

Physicochemical Characteristics of Malaysian Stingless Bee Honey from *Trigona* Species

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ABSTRACT

The physicochemical properties of stingless bee honey are diverse according to origin of geographical or botanical and fruit or flower season. These factors result in production of honey with different colour, pH, ash and water content although the bee species is similar. To date, the physicochemical properties of honey from Malaysian *Trigona* species are unknown. Therefore, determination of physicochemical properties of honey from stingless bee is crucial for Malaysian researchers in order to analyse the purity of the honey. Five honey samples were used for the current study. Four samples were stingless bee honey from two of the most common domesticated Malaysian stingless bee species; *Trigona thorasica* and *Trigona itama*. Meanwhile, one sample was from Tualang honey. For stingless bee honey, the values for pH, moisture content, electrical conductivity, ash content and hydroxymethylfurfural (HMF) were 2.79 ± 0.04 to 2.95 ± 0.02 , 28.3 ± 0.68 to 33.7 ± 0.51 , 0.47 ± 0.22 to 0.55 ± 0.38 , 0.132 ± 0.03 to 0.532 ± 0.83 and 0.080 ± 0.16 to 3.42 ± 1.03 , respectively. The colour of stingless bee honey varies based on observation or absorbance assay. The study described the physicochemical properties derived from Malaysian bees of *Trigona thorasica*, *Trigona itama*, and *Apis Dorsata*.

KEYWORDS: Malaysian Stingless Bee; Physicochemical properties

INTRODUCTION

Stingless bee honey is a desirable bee product, which has been consumed for ages in its natural form.^{1,2} It has been practiced traditionally in the Malay, Arab, Hebrew, Persian, Indian, Roman and Chinese communities. Previously, it is referred as pot-honey, a legacy of stingless bee. However, the Malay addresses it as “madu kelulut”. This stingless bee honey is composed of carbohydrates, amino acids, phenolic compounds, organic acids, vitamins, minerals, lipids and enzymes. These forms the major constituents of the honey.³ The honey of stingless bee and *Apis* are similar in their compositional property such as ash and nitrogen content.⁴ However, they are different in term of moisture content, acidity, viscosity, and sucrose and mineral composition.^{5,6} The stingless bees forage on broad-spectrum of flowers that grow at different types of habitat, hence they produce different honey composition. Most of the physicochemical properties of honey mainly depend on the botanical source of the nectar, the environmental and climatic conditions as well as the species of the stingless bee that produce the honey. Before the introduction of sugarcane and *Apis* honey, stingless bee honey was the first and foremost source of sweetener.^{7,8} It has

been utilized as food, sweetener or for production of traditional fermented drink such as balche. The Mayan civilization has used this drink for its ritual activity.^{8,9}

The native stingless bees found in Malaysia are primarily known for their great ecological significance as plant pollinators.¹⁰ Products of the Malaysian stingless bee such as honey and propolis are of significant nutritional and therapeutic value.¹¹ The utilization of stingless bee honey as a traditional natural remedy has been in practice for the past few decades among Malay people. Currently, demands on stingless bee products including honey, propolis and hive are increasing. In addition, interest on farming the stingless bee is increasing among people in the rural residence especially in Kelantan, Terengganu, Perak and Kedah to suffice the high demand on crude honey and honey-based products.

Honey as a medicine has been mentioned by Allah Almighty in the Quran; (Chapter 16, sentence 69) “There comes from their bellies a drink of many colours in which there is healing for mankind. Abu Hurairah said “Whoever eats honey three times a month will not fit with any great disaster. Aisha said “The Prophet of Allah did indeed love to eat sweetmeats and honey”. The prophet of Allah used to drink a cup of water and honey on an empty stomach every day. This is a tremendously prudent thing to do to preserve everyone’s health. Health treatment using honey absolutely has long been

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practiced among Muslim community since more than one thousand years ago.

Determination of the physicochemical properties of stingless bee honey from *Trigona thorassica* and *Trigona itama* which are the most commonly available and utilized in Malaysia is very crucial. Ultimately, the finding is beneficial for setting the quality of food standard and honey characterization especially in the identification of Malaysian stingless bee honey. Therefore, the purity of honey can be determined and artificial or adulterated

honey may be easily identified.¹² To date, this type of finding is unknown although many research on stingless bee have been done in our country. The main purpose for this study was to investigate the physicochemical characteristics of Malaysian stingless bee honey.

MATERIALS AND METHODS

Honey Bee

Five fresh honey samples were collected directly from the bee farms. Information of honey samples were shown in Table 1.

Table 1: The identification of honey samples

Honey code	Bee species	Farm location	Main nectar	Time of collection
Honey A	<i>Trigona thorassica</i>	Kelantan	Monofloral	Durian season
Honey B	<i>Trigona itama</i>	Kelantan	Monofloral	Durian season
Honey C	<i>Trigona thorassica</i>	Terengganu	Multifloral	Normal season
Honey D	<i>Trigona itama</i>	Terengganu	Multifloral	Normal season
Honey E	<i>Apis dorsata</i>	Terengganu	Multifloral	Normal season

pH

The pH was determined by following the method of Belay *et al.* (2013) which was adapted from the AOAC, 1990 method number 962.19. Gently 10 g of each of the honey sample were separately measured in clean beakers and labeled accordingly. Each of the labeled sample was well mixed with 75 mL of sterile distilled water until a homogenous solution was achieved. After calibrating the pH meter with a standard buffer solution with pH of 4, 7 and 10, the glass electrode was gently immersed into the honey solution. Readings were recorded, and the procedure was repeated three times in order to achieve accurate result.¹³

Ash Content

The ash content was determined using the adopted method of Chuttong *et al.* (2016) from the AOAC 2006 number 962.19. Clean crucibles were oven dried at 105 °C for six hours. The crucibles were then transferred to desiccators with a porcelain plate and desiccant. The desiccators were then covered and the crucibles were allowed to cool before being weighed. The empty dried crucibles were then labeled, weighed and recorded accordingly. Five grams of each of the honey samples was weighted into the respective labeled crucible and carefully placed in a muffle furnace at 550 °C for 12 hours. After cooling, the crucibles were weighed and recorded accordingly. The ash content was calculated as follows:

Ash content (g) = $\frac{\text{Weight after ashing} - \text{Tare weight of crucible}}{\text{Original sample weight}} \times 100$

Moisture Content

The moisture content was determined using a digital Atago handheld refractometer (Kruss, HRH30). A few drops of honey was gently placed in the sample plate and then covered. Displayed readings were recorded and the procedure was repeated three times. The moisture content was calculated as 100-Brix = moisture content (0 to 85% Brix range with an accuracy of $\pm 0.2\%$).¹⁴

Electrical Conductivity

Electrical conductivity (EC) was measured using a conductivity meter HI 98311 (Hanna Instruments). A 20% (w/v) solution of honey was prepared by weighing 2 g of honey to 10 mL distilled water. After calibrating the EC meter, the conductance cell was suspended into the honey solution and the readings were recorded (Chuttong *et al.*, 2016). The procedure was repeated for each sample three times.

Colour Intensity

Using the method of Pontis *et al.* (2014), 5 g of each of the honey sample was dissolved in 10ml demonized water and gently mixed with a vortex mixer. About 2ml of the solution was transferred in to curvets and the absorbance was measured using a spectrophotometer at 635 nm. The recorded absorbance was matched with the Pfund scale.¹⁵

Hydroxymethylfurfural

Gas chromatography mass spectrometry was used to identify the hydroxymethylfurfural. The samples

were send to Universiti Malaysia Pahang.

RESULTS

The pH values of the stingless bee honey A, B, C and D ranged between 2.79 ± 0.04 to 2.95 ± 0.02 , while the pH of honey E was 3.64 ± 0.04 . All of the honey types are characterized as acidic. The results showed that although with different geographical and botanical source of the honey samples, their pH were almost within the same range. The finding may suggest that stingless bee honey has similar range of pH regardless their origin. The ash content value obtained for all stingless bee honey ranged between $0.132\text{g}/100\text{g}$ to $0.532\text{g}/100\text{g}$, while the ash content of Tualang honey was 0.668g . The highest moisture content of 33.7% was determined from honey A and the lowest of 28.3% from honey D. Meanwhile, the moisture content for honey E was 24.8% . The high moisture content of stingless bee honey is important and differs from the *Apis* honey. Dissimilar to the stingless bee honey, *Apis* honey contains very less water and this results in its high viscosity. The electrical conductivity (EC) results ranges from $0.47\text{ mS}/\text{cm}$ to $0.55\text{ mS}/\text{cm}$ for stingless bee honey and $0.23\text{ mS}/\text{cm}$ for Tualang

honey. The results for color analyses of each honey were categorized based on the absorbance measurement. The absorbance of honey A was 0.457 and categorized as extra light amber. Honey B was 1.056 and honey D was 0.694 both of which were categorized as light amber. Honey C was 0.239 , and honey E was 0.385 and were both categorized as white. After absorbance measurement, both were characterized as being in similar category as expected. In contrast, honey C and honey E, by direct observation indicates the yellow to golden color and darker than golden color respectively. Meanwhile, after absorbance measurement, both of them were identified as white color. Therefore, absorbance measurement is very crucial rather than completely depends on direct observation. All color of the honey samples were in accordance to the Pfund scale.

Low hydroxymethylfurfural (HMF) values were determined from three honey samples. The lowest HMF was determined in honey B ($0.80\text{ mg}/\text{kg}$) and the highest in honey D ($3.42\text{ mg}/\text{kg}$). Meanwhile, HMF value for Tualang honey is $0.75\text{ mg}/\text{kg}$. The detailed psychochemical properties of stingless bee honey and Tualang honey are recorded on Table 2.

Table 2: The psychochemical properties of Malaysian Trigona and Apis honey (n=3).

Honey code	pH	Moisture Content (%)	Colour (Abs)	Electric Conductivity (mS/cm)	ASH Content (g/100g)	HMF (mg/kg)
Honey A	2.79 ± 0.04	33.7	0.457 ± 0.64	0.47 ± 0.22	0.132 ± 0.03	2.56 ± 0.96
Honey B	2.93 ± 0.03	31.7	1.056 ± 0.30	0.55 ± 0.06	0.268 ± 0.21	0.080 ± 0.16
Honey C	2.95 ± 0.02	28.9	0.239 ± 0.09	0.53 ± 0.10	0.4 ± 0.03	0.92 ± 0.17
Honey D	2.92 ± 0.01	28.3	0.694 ± 0.88	0.55 ± 0.38	0.532 ± 0.83	3.42 ± 1.03
Honey E	3.64 ± 0.04	24.8	0.385 ± 0.61	0.23 ± 0.33	0.668 ± 0.28	0.75 ± 0.44

DISCUSSION

The common pH for Malaysian stingless bee honey is undetermined. The pH values obtained from the current study were almost similar with the findings of Silva *et al.* that ranges between 2.90 ± 0.0 to 3.83 ± 0.1 . They analyzed using nine Brazilian Jandaira honey (*Melipona subnitida*).¹⁷ Moreover, similar results were also shown in previous study that utilized 152 stingless bee honeys.¹⁸ However, recent studies on stingless bee honey have indicated the mild pH range of 4.1 ± 0.0 to 4.5 ± 0.0 for Colombian stingless bee honey¹⁹ and 3.06 ± 0.12 to 5.18 ± 1.35 for Guatemalan stingless bee honey.²⁰ Low pH value usually indicates more acidity and more hydrogen ion, that involved in the formation of other compounds in stingless bee honey.²¹ The lower the pH of the honey, the higher ability of the honey to inhibit the presence and growth of microorganisms.¹⁷

All the ash content obtained from the stingless bee honey were aligned within the suggested standards (max $0.5\text{g}/100\text{g}$) for *Melipona favosa* honey from Venezuela. The values were almost similar with the previous study, $0.03\text{g}/100\text{g}$ to $0.40\text{g}/100\text{g}$ ²² and with study conducted in Brazil, $0.52\text{ g}/100\text{g}$ to $0.03\text{ g}/100\text{g}$.²³

The water content of stingless bee honey depends on various factors such as fruit or flower season, the geography that affect the temperature, bee species and maturity of the honey. The moisture content of our stingless bee honey was almost similar to the findings of Nascimento and co-workers. Their research team have used the Brazilian honey and obtained the moisture content ranging between 25.99% to 36.99% .²⁴ Some stingless bee honey indicated a mild water content as shown in previous studies, 23.40% to 25.60% ,²⁵

23.70 %²⁶ and 22.7% to 35.4%²⁷.

The EC result ranges from 0.47 mS/cm to 0.55 mS/cm for stingless bee honey and 0.23 mS/cm for Tualang honey. The EC values are almost similar with the study from Thailand, 0.53 mS/cm to 0.71 mS/cm.²⁸ However, our EC are lower than the previous findings which reported 1.1 mS/cm.^{29,30} The EC of honey E was in the range of 0.26 mS/cm as previously reported for the *Apis* honey.³¹ The EC is an important criteria for determination of floral origin and also to regulate the concentration of organic acids, minerals, and proteins.^{2,32}

Similar to other physicochemical parameters, the color of honey is affected by mineral content and also geographical and botanical origin. Diverse condition may produce different honey color either white, colorless, pale yellow, golden or amber.³³ Storage duration and light exposure are affecting the color of honey.²³ Previous study has characterized that darker honey has higher mineral content than the light honey.^{34,35}

The HMF specifies the freshness of the honey and may present in a small proportion. Initially, the formation of HMF is due to poor handling, storage or processing, honey degradation and also prolonged exposure of high temperature or hitting. Ultimately, fructose in the honey is decomposing and reacting with acid.^{36,37,38} For stingless bee honey, maximum HMF suggested by previous study is 40 mg/kg.³⁹ Souza and co-workers obtained HMF value ranging from 2.4 mg/kg to 16.0 mg/kg for stingless bee honey.¹⁸ Meanwhile, HMF values below than 2.4mg/kg were obtained from Guatemalan stingless bees honey.²⁰

CONCLUSION

Our study revealed for the first time the range of physicochemical characteristics of Malaysian stingless bee honey from the *Trigona* species. Our finding was comparable with the other groups in term of pH, ash content, moisture content, electrical conductivity, color intensity and hydroxymethylfurfural content. Higher moisture content and pH are the principal characteristics of the stingless bee honey compared to the *Apis Dorsata* honey.

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