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EVALUATION OF PHYSICOCHEMICAL PROFILE, MULTI ELEMENTAL COMPOSITION AND ANTIOXIDANT PROPERTY OF *Heterotrigona itama* FROM NORTHERN REGION IN MALAYSIA

(Penilaian Profil Fizikokimia, Komposisi Berbilang Unsur dan Sifat Antioksidan *Heterotrigona itama* Dari Wilayah Utara Di Malaysia)

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Abstract

Studies on physical and chemical compositions of stingless bee honey from northern region in Malaysia are still limited. Therefore, this study evaluates the physicochemical profile [pH, free acidity, ash content, total dissolved solids (TDS) and 5-hydroxymethylfurfural (5-HMF)], multi-elemental composition and antioxidant property of fresh stingless bee honey of *Heterotrigona itama*. Twenty honey samples were collected from the Northern regions (Kedah and Penang) of Malaysia. The results revealed that the values for pH, free acidity, ash content, and TDS were pH 3.01-3.43, 65.42-184.66 meq/kg, 0.07-0.61 g/100g, and 258.83-365.67 ppm, respectively. The concentration of 5-HMF in the honey samples elevated from not detected (fresh) to a maximum of 214.38 mg/kg (after 6- and 15-months storage). For multi-elemental composition, potassium was the highest concentration (16.13-1623.75 mg/kg) followed by calcium (36.23-272.5 mg/kg), sodium (31.25-148.25 mg/kg), aluminum (25.35-62.25 mg/L) and magnesium (11.14-179.75 mg/kg). Iron (< 0.02-41.55 mg/kg), copper (< 0.05-23.15 mg/kg), manganese (< 0.02-13.85 m/kg) and zinc (< 0.01-4.63 mg/kg) were found in low concentrations. Arsenic, lead, nickel, barium, cadmium and chromium were found in lower concentrations than the limit of detection, which indicates no contamination or environmental pollution on the studied areas. The total phenolic content (TPC) of the samples was 95.0-364.9 mg GAE/100g, showed relevant amounts of antioxidant properties, suggesting a source of natural antioxidants. In conclusion, the ash content, pH, and 5-HMF of fresh honey samples were compliant with the limit set by the Malaysian Standards. The 5-HMF concentration of all the honey samples after prolonged storage for six months exceeded the permitted range, which indicates that the honey samples deteriorated. Therefore, it is advised to consume the honey in less than six months.

Keywords: Stingless bee honey, *Heterotrigona itama*, northern Malaysia, physicochemical properties, multi-element

Abstrak

Kajian tentang komposisi fizikal dan kimia madu lebah tanpa sengat dari wilayah utara di Malaysia masih terhad. Oleh itu, kajian ini menilai profil fizikokimia (pH, keasidan bebas, kandungan abu, jumlah pepejal terlarut (TDS) dan 5-hidroksimetilfurfural (5-HMF)), komposisi pelbagai unsur dan sifat antioksidan madu lebah tanpa sengat segar iaitu *Heterotrigona itama*. Dua puluh sampel madu telah dikumpul dari wilayah Utara (Kedah dan Pulau Pinang) Malaysia. Keputusan menunjukkan bahawa nilai pH, keasidan bebas, kandungan abu, dan TDS masing-masing adalah pH 3.01-3.43, 65.42-184.66 meq/kg, 0.07-0.61 g/100g dan 258.83-365.67 ppm. Kepekatan 5-HMF dalam sampel madu adalah daripada tidak dikesan (segar) kepada maksimum 214.38 mg/kg (selepas penyimpanan 6 dan 15 bulan). Bagi komposisi berbilang unsur, kalium adalah berkepekatan tertinggi (16.13-1623.75 mg/kg) diikuti kalsium (36.23-272.5 mg/kg), natrium (31.25-148.25 mg/kg), aluminium (25.35-62.25 mg/L) dan magnesium (11.14-179.75 mg/kg). Besi (< 0.02-41.55 mg/kg), kuprum (< 0.05-23.15 mg/kg), mangan (< 0.02-13.85 m/ kg) dan zink (< 0.01-4.63 mg/kg) didapati dalam kepekatan rendah. Arsenik, plumbum, nikel, barium, kadmium dan kromium didapati mempunyai kepekatan lebih rendah daripada had pengesanan yang menunjukkan tiada pencemaran atau pencemaran alam sekitar di kawasan yang dikaji. TPC sampel ialah 95.0-364.9 mg GAE/100g, menunjukkan jumlah sifat antioksidan yang relevan, mencadangkan sumber antioksidan semulajadi. Kesimpulannya, kandungan abu, pH, dan 5-HMF sampel madu segar adalah mematuhi had yang ditetapkan oleh Piawaian Malaysia. Kepekatan 5-HMF bagi semua sampel madu selepas penyimpanan berpanjangan selama 6 bulan telah melebihi julat yang dibenarkan, menunjukkan sampel madu semakin merosot. Oleh itu, adalah dinasihatkan untuk mengambil madu kurang daripada 6 bulan.

Kata kunci: madu lebah tanpa sengat, *Heterotrigona itama*, utara Malaysia, sifat fizikokimia, pelbagai unsur

Introduction

Honeybees are categorized into two main groups: sting bees and stingless bees [1]. The name of the respective groups indicates the presence of sting on the bees. Stingless bees live in forests that have warm and humid conditions [2]. Hence, they can be found in Australia, Africa, Southeast Asia, and South America [3]. The honey produced by stingless bees is known as stingless bee honey. Other than honey, stingless bees are able to produce propolis, royal jelly, pollen, and beeswax. There are about 600 species with 56 genera of stingless bee honey identified worldwide [4]. In Malaysia, there are 33 species of stingless bee from the genus of *Trigona* and *Melipona* such as *Heterotrigona itama*, *Tetrigona apicallis*, *Geniotrigona thoracica*, *Lepidotrigona terminata*, and *Trigona binghami* [5, 6]. On the other hand, as the species of stingless bee vary, the chemical and physical compositions of stingless bee honey also change. Besides the bees' species, there are other factors that influence the compositions of honey such as botanical origin, extraction season, number of nectar source(s), and geographical origin [7].

Stingless bee honey mainly contains sugars (fructose and glucose), water and minor constituents of vitamins, enzymes, amino acids, minerals, and phenolic compounds (flavonoids and phenolic acids) [8]. In conjunction with the constituents of honey, there has been growing interest in the potential use of local and overseas stingless bee honeys for the food, pharmaceutical, and cosmetic industries in recent years [9, 10]. Therefore, the nutritional, therapeutic, and medical benefits as well as biological properties of stingless bee honey have been studied all over the world. Even though there are studies about the Malaysian stingless bee honey, they are limited to stingless bee honey from the northeast and southern end of Peninsular Malaysia [11, 12]. Therefore, this study is vital as it covers the investigation of various physical and chemical compositions of the Malaysian stingless bee honey belonging to *Heterotrigona itama* species from the northern region of Peninsular Malaysia. The physical and chemical compositions were physicochemical properties, total phenolic content, multi-elemental composition, and 5-hydroxymethylfurfural (5-HMF) content. These parameters have been chosen for this study because they have their respective significance as briefly discussed below. The investigated parameters were compared to the Malaysian Standard for stingless bee honey (Table 1) established by the Department of Standards Malaysia, in line with the international standards established by the Codex Alimentarius Commission and European Council Directive with the advice of the International Honey Commission [13, 14].

Table 1. Malaysian Standard for stingless bee honey

Parameter	Malaysian Standard
Moisture (g/100g)	Max 35
Sum of fructose and glucose (g/100g)	Max 35
Sucrose (g/100g)	Max 7.5
Maltose (g/100g)	Max 9.5
Free acidity (meq/100g)	Not applicable
Ash content (g/100g)	Max 1.0
5-HMF (mg/kg)	Max 30
Diastase activity	Not applicable
pH	2.5-3.8
Presence of plant phenolics	Present

In this study, there are four physicochemical properties that were investigated (pH, free acidity, total dissolved solids, ash). Stingless bee honey is naturally acidic; its acidity helps to minimize the growth of bacteria which can improve honey shelf life [15]. The acidity of stingless bee honey is caused by the presence of organic acids, for example, formic acid, oxalic acid, acetic acid, tartaric acid, etc. [5]. On the other hand, when the storage period of honey increases, the acidity of honey also increases due to the fermentation process which converts alcohol into organic acid [16]. Thus, the degree of deterioration of stingless bee honey can be investigated by measuring their pH and free acidity [17]. By measuring the total dissolved solids of stingless bee honey, the total content of inorganic and organic elements can be associated [18]. The ash content analysis of stingless bee honey indicates the total inorganic elements, including sodium, potassium, magnesium, calcium, iron, zinc, manganese, chromium, copper, and aluminum in the samples [19]. In short, all these four properties are well-known criteria for the determination and evaluation of stingless bee honey quality [20].

Polyphenols are one of the most abundant and extensively dispersed chemical groups in plants since the nectar is obtained from blossoms of plants and hence it contains polyphenols too [21]. The common polyphenol compounds in stingless bee honey are flavonoids and simple phenolic derivatives [22]. According to the Malaysian Standards, the presence of phenolic compounds shows the good quality of stingless bee honey [13]. The higher the total phenolic content, the higher the quality of the honey [23]. By determining the total phenolic content of honey, the antioxidant properties of honey can be identified [24]. This is because the total phenolic content has a strong correlation with redox and antioxidant capacities [25]. Other than TPC assay, there are a few other tests normally used to determine the antioxidant properties of the honey which are the DPPH, β-carotene linoleic acid emulsion method, and iron reduction power [26, 27, 28].

Minerals in the honey are particularly important since the mineral contents are dependent on the plant uptake from the soil that the source of the nectar-bearing plant is located and the environment where the bees will collect the nectar for the production of the honey [29]. Furthermore, the content of heavy metals in honey is an indicator of surrounding environmental pollution [30]. As honeybees interact with water, soil, air, and plants located within a particular radius from beehives, pollutants will consequently be brought by honeybees into beehives [31].

During the manufacturing and storage process of foods that are rich in sugar, the chemical alteration of carbohydrates can occur [32]. The chemical alteration of carbohydrates will be triggered if the food is in acidic condition; thus, the formation of furfural compounds via the dehydration process of carbohydrates in honey is unavoidable [32]. Furfurals are organic compounds; and

the common furfural compounds found in foodstuffs are 5-hydroxymethylfurfural (5-HMF), 2-furfural (2-FAL), 2-furfurol (2-FOL), 5-methylfurfural (5-MF), and 2-acetylfuran (2-AF) [33]. The concentration of 5-HMF is used as a quality indicator of heat processing and freshness of various foodstuffs such as sauces, honey, oils, jams, vegetable products, and fruit-based products [32]. This is because its concentrations tend to elevate during the heating process or long storage period [34]. There are several studies that reported that 5-HMF can be detrimental to human bodies and health due to its cytotoxicity, carcinogenicity, and mutagenicity [35].

Materials and Methods

Chemicals and reagents

All the chemicals and reagents used were of analytical grade. Methanol was purchased from Merck (Darmstadt, Germany). Methanol HPLC grade was purchased from Elite Advanced Material (EAM) (Selangor, Malaysia). Hydrochloric acid (HCl) (37%), nitric acid (HNO_3) (65%), sodium hydroxide (NaOH) pellets, and sodium carbonate were from QRëC (Asia) Sdn. Bhd.; gallic acid was from Fluka (Buchs, Switzerland); and Folin-Ciocalteu's phenol reagent (2N), and 5-HMF standard were from Sigma-Aldrich (St. Louis, MO, USA). Quality control standard 7A elements, 5% HNO_3/trace tartaric acid/trace HF: K (1,000 mg/L); Ag (50 mg/L); Al, B, Ba, Na (100 mg/L); and Si (500 mg/L) in 5% HNO_3/trHF; and quality control standard TruQ™ms, 5% HNO_3/trace tartaric acid/trace HF (1,000 mg/L): As, Be, Ca, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, Tl, V, and Zn in 5% HNO_3 were purchased from PerkinElmer (USA). Ultrapure water used in the experiments was purified using a Direct-Q®RƐT Direct-Q UV-R water purification system (Millipore, MA, USA).

Honey samples

Twenty honey samples of *Heterotrigona itama* were obtained directly from the beekeepers in Penang and Kedah, Malaysia. Their geographical origins are listed in Table 2. The evaluation of physicochemical profile (pH, free acidity, ash content, TDS, 5-HMF), multi-elemental composition, and antioxidant property were only carried out using the fresh honey samples. The honey samples were stored in the dark at room temperature for six and fifteen months to figure out the consequence of storage duration on the concentration only for 5-HMF.

Table 2. The stingless bee honey species and geographical origins

Honey Sample ID	Geographical Origin
1	Kampung Pondok Upih, Balik Pulau, Per
2	Kampung Pondok Upih, Balik Pulau, Penang
3	Kampung Pondok Upih, Balik Pulau, Penang
4	Kampung Pondok Upih, Balik Pulau, Penang
5	Bukit Berapit, Bukit Mertajam, Penang
6	Kubang Hulu, Bukit Mertajam, Penang
7	Jitra, Kedah
8	Merbok, Kedah
9	Merbok, Kedah
10	Merbok, Kedah
11	Merbok, Kedah
12	Merbok, Kedah
13	Merbok, Kedah
14	Sungai Petani, Kedah
15	Sungai Petani, Kedah
16	Sungai Petani, Kedah
17	Sik, Kedah
18	Sik, Kedah
19	Sik, Kedah
20	Sik, Kedah

Preparation of gallic acid standard solutions

Approximately 0.11 g of gallic acid was dissolved in ultrapure water in a 100 mL volumetric flask to produce a stock solution. Then, the stock solution was further diluted to a series of concentrations between 100-1,000 mg/L and marked as standard solutions prior to UV-Vis analysis. A control sample with a concentration of 0.5 mg/L was also prepared to ensure the analysis is properly performed to obtain reliable results.

Preparation of 5-HMF standard and honey sample solutions

Roughly 2,000 mg/L of stock solution was prepared by dissolving 50 mg of 5-HMF with ultrapure water in a 25 mL volumetric flask. The standard solutions (0.05, 0.5, 25, 50 mg/L) were freshly prepared to construct a calibration curve. The calibration curve was obtained by plotting the peak area against the concentrations. The preparation of honey sample was performed according to Moniruzzaman et al.'s study, with slight modification [18]. One gram of honey sample was diluted to 10 mL with ultrapure water. The solution was filtered through a 0.45 μm nylon membrane filter prior to injection into the HPLC.

Multi-elemental analysis

One gram of honey sample was mixed with 10 mL HNO_3 (65%), and the mixture was heated using a hot plate until it was nearly dry. The sample was then diluted to 50 mL with ultrapure water and analyzed using ICP-OES. The following 15 elements were determined using ICP-OES: Ca, K, Na, Ba, Mg, Cd, Cr, Cu, Fe, Ni, Pb, Zn, As, Mn, and Al.

pH analysis

The pH of the honey sample was analyzed based on AOAC Official Method 962.19 (2005) [36]. Two grams of honey sample was dissolved in 15 mL of distilled water, and the pH was measured using a pH meter (Oakton® pH 700 Benchtop). The pH meter was calibrated with a standard buffer solution of pH 1, 4, and 7 prior to the pH determination.

Free acidity

Free acidity was performed according to AOAC Official method 962.19 (2005) [36]. The titrimetric method was used to determine the free acidity of the honey sample. One gram of honey sample was dissolved in 7.5 mL of distilled water. Then, the solution was titrated with 0.1 M NaOH until the pH reached 8.0, and the amount of NaOH used was recorded. Free acidity was expressed as milliequivalent acid per kg of honey (meq/kg).

Ash content

Ash content was measured according to AOAC methods 920.181 (2006) [37]. The crucible was washed with 10% concentrated HCl, rinsed with distilled water, and dried in an oven at 100 ^{o}C for one hour. Then, the crucible was cooled down to room temperature and weighed. Next, 2.5 g of honey sample was weighed in the crucible and heated at 500 ^{o}C in the furnace for two hours. The sample was then cooled and reweighed. The ash content was calculated using the formula below:

$$\text{Ash content (\%)} = (Z - X / Y - X) \times 100 \quad (1)$$

Where X is a weight of empty crucible in g, Y is a sample before ashing + weight of crucible in g, and Z is a after complete ashing, weight of crucible + ash in g

Total dissolved solids

The total dissolved solids were determined by weighing 5 g of honey sample and diluted with 50 mL of distilled water, and measured using conductometer.

Antioxidant property

The Folin-Ciocalteu method based on Gul and Pehlivan [38] was used to determine the total phenolic compound (TPC). Briefly, 1 g of honey sample was dissolved in 5 mL methanol and filtered through Whatman No 1 filter paper. Forty microliter of this solution was mixed with 2.4 mL of ultrapure water and 200 μL of non-diluted Folin-Ciocalteu reagents for three minutes. Then, 0.6 mL of Na_2CO_3 (20%) was added to this mixture, and the mixture was incubated in the dark for two hours at room temperature. The incubated samples were used for TPC determination by using UV-Vis spectrometer. Ultrapure water was used as a blank and reference in UV-Vis analysis. Each sample was scanned at a wavelength of 244.4 nm for one minute. Gallic acid (100-1,000 mg/L) was used to plot a standard calibration curve. The maximum absorbance of the samples and the gallic acid

standards were determined through scanning at a range of 200 nm to 1,100 nm. The TPC was expressed as mg gallic acid equivalents (GAE) per 100 g of honey and calculated using the equation below:

$$\text{TPC (mg } GAE/100g) = \frac{C_{GA}\ x\ V\ x\ DF}{weight\ of\ honey\ sample\ (g)} \quad (2)$$

Where C_{GA} is the concentration of gallic acid from the curve (mg/L), V is the volume of honey sample solution (L), and DF is the dilution factor.

The evaluation of physicochemical profile (pH, free acidity, ash content, TDS, 5-HMF), multi-elemental composition, and antioxidant property were carried out in triplicates.

Instrumentation: UV-Vis spectrophotometer

Lambda 35 UV-Vis Spectrometer used for the TPC determination was from PerkinElmer (Singapore). Oakton® pH 700 Benchtop (USA) pH meter was used to evaluate free acidity and pH measurement. Alpha 800 conductometer was used for the determination of total dissolved solid, while WiseTherm furnace was used for the determination of ash content.

High performance liquid chromatography

Quantitative analysis of 5-HMF was carried out using HPLC unit (Shimadzu, Tokyo, Japan) consisting of a pump (LC-10AT), UV-Vis detector (SPD 10A), injector, and 20 µL sample loops. The HPLC analytical column was an Inertsil ODS C18 column (150 mm, 4.6 mm i.d., 10 µm particle size). Isocratic elution was performed using methanol-water (10:90, v/v) at a flow rate of 1 mL/min and the detector was monitored at a wavelength of 285 nm. The injection volume was 20 µL. The 5-HMF concentrations in the honey samples were calculated by comparing the peak area with the standard solutions after correcting the dilution of the honey samples.

ICP-OES

The ICP-OES used was an Axial Viewed Perkin Elmer Optima 8000 ICP-OES equipped with a Meinherd nebulizer, a cyclonic spray chamber, and ICP Winlab software data system.

HPLC instrumental validation

The instrumental validation (linear range, linearity, precision, limit of detection and quantification, and recovery) in this study is based on the ICH Guidelines [39].

Results and Discussion

HPLC instrumental validation

The instrumental validation for the analysis 5-HMF using HPLC was performed by determining the linear range, linearity, precision, limit of detection (LOD), limit of quantification (LOQ), and recovery. The results are summarized in Table 3.

Table 3. Analytical characteristics of the HPLC method

Parameter	Value
Linear range (mg/L)	0.05-50
Linear equation	y = 493900x + 370074
Correlation coefficient (R^2)	0.9992
Limit of detection (LOD) (µg/L)	0.32
Limit of quantification (LOQ) (µg/L)	0.96
Intra-day precision (% RSD)	0.05-2.70
Inter-day precision (% RSD)	1.38-3.97
Recovery (%)	96.8-99.4

Linear range and linearity

The linearity of the calibration curve of 5-HMF was determined by injecting five standard solution concentrations in the range of 0.05 mg/L to 50 mg/L. The linear regression was obtained by plotting the peak area against the concentration of standard solutions. Good linearity with R^2 of 0.9992 was obtained between the peak area and analyte concentrations.

Precision

The precision of the method was assessed by injecting five times each of the standard solutions (0.05, 5, and 50 mg/L) on the same day (intra-day) and over seven days (inter-day), respectively. Good precision of both intra-day (0.05 to 2.70%) and inter-day (1.38 to 3.97%) was found.

Limit of detection and quantification

The LOD and LOQ values were calculated by dividing the standard deviation of the lowest concentration of 5-HMF (0.05 mg/L) to the slope of regression equation and multiplying the result by 3 and 9, respectively. The LOD and LOQ for 5-HMF were 0.32 mg/L and 0.96 mg/L, respectively.

Recovery

Recovery studies were carried out by spiking two concentrations (0.05 and 5 mg/L) of 5-HMF standards to the honey samples. The data obtained were compared with the blank sample, and the percent recoveries were calculated for both spiked samples. Satisfactory recoveries were obtained (96.8-99.4%).

ICP-OES instrumental validation

The instrumental validation for multi-element analysis using ICP-OES was performed by determining the R^2 of the calibration curve, LOD, and LOQ. Results are summarized in Table 4.

Table 4. Analytical characteristics of the ICP-OES

Element	Range of Calibration Curve (mg/L)	Correlation Coefficient (R^2)	LOD (mg/L)	LOQ (mg/L)
Ca	1-5	0.9996	0.03	0.10
Cd	1-5	0.9991	0.01	0.03
Cr	1-5	0.9990	0.04	0.11
Cu	1-5	0.9998	0.05	0.16
Fe	1-5	0.9993	0.02	0.06
Mg	1-5	0.9992	0.02	0.06
Ni	1-5	0.9994	0.03	0.09
Pb	1-5	0.9996	0.02	0.05
Zn	1-5	0.9993	0.01	0.02
As	1-5	0.9993	0.02	0.07
Mn	1-5	0.9993	0.02	0.07
Al	1-5	0.9820	0.13	0.39
K	5-25	0.9983	0.20	0.59
Na	0.5-2.5	0.9977	0.02	0.05
Ba	0.5-2.5	0.9962	0.01	0.02

Linearity

The linearity of the ICP-OES analysis was determined by injecting five standard concentrations as stated in Table 4. The linear regression of multi-element was obtained by plotting the mean intensity of standard solutions against the concentration of standard solutions. All elements have good linearity with R^2 between 0.9820-0.9998.

Limit of detection and quantification

The LODs and LOQs for all elements were in the range of 0.01 to 0.20 mg/L and 0.02 to 0.59 mg/L, respectively.

Physicochemical properties of stingless bee honey

Table 5 shows the results of the selected physicochemical property of pH, free acidity, ash content, and total dissolved solids. All the measurements were determined in triplicates.

Table 5. Physicochemical properties of the stingless bee honey

Sample	pH	Free Acidity (meq/kg)	Ash Content (g/100g)	Total Dissolved Solid (ppm)	TPC (mg GAE/100 g)
1	3.16 ± 0.09	150.01 ± 21.20	0.21 ± 0.01	289.83 ± 29.51	226.5 ± 8.5
2	3.43 ± 0.07	162.64 ± 24.55	0.61 ± 0.10	519.83 ± 66.46	304.2 ± 9.9
3	3.24 ± 0.08	184.66 ± 14.62	0.20 ± 0.05	277.00 ± 6.08	202.4 ± 8.6
4	3.21 ± 0.03	151.23 ± 12.40	0.25 ± 0.02	322.33 ± 5.53	262.3 ± 8.0
5	3.18 ± 0.04	103.54 ± 9.13	0.25 ± 0.10	308.82 ± 113.11	265.1 ± 2.2
6	3.01 ± 0.17	99.08 ± 8.37	0.07 ± 0.06	313.83 ± 7.01	172.1 ± 0.5
7	3.01 ± 0.38	161.04 ± 12.66	0.18 ± 0.04	267.17 ± 11.73	177.0 ± 9.7
8	3.29 ± 0.05	160.84 ± 20.01	0.42 ± 0.06	362.33 ± 45.74	272.5 ± 5.0
9	3.29 ± 0.11	178.46 ± 16.32	0.33 ± 0.01	312.17 ± 15.00	322.3 ± 7.0
10	3.28 ± 0.14	93.07 ± 9.80	0.40 ± 0.11	303.33 ± 17.21	135.0 ± 4.9
11	3.23 ± 0.13	154.71 ± 7.48	0.21 ± 0.06	275.00 ± 17.32	95.0 ± 0.3
12	3.32 ± 0.11	145.48 ± 6.40	0.46 ± 0.18	330.33 ± 22.67	212.9 ± 2.2
13	3.18 ± 0.14	123.88 ± 1.59	0.20 ± 0.02	302.83 ± 29.75	327.6 ± 9.4
14	3.26 ± 0.14	129.07 ± 29.60	0.24 ± 0.20	320.50 ± 56.25	204.7 ± 4.9
15	3.27 ± 0.13	120.54 ± 6.31	0.20 ± 0.04	333.83 ± 25.97	115.4 ± 6.5
16	3.22 ± 0.12	85.59 ± 6.24	n.c.o.	302.00 ± 55.33	364.9 ± 4.8
17	3.28 ± 0.03	65.42 ± 13.53	n.c.o.	282.67 ± 24.19	210.6 ± 1.5
18	3.22 ± 0.11	114.07 ± 1.32	n.c.o.	258.83 ± 57.93	n.c.o.
19	3.29 ± 0.16	149.57 ± 14.75	n.c.o.	365.67 ± 36.43	n.c.o.
20	3.18 ± 0.14	96.02 ± 5.62	n.c.o.	315.00±33.06	n.c.o.

n.c.o. refer as not carried out

pH analysis

All of the honey samples analyzed in this study were found to be acidic with a range of 3.01 to 3.43 (Table 5). The range of the pH was compliant with the Malaysian Standards for stingless bee honey (pH 2.5-3.8). The pH values obtained from the current study were almost similar to the finding of other regions in Malaysia (pH 2.92-3.71) (Table 6). However, based on the previous study by Selvaraju et al. [40], they found a sample in Penang that has a pH (pH 4.03) higher than the maximum value stated by the Malaysian Standards. The findings proved that despite the different geographical and

botanical sources of the honey samples, their pH was almost within the same range and had no significant difference ($p > 0.05$). This was confirmed by running the pH reading of 20 samples in One-Way ANOVA Test, and the p-value was 0.4028. The result might suggest that stingless bee honey has a similar range of pH regardless of their origin [11]. As reported by Moniruzzaman et al. [41], the high acidity of the honey is caused by the fermentation of sugar into organic acid, which is responsible for honey flavor and stability against microbial spoilage. A low pH value also indicates that the stingless bee honey is more acidic and has more hydrogen ions, which are involved in the formation of other compounds in the honey [11].

Table 6. Physicochemical properties of stingless bee honey produced by *Heterotrigona itama* at different regions in Malaysia

Location	**pH**	**Free Acidity (meq/kg)**	**Ash (g/100g)**	**Total Dissolved Solids**	**TPC (mg GAE/100g)**	**Ref.**
Pahang	3.71		0.41			[42]
Kedah	3.71		0.23			
Selangor	3.29		0.22			
Johor	3.24-3.42		0.15-0.67		357.1 - 520.8	[29]
Melaka	3.27-3.36		0.18-0.47			
Perak	3.26	136.8	0.08			[43]
Kelantan	2.93		0.27			[11]
Terengganu	2.92		0.53			
Kedah			0.39		228.1-235.3	[44]
Johor			0.29			
Melaka	3.18	176.3		74.9 °Brix		[5]
Johor	3.27	107.5		74.7 °Brix		
Pahang	3.00	246.3		73.9 °Brix		
Penang	4.03					[40]
Kedah	3.22					
Pahang	3.17					[45]
Sarawak	2.92-3.58		0.29 %			[46]
Penang Kedah Kelantan Terengganu Selangor Negeri Sembilan Melaka	3.17	220.6	0.28 %	0.28%		[47]
Sarawak Selangor Johor Kedah	2.86-3.42	17.0-336.2	0.05-0.15 %		251.1-541.8	[48]
Penang	3.01-3.43	99.08-184.66	0.07-0.61	277.00-519.83	95.0-364.9	Present study
Kedah	3.01-3.32	65.42-178.46	0.18-0.46	258.83-365.67	95.0-364.9	

Free acidity

The free acidity of the honey samples was ranged from 65.42 to 184.66 meq/kg (Table 5). The values detected were in a similar range with the previous studies from other regions in Malaysia (Table 6), except for the sample studied by Shamsudin et al. [5] and Zawawi et al. [48] which contained a relatively higher free acidity value (246.3 and 336.2 meq/kg, respectively). The Malaysian Standards did not set a limit for the free acidity in stingless bee honey. Based on the Codex Alimentarius, the free acidity of honey shall not exceed 50 meq/kg. The free acidity of the samples studied were significantly high and did not comply with the Codex standard of honey. Free acidity is a quality parameter related to honey fermentation of sugars to organic acids which depends on the nectar of different floras [43, 49, 50]. Therefore, it can be concluded that the high fermentation of sugars creates the organic acids in the stingless bee honey.

Ash content

The ash content is depending on the amount of minerals present in the honey. The higher the value of ash content, the greater the amount of mineral present in the nectar. The percentage of the ash was measured based on the remaining inorganic residue found after the incineration of the honey sample [46]. The ash content obtained from this study was between 0.07 and 0.61 g/100g. However, analysis on samples 16 to 20 have not carried out due to the constraint of instruments. Nonetheless, for samples 1 to 15, the ash content obtained was aligned with the Malaysian Standards (max 1.0 g/100g). The values were also similar to the previous studies from other regions in Malaysia (0.08-0.47 g/100g) (Table 6) which also complied with the Malaysian Standards.

Total dissolved solids

The total dissolved solids are related to the moisture and sugar content in the honey. In general, honey with high total dissolved solids possesses high sugar content and low moisture content [5]. The total dissolved solids from this study were in the range of 258.83 to 365.67 ppm, except for sample 2 which had a very high value (519.83 ppm). The high value of total dissolved solids of sample 2 is probably due to the high content of organic and inorganic substances, mainly sugars such as glucose and fructose [51]. In other words, the probability of the honey being adulterated with syrup or honeybees which have been fed with sugar syrup is high. From the previous studies, only Moniruzzaman et al. [51] reported the total dissolved solids of Malaysian stingless bee honeys in ppm unit, whereas Shamsudin et al. [5] and Ng et al. [52] reported the total dissolved solids in percent °Brix. In order to record the total dissolved solids in percent °Brix, a digital refractometer is used. The total dissolved solids of Malaysian stingless bee honey from Moniruzzaman et al. [51] ranged from 138.00 to 395.00 ppm.

TPC analysis

The gallic standard solutions and all the samples were scanned from the wavelength of 200 nm to 1100 nm by using UV-Vis spectrometer in order to determine the maximum absorbance. The maximum wavelength was roughly scanned at 244.4 nm for the standard solutions and the sample solutions against the blank solution.

From Table 5, the TPC values varied from 95.0 mg GAE/100 g to 364.9 mg GAE/100 g. The highest TPC was found in sample 16, while the lowest TPC was found in sample 11. However, the TPC found by Abu Bakar et al. [29] (Table 6) showed higher TPC values which were between 357.14 and 520.83 mg GAE/100 g. As the TPC assay is able to identify the total amount of phenolic content in the samples, a high TPC value indicates a greater amount of phenolic content. When the amount of phenolic content of honey is greater, then honey will have better redox properties which leads to higher antioxidant activity.

Analysis of 5-HMF

The concentration of 5-HMF in the honey samples was quantified based on the external standard method using the calibration curve. The standard calibration curve of 5-HMF was constructed using four different concentrations which included 0.05, 1, 10, 50 mg/L of the standard solutions. The baseline separation for 5-HMF was achieved in less than four minutes under the stated experimental conditions.

5-HMF is known as the best indicator for the freshness and quality of the honey which provides information on

degradation, adulteration, and deterioration of the honey [53]. The honey samples used in this study were obtained freshly from the local beekeepers in Kedah and Penang, Malaysia in December 2019. The concentration of 5-HMF present in the fresh honey samples was analyzed after receiving the honey samples from the beekeepers. From the analysis, only honey sample 10 contained a very low concentration (0.31 ± 0.01 mg/kg) of 5-HMF, while the other 19 honey samples were not detected (Table 7). The results obtained from the previous study by our research group are congruent with other studies of different regions and countries where the 5-HMF was not detected or present in a very little amount in fresh stingless bee honey samples (Table 8).

To investigate the consequence of prolonged storage on the concentration of 5-HMF, the honey samples were stored for six months in the dark at room temperature. The honey samples were kept at room temperature to stimulate the common practice that honey is normally stored at room temperature. Besides, the honey samples were stored in the dark in order to prevent excess heat from sunlight; otherwise, the temperature of the honey samples would increase when exposed to sunlight. The increase in temperature of the honey samples would lead to an escalation in the concentration of 5-HMF which will affect the results of the study [54]. After storage for six months, the honey samples were analyzed in July 2020, and the concentrations of 5-HMF were found to have increased to the range of 48.18 ± 1.11 to 192.99 ± 10.39 mg/kg (Table 7). These values exceeded the maximum allowed limit of both Codex Alimentarius Standard (80 mg/kg) and the Malaysian Standard (30 mg/kg). Sample 6 showed a significantly higher concentration of 5-HMF, which might have been influence by the floral source of the honey [53].

Table 7. Concentrations of 5-HMF in honey samples of fresh and different storage durations

Sample	Concentration of 5-HMF (mg/kg)		
	December 2019 (Fresh)	**July 2020 (6 months storage)**	**May 2021 (15 months storage)**
1	N.D.	70.84 ± 0.23	65.10 ± 3.11
2	N.D.	56.20 ± 0.06	38.71 ± 0.91
3	N.D.	49.95 ± 2.13	45.98 ± 1.41
4	N.D.	52.26 ± 0.68	62.45 ± 0.74
5	N.D.	72.97 ± 0.51	75.82 ± 0.65
6	N.D.	192.99 ± 10.39	214.38 ± 4.03
7	N.D.	55.77 ± 5.24	56.31 ± 1.81
8	N.D.	61.95 ± 4.96	44.95 ± 0.65
9	N.D.	73.25 ± 0.34	66.04 ± 2.50
10	0.31 ± 0.01	81.29 ± 0.94	74.29 ± 2.32
11	N.D.	92.10 ± 1.33	54.72 ± 1.28
12	N.D.	57.48 ± 1.01	74.07 ± 1.49
13	N.D.	78.32 ± 0.08	91.00 ± 2.87
14	N.D.	68.50 ± 0.59	79.52 ± 0.59
15	N.D.	48.18 ± 1.11	95.33 ± 2.12
16	N.D.	61.39 ± 0.25	127.36 ± 5.43
17	N.D.	66.10 ± 1.96	117.85 ± 0.02
18	N.D.	61.97 ± 0.54	56.74 ± 2.28
19	N.D.	109.05 ± 1.46	64.01 ± 1.85
20	N.D.	62.76 ± 0.62	80.59 ± 2.87

N.D. refer to not detected

Table 8. Concentration of 5-HMF in fresh stingless bee honey of different regions and countries

Region/Country	Concentration of 5-HMF (mg/kg)	Ref.
Guatemala	N.D.	[55]
Australia	0.4 – 2.1	[56]
Thailand	0.25 – 1.89	[57]
Thailand	2.2 – 68.8	[58]
Brazil	N.D.	[59]
Pakistan	14.54 – 37.09	[60]
Brazil	17.81 – 34.62	[61]
Colombia	N.D.	[62]
Kelantan	3.42 ± 1.03	[11]
Terengganu	0.080 ± 0.16	[11]
Pahang	74.3 – 85.9	[63]
Pahang	0.07 ± 0.06	[5]
Malacca	0.05 ± 0.02	[5]
Johor	N.D.	[5]
Kelantan	0.42 – 0.95	[64]
Johor	0.02 – 48.68	[12]
Sarawak	0.21 – 0.48	[46]
Pahang	N.D.	[45]
Sarawak	0.11 – 0.91	[65]

N.D. refer to not detected

The honey samples were then kept for another nine months in the dark at room temperature in order to study the consequence of prolonged storage on the concentration of 5-HMF. The honey samples were analyzed in May 2021, and the results obtained are presented in Table 9. It was observed that all the honey samples contained a high concentration of 5-HMF. The concentration of 5-HMF present in the honey samples were ranging from 38.71 ± 0.91 to 214.38 ± 4.03 mg/kg. This situation shows that the honey samples have violated the allowed limit for the concentration of 5-HMF in the Malaysian Standard and Codex Alimentarius Standard. Thus, this indicates that the honey samples have deteriorated.

Malaysia is a tropical country where the climate is warm and humid all year round [66]. Thus, the concentration of 5-HMF in the honey originating from Malaysia should not exceed 80 mg/kg according to the Codex Alimentarius Standard. The results demonstrate that all the honey samples were within the maximum allowed limit of 5-HMF established by the Codex Alimentarius Standard, except for honey samples 6, 13, 15, 16, 17, and 20. Therefore, this indicates that honey samples 6, 13, 15, 16, 17, and 20 have deteriorated after prolonged storage of another nine months and are not suitable to be consumed.

On the other hand, it was found that the 5-HMF concentration in the honey samples determined in May 2021 fluctuated as compared to the honey samples determined in July 2020. It was noticed that the 5-HMF concentration in some of the honey samples increased, while some decreased. The concentration of 5-HMF increased for honey samples 4, 5, 6, 7, 12, 13, 14, 15, 16, 17, and 20 after prolonged storage. The escalation in the concentration of 5-HMF in the honey samples after prolonged storage is consistent with other earlier studies [54, 58].

The escalation in the concentration of 5-HMF in the honey samples is due to the degradation of the glucose

and fructose under acidic conditions [67, 68]. The longer the duration of storage for the honey samples, the more glucose and fructose are converted to 5-HMF, as stingless bee honey is acidic in nature. Hence, this situation would lead to a higher concentration of 5-HMF [33]. The high concentration of 5-HMF in the honey samples indicates that the honey samples degraded due to prolonged storage. As a result, the high concentration of 5-HMF in the honey samples would decrease the commercial value of the honey.

For honey samples 1, 2, 3, 8, 9, 10, 11, 18, and 19, it was found that the concentration of 5-HMF decreased after prolonged storage. However, large decrease in the concentration of 5-HMF was noticed for honey samples 11 and 19. The concentration of 5-HMF decreased to half when compared with the value quantified in July 2020. The concentration of 5-HMF's decrease in the honey samples could be due to the augmentation of complex processes which favor the decomposition of 5-HMF, whereby it will reduce the formation of 5-HMF [69].

Besides, the concentration of 5-HMF's decrease in the honey samples could also be due to the decomposition of 5-HMF from other products in the diluted honey samples, specifically for the diluted honey samples stored at room temperature. This is proven by the study of Wunderlin et al. [70] whereby the decrease in the 5-HMF band at 284 nm had a simultaneous increase of a new band at 252 nm. Thus, they suggested that the sample solutions containing 5-HMF should be stored in the dark at temperatures ranging from 4 to 8 °C before the sample solutions are analysed. Wunderlin et al. [70] also suggested that the analysis of 5-HMF should be made within six hours after sample preparation in order to prevent the decomposition of 5-HMF in sample solutions.

Furthermore, fructose is known to have a protective effect on the decomposition of 5-HMF [70]. The decomposition of 5-HMF is significant in honey samples without fructose. Therefore, the decrease in 5-HMF observed in a few honey samples in this study could be explained by the lesser amount of fructose in the honey samples which reduces the protective effect of fructose on the decomposition of 5-HMF. This eventually led to the decomposition of 5-HMF in the honey samples. As observed in this study, the 5-HMF concentration in some of the honey samples reduced after prolonged storage in the dark at room temperature for another nine months.

Multi-elemental composition

For the multi-elemental analysis, it was found that the highest concentration of elements was potassium (Table 9). Sample 2 exhibited a very high concentration of potassium (1,623.75 mg/kg), while sample 6 exhibited a very low concentration of potassium (16.13 mg/kg). By excluding these two samples, the average concentration range of potassium was 255.40 mg/kg to 915.50 mg/kg. Calcium, sodium, and aluminum were present in moderate concentrations in the honey with an average concentration range of 36.23 to 272.5 mg/kg, 31.25 to 148.25 mg/kg, and 25.35 to 62.25 mg/kg, respectively. For magnesium, the average range of concentration was 11.14 to 78.38 mg/kg, excluding one sample from Penang (sample 2) that had a relatively high concentration (179.75 mg/kg).

Table 9. Elemental analysis of honey samples (n=2, mean±SD) (mg/kg)

Sample	Element					
	K	Na	Al	Ca	Mg	Fe
1	469.63 ± 34.64	94.88 ± 0.03	176.25 ± 0.02	125.50 ± 0.01	43.88 ± 0.02	2.90 ± 0.00
2	1623.75 ± 0.22	148.25 ± 0.02	79.50 ± 0.01	205.75 ± 0.01	179.75 ± 0.02	4.28 ± 0.00
3	426.50 ± 28.82	95.13 ± 1.24	463.50 ± 0.08	191.00 ± 0.02	60.25 ± 1.66	6.05 ± 0.00
4	425.75 ± 0.06	77.44 ± 4.00	450.00 ± 0.06	178.75 ± 0.01	41.00 ± 0.01	3.73 ± 0.00
5	478.31± 36.33	140.00 ± 0.00	662.25 ± 0.06	272.50 ± 0.01	58.00 ± 0.01	5.53 ± 0.00
6	16.13 ± 0.00	76.94 ± 4.33	114.53 ± 0.02	36.23 ± 0.01	11.14 ± 0.37	3.35 ± 0.00

7	532.69 ± 42.69	113.50 ± 0.02	229.00 ± 0.02	234.90 ± 0.06	78.38 ± 0.03	7.68 ± 0.00
8	612.25 ± 0.06	74.75 ± 0.01	227.00 ± 0.01	129.50 ± 0.01	70.73 ± 0.04	1.65 ± 0.00
9	588.00 ± 14.85	105.75 ± 0.00	103.85 ± 0.06	139.50 ± 0.06	23.63 ± 0.02	1.60 ± 0.00
10	512.00 ± 16.26	112.93 ± 4.07	112.75 ± 0.02	163.95 ± 6.01	65.18 ± 1.66	41.55 ± 0.01
11	474.45 ± 38.25	79.75 ± 0.02	137.65 ± 0.03	81.90 ± 0.02	34.88 ± 2.65	< 0.02
12	376.25 ± 0.07	87.70 ± 0.01	73.50 ± 0.04	79.50 ± 0.02	28.25 ± 0.00	< 0.02
13	351.65 ± 0.15	31.25 ± 0.01	42.35 ± 0.01	52.05 ± 0.01	34.25 ± 0.01	< 0.02
14	560.63 ± 14.67	108.625 ± 0.01	67.13 ± 0.05	144.69 ± 4.33	23.41 + 0.44	1.10 ± 0.00
15	410.75 ± 0.06	107.31 ± 5.75	109.98 ± 7.88	136.15 ± 7.11	21.40 ± 1.13	1.15 ± 0.00
16	612.75 ± 2.48	129.75 ± 0.01	101.48 ± 0.07	154.18 ± 5.69	34.08 ± 0.11	1.00 ± 0.00
17	915.50 ± 0.11	150.13 ± 11.84	270.58 ± 1.24	166.13 ± 5.20	44.15 ± 0.02	1.45 ± 0.21
18	489.48 ± 10.15	102.00 ± 0.02	209.35 ± 0.04	159.18 ± 5.48	34.95 ± 0.92	1.70 ± 0.00
19	594.50 ± 0.06	86.25 ± 0.02	136.50 ± 0.03	121.05 ± 0.07	22.05 ± 0.00	4.50 ± 0.00
20	255.40 ± 0.06	47.75 ± 0.03	25.35 ± 0.01	78.95 ± 0.04	22.00 ± 0.01	< 0.02

Elements found in low concentrations were iron (< 0.02 to 41.55 mg/kg), copper (< 0.05 to 23.15 mg/kg), manganese (< 0.02 to 13.85 mg/kg), and zinc (< 0.01 to 4.63 mg/kg) (Table 10). The concentration of heavy metals such as arsenic, lead, nickel, barium, cadmium, and chromium were found lower than the LODs values (Table 4). The differences in the multi-element concentrations of the same honey species collected from different locations might be due to the type of soil in which the nectar of the flower was located [71].

Table 10. Elemental analysis of honey samples (n=2, mean±SD) (mg/kg)

Sample	Element								
	Cu	**Zn**	**Mn**	**As**	**Pb**	**Ni**	**Ba**	**Cd**	**Cr**
1	6.28 ± 0.00	1.95 ± 0.00	1.43 ± 0.00	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
2	20.85 ± 0.01	1.43 ± 0.00	5.90 ± 0.11	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
3	13.83 ± 0.01	1.70 ± 0.00	1.70 ± 0.00	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
4	14.45 ± 0.01	1.33 ± 0.00	< 0.02	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
5	12.05 ± 0.01	2.03 ± 0.00	0.80 ± 0.00	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
6	8.85 ± 0.01	0.70 ± 0.00	< 0.02	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
7	10.80 ± 0.00	4.63 ± 0.00	2.55 ± 0.00	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
8	23.15 ± 0.01	2.25 ± 0.00	2.63 ± 0.00	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
9	19.88 ± 0.02	1.40 ± 0.00	2.90 ± 0.04	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
10	< 0.05	1.05 ± 0.00	1.15 ± 0.07	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
11	< 0.05	< 0.01	< 0.02	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
12	< 0.05	< 0.01	< 0.02	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
13	< 0.05	< 0.01	4.25 ± 0.00	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
14	7.80 ± 0.00	2.09 ± 0.09	2.73 ± 0.00	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
15	11.30 ± 0.32	1.08 ± 0.00	< 0.02	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
16	< 0.05	3.50 ± 0.00	1.90 ± 0.00	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
17	< 0.05	< 0.01	13.85 ± 0.00	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
18	< 0.05	2.10 ± 0.00	< 0.02	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
19	< 0.05	< 0.01	< 0.02	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04
20	< 0.05	< 0.01	< 0.02	< 0.02	< 0.02	< 0.03	< 0.01	< 0.01	< 0.04

Our findings for this species were similar to the values from the study performed by Abu Bakar et al. [29] in Melaka and Johor. The concentrations found for potassium, calcium, magnesium, and zinc were 236.33 to 701.33 mg/kg, 51.83 to 292.67 mg/kg, 18.53 to 50.30 mg/kg, and 4.37 to 5.33 mg/kg, respectively. Another study by Cheng et al. [72] in Selangor, Johor, Terengganu, and Sabah found that the concentrations of potassium, sodium, calcium, magnesium, iron, and zinc were 484.11 mg/L to 761.22 mg/L, 381.47 mg/L to 589.46 mg/L, 141.22 mg/L to 205.98 mg/L, 14.56 mg/L to 41.06 mg/L, 5.98 mg/L to 7.81 mg/L, and 1.02 mg/L to 3.03 mg/L, respectively. They also found that the concentrations of arsenic, lead, and cadmium were below the LODs.

From this study, the highest concentration of potassium was 1623.75 mg/L and the lowest was 16.13 mg/L. The daily recommended intake (Recommended Daily Allowance, RDA) of calcium is based on age, sex, and physio-pathological conditions, generally ranging from 1,000 to 1,200 mg [73]. The World Health Organization (WHO) recommends a potassium daily intake of more than 3,500 mg and a sodium daily intake of less than 2,000 mg [74]. The concentration obtained for these three elements was below the maximum daily intake, therefore it is safe for daily consumption for nutritional values. The concentration of the toxic elements was below the LOD. In terms of food safety, the multi-elemental profile, especially on the toxic elements in the honeys, gave information on the environmental conditions of the harvesting region. Based on the results obtained, our study indicated that there is neither contamination nor environmental pollution in the studied areas.

Conclusion

In this study, the Malaysian stingless bee honey which originated from a particular bee species (*Heterotrigona itama*) from the northern region of Malaysia (Kedah and Penang) was successfully investigated for its physicochemical profile, multi-elemental composition, and antioxidant activity. The antioxidant activity of honey was studied by evaluating their TPC. The pH, free acidity, ash content, total dissolved solids, minerals, and TPC were studied for the fresh honey samples only; whereas the 5-HMF content was investigated for fresh samples, after 6 and 15 months. The findings of ash content, pH, and TPC of all the honey samples were compliant with the advised limit set by Malaysian Standards and previous studies. The free acidity of all the samples and total dissolved solids of sample 2 exceeded the permitted range. The concentration of minerals was observed that their concentration is below the advised daily intake. At the same time, it was observed that the concentration of toxic elements is below the LOD; thus, it is safe to consume these honey samples. The 5-HMF content of all the fresh honey samples was not detected, except sample 10 (0.32 mg/kg). Although 5-HMF was detected in fresh sample 10, it is still low and within the permitted range by Malaysian Standards. Unfortunately, the 5-HMF content of all the honey samples (after 6 and 15 months) exceeded the permitted range. Therefore, it is advised to consume the honey in less than six months. Alternatively, a new study regarding the 5-HMF content of honey samples stored at 4 °C (refrigerator) for different storage periods has to be carried out in order to visualize the change pattern of 5-HMF content in honey. Additionally, the new study has to include the influence of storage period on the physicochemical profile and antioxidant activity of honey, so that the correlation of the parameters can be studied too.

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