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COMPARISON OF PHYSICOCHEMICAL, TOTAL PROTEIN AND ANTIOXIDANT PROFILES BETWEEN MALAYSIAN *Apis* AND *Trigona* HONEYS

(Perbandingan Profil Fizikokimia, Jumlah Protin dan Antioksidan antara Madu *Apis* dan *Trigona* Malaysia)

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Abstract

The properties of honeys are highly influenced by botanical sources, geographical origins, seasonal, processing, and bee species. The aims of this study are to characterize and compare the physicochemical, total phenolic, total flavonoids, total protein and antioxidant profiles between several Malaysian *Apis* and *Trigona* honeys. pH, free acidity, total soluble solids, ash content, electrical conductivity, density, colour, hydroxymethylfurfural and sugar content were the observed physicochemical parameters. All honey samples were also analyzed for their total phenolic (TPC), flavonoid (TFC) and protein contents, DPPH radical scavenging and total antioxidant activities. The physicochemical results of Malaysian *Apis* and *Trigona* honeys were noticeably different in terms of free acidity and electrical conductivity (EC), with extremely high free acidity (271.1 – 553.2 meq/kg) and higher EC (0.92 – 1.29 mS/cm) were observed for the latter. The results from TPC (60.21 mg GAE/100 g), TFC (65.86 mg QE/100 g), DPPH radical scavenging IC_{50} (10.57 mg/mL) and total antioxidant activities (713.82 µM Fe(II)) revealed that *Trigona* K1 honey was rich with polyphenols and other antioxidants compared to other *Trigona* and *Apis* honeys. It can be concluded from the present study that Malaysian *Trigona* honeys have distinguished physicochemical and antioxidant profiles than *Apis* honeys.

Keywords: *Apis* honey, *Trigona* honey, stingless bee, physicochemical, total phenolic content, antioxidant

Abstrak

Sifat-sifat madu adalah sangat dipengaruhi oleh sumber botani, asal geografi, musim, pemprosesan dan jenis lebah. Matlamat kajian ini adalah untuk mencirikan dan membandingkan profil fizikokimia, jumlah fenolik, jumlah flavonoid, jumlah protin dan antioksidan antara beberapa madu *Apis* dan *Trigona* Malaysia. pH, asid bebas, jumlah pepejal larut, kandungan abu, kekonduksian elektrikal, ketumpatan, warna, kandungan hidroksimetilfurfural dan kandungan gula adalah parameter-parameter yang dilihat.

Kesemua sampel madu juga dianalisa untuk jumlah kandungan fenolik (TPC), kandungan flavonoid (TFC) dan kandungan protin, aktviti memerangkap radikal DPPH dan aktiviti jumlah antioksidan. Keputusan fizikokimia bagi madu *Apis* dan *Trigona* Malaysia telah menunjukkan perbezaan yang ketara dari segi asid bebas dan kekonduksian elektrikal (EC), dengan asid bebas yang amat tinggi (271.1 – 553.2 meq/kg) dan EC yang tinggi (0.92 – 1.29 mS/cm) diperhatikan untuk jenis madu kedua. Keputusan TPC (60.21 mg GAE/100 g), TFC (65.86 mg QE/100 g), IC_{50} memerangkap radikal DPPH (10.57 mg/mL) dan aktiviti jumlah antioksidan (713.82 µM Fe(II)) mendedahkan bahawa madu *Trigona* K1 adalah kaya dengan polifenol-polfenol dan antioksidan-antioksidan lain berbanding dengan madu *Trigona* dan *Apis* yang lain. Dapat disimpulkan daripada kajian ini bahawa madu *Trigona* Malaysia mempunyai profil fizikokimia dan antioksidan yang berbeza daripada madu *Apis*.

Kata kunci: madu *Apis*, madu *Trigona*, lebah kelulut, fizikokimia, jumah kandungan fenolik, antioksidan

Introduction

Honey composition is greatly influenced by its botanical and geographical origins [1, 2], therefore justifying the presence of a variety of honey in the global market. Known to have carbohydrates and water as its major compositions, honey also comprises of other constituents including enzymes, amino acids, organic acids, vitamins, minerals, polyphenols (phenolic compounds), carotenoids and Maillard reaction products which are contributed by plants and bees themselves [1, 2]. Enzymes such as catalase, glutathione-*S*-transferase and superoxide dismutase are among antioxidant enzymes found in honeybees *A. mellifera* [3]. During their visit on flowering plants, bees could have visit various different plants and thus take up the antioxidant compounds produced by the plants in the form of nectar and/or pollen. Due to this, honey exhibits a wide range of free-radical scavenging phytochemicals such as flavonoids, phenolic acids, ascorbic acid, α-tocopherol, carotenoids-like substances as well as other non-enzymatic antioxidants such as Maillard reaction products, organic acids, amino acids and proteins [2, 4-7]. Particularly, polyphenols mainly flavonoids and phenolic acids have been directly linked to the antioxidant activities in honey through both direct and indirect scavenging of free radicals [2, 6, 8].

Sensory properties of honey (i.e. colour, texture, flavor, odor) may influence consumption predisposition and commercialization. In fact, other than sensory, honey quality can be determined through its physical, chemical and microbiological characteristics [9]. Considering the importance of ensuring quality of honey, two guidelines namely Codex Alimentarius Standard 1981/2001 [10] and European Union (EU) Directive 2001/110 [11] have outlined several honey physicochemical quality criteria to evaluate honey quality including moisture content, free acidity, ash content, electrical conductivity, reducing and non-reducing sugars, diastase activity and hydroxymethylfurfural (HMF) content. Although microbiological properties can also be used to evaluate honey quality, lacks of specifications with regard to microbial contamination and hygiene of honey except for *Clostridium botulinum* making physicochemical analysis of honey is the most frequently studied all over the world [9].

Even though honey can be produced by honey bee (Apidae, Apini) and stingless bee (Apidae, Meliponini), physicochemical properties of honeys were shown to differ between different bee genera (e.g. *Apis*, *Melipona*, *Trigona*) [12-14]. Specifically, higher moisture content, lower diastase activity and reducing sugars, higher electrical conductivity and acidity were the characteristics observed in sour-sweet (acidic) taste stingless bee honey as compared to *Apis mellifera* honey [15]. Considering the difference in the physicochemical properties of stingless bee honeys, the influence of bee species on honey composition and properties should be considered when assessing honey quality [12, 13, 16, 17]. However, there are no or limited published quality control regulations pertaining to stingless bee honey are made available. The two mentioned guidelines Codex Alimentarius and EU Directive particularly address honey produced by honeybees (*Apis* spp.) and *A. mellifera* (European or western honeybees), respectively [10, 11]. Thus, it is noteworthy that honey produced from different bee genera than specified in EU Directive and Codex Alimentarius may not meet the outlined criteria.

Insufficient information pertaining to honeys from stingless bee could be the reason it is not included in the international standards for honey (Codex Alimentarius) [15]. The aims of this study are to characterize the physicochemical, total phenolic, total flavonoids, total protein and antioxidant properties of several Malaysian *Apis* and *Trigona* honeys and to differentiate these profiles between these two bee genera. The information from this study is useful and can be considered for the establishment of national and international standards for stingless bee honey.

Materials and Methods

Chemicals

D(-)-Fructose, D(+)-sucrose, analytical and HPLC-grade methanol were purchased from Merck (Darmstadt, Germany). Bovine serum albumin was procured from Vivantis Inc. (USA). D(+)-glucose and 5-hydroxymethylfurfural (HMF) were supplied by Sigma Chemicals Co. (St. Louis, MO, USA). Folin & Ciocalteu's phenol reagent, sodium nitrite, sodium carbonate, aluminum chloride, iron (III) chloride hexahydrate, iron (II) sulfate heptahydrate, Bradford reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ) were purchased from Sigma-Aldrich Co. (Steinheim, Germany).

Collection of honey samples

Seventeen natural honeys from *Apis* spp. and *Trigona* spp. were collected from several areas in Malaysia. Three types of unifloral honey (gelam, nanas and acacia) and two types of multifloral honey (tualang and kelulut) were analyzed. Details of honey samples are provided in Table 1. All honey samples were identified by authorized bee keepers and personnel from Johor Department of Agriculture, Terengganu Honey Collector Corporation, as well as Sabah Rural Development Corporation. All honey samples were kept in the dark at room temperature between 24-28 °C prior to analysis. All physicochemical analyses were conducted in less than nine months. Samples were tested for biochemical and antioxidant analyses within one year of storage.

Physicochemical profiles

pH and free acidity

pH of samples was measured in a solution prepared with 10 g of sample in 75 mL of distilled water using a pH meter (Delta 320, Mettler Toledo, USA) whereas free acidity was determined by dissolving 10 g of samples in 75 mL of distilled water and titrated with 0.1 M NaOH to pH 8.3 [18].

Total soluble solids and density

Total soluble solids (TSS) of 20% (w/v) samples were measured using a refractometer (E-line ATC 44-803, Bellingham-Stanley, UK). TSS measurements were further corrected for a standard temperature of 20 °C by including the correction factor 0.00023/°C [19]. Density was calculated using Equation 1.

Table 1. Honey samples

Sample	Bee species	Botanical origin	Location
A1	*Apis mellifera*	*Acacia mangium*	Johor, Peninsular Malaysia
A2, A3, A4	*Apis cerana indica*	*Acacia mangium*	Sabah, West Malaysia
G1, G2, G3	*Apis mellifera*	*Melaleuca cajuputi*	Terengganu, Peninsular Malaysia
N1, N2, N3	*Apis mellifera*	*Ananas comosus*	Johor, Peninsular Malaysia
T1	*Apis dorsata*	Mixed source	Sabah, West Malaysia
T2, T3	*Apis dorsata*	Mixed source	Terengganu, Peninsular Malaysia
K1	*Trigona spp.*	Mixed source	Sabah, West Malaysia
K2, K3, K4	*Trigona itama*	Mixed source	Kelantan, Peninsular Malaysia

A = acacia, G = gelam, N = nanas, T = tualang, K = kelulut

$$\text{Density} = \frac{[(\text{weight of sample+cylinder}) - \text{weight of cylinder}]}{\text{volume of sample}} \quad (1)$$

Ash content and electrical conductivity

One gram of samples was ignited until completely dried. The sample crucibles were then placed in a furnace (Thermolyne-Barnstead, USA) and incinerated at 600 °C for 6 hours. Ash content (g ash/100 g of honey) was calculated using the following formula [18]:

$$\text{Ash} = \frac{[(m_1 - m_2)]}{m_0}\ x\ 100 \quad (2)$$

where m_0 = sample weight, m_1 = weight of crucible + ash, and m_2 = weight of empty crucible. Electrical conductivity (EC) of 20% w/v (dry weight basis) sample was determined using a conductivity meter (HI 98311, Hanna Instruments, Mauritius) in ultrapure water [18].

Hydroxymethylfurfural content

HMF content was determined using a HPLC according to methods described by the IHC [18]. Ten grams of sample was dissolved in 50 mL ultrapure water, filtered through 0.45 µm nylon membrane filter, and injected into an HPLC system (Agilent 1100, Agilent Technologies, USA) equipped with a photodiode array detector. The analytical column was a ZORBAX Eclipse XDB-C18 (4.6 x 150 mm, 5 µm; Agilent Technologies, USA). The mobile phase was methanol: water (90:10, v/v) at a flow rate of 1.0 mL/min. The detection wavelength was set at 285 nm. The standard curve was prepared using standard HMF (0 – 10 mg/L, Y = 145.77x + 0.8048, R^2 = 0.999). Results were expressed as mg of HMF/1000 g of honey (mg/kg).

Colour

Colour of samples was measured using a colour photometer (Hanna HI 96785, Hanna instruments, Romania) with reference to analytical grade glycerol and classified according to United States Department of Agriculture (USDA) colour standard designation [20].

Sugar content

Sugar content of honey samples was determined using HPLC system (Agilent 1100, Agilent Technologies, USA) equipped with a refractive index detector [18]. Honey solution 5% (w/v) was prepared, filtered through 0.45 µm nylon membrane filter and 20 µL was injected into the HPLC system equipped with Phenomenex NH_2 column (5 µm, 250 mm x 4.6 mm) kept at 30 ºC. Mobile phase was ultrapure water maintained at a flow rate 0.6 mL/min. Calibration curves were produced for glucose, fructose, and sucrose solutions (0 - 10 mg/ mL, R^2 = 0.999). Results were expressed as mg of saccharide/100 g of honey (mg/100 g).

Total protein and antioxidant profiles

Determination of total phenolic content

One milliliter of Folin-Ciocalteu reagent was mixed with 1 mL of diluted honey samples (0.2 g/mL). After 3 min, 1 mL of 10 % sodium carbonate solution was added to the reaction mixture, and the volume was adjusted to 10 mL with distilled water [21]. The mixture was incubated in the dark for 90 min at ambient temperature and the absorbance was read at 725 nm (Epoch, BioTek Instruments Inc., USA). The standard curve was prepared using gallic acid (0 - 140 µg/mL, Y = 0.0066x - 0.0098, R^2 = 0.999). Results were expressed as mg of gallic acid equivalents (mg GAE)/100 g of honey.

Determination of total flavonoid content

Four milliliters of distilled water were added to 1 mL of diluted honey (0.2 g/mL), followed by addition of 0.3 mL of 5 % w/v sodium nitrite. After 5 minutes, 0.3 mL of 10 % w/v aluminum chloride was added. After 6 minutes, 2 mL of 1 M NaOH was added, and the volume was increased to 10 mL by adding 2.4 mL of distilled water [21]. The solution was mixed well and read at 510 nm. A calibration curve was prepared using standard solutions of quercetin (0 - 100 µg/mL, Y = 0.0004x + 0.0007, R^2 = 0.998). Results were expressed as mg of quercetin equivalents (mg QE)/100 g of honey.

Determination of total protein content

Honey sample (5 µL, 50 % w/v) was mixed with 250 µL of Bradford reagent [22]. The mixture was incubated for 5 minutes and the absorbance was taken at 595 nm. Bovine serum albumin (0 - 1.4 mg/mL, Y = 0.3739x – 0.0183, R^2 = 0.997) was used as a standard for preparing

the calibration curve. Results were expressed as mg of protein/kg of honey.

DPPH radical scavenging activity

The DPPH radical scavenging activity was measured as described by [23] and with some modifications as made by Ferreira et al. [24]. Pure honey samples in different concentrations (0.98 - 62.5 mg/mL), ascorbic acid (0.24 - 31.25 µg/mL) and DPPH (0.04 mg/mL) were prepared in methanol. In 96-well plates, 100 µL of sample was mixed with 100 µL of methanolic solution containing DPPH radical. The mixture was homogenized and left to stand in the dark for 30 minutes. Absorbance was measured at 517 nm against a blank to eliminate the influence of honey colour. The blank was honey sample without DPPH radical. Ascorbic acid was used as a control. Radical scavenging activity (RSA) was calculated using the following formula:

$$\text{RSA}\ (\%) = \frac{[(A_{DPPH} - A_S)]}{A_{DPPH}}\ x\ 100 \qquad (2)$$

where A_S is the absorbance of the solution when the sample has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. IC_{50} is the concentration of sample at which 50% of DPPH radicals were scavenged. IC_{50} was calculated from the relationship curve of RSA (%) against sample concentrations.

Estimation of total antioxidant activity

Total antioxidant activity was determined using ferric reducing antioxidant power (FRAP) assay. FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ at the volume of 10:1:1 and pre-warmed to 37 ˚C prior to use. Diluted honey (200 µL, 0.2 g/mL) was mixed with 1.5 mL of FRAP reagent. The absorbance of the reaction mixture was measured at 593 nm after incubation at 37 ˚C for 4 minutes [21]. Aqueous standard solution of $FeSO_4.7H_2O$ (0 - 1000 µM, R^2 = 0.997) were used for the calibration curve. Results were expressed as the FRAP value (µM Fe (II)) of the 20% honey solution.

Statistical analysis

Results were presented as mean values ± standard deviation (SD). All physicochemical, total protein and antioxidant analyses were conducted in three independent experiments in triplicates (n = 9) except for HMF (two independent experiments, n = 2) and sugar content (three independent experiments, n = 3). The statistical differences represented by letters were obtained through one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test ($p < 0.05$). These were carried out using SPSS version 16.0 program (IBM Corporation, New York, USA).

Results and Discussion

Physicochemical profiles

Trigona honeys showed lower pH but higher free acidity and EC than *Apis* honeys (Table 2). Similarly, earlier study reported that *Trigona* (*Heterotrigona and Geniotrigona spp*.) honey had lower pH but higher free acidity than *A. mellifera* honey [17]. pH of all honey samples (2.88 - 3.65) were lower compared to the range set for blossom honeys (pH 3.5 - 4.5) and none fall within honeydew honey classification (pH 4.5 - 6.5) (Table 2). pH of Malaysian *Apis* honeys obtained in this study were comparable to those reported by literatures [25-27] with pH ranged from 3.44 - 3.89, 3.21 - 3.50, and 3.53 - 4.03, respectively. Evidence also pointed out that some Malaysian and Thailand *Trigona* honeys exhibited slightly higher pH (3.70 - 4.05) [15, 28, 29] than observed in this study. pH values of some Indian and Algerian honeys ranged from 3.7 - 4.4 and 3.49 - 4.43, respectively [19, 30]. pH differences in honey could be affected by harvest and storage conditions [31] and bee species [15-17]. The acidic pH contributes to honey antibacterial properties [32] and subsequently prolongs the shelf-life of honey due to stability against microbial spoilage [27]. It was reported by Al-Kafaween [33] that 25% (w/v) *Trigona* honey was the minimum bactericidal concentration (MBC) needed to kill bacteria *Pseudomonas aeruginosa* and *Streptococcus pyogenes*, thus indicating the antibacterial activity of *Trigona* honey.

Free acidity of honeys ranged from 10.80 - 553.20 meq/kg (Table 2). Gelam (G1) sample was the only honey sample that showed free acidity within limit 50 meq/kg which indicates absence of undesirable fermentation [10]. Honeys from Portugal were reported to contain free acidity between 16 - 32 meq/kg [9]. The higher free acidity in nearly all analyzed Malaysian honey samples could possibly be the results of increased glucose oxidation and fermentation activity. Considered the seasonal factor, diluted honey resulted in activation of glucose oxidase, the enzyme that converts glucose to gluconic acid which is the major organic acid in honey [17, 32]. Fermentation is also expected to occur within the naturally higher moisture content Malaysian honeys upon storage. This condition favors action of yeasts that convert glucose and fructose into ethyl alcohol and carbon dioxide. The alcohol is then converted into acetic acid and water in the presence of oxygen, resulting in a sour taste [19, 25]. On top of that, this study suggested that the extremely high free acidity seen in *Trigona* honeys (Table 2) is most likely be due to the influence of bee species and floral composition. Similar findings were observed by Chuttong et al. [15] in which their honey samples from three *Trigona* spp. exhibited total acidity between 440 and 592 meq/kg. Another study by Shamsudin et al. [17] pointed out that free acidity between *Heterotrigona itama and Geniotrigona thoracica* honeys differ between nectar sources. Also, their *Trigona* honey samples showed higher free acidity than *A. mellifera* honey with four organic acids namely gluconic, lactic, acetic and citric acids were found in all these stingless bee honey samples.

Total soluble solids (TSS) of honeys was in the range of 63.33 - 84.11 ˚Brix (Table 2), in accordance to the values of 60.9 - 76.7 ˚Brix, 76.2 - 80.4 ˚Brix, 79.0 - 82.2 ˚Brix and 70.0 - 85.0 ˚Brix found in some Malaysian, Indian, Portugal and Saudi Arabia honeys, respectively [17, 19, 34, 35]. Earlier study demonstrated that the TSS values found in stingless bee honey were lower than in *A. mellifera* honey [17]. However, two *A. mellifera* honeys (gelam G2 and G3) also showed lower TSS values (Table 2). It was reported that Saudi Arabia honeys with low TSS contents showed low density values and high water content [35]. The Brix values are related to the sugar content in honey [17]. Density of analyzed honey samples ranged from 1.27 - 1.57 g/mL (Table 2) whereas some Saudi Arabia honeys demonstrated density between 1.35 - 1.44 [35]. Kelulut (K3 and K4) and gelam (G2) honeys showed lower density than other samples (Table 2). Honeys with lowest density have high moisture content and vice versa [30, 35]. Due to the high moisture content, stingless bee honeys such as *Melipona subnitida* honeys appear more fluid [14]. In fact, several factors are known to affect the honey water content including harvesting season, climatic factors and degree of maturity reached in the hive [34].

Ash content and EC showed some interrelation with mineral content of honey [26]. Ash content of samples ranged from 0.03 - 0.45 g/100 g (Table 2). Some Algerian, Portugal and Indian honeys exhibited ash content between 0.06 - 0.23%, 0.07 - 0.35% and 0.03 - 0.43%, respectively [9, 19, 30]. Similar to earlier findings, all analyzed honey samples were of nectar origin where the ash content ≤ 0.6% [26]. A number of mineral elements were reported to be present in Malaysian honeys including potassium (70%), sodium, magnesium, iron, aluminium, zinc, chromium, etc. [26]. The ash content of *Trigona* honeys (0.13 - 0.45 g/100 g) measured in this study was in accordance to the previous study which reported ash content in between 0.15 and 0.67 g/100 g [36]. Some Thailand stingless bee honeys were shown to have ash content ranged from 0.04 - 3.1 g/100g [15]. Trigonini are known as the most generalized bees due to the wide spectrum of pollen they collect, possibly due to their small size that gives them more flexibility in collective sampling as well as living in populous to very populous colonies [37, 38], thus could explain the higher ash contents observed for some *Trigona* honeys in this study. Amazingly, it was reported that *Trigona* bees were shown to collect a very high number of total pollen (3289 - 58,994) compared to *A. mellifera* bees (53 - 485) [29].

EC values of samples ranged between 0.25 and 1.29 mS/cm (Table 2). EC relies on ash and acid contents of honey. Specifically, the higher their contents, the higher the resulting conductivity [9, 19]. Higher EC values ranged from 1.07 - 1.80 mS/cm and > 2.0 mS/cm have been reported for respective Malaysian *Apis* honeys and

Thailand stingless bee honeys [15, 26]. Similarly, some *Apis* honeys and all *Trigon*a honeys in this study exhibited high EC values (> 0.8 mS/cm), which do not fall within the guideline for nectar honey by previous studies [10,11].

HMF content in honey samples ranged from 0.70 to 163.15 mg/kg honey (Table 2). HMF is normally absent in fresh honeys but tends to increase during processing and/or due to aging, thus it is widely recognized as a parameter of honey freshness [9]. According to Codex Alimentarius Commission [10], honey originated from countries or regions with tropical ambient temperatures and blends of these honeys shall contain HMF not more than 80 mg/kg honey. However, honeys acacia (A1) and kelulut (K1) showed HMF values higher than permitted limit of 80 mg/kg honey. This indicates that these two honey samples have lost their freshness due to much longer storage time compared to other samples prior to analysis. Samples with low HMF values indicate freshness of samples [35] and proven unheated as claimed by the honey suppliers. Higher HMF content could also be the results of heating where acid-catalyzed dehydration of hexose sugars such as fructose and glucose takes place [25]. Singh and Bath [39] stated that fructose is highly susceptible to the acid-catalyzed dehydration reaction as it is found unstable at pH 4.6 and is five times more reactive than glucose in its most stable acid environment. Earlier study by Khalil et al. [25] reported that five Malaysian honey samples stored for up to six months at room temperature yielded low HMF values ranged from 2.80 - 24.87 mg/kg honey, which is within the permitted maximum level of 40 mg/kg honey as recommended by [10] for honey after processing and/or blending. The study also revealed that two tualang honey samples stored for 24 months at room temperature had the highest HMF values (986.57 - 1131.76 mg/kg honey) regardless of low pH content. Their study demonstrated that storage duration had a strong positive correlation with HMF formation whereas pH showed only moderate correlation with HMF content. Thus, HMF content in honey could be influenced by poor storage conditions (e.g. temperature), higher temperature and longer duration of heating process, pH and floral sources.

Colour of honeys ranged from 51 - 150 mm Pfund (Table 2). Darker colour was observed for *Trigona* honeys, dissimilar to most of monofloral *Apis* honeys (Table 2). Dark-colored honeys were reported to have higher mineral content [26], however, this pattern was inconsistent in this study. Earlier study revealed that dark honeys exhibited higher phenolic contents and were rich in pigments such as carotenoids than the light ones [24, 35].

The sugar content in analyzed honey samples are shown in Figure 1. It can be seen that all *Apis* honeys exhibited higher concentration of sugars (43.5 - 61.15 g/100 g) than *Trigona* honeys (12.15 - 38.00 g/100 g). This could explain the less sweetness taste of *Trigona* honeys and one of the reasons for their low sugar content could be due to the conversion of sugars into organic acids [32] resulting in a sour taste. Similarly, these findings were supported by Omar et al. [28] who reported low concentrations of fructose and glucose (4.5 - 24.6%) and sucrose (0.5 - 2.2%) in four Malaysian stingless bee honeys. Much recent study also demonstrated low reducing sugars [fructose and glucose] (15.4 - 24.7%) in Malaysian *Trigona* honeys compared to *A. mellifera* honey (51.0%) [17].

EU Council [11] pointed out that total fructose and glucose (reducing sugars) content in blossom honey must not be less than 60 g/100 g of honey. Higher reducing sugars were reported in some Malaysian *Apis* honeys between 61.17 - 63.89% [27], Algerian honeys between 67.83 - 80.25% [30] and Portugal honeys between 67.7 - 73.7% [9]. Nonetheless, the lower reducing sugar values of *Apis* honeys seen in this study were supported by Saxena et al. [19] who demonstrated variations in the reducing sugar content of some Indian honey samples ranging from 43.3 - 65.5%. Comparisons between all honey samples revealed that Acacia (A2) and gelam (G1) honeys exhibited highest fructose (29.6 %) and glucose (30.2 %), respectively (Figure 1). Sucrose contents in analyzed honey samples ranged from 0.3 - 5.6 g/100 g (Figure 1). Codex Alimentarius Commission [10] specified that sucrose content in honey should be less than 5%. Except for acacia (A1), other samples showed lower sucrose content within the permitted limit. The higher sucrose content observed in

acacia (A1) honey sample could be due to the early harvest of honey where sucrose conversion to fructose and glucose by the bees' invertase enzymes has not completely taken place [19]. Additionally, nanas honeys (N1-N3) exhibited nearly equivalent composition of reducing sugars (Figure 1). It is suggested that bee species and sources of nectar are most likely to have some influence on these observed results.

Total protein and antioxidant profiles

Apis honeys showed total phenolic content (TPC) between 12.6 - 47.1 mg GAE/100 g of honey (Table 3). These results were comparable to the earlier studies by Khalil et al. [21] (22.32 - 41.99 mg GAE/100 g), Moniruzzaman et al. [27] (18.67 - 35.27 mg GAE/100 g) and Ranneh et al. [40] (13.94 - 18.39 mg GAE/100 g) for Malaysian *Apis* honeys. In contrast, other studies reported higher (110.39 - 196.50 mg GAE/100 g) [41] and lower TPC (1.52 – 4.22 mg GAE/100 g) [42] for some Malaysian *Apis* honeys. *Trigona* honeys showed higher TPC than *Apis* honeys in the range of 33.2 – 60.2 mg GAE/100 g (Table 3), in accordance to the TPC results reported by Bakar et al. [36]. Foraging differences between these bee species on multiple plant sources could possibly be one of the reasons [16]. Also, it is noteworthy that the TPC results for *Trigona* honeys in this study were much higher than reported by Ranneh et al. [40] (22.81 - 23.53 mg GAE/100 g). This observation may be due to the influence of several factors including floral origin and seasonal factors. Floral origin affects concentration and type of phenolic compounds found in honey [8, 19]. Seasonal factors such as monsoon season may restrict the norm of bee foraging activities [16].

Estimated total flavonoid content (TFC) obtained for *Apis* honeys in this study (Table 3) were higher (14.5 - 70.3 mg QE/100 g of honey) than other reported studies for Malaysian *Apis* honeys by Khalil et al. [21] (13.53 – 31.89 mg catechin equivalent (CE)/100 g), Moniruzzaman et al. [27] (2.20 – 6.57 mg CE/100 g), Ranneh et al. [40] (6.47 – 6.70 mg CE/100 g) and Chua et al. [41] (18.51 – 32.89 mg rutin equivalent (RE)/100 g). *Trigona* honeys in this study exhibited TFC in the range of 43.2 - 65.9 mg QE/100 g of honey (Table 3). These results were higher than TFC values of *Trigona* honeys demonstrated by earlier studies which were in the range of 5.38 - 30.86 mg RE/100 g [36], 9.79 - 10.15 mg CE/100 g [40] and 2.38 - 9.31 mg QE/100 g [17]. Variations between the present and previous findings could be due to different floral origins, seasonal factors, and bee species preference [4, 16, 17].

The protein contents ranged from 274.4 - 1058.9 µg/g (Table 3). Protein in honeys can be attributed to the presence of enzymes as the main contributors from introduction by bees or from nectar of plants [19, 27]. In fact, total protein and amino acid in honey are also influenced by the geographical and botanical origins as well as storage time [27]. Normally, honey contains less than 5 mg protein/g honey and amino acid proline dominates in honey [1]. Moniruzzaman et al. [27] reported that the protein content in their Malaysian *Apis* honey samples ranged from 2040 - 4830 µg/g, higher than observed in this study.

The IC_{50} for honey samples ranged from 10.6 – 52.7 mg/mL, lowest in kelulut (K1) and highest in gelam (G1) samples, respectively (Table 3). Earlier findings reported that IC_{50} of Malaysian *Apis* honeys were between 5.24 and 17.51 mg/mL [42] whereas Malaysian *Trigona* honeys exhibited IC_{50} between 32.58 and 105.53 mg/mL [17]. The lower IC_{50} for some *Apis and Trigona* honeys in this study indicated the high radical scavenging activity of these honeys (Table 3). From Table 3, it can be seen that monofloral acacia (A1 and A2), multifloral kelulut (K1-K4) and tualang (T1) showed 50% DPPH inhibition at less than 20 mg/mL honey. The unpaired electron of DPPH forms a pair with an electron donated by antioxidants (e.g. from honey), causing a colour changes from deep purple to yellow as a result of conversion of DPPH radical to its reduced form (1,1-diphenyl-2-picryl-hydrazine) [19]. However, DPPH only measures the activity of water-soluble antioxidants [43]. Radical scavenging activity of phenolics is positively correlated with the number of OH groups [44]. Thus, it is assumed that honeys with lower IC_{50} may contain phenolics with more OH groups in addition to other water-soluble antioxidants.

Reducing power of honeys ranged from 196.3 – 713.8 µM Fe(II) (Table 3). FRAP assay is a simple, fast and precise assay [45] that gives a direct estimation of the

antioxidants (reductants) present in a sample based on their ability to reduce ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous form (Fe^{2+}-TPTZ) which resulted in a blue product [21, 27]. Aljadi et a. [5] reported that the FRAP values for Malaysian gelam and coconut honeys were 1350 and 961 µM Fe(II), respectively. These values are higher than those obtained in this study. Variations in the antioxidant activities of honeys are likely due to the different types and concentrations of polyphenols in each honey sample [16, 44]. However, total antioxidant activity is not solely contributed by the polyphenols where the presence of constituents other than the phenolic compounds such as vitamin C (ascorbic acid), E (α-tocopherol) and carotenoids may as well have some contributions [2, 4].

Table 2. Physicochemical profiles of honey samples

Sample	**pH**	**Free Acidity (meq/kg)**	**TSS (°Brix)**	**Density (g/mL)**	**Ash (g/100 g)**	**EC (mS/cm)**	**HMF (mg/kg)**	**Colour[c] (mm Pfund)**
A1	3.24 ± 0.01	84.00 ± 0.53	82.11 ± 1.90	1.40 ± 0.00	0.22 ± 0.03	0.84 ± 0.02	117.73 ± 9.60	150.00 ± 0.00
A2	3.64 ± 0.03	64.93 ± 4.27	77.67 ± 0.58	1.38 ± 0.00	0.17 ± 0.03	0.74 ± 0.01	5.55 ± 0.23	81.00 ± 1.00
A3	3.59 ± 0.02	61.27 ± 5.83	74.78 ± 2.04	1.39 ± 0.00	0.21 ± 0.06	0.81 ± 0.01	4.08 ± 2.18	80.00 ± 1.00
A4	3.40 ± 0.04	77.93 ± 4.46	77.00 ± 1.00	1.36 ± 0.00	0.17 ± 0.03	0.74 ± 0.02	6.11 ± 0.42	81.00 ± 1.00
G1	3.65 ± 0.06	10.80 ± 0.35	84.11 ± 0.19	1.55 ± 0.00	0.03 ± 0.02	0.25 ± 0.03	45.42 ± 0.02	51.00 ± 1.00
G2	3.12 ± 0.02	88.67 ± 0.83	63.33 ± 2.08	1.28 ± 0.00	0.04 ± 0.03	0.46 ± 0.00	nd	64.00 ± 2.00
G3	3.38 ± 0.02	76.13 ± 4.57	68.56 ± 1.26	1.35 ± 0.00	0.15 ± 0.04	0.57 ± 0.01	0.70	120.00 ± 1.00
N1	3.33 ± 0.01	57.60 ± 1.44	77.89 ± 0.84	1.57 ± 0.00	0.10 ± 0.03	0.37 ± 0.01	13.97 ± 1.52	101.00 ± 5.00
N2	3.31 ± 0.01	56.20 ± 0.00	77.67 ± 0.58	1.39 ± 0.00	0.11 ± 0.02	0.41 ± 0.02	13.20 ± 0.93	66.00 ± 4.00
N3	3.26 ± 0.04	59.87 ± 2.16	79.67 ± 1.15	1.32 ± 0.00	0.11 ± 0.03	0.40 ± 0.02	14.42 ± 0.76	67.00 ± 4.00
T1	3.29 ± 0.01	84.67 ± 0.42	75.67 ± 1.15	1.35 ± 0.00	0.13 ± 0.01	0.84 ± 0.02	46.54 ± 3.56	130.00 ± 1.00
T2	3.30 ± 0.02	70.00 ± 1.22	73.33 ± 1.53	1.47 ± 0.00	0.16 ± 0.02	0.89 ± 0.02	nd	75.00 ± 1.00
T3	3.28 ± 0.02	73.83 ± 0.98	74.33 ± 1.34	1.46 ± 0.00	0.16 ± 0.01	0.75 ± 0.01	nd	107.00 ± 2.00
Mean *Apis*	3.37 [a]	66.61 [a]	75.85 [a]	1.41 [a]	0.13 [a]	0.62 [a]	-	90.00 [a]

Table 2 (cont'). Physicochemical profiles of honey samples

Sample	pH	Free Acidity (meq/kg)	TSS (˚Brix)	Density (g/mL)	Ash (g/100 g)	EC (mS/cm)	HMF (mg/kg)	Colour[c] (mm Pfund)
K1	2.98 ± 0.02	271.13 ± 1.51	70.00 ± 1.73	1.35 ± 0.00	0.13 ± 0.01	0.92 ± 0.00	163.15 ± 14.08	118.00 ± 0.00
K2	3.24 ± 0.02	466.60 ± 4.87	72.45 ± 0.69	1.36 ± 0.00	0.45 ± 0.01	1.29 ± 0.02	5.87 ± 1.26	91.00 ± 3.00
K3	2.88 ± 0.01	553.20 ± 6.04	68.67 ± 0.58	1.27 ± 0.00	0.15 ± 0.01	0.93 ± 0.00	8.66 ± 1.28	100.00 ± 2.00
K4	3.02 ± 0.03	501.47 ± 4.17	67.33 ± 1.15	1.27 ± 0.00	0.25 ± 0.01	1.08 ± 0.01	6.03 ± 2.26	121.00 ± 1.00
Mean *Trigona*	3.03 [b]	448.10 [b]	69.61 [b]	1.31 [b]	0.25 [b]	1.05 [b]	-	107.00 [a]

[a] A = acacia, G = gelam, N = nanas, T = tualang, K = kelulut, TSS = total soluble solids, EC = electrical conductivity, HMF = hydroxymethylfurfural, nd = not detected

[b] Values are means ± SD of three independent experiments in triplicates (n = 9) except for HMF (two independent experiments, n = 2)

[c] USDA colour standard for honey; 51 – 85 = light amber, 86 - 114 = amber, > 114 = dark amber

[d] Values with different letters (superscripts) indicate significant differences ($p < 0.05$)

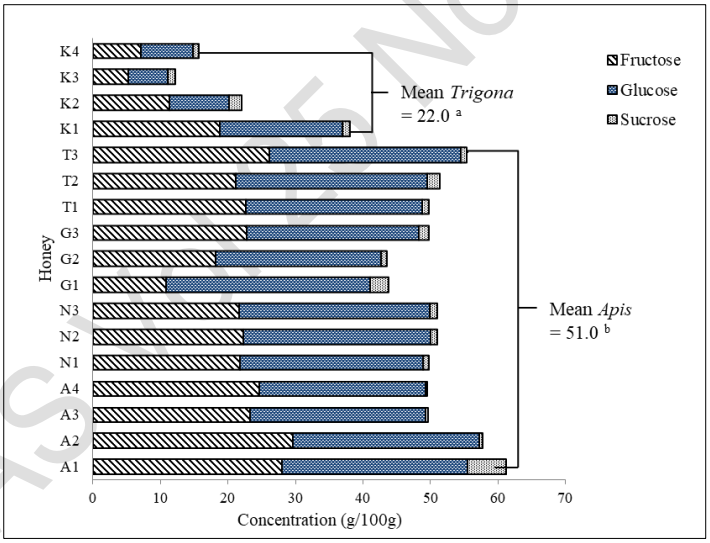

Figure 1. Sugar content of honeys: acacia (A), nanas (N), gelam (G), tualang (T) and kelulut (K). Values with different letters (superscripts) indicate significant differences ($p < 0.05$)

Table 3. Total protein and antioxidant profiles of honey samples

Sample	TPC (mg GAE/100 g)	TFC (mg QE/100 g)	Protein (µg/g)	IC_{50} (mg/mL)	FRAP (µM Fe(II))
A1	47.1 ± 2.3	48.1 ± 0.7	577.5 ± 21.6	12.9 ± 1.0	529.8 ± 6.4
A2	23.2 ± 1.3	34.2 ± 1.3	556.1 ± 47.9	12.2 ± 3.9	285.3 ± 1.9
A3	24.8 ± 0.8	32.3 ± 3.6	556.1 ± 34.4	21.9 ± 0.2	285.6 ± 10.2
A4	24.6 ± 0.8	29.3 ± 4.5	543.6 ± 66.5	26.8 ± 3.4	276.8 ± 2.8
G1	12.6 ± 0.1	14.5 ± 0.5	274.4 ± 32.5	52.7 ± 2.1	196.3 ± 3.3
G2	20.5 ± 0.9	30.0 ± 0.4	1058.9 ± 103.2	44.8 ± 8.4	201.6 ± 43.5
G3	20.1 ± 1.6	41.4 ± 2.1	598.9 ± 31.3	32.8 ± 3.9	234.2 ± 47.7
N1	25.6 ± 3.1	20.5 ± 1.6	481.2 ± 31.3	34.6 ± 2.4	263.0 ± 4.1
N2	27.6 ± 0.3	19.3 ± 0.8	491.9 ± 22.3	33.8 ± 3.4	263.5 ± 2.1
N3	24.8 ± 2.2	18.9 ± 1.4	468.8 ± 6.2	35.7 ± 3.3	265.4 ± 1.2
T1	46.3 ± 1.6	70.3 ± 1.1	495.5 ± 42.9	13.9 ± 1.2	529.8 ± 16.9
T2	26.9 ± 0.3	26.8 ± 2.4	351.1 ± 18.8	29.1 ± 0.7	269.1 ± 8.5
T3	22.3 ± 0.4	24.4 ± 1.3	352.9 ± 29.5	33.6 ± 2.3	253.3 ± 13.1
Mean *Apis*	26.6 [a]	31.5 [a]	523.6 [a]	29.6 [a]	296.4 [a]
K1	60.2 ± 2.2	65.9 ± 4.8	335.0 ± 21.6	10.6 ± 0.6	713.8 ± 20.1
K2	41.8 ± 1.2	53.4 ± 2.7	461.6 ± 42.5	11.2 ± 0.6	624.7 ± 4.3
K3	33.2 ± 1.2	43.2 ± 3.3	440.2 ± 14.2	19.7 ± 1.6	334.9 ± 4.4
K4	35.0 ± 1.1	46.6 ± 2.6	682.7 ± 40.5	14.8 ± 0.5	428.7 ± 1.0
Mean *Trigona*	42.6 [b]	52.3 [b]	479.9 [a]	14.1 [b]	525.5 [b]

[a] A = acacia, G = gelam, N = nanas, T = tualang, K = kelulut, TPC = total phenolic content, TFC = total flavonoid content, IC_{50} = concentration of sample at which 50% of DPPH radicals were scavenged, FRAP = ferric reducing antioxidant power assay, GAE = gallic acid equivalent, QE = quercetin equivalent.

[b] Values are means ± SD of three independent experiments in triplicates (n = 9).

[c] Values with different letters (superscripts) indicate significant differences ($p < 0.05$).

Conclusion

The physicochemical properties of Malaysian honeys were influenced by many factors including bee species, floral sources, seasonal factors, processing, geographical distribution, etc. The Malaysian *Apis* and *Trigona* honeys were remarkably different in several physicochemical parameters and therefore this study strongly supported the establishment of different standards for stingless bee honeys as a guideline for the consumers as well as the authorities in assessing honey quality. Considering the limited number of samples analyzed in this study, it is suggested that more studies should be conducted in the future with variety of honeys from different bee genera to obtain comprehensive information. It was observed from the antioxidant profiling that *Trigona* honeys exhibited significantly higher TPC, TFC and reducing power as well as lower IC_{50} than *Apis* honeys. However, two of the studied *Apis* honeys namely monofloral acacia (A1) and multifloral tualang (T1) honeys showed resemblance to *Trigona* honeys, indicating the influence of other factors such as floral sources to the honeys' antioxidant properties. It can be concluded from this study that both Malaysian *Apis* and *Trigona* honeys exhibited antioxidant activities at varying levels and thus can serve as good sources of natural antioxidants.

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References

1. Anklam, E. (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry,* 63(4): 549-562.
2. Gheldof, N., Wang, X. H. and Engeseth, N. J. (2002). Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry*, 50(21): 5870-5877.
3. Weirich, G. F., Collins, A. M. and Williams, V. P. (2002). Antioxidant enzymes in the honey bee, *Apis mellifera*. *Apidologie*, 33(1): 3-14.
4. Al-Mamary, M., Al-Meeri, A. and Al-Habori, M. (2002). Antioxidant activities and total phenolics of different types of honey. *Nutrition Research*, 22(9): 1041-1047.
5. Aljadi, A. M. and Kamaruddin, M. Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry*, 85(4): 513-518.
6. Beretta, G., Granata, P., Ferrero, M., Orioli, M. and Facino, R. M. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta*, 533(2): 185-191.
7. Baltrušaitytė, V., Venskutonis, P. R. and Čeksterytė, V. (2007). Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chemistry*, 101(2): 502-514.
8. Tenore, G. C., Ritieni, A., Campiglia, P. and Novellino, E. (2012). Nutraceutical potential of monofloral honeys produced by the Sicilian black honeybees (*Apis mellifera* ssp. sicula). *Food and Chemical Toxicology*, 50 (6): 1955-1961.
9. Gomes. S., Dias, L. G., Moreira, L. L., Rodrigues, P. and Estevinho, L. (2010). Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. *Food and Chemical Toxicology*, 48(2): 544-548.
10. Codex Alimentarius Commission (2001). Codex Standard for Honey: CODEX STAN 12-1981, Rev. 1 (1987), Rev. 2 (2001). FAO, Rome: pp. 1-8.
11. EU (European Union) Council. (2002). Council Directive 2001/110/EC of 20 December 2001 relating to honey. *Official Journal of the European Union*, L10: pp. 47-52.
12. Vit, P., Bogdanov, S. and Kilchenmann, V. (1994). Composition of Venezuelan honeys from stingless bees (Apidae : Meliponinae) and *Apis mellifera* L. *Apidologie*, 25(3): 278-288.
13. Bogdanov, S., Vit, P. and Kilchenmann, V. (1996). Sugar profiles and conductivity of stingless bee honeys from Venezuela. *Apidologie*, 27(6): 445-450.
14. Silva, T. M. S., dos Santos, F. P., Evangelista-Rodrigues, A., da Silva, E. M. S., da Silva, G. S., de Novais, J. S., dos Santos, F. d. A. R. and Camara, C. (2013). A phenolic compound, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (*Melipona subnitida*) honey. *Journal of Food Composition and Analysis*, 2 (1): 10-18.
15. Chuttong, B., Chanbang, Y., Sringarm, K. and Burgett, M. (2016). Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South East Asia (Thailand). *Food Chemistry*, 192: 149-155.
16. Ismail, N. I., Kadir, M. R. A., Mahmood, N. H., Singh, O. P., Iqbal, N. and Zulkifli, R. M. (2016). Apini and Meliponini foraging activities influence the phenolic content of different types of Malaysian honey. *Journal of Apicultural Research*, 55(2): 137-150.

17. Shamsudin, S., Selamat, J., Sanny, M., Razak, S. B. A., Jambari, N. N., Mian, Z. and Khatib, A. (2019). Influence of origins and bee species on physicochemical, antioxidant properties and botanical discrimination of stingless bee honey. *International Journal of Food Properties*, 22(1): 239-264.
18. Bogdanov, S. (2009). Harmonised method of the international honey commission. Access from http://www.bee-hexagon.net/en/network.htm. [Access online 20 January 2013].
19. Saxena, S., Gautam, S. and Sharma, A. (2010). Physical, biochemical and antioxidant properties of some Indian honeys. *Food Chemistry*, 118(2): 391-397.
20. United States Department of Agriculture (1985). United States standards for grades of extracted honey. Washington D.C., United States of America: Agricultural Marketing Service.
21. Khalil, M. I., Sulaiman, S. A., Alam, N., Ramli, N., Mohamed, M., Bai'e, S. and Hua G. S. (2012). Content and antioxidant properties of processed tualang honey (Agromas®) collected from different regions in Malaysia. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4: 214-219.
22. Bradford, M. M. (1976). A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2): 248-254.
23. Wang, H., Gao, X. D., Zhou, G. C., Cai, L. and Yao, W. B. (2008). In vitro and in vivo antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. *Food Chemistry*, 106(3): 888-895.
24. Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M. and Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry*, 114(4): 1438-1443.
25. Khalil, M. I., Sulaiman, S. A. and Gan, S. H. (2010). High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year. *Food and Chemical Toxicology*, 48(8-9): 2388-2392.
26. Chua, L. S., Abdul-Rahaman, N. L., Sarmidi, M. R. and Aziz, R. (2012). Multi-elemental composition and physical properties of honey samples from Malaysia. *Food Chemistry*, 135(3): 880-887.
27. Moniruzzaman, M., Khalil, M. I., Sulaiman, S. A. and Gan, S. H. (2013). Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*. *BMC Complementary and Alternative Medicine*, 13(1): 43.
28. Omar, S., Enchang, F. K., Nazri, M. U. I. A., Ismail, M. M. and Ismail, W. I. W. (2019). Physicochemical profiles of honey harvested from four major species of stingless bee (kelulut) in north east peninsular of Malaysia. *Malaysian Applied Biology*, 48(1): 111-116.
29. Selvaraju, K., Vikram, P., Soon, J. M., Krishnan, K. T. and Mohammed, A. (2019). Melissopalynological, physicochemical and antioxidant properties of honey from West Coast of Malaysia. *Journal of Food Science and Technology*, 56(5): 2508-2521.
30. Ouchemoukh, S., Louaileche, H. and Schweitzer, P. (2007). Physicochemical characteristics and pollen spectrum of some Algerian honeys. *Food Control*, 18(1): 52-58.
31. Terrab, A., Díez, M. J. and Heredia, F. J. (2002). Characterisation of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chemistry*, 79(3): 373-379.
32. Tan, H. T., Rahman, R. A., Gan, S. H., Halim, A. S., Hassan, S. A., Sulaiman, S. A. and Kirnpaul-Kaur, B. S. (2009). The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC Complementary and Alternative Medicine*, 9 (34): 1-8.
33. Al-Kafaween, M. A., Hilmi, A. B. M., Jaffar, N., Al-Jamal, H. A. N., Zahri, M. K. and Jibril, F. I. (2020). Antibacterial and antibiofilm activities of Malaysian *Trigona* honey against *Pseudomonas aeruginosa* ATCC 10145 and *Streptococcus pyogenes* ATCC 19615. *Jordan Journal of Biological Sciences*, 13(1): 69-76.

34. Silva, L. R., Videira, R., Monteiro, A. P., Valentão, P. and Andrade, P. B. (2009). Honey from Luso region (Portugal): physicochemical characteristics and mineral contents. *Microchemal Journal*, 93(1): 73-77.
35. Alqarni, A. S., Owayss, A. A. and Mahmoud, A. A. (2016). Physicochemical characteristics, total phenols and pigments of national and international honeys in Saudi Arabia. *Arabian Journal of Chemistry*, 9(1): 114-120.
36. Bakar, M. F. A., Sanusi, S. B., Bakar, F. I. A., Cong, O. J. and Mian, Z. (2017). Physicochemical and antioxidant potential of raw unprocessed honey from Malaysian stingless bees. *Pakistan Journal of Nutrition*. 16(11): 888-894.
37. Roubik, D. W. (1979). Nest and colony characteristics of stingless bees from French Guiana (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, 52(3): 443-470.
38. Sommeijer, M. J., De Rooy, G., Punt, W. and De Bruijn, L. L. M. (1983). A comparative study of foraging behavior and pollen resources of various stingless bees (Hym., Meliponinae) and honeybees (Hym., Apinae) in Trinidad, West-Indies. *Apidologi*e, 14(3): 205-224.
39. Singh, N. and Bath, P. K. (1997). Quality evaluation of different types of Indian honey. *Food Chemistry*, 58(1-2): 129-133.
40. Ranneh, Y., Ali, F., Zarei, M., Akim, A. M., Hamid, H. A. 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-Food Science and Technology*, 89: 1-9.
41. Chua, L. S., Rahaman, N. L. A., Adnan, N. A. and Tan, T. T. E. (2013). Antioxidant activity of three honey samples in relation with their biochemical components. *Journal of Analytical Methods in Chemistry*, 2013: 313798.
42. Khalil, M. I., Alam, N., Moniruzzaman, M., Sulaiman, S. A. and Gan, S. H. (2011). Phenolic acid composition and antioxidant properties of Malaysian honeys. *Journal of Food Science*, 76(6): 921- 928.
43. Frankel, S., Robinson, G. E. and Berenbaum, M. R. (1998). Antioxidant capacity and correlated characteristics of 14 unifloral honeys. *Journal of Apicultural Research*, 37(1): 27-31.
44. Ismail, N. I., Sornambikai, S., Kadir, M. R. A., Mahmood, N. H., Zulkifli, R. M. and Shahir, S. (2018). Evaluation of radical scavenging capacity of polyphenols found in natural Malaysian honeys by voltammetric techniques. *Electroanalysis*, 30(12): 2939-2949.
45. Benzie, I. F. F. and Strain, J. J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, 299: 15-27.