Botanical discrimination of Greek unifloral honeys with physico-chemical and chemometric analyses. *Food Chem.* 165:181-190.
- Kek SP, Chin NL, Yusof YA, Tan SW and Chua LS, 2018. Classification of entomological origin of honey based on its physicochemical and antioxidant properties. *Int. J. Food Prop.* 20(3): S2723–S2738.
- Kelly N, Farisyah MSN, Kumara TK and Marcela P, 2014. Species diversity and external nest characteristics of stingless bees in meliponiculture. *Pertanika J. Trop. Agric. Sc.* 37(3):293-298.
- Khalil MI, Mahaneem M, Mohsin S, Jamalullail S and Alam N, 2011. Evaluation of radical scavenging activity and colour intensity of nine Malaysian honeys of different origin. *J. ApiProd. ApiMed. Sc.* 3(1):4-11.
- Lavinias FC, Macedo EHBC, Sá GBL, Amaral ACF, Silva JRA, Azevedo MMB, Vieira BA, Domingos TFS, Vermelho AB, Carneiro CS and Rodrigues IA, 2019. Brazilian stingless bee propolis and geopropolis: Promising sources of biologically active compounds. *Braz. J. Pharmacog.* 29(3):389-399.
- Lim DCC, Abu Bakar MF and Majid M, 2019. Nutritional composition of stingless bee honey from different botanical origins. *Int. Conf. Biodiversity.* 269:012025.
- Majid M, Fadzelly M, Bakar A and You KY, 2019. Variations of physicochemical properties of stingless bee honey from different botanical origin in state of Johor, Malaysia. *Int. Conf. Biodiversity.* 269:012028.
- Moniruzzaman M, Sulaiman SA, Khalil MI and Gan SH, 2013. Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: A comparison with manuka honey. *Chem. Centr. J.* 7(1):1-12.
- Muruke MH, 2014. Assessment of antioxidant properties of honeys from Tanzania. *J. Bio. Agric. Healthcare.* 4(27):22-33.
- Nielsen SS, 1998. Compositional analysis of food. *Instructor's Manual for Food Analysis: Second Edition.* Springer. New York.
- Nordin A, Sainik NQAV, Chowdhury SR, Saim A and Binand Idrus RBH, 2018. Physicochemical properties of stingless bee honey from around the globe: A comprehensive review. *J. Food Comp. Anal.* 73:91-102.
- Omar S, Enchang FK, Nazri MUIA, Ismail MM and Ismail WIW, 2019. Physicochemical profiles of honey harvested from four major species of stingless bee (Kelulut) in north east peninsular of Malaysia. *Malays App. Bio.* 48(1):111-116.
- Pathare PB, Opara UL and Al-said FA, 2013. Colour measurement and analysis in fresh and processed foods: A review. *Food Bioprocess Technol.* 6:36-60.
- Pontara LP, de M Clemente E, Oliveira DM, Kwiatkowski A, Rosa CILF and Saia VE, 2012. Physicochemical and microbiological characterization of cassava flower honey samples produced by africanized honeybees. *Food Sc.*



- Technol. 32(3):547-552.
- Ramly NS, Rosidi Sujanto IS, Abd Ghani A, Tang YHJ, Alias N and Ngah N, 2021a. The impact of processing methods on the quality of honey: A review. *Malays J. Appl. Sc.* 6(1):99-110.
- Ramly NS, Rosidi Sujanto IS, Abd Ghani A, Tang YHJ, Alias N, Salmah M, Zakaria AJ and Ngah N, 2021b. Physicochemical properties and mineral elements in honey from various species of Malaysian Stingless Bee. *Biosc. Res.* 18(SI-2): 34-44.
- Ramly NS, Rosidi Sujanto IS, Abd Ghani A, Tang YHJ, Alias N, Abu Bakar MF and Ngah N, 2021c. correlations between colour lightness and the sweetness of stingless bee honey with its minerals content. *J. Agrobiotechnology.* 12(2):88-96.
- Razali MTA, Zainal ZA, Maulidiani M, Shaari K, Zamri Z, Idrus MZM, Khatib A, Abas F, Yee SL, Lim LR and Ismail IS, 2018. Classification of raw stingless bee honeys by bee species origins using the NMR- and LC-MS-based metabolomics approach. *Molecules.* 23:2160.
- Ranneh Y, Ali F, Zarei M, Akim AM, Abd Hamid H and Khazaai H, 2018. Malaysian stingless bee and Tualang honeys: A comparative characterization of total antioxidant capacity and phenolic profile using liquid chromatography-mass spectrometry. *LWT.* 89:1-9.
- R Core Team, 2020. The R project for statistical computing. <https://www.r-project.org>.
- Rosidi Sujanto IS, Ramly NS, Abd Ghani A, Tang YHJ, Alias N and Ngah N, 2021a. The composition and functional properties of stingless bee honey: A review. *Malays Appl. Sc.* 6(1):111-127.
- Rosidi Sujanto IS, Ramly NS, Abd Ghani A, Tang YHJ, Alias N, Abu Bakar MF and Ngah N, 2021b. The physicochemical properties and nutritional value of stingless bee honey at Gelam forest in Telaga Papan, Terengganu, Malaysia. *J. Agrobiotechnology.* 12(2): 108-120.
- Rosli NF, Hazemi MHF, Akbar MA, Basir S, Kassim H and Bunawan H, 2020. Stingless bee honey: Evaluating its antibacterial activity and bacterial diversity. *Insects.* 11:500.
- Shamsudin S, Selamat J, Sanny M, Abd. Razak SB, Jambari NN, Mian Z and Khatib A, 2019. Influence of origins and bee species on physicochemical, antioxidant properties and botanical discrimination of stingless bee honey. *Int. J. Food Prop.* 22(1):239-264.
- Souza BA, Roubik D, Barth OM, Heard T, Enríquez E, de Carvalho CAL, Marchini C, Locatelli J, Persano-Oddo L, Almeida-Muradian L, Bogdanov S and Vit P, 2006. Composition of stingless bee honey: Setting quality standards. *Interciencia,* 31(12): 867-875.
- Tuksitha L, Chen YLS, Chen YL, Wong KY and Peng CC, 2018. Antioxidant and antibacterial capacity of stingless bee honey from Borneo (Sarawak). *Journal of Asia-Pacific Entomology.* 21:563-570.
- Weston RJ, Brocklebank LK and Lu Y, 2000. Identification and quantitative levels of antibacterial components of some New Zealand honeys. *Food Chem.* 70:427-435.
- Wong P, Ling HS, Chung KC, Yau TMS and Gindi SRA, 2019. Chemical analysis on the honey of *Heterotrigona itama* and *Tetrigona binghami* from Sarawak, Malaysia. *Sains Malaysiana.* 48(8): 1635-1642.
- Ya'akob H, Norhisham N, Mohamed M, Sadek N and Endrini S, 2019. Evaluation of physicochemical properties of *Trigona sp.* stingless bee honey from various districts of Johor. *J. Kejuruteraan SI.* 2(1):59-67.
- Yücel Y and Sultanoğlu P, 2013. Characterization of Hatay honeys according to their multi-element analysis using ICP-OES combined with chemometrics. *Food Chem.* 140:231-237.

Contribution of Authors

Sujanto ISR & Ramly NS: Planned and managed the study, performed the experiment, collected and analysed data and manuscript write up
Tang JYH, Ghani AA & Alias N: Conceived the idea, interpreted data and manuscript write up
Ngah N: Conceived the idea, designed the research methodology, supervised the project, interpreted the data and manuscript write up and

