# PHYSICOCHEMICAL CHARACTERISTICS OF STINGLESS BEE HONEY FROM *Heterotrigona itama*

Hendrie Johann Muhamad Ridzwan<sup>1\*</sup>, Nur Syamimi Ismail<sup>1</sup>, Mohamad Amir Shah Yusop<sup>2</sup>, Wan Aizuddin Wan Razali<sup>1</sup>, Anisah Mohammed<sup>2</sup>

> <sup>1</sup>Faculty of Applied Sciences, Universiti Teknologi MARA Pahang, Bandar Tun Abdul Razak Jengka, Pahang, Malaysia

<sup>2</sup>Faculty of Plantation & Agrotechnology, Universiti Teknologi MARA Pahang, Bandar Tun Abdul Razak Jengka, Pahang, Malaysia

\*Corresponding author: hendrie@uitm.edu.my

#### Abstract

Stingless bee honey from *Meliponini* species is less common than honey produced by honey bee from *Apis* species. However, *Meliponini* consists of more than 50 species than *Apis*. This study aims to investigate the physicochemical characteristics of Malaysian stingless bee honey from *Heterotrigona itama* species. The samples of stingless bee honey were collected from UiTM Jengka, Pahang. The honey produced by *Heterotrigona itama* was selectively taken from five different logs. The physicochemical parameters tested were pH, ash content, acidity value, colour intensity, and moisture content. The pH of the stingless bee ranging between  $3.32\pm0.01$  to  $3.60\pm0.01$ . The colour of the honey is in the range of light amber to extra light amber. The ash content and acidity value of the stingless bee honey samples ranging from  $0.127\pm0.061$  g/100g to  $0.413\pm0.022$  g/100g and  $109\pm7.81$  meq/kg to  $135\pm8.66$  meq/kg, respectively. The moisture content value ranging from  $23\pm0.50$  % to  $26.83\pm0.29$  %. Except for the ash content, the physicochemical characteristic result of the honey obtained from different logs was nearly similar.

Keyword: Heterotrigona itama, Physicochemical Characteristics, Stingless Bee Honey

# Introduction

Honey that is widely available was obtained from honey bee in *Apis* species. Meanwhile, a stingless bee from *Meliponini* species produces stingless bee honey. Interestingly, researchers first discovered *Meliponini* species before realising the existence of *Apis* species. Both species have different properties, however, *Meliponini* has 50 times more species than *Apis*. In general, a stingless bee produces less honey compared to the normal bee like honey bee (Roubik, 2006). This makes it less favourable to a beekeeper. However stingless bee honey is still in demand due to its rareness and unique properties. The completely different setup of stingless bee farm compared to the common honey bee makes the market price for stingless bee honey much higher. Kek et al. (2018) revealed that beekeeper in tropical regions such as Africa, Bolivia, and Columbia demand the price of the stingless bee honey ten times higher than the honey from normal bees.

Moreover, the flavonoids and polyphenols compounds with antioxidant properties that are present in stingless bee honey are much higher than *Apis* species. Stingless bee honey also has higher water content compared to the normal bees (Se et al., 2018). In contrast to the honey from *Apis* species, stingless bee honey has a sour and an acidic taste (Bijlsma et al., 2006).

Currently, there is a growing research interest in stingless bee honey in Malaysia. Published by Universiti Teknologi Mara (UiTM) Cawangan Pahang – September 2020 | **53**  *Heterotrigona itama* is one of the main stingless bee species in Malaysia known for its honey production (Cheng et al., 2019). The composition of honey from stingless bee depends on the type of flowers the bee visited and the condition of the weather in a certain geographical area (Chakir et al., 2016). Therefore, the aim of this study is to determine the physicochemical characteristics of stingless bee honey from *Heterotrigona itama* species at a bee farm in UiTM Pahang, Jengka Campus.

# **Materials and Methods**

# Sample collection

The samples of stingless bee honey were collected from a farm located at UiTM Pahang, Jengka Campus. Five different logs of stingless bee from the same species of *Heterotrigona itama* were selected randomly for this study. A syringe was used to extract the honey from the honey pot. The sample was sealed in a glass jar and kept in the fridge for further analysis.

# Parameter of study (Physicochemical characteristic of the honey sample) pH

The pH determination was conducted by following the method previously described by Fatima et al. (2017). The test was done by immersing pH meter electrode in a solution that contains 10 g of the honey sample from *Heterotrigona itama* species dissolved in 75 mL of distilled water. The procedure was replicated three times. A standard buffer solution of pH 4 and 7 were used to calibrate the pH meter before the experiment.

# Ash content

The ash content was determined by replicating the method as stated by Chuttong et al. (2016). After a drying process in the oven at  $105\Box$  for six hours and the cooling process in the desiccator, the empty crucibles were weighed and data was recorded. Crucibles were filled with 5 g of the sample and placed into the muffle furnace at  $550\Box$  for 12 hours. The crucibles were weighed again with ashes in it after the cooling process was done in a desiccator. The procedure was duplicated three times.

### **Moisture content**

A portable handheld refractometer was used to measure the moisture content. Only a few drops of the sample were needed. The procedure was replicated three times.

### **Colour Intensity**

The colour intensity was determined by following the method described by Fatima et al. (2017). The absorbance value of the sample was measured by using Ultraviolet visible spectrophotometer. A 5 g of honey sample was dissolved in 10 mL of deionized water and gently mixed by vortexing the solution. 2 mL of honey solution was transferred into a cuvette. The absorbance at 635 nm was recorded. To determine the colour intensity, a scale called Pfund scale was used. The procedure was replicated three times.

# **Total acidity**

A titrimetric method was applied to determine the acidity of the sample. A 10 g of sample was dissolved in a beaker containing 75 mL distilled water. Titration was done using 0.05 M of sodium hydroxide solution until the pH reached 8.5. 10 mL of 0.05 M of sodium hydroxide solution was immediately added in the titrated solution. The solution was back-titrated using 0.05 M of a hydrochloric acid solution until the pH reached 8.3. The procedure was replicated three times.

#### **Result and Discussion**

The results of the tested parameters on stingless bee honey such as pH, ash content, colour intensity, total acidity, and moisture content were tabulated in **Table 1** until **Table 5**.

**Table 1** represents the pH value for *Heterotrigona itama* honey. From the table, the range of pH value is from  $3.32\pm0.01$  to  $3.60\pm0.01$ . The highest pH value was recorded from log 4 with  $3.60\pm0.02$  whereas log 5 indicates the lowest pH value at  $3.32\pm0.01$ . The result showed that *Heterotrigona itama* honey was considered as acidic honey. The content of organic acids in the honey could contribute to the difference in pH value as it can reduce the value of the pH. Organic acids are produced by the fermentation of sugars content in the honey (El Sohaimy et al., 2015).

Heterotrigona itama (Honey)	pH value
Log 1	3.54±0.01
Log 2	$3.54{\pm}0.01$
Log 3	3.38±0.01
Log 4	3.60±0.02
Log 5	3.32±0.01

Table 1 pH value of Heterotrigona itama honey

From the analysis of the ash content of stingless bee honey from *Heterotrigona itama*, less than 1 g/100 g of honey was obtained. From **Table 2**, the ash content was at the range of  $0.127\pm0.061$  to  $0.413\pm0.022$  g/100g. Log 1 has the highest value ( $0.413\pm0.022$  g/100g) where as Log 4 shows the lowest ash content ( $0.127\pm0.061$  g/100g). The ash content mostly associated with the amount of mineral content in the honey. The source of nectar from the plant types and soil area may contribute to the different amount of mineral content in the honey (Nascimento et al., 2015).

 Table 2 Ash content of Heterotrigona itama honey

Heterotrigona itama (Honey)	Ash content (g/100 g)
Log 1	0.413±0.022
Log 2	$0.271 \pm 0.090$
Log 3	$0.279 \pm 0.027$
Log 4	$0.127{\pm}0.061$
Log 5	0.241±0.009

The colour intensity for all sample of stingless bee honey was determined by comparing the absorbance measurement using Pfund scale. **Table 3** shows that the range scale of Pfund readings is between 35-85mm. Log 1, 2, and 3 showed Pfund scale within 51-85 mm, which is classified as light amber. Meanwhile, Pfund scale for Log 4 and 5 was 35-50 mm and considered as extra light amber. The colour intensity of stingless bee honey is affected by the mineral content in the honey in which higher mineral content will give a darker colour to the honey (Nascimento et al., 2015). However, other factors such as the types of flower it harvest and the resin used for the honey pot will also affect the colour of the honey. Therefore, darker honey is not always associated with high mineral content.

<i>Heterotrigona itama</i> (Honey)	Absorbance	Pfund scale (mm)	Colour of honey
Log 1	$0.6715 \pm 0.008$	51 - 85	Light amber
Log 2	$1.1836 \pm 0.005$	51 - 85	Light amber
Log 3	$0.6234 \pm 0.004$	51 - 85	Light amber
Log 4	0.5881±0.009	35 - 50	Extra light amber
Log 5	$0.5574 \pm 0.002$	35 - 50	Extra light amber

**Table 3** Colour Intensity of *Heterotrigona itama* honey

**Table 4** shows the total acidity of *Heterotrigona itama* honey. The range of the total acidity result was from 109.00 meq/kg to 135.00 meq/kg. Log 2 recorded with the highest total acidity at  $135.00\pm 8.66$  meq/kg whereas Log 5 shows the lowest value at  $109.00\pm 7.81$  meq/kg. According to Biluca et al. (2016), the acidity value represents the amount of organic acids present in the honey. There is a slight difference in the total acidity value between the logs. It may be caused by the fermentation process that could occur during the storage period of the honey sample. Acetic acid and lactic acid are the common organic acids produced in the honey fermentation process (Razali et al., 2018).

Table 4 Total acidity of Heterotrigona itama honey

Heterotrigona itama (Honey)	Total acidity (meq/kg)
Log 1	129.67±4.16
Log 2	135.00±8.66
Log 3	111.67±7.02
Log 4	134.33±1.15
Log 5	109.00±7.81

The moisture content of *Heterotrigona itama* was observed using a handheld refractometer. **Table 5** shows that the moisture content of *Heterotrigona itama* honey is in between  $23.33\pm0.02$  % to  $26.83\pm0.01$ %. Log 4 recorded the lowest moisture content at  $23.00\pm0.29$ % whereas Log 2 recorded the highest moisture content at  $26.83\pm0.01$ %. The result obtained is lower compared to the previous studies. Studies by Fatima et al. (2017) and Nascimento et al. (2015) recorded a higher moisture content in the stingless bee with 33.73 % and 36.89%, respectively. The higher moisture content will cause a greater degree of the fermentation process that will affect the quality of the honey (Nordin et al., 2018).

Table 5 Moisture content of Heterotrigona itama ho	oney
--	------

Heterotrigona itama (Honey)	Moisture content (%)
Log 1	23.33±0.02
Log 2	23.00±0.29
Log 3	26.67±0.29
Log 4	26.83±0.01
Log 5	26.67±0.29

#### Conclusion

The physicochemical characteristic result of the honey obtained from different logs was nearly similar with slight differences except for the ash content value. The result of the pH value and the total acidity showed that the fermentation process may have occurred. This study shows that the quality of the honey from the same stingless bee species in different logs may not be identical.

#### Acknowledgement

The authors would like to thank Universiti Teknologi MARA Pahang for the services and facilities to carry out the work.

#### **Conflict of interests**

Authors hereby declares that there is no conflict of interests with any organization or financial body for supporting this research.

#### References

Bijlsma, L., de Bruijn, L. L. M., Martens, E. P., & Sommeijer, M. J. (2006). Water content of stingless bee honeys (Apidae, Meliponini): interspecific variation and comparison with honey of *Apis mellifera*. *Apidologie*, *37*(4), 480–486.

Biluca, F. C, Braghini, F., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (*Meliponinae*). Journal of Food Composition and Analysis, 50, 61-69.

Chakir, A., Romane, A., Marcazzan, G. L., & Ferrazzi, P. (2016). Physicochemical properties of some honeys produced from different plants in Morocco. *Arabian Journal of Chemistry*, *9*, 946–954.

Cheng, M. Z. S. Z., Ismail, M., Chan, K. W., Ooi, D. J., Ismail, N., Zawawi, N., & Esa, N. M. (2019). Comparison of sugar content, mineral elements and antioxidant properties of *Heterotrigona itama* honey from suburban and forest in Malaysia. *The Malaysian Journal of Medical Sciences*, 15(SP1), 104-112.

Chuttong, B., Chanbang, Y., Sringarm, K., & Burgett, M. (2016). Physicochemical profiles of stingless bee (*Apidae: Meliponini*) honey from South East Asia (Thailand). *Food Chemistry*, *192*, 149–155.

El Sohaimy, S. A., Masry, S. H. D., & Shehata, M. G. (2015). Physicochemical characteristics of honey from different origins. *Annals of Agricultural Sciences*, 60(2), 279-287.

Fatima, I. J., Ab, M. H., Salwani, I., & Lavaniya, M. (2017). Physicochemical Characteristics of Malaysian Stingless Bee Honey from *Trigona* Species. *IIUM Medical Journal Malaysia*, 17(1).

Kek, S. P., Chin, N. L., Yusof, Y. A., Tan, S. W., & Chua, L. S. (2018). Classification of entomological origin of honey based on its physicochemical and antioxidant properties. *International Journal of Food Properties*, *20*, 2723–2738.

Nascimento, A., Marchini, L., Carvalho, C., Araujo, D., Olinda, R., & Silveira, T. (2015). Physical-chemical parameters of honey of stingless bee (*Hymenoptera: Apidae*). *American Chemical Science Journal*, 7(3), 139-149.

Nordin, A., Sainik, N. Q. A. V., Chowdhury, S. R., Bin Saim, A., & Idrus, R. B. H. (2018). Physicochemical properties of stingless bee honey from around the globe: A comprehensive review. *Journal of Food Composition and Analysis*, 73, 91–102.

Razali, M., Zainal, Z. A., Maulidiani, M., Shaari, K., Zamri, Z., Mohd Idrus, M. Z., . . . Ismail, I. S. (2018). Classification of raw stingless bee honeys by bee species origins using the NMR- and LC-MS-based metabolomics approach. *Molecules*, *23*(9), 2160.

Roubik, D. W. (2006). Stingless bee nesting biology. Apidologie, 37(2), 124–143.

Se, K. W., Ghoshal, S. K., Wahab, R. A., Ibrahim, R. K. R., & Lani, M. N. (2018). A simple approach for rapid detection and quantification of adulterants in stingless bees (*Heterotrigona itama*) honey. *Food Research International*, *105*, 453–460.