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PHYSICOCHEMICAL PROFILES OF HONEY HARVESTED FROM FOUR MAJOR SPECIES OF STINGLESS BEE (KELULUT) IN NORTH EAST PENINSULAR OF MALAYSIA

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ABSTRACT

Stingless bee honey is one of the high demand honey in Malaysia. However, honey characters particularly from different species is lacking in order to increase its commercial value. Thus, physicochemical profile analysis of honey harvested from four major species of stingless bee in Kelantan, Malaysia, *Geniotrigona thoracica, Heterotrigina itama, Lepidotrigona terminata* and *Tetragonula laeviceps* was conducted to investigate the honey character. Character of the stingless bee honey harvested from various species from the same geographic area was compared. The results were then compared to stingless bee honey from other countries. Most of physicochemical data of stingless bee honey from different species collected from Kelantan showed almost similar values. However, value of pH, free acidity and total acidity displayed significant different results. This indicates species of stingless bee may affect the results. In order to compare local stingless bee honey with international stingless bee honey, the data was represented as mean regardless of their species. From the results, moisture content was consistently high in stingless bee honey harvested from Malaysia, Thailand, Brazil and Venezuela while, other data showed different values. The results demonstrated that species of stingless bee particularly from other countries is crucial in order to characterize the honey. However, Malaysian stingless bee honey has unique character based on physicochemical data. The data can be used as a marketing strategy in increasing commercial value of local stingless bee honey.

Key words: Kelulut honey, physicochemical analysis, stingless bee honey, *Geniotrigona thoracica, Heterotrigina itama, Lepidotrigona terminata*, *Tetragonula laeviceps*

INTRODUCTION

Stingless bee or locally known as kelulut is one of the bee species which has the ability to produce honey (Jaafar, 2012). It is classified under the tribe *Meliponine* from the Apidae family. Their sizes vary, but are smaller than other bees in the same family. Due to their small size, honey produced by them is about five times less compared to honey produced by other members of genus *Apis* (Jaafar, 2012). The morphology of stingless bee is known to be different with reduced sting and the absence of venom, making them a preferable choice for commercial beekeeping. Apart from their morphology, stingless bee constructs honey pots, made from propolis and beeswax (also called cerumen) to store honey (Vit *et al*., 2004) and other products including beebread and larvae as opposed to honey combs constructed by bees of *Apis* (Almeida-Muradian *et al.,* 2013).

The practice of rearing stingless bees has been established earlier in many countries such as Brazil, Venezuela, Thailand, Indonesia and Philippines. Their research focused mainly on their own native stingless bee, particularly on the characteristic of honey. Most of the studies concluded that stingless bee honey have distinctive characteristic compared to the other honey produced from different species

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in particular from genus *Apis* (Souza *et al.,* 2006; Chuttong *et al.,* 2016).

In Malaysia, research on the characteristic of local kelulut honey is still lacking. At present, approximately 50 stingless bee species have been identified in Malaysia. Four major stingless bees in Malaysia with high commercial potency have been identified; *Geniotrigona thoracica, Heterotrigona itama, Lepidotrigona terminata* and *Tetragonula laeviceps* (Jaafar, 2012). These four species differ in term of size, the structure of honey pot and volume of honey produced per hive.

In order to identify the characteristic of stingless bee honey, present study was conducted to analyse the physicochemical properties of honey collected from four different species of stingless bee in Kelantan. Data obtained from the study was also compared to the value stated in the other tropical countries. By the end of this study, the data obtained could be used as a guide for further characterization of stingless bee honey from other regions throughout Malaysia and subsequently, could be used as a basis for increasing commercial value of our local stingless bee honey internationally.

MATERIALS AND METHODS

Sample collection

Honey samples were harvested from the Min House Camp Beekeeping Farm, Kota Bharu, Kelantan in July 2014. Matured honey from sealed honey pot was selected for harvesting using a sterile 30 ml syringe before being stored in a sealed test tube and kept away from sunlight at temperatures between 20–25°C. Several adult stingless bee workers from each species were captured and preserved in cryovials filled with 70% of ethanol and stored at 4°C for species identification.

Physicochemical analysis

Physicochemical analysis was carried out according to the methods outlined in the CODEX Alimentarius Commission (2001) and International Honey Commission (IHC) (2009). These included moisture and ash content, electrical conductivity, pH, total acidity, diastase activity, hydroxymethylfurfural (HMF) and sugar composition. All analyses were performed in triplicates. The results were compared with several studies from other countries.

Moisture content

The moisture content of honey was measured using MISCO BKPR-2 digital refractometer (MISCO refractometer, United States) at 20°C immediately after harvesting from the honey pot.

Ash content

Ash content was determined using a Phoenix electrical furnace (CEM, United states) adjustable up to 600°C (± 25°C). Firstly, 5g of honey was preheated to approximately 300°C until it was completely carbonized before the temperature inside the furnace was raised to 600°C for at least an hour to complete the ashing process. The resulting ash was weighed and expressed as ash content in g/100g honey.

Electrical conductivity

Honey solution of 20% (w/v) was prepared by dissolving 20g of honey in 100ml ultrapure water prior to measuring the electrical conductivity using HI-98311 electrical conductivity meter (Hanna Instruments, United States).

pH and total acidity

pH and acidity were measured using a Delta 320 pH meter (Mettler Toledo, Switzerland), accurate to 0.01 unit. Firstly, 10g of honey was dissolved in 75ml of distilled water before the pH was measured. Meanwhile, total acidity was determined via the titrimetric method. The dissolved honey solution was firstly titrated with 0.05M sodium hydroxide (NaOH) until pH 8.5 to obtain free acidity value of honey. Subsequently, the titration was continued with the addition of 10ml 0.05M NaOH and 0.05M hydrochloric acid (HCl) to pH 8.3 in order to obtain the lactone acidity (LA). Total acidity was expressed in milliequivalents per kilogram (meq/kg) by adding free acidity and lactone acidity value.

Diastase activity

The diastase activity was determined using Ultrospec 2100 Pro spectrophotometer (Amersham Biosciences, United Kingdom) with a small band interference filter at 660nm. 10g honey was dissolved with 15ml of water and 5ml acetate buffer without any heating. Then, the solution was mixed with 3ml of sodium chloride (NaCl) and distilled water in 50ml volumetric flask. Two flasks each containing 10ml honey solution and 10ml starch solution were prepared separately and placed in 40pC water bath. After 15 minutes, 5ml starch solution was transferred into a flask containing honey solution. After 5 minutes, 0.5ml of honey solution was transferred and mixed directly with 5ml diluted iodine solution. Sample absorbance was immediately measured against water blank. A graph of absorbance against time was then plotted to calculate diastase activity (Schade *et al*., 1958).

Hydroxymethylfurfural (HMF)

HMF was determined using the spectrophotometric method outlined by White (1979). A total of 5g honey was diluted with 25ml distilled water and transferred into a 50ml volumetric flask. The Carrez solution I (0.5ml) was added followed by 0.5ml Carrez II solution and mixed homogeneously. The solution was then filtered using a Whatman filter paper with 0.45µm pore size. Then 5ml initial honey solution was mixed with 5ml water before measuring its absorbance against reference solution of 5ml initial honey solution and 5ml 0.2% sodium bisulphite solution. The absorbance was measured using Ultrospec 2100 Pro spectrophotometer (Amersham Biosciences, United Kingdom) with the ultraviolet (UV) absorbance at 284nm and 336nm, and the values were expressed in (mg/kg) to measure the HMF of honey.

Determination of sugar

Total sugar content was measured using Gas Chromatography (Agilent, USA) equipped with a Mass Spectrometry detector. Briefly, 1mg sugar standard (fructose, glucose, maltose, sucrose) and 5mg honey samples were prepared in different glass vials. After that, 0.45ml of pyridine was added into each glass vials prior to immersion in a water bath at 70p C for 10 minutes. Later, 0.5ml of hexamethyldisilazane (HMDS) was added and mixed well with the solution. Trifluoroacetic acid (0.05ml) was then added carefully before vortexing for 30 seconds and left for 15 minutes before the screw cap was tightened. All prepared samples in the vials were left for at least 24 hours to stabilize before being injected into the GCMS system equipped with Agilent CP8912, VF-1ms 30m x 0.25mm x 0.25um column (Agilent, USA). The results were expressed in percentage of sugar in honey.

Statistical analysis

Statistical analyses for physicochemical profiles from local stingless bee honey was done by One-way ANOVA while Tukey's test was used for the *post-hoc* test. Analyses was done using SPSS 22.0. Differences between groups were considered significant if $p < 0.05$. All data points showed the mean of standard deviation (SD). Statistical analysis for the international stingless bee honey could not be performed, due to lack of raw data. However some values were recovered from literature review (Souza *et al.,* 2006; Chuttong *et al.,* 2016).

RESULTS AND DISCUSSION

Moisture

Honey was harvested from honey pot of *G. thoracica, H. itama, L. terminata* and *T. leaviceps*. According to CODEX Standard 12-1981 (1987), the maximum moisture content allowed in honey is not more than 20%. However, the moisture content from all stingless bees in this study was higher than the amount allowed even though measurements of the moisture content was taken immediately after the honey was harvested from the honeypot. *L. terminata* displayed the lowest moisture content (23.93 ± 1.46%), while *G. thoracica* demonstrated the highest value (27.63 ± 0.83%) (Table 1). Overall, the average moisture content of honey from all four stingless bee species was 26.30 ± 1.14%.

The moisture content of stingless bee honey from other countries such as Thailand and Brazil was the highest, with an average of 31% (Table 2)

Table 1. Summary of physicochemical analysis (moisture content, ash, electrical conductivity, pH, free acidity, total acidity, diastase and HMF level) and sugar composition of honey harvested from four major species of stingless bees in Kelantan

Parameter	CODEX Std.	Species			
		Geniotrigona thoracica	*Heterotrigona itama*	*Lepidotrigona terminata*	*Tetragonula laeviceps*
Moisture (%)	< 20%	27.63 ± 0.83	26.60 ± 1.47	23.93[!] ± 1.46	27.05 ± 0.78
Ash (g/100g)	–	0.23 ± 0.06	0.25 ± 0.15	0.23 ± 0.05	0.18 ± 0.02
Electrical Conductivity (mS/cm)	< 0.8	0.29	0.26	0.24	0.18
pH	–	3.72 ± 0.02	3.40[!] ± 0.11	4.05[!] ± 0.05	3.92 ± 0.02
Free acidity (meq/kg)	–	262.50 ± 75.55	87.00[!] ± 27.06	102.67[!] ± 5.69	156.50[!*#] ± 4.95
Total acidity (meq/kg)	–	281.98 ± 1.26	100.63[!] ± 31.47	121.87[!] ± 5.74	175.40[!*#] ± 5.09
Diastase (schade)	> 8	1.22	1.97	1.43	1.85
HMF (mg/kg)	< 80	0.60	0.42	0.58	0.95
Fructose and glucose (%)	> 60% (Blossom)	4.46 ± 4.82	7.85 ± 1.98	24.6 ± 26.54	9
Sucrose (%)	< 5%	1.23 ± 1.45	0.45 ± 0.50	0.65 ± 0.26	2.2
Maltose (%)	–	11.29 ± 10.37	5.73 ± 3.62	9.76 ± 10.35	25.2

Mean ± SD, n=3 (! compare to *G. thoracica*; * compare to *H. itama*; # compare to *L. terminate*). $p < 0.05$. Data without SD obtained from n=1 because of sample limitation.

Table 2. Comparison of physicochemical analysis and sugar composition of Malaysian stingless bee honey with the data obtained from other tropical countries (regardless of species but from the same genus)

Parameter	CODEX Std.	Country			
		Kelantan (Malaysia)	Thailand	Brazil	Venezuela
Moisture (%)	< 20	26.90 ± 1.78	31 ± 5.4	29	22
Ash (g/100g)	–	0.22 ± 0.03	0.53 ± 0.63	0.18	0.67
Electrical Conductivity (mS/cm)	< 0.8	0.24	1.10 ± 0.78	5.23	3.10
pH	–	3.89 ± 0.15	3.6 ± 0.20	3.9	ND
Free acidity (meq/kg)	–	122.95 ± 55.41	ND	ND	ND
Total acidity (meq/kg)	–	138.33 ± 57.10	164 ± 162	49	46
Diastase (schade)	> 8	1.62	1.5 ± 1.6	10.27	5.30
HMF (mg/kg)	< 80	0.64	8.7 ± 12	21.4	1.1
Fructose and glucose (%)	> 60% (Blossom)	11.08 ± 11.11	31	78.2	60.5
Sucrose (%)	< 5%	1.13	1.2	3.3	2.0
Maltose (%)	–	11.5 ± 8.11	41 ± 15	ND	ND

Mean ± SD, n=3. ND: not determined. Data without SD was obtained either because of sample limitation (n=1) or not mentioned in the literature.

(Chuttong *et al.,* 2016) compared to the Malaysian stingless bee honey. However, Venezuela reported the lowest moisture content of stingless bee honey at 22% (Souza *et al.,* 2006). Moisture is one of the important criteria used to determine the quality of honey. Matured honey harvested from *Apis* species and from the temperate climates commonly displayed moisture content below 20% (Bijlsma *et al*., 2006). In contrast, honey collected from tropical countries, both from genus *Apis* and stingless bees, showed a higher moisture content. Moisture content is affected by many factors, such as the maturity of honey in the hive, harvesting season and climate change which is heavily influenced by humidity and temperature (Finola *et al.,* 2007). High water content can expose and accelerate the honey into fermentation process. Thus, high water content in Kelulut honey is one of major issues that needs to be encountered in order to retain the freshness and the quality of honey.

Ash content

Average of ash content from the honey of all species was 0.22 ± 0.07 g/100g (Table 1) with the lowest value observed from *T. laeviceps* (0.18 ± 0.02 g/100g) and the highest value was obtained from *H. itama* (0.25 ± 0.15 g/100g). Ash content from all honey samples was lower than the ash content reported for stingless bee honey from Thailand and Venezuela (Table 2). The differences were as expected since the ash content depends on the dominant nectar plant composition, which is influenced by the type of soil, geographical and botanical origin (Felsner *et al.,* 2004).

Electrical conductivity

The average value of electrical conductivity from all honey samples was 0.24 mS/cm (Table 1), ranging from 0.18 mS/cm (*L. terminata*) to 0.29 mS/cm (*G. thoracica*). The values were below than the standard set by the CODEX Standard (Table 1). Other studies showed a similar wide-ranging electrical conductivity results. For instance, Thailand, Brazil and Venezuela reported a mean value of 1.10 mS/cm, 5.23 mS/cm and 3.10 mS/cm respectively (Table 2). Electrical conductivity varies greatly and it is caused by difference in protein content, organic acids and mineral concentration of nectar extracted from the floral source.

pH and total acidity

Honey collected from *H. itama* exhibited the lowest pH value (pH 3.40 ± 0.11), while honey from *L. terminata* displayed the highest pH value (pH 4.05 ± 0.05) (Table 1). The mean pH value of Malaysian stingless bee honey (pH 3.77 ± 0.05) was almost similar to honey from Thailand, Brazil and Venezuela (Table 2). The pH value by far is the most consistent data in this study and must not exceed pH 4.1 as mentioned in most similar studies (Moniruzzaman *et al.,* 2013). Also, *H. itama* displayed the lowest free acidity and total acidity (87.00 ± 27.06 meq/kg and 100.63 ± 31.47 meq/kg respectively) while *G. thoracica* had the highest level of free acidity and total acidity (262.50 ± 75.55 meq/kg and 281.98 ± 1.26 meq/kg respectively. Malaysian stingless bee honey has an average of 152.17 ± 28.31 meq/kg of free acidity. However, no comparison could be made with other tropical countries as the free acidity value is not reported (Table 2). Overall, Malaysian stingless bee honey exhibited total acidity with a mean value of 169.97 ± 10.89 meq/kg (Table 1). The value was lower as compared to the honey collected from Thailand, but higher in comparison to Brazil and Venezuela (Table 2). The pH value and acidity of honey can be used to determine the purity and quality of honey as it is can be used as an indicator for honey maturity (Dimins *et al.,* 2006).

Diastase activity

The stingless bee honey from all four species had low diastase activity, with an average of 1.62 schade and ranging from 1.22 schade in *G. thoracica* to 1.97 schade in *H. itama* (Table 1). Diastase enzyme activity of stingless bee honey from Thailand showed almost similar result to the Malaysian honey (Table 2). However, diastase activity value of stingless bee honey from Brazil and Venezuela demonstrated vast differences, which are 5.3 and 10.3 schade respectively. According to the IHC, the minimum value of diastase activity in honey should not be less than 3 schade unit (CODEX Alimentarius Commission, 2001). This test is done to determine the presence of diastase in honey and its activity, as it is found naturally in honey. The enzyme is found in the stomach of bees and it is involved in sugar conversion. The activity of diastase is not only important in determining the quality of honey but also its purity.

Hydroxymethylfurfural (HMF)

HMF can be found in a very low amount in fresh and untreated honey, and showed a variation in every batch (Khalil *et al.,* 2010). It is used not only to determine the freshness of honey but also an indicator to identify adulterated honey because HMF will increase over time or has been introduced to heat (LeBlanc *et al.,* 2009). HMF content in all Malaysian stingless bee honey showed low values with a mean value of 0.64 mg/kg (Table 1). Highest HMF value of honey was reported from Brazil (Table 2). Nevertheless, all the results were far below the maximum limit of HMF (80 mg/kg) as stated in the CODEX Alimentarius.

Determination of sugar

A major difference in honey harvested from stingless bee compared to other species and geography can be observed in the sugar composition and total sugar content. Honey harvested from four species of Malaysian stingless bees showed high value of maltose in *G. thoracica* (11.29 ± 10.37%) and *T. laeviceps* (25.2%) (Table 1). Similar observation was recorded in honey from Thailand (Table 2) but with a higher value (41%). The value was higher when compared to honey from *A. mellifera* species recorded by White (1979) and honey from stingless bee in South America where they found only a trace amount of maltose (Oddo *et al.,* 2008).

According to CODEX Stan.12-1981, (1987), monosaccharide (total fructose and glucose) content in honey must not be less than 60% to be recognized as a natural honey. However, it was found that all of the tested honey did not meet the criteria even though the honey collected was directly from honey pot. Honey produced by *L. terminata* gave the highest mean total value of fructose and glucose with 24.6 ± 26.54% and mean total sugar of 35.01%. The other three species namely, *G. thoracica*, *T. laevicept* and *H. itama* had a mean total fructose and glucose values of 4.46 ± 4.82%, 9.00%, 7.85 ± 1.98% and total sugar content of 16.98%, 36.4% and 14.03% respectively (Table 1). Meanwhile, various results were observed when we compared local stingless bee honey with the honey from Thailand, Brazil and Venezuela (Table 2).

Sucrose content of Malaysian stingless bee honey was within the international standards with the mean value of 1.13% (Table 2). All stingless bee honey from Thailand, Brazil and Venezuela also displayed similar results. Distinct results were reported by Chuttong *et al*. (2016) where the mean total sugar was 31%, with markedly low sucrose at 1.2% but similar on maltose dominancy (Table 2). The low total sugar content in this study may be affected by different floral sources and sugar being converted into inorganic acid (Moniruzzaman *et al.,* 2013). It is suggested that further investigation into the sugar composition and its total sugar should be conducted with consideration of different species of stingless bee, floral sources, climate and geographic area.

CONCLUSION

Honey harvested from stingless bees: *G. thoracia*, *H. itama, L. terminata,* and *T. laevicept,* in Kelantan showed almost similar values when compared to each other except for the value of pH, free acidity and total acidity. It was predictable since the honey even though was harvested from different species, was collected from the same location. Physico-chemical data of local stingless honey displayed different values when compared to stingless bees found in Thailand and South American countries (Brazil and Venezuela). Moreover, all stingless bee honey from different countries exhibited major differences when compared to values set in the CODEX Standard for Honey (CODEX Stan 12-1981, 2001). It was expected since the standard was based on honey collected from *Apis* species, which is stinging bee. Distinct character showed in local stingless bee honey when compared to international stingless bee honey and data from CODEX, can be used to highlight uniqueness of our honey. The data may be used to increase commercial value of our kelulut (stingless bee) honey.

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