



**UNIVERSITI PUTRA MALAYSIA**

***EFFECTS OF CONVENTIONAL, DIFFUSION AND MODERN MOISTURE  
REMOVAL METHODS ON QUALITY OF STINGLESS BEE (*Heterotrigona  
itama Cockerell*) HONEY***

**SYAHRUL ANIS HAZWANI BINTI MOHD BAROYI**

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By

**SYAHRUL ANIS HAZWANI BINTI MOHD BAROYI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the Degree of Master of  
Science**

**May 2021**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirement for the degree of Master of Science

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**May 2021**

**Chair : Prof. Ir. Yus Aniza Yusof, PhD**  
**Faculty : Engineering**

Stingless bee honey produced from *Heterotrigona itama* is a popular natural sweetener that exhibits numerous bioactivities and health benefits. However, the honey revealed high moisture content (>27.0%), making it highly susceptible to undesirable microbial fermentation. The conventional processing methods practised by the beekeepers in Malaysia were compared, in which the stingless bee honey was subjected to chiller storage (CT), room temperature storage (RT), double-boiled (DB) and open tray drying (OT) for five days. Interestingly, although honey stored at OT was found to reduce a more significant amount of moisture (~38% within five days), the increase of colour intensity and viscosity in OT honey should not be neglected. The colour intensity and viscosity increased from 284 to 314 mm Pfund and 0.13 to 6.80 Pa.s, respectively, in five days, indicating the honey at risk of oxidation and might not reach consumers' acceptance. Meanwhile, reducing the moisture levels to below 20% without deteriorating honey quality is contemporary urge research. Therefore, this study aimed to evaluate the feasibility of alternative processing methods by utilizing custom-designed clay pots to reduce the moisture content of the stingless bee honey and investigate the physicochemical properties and storage stability of honey. On the other hand, 50 mL of freshly harvested honey were placed in custom-designed clay pots (5 cm diameter x 6 cm height), covered and stored at either  $25 \pm 1^\circ\text{C}$  (RT) (maintained at 60% relative humidity) or  $35 \pm 1^\circ\text{C}$  (ET) (maintained at 25% relative humidity). Results showed that the honey's moisture content and water activity after ET storage in clay pots were significantly ( $p \leq 0.05$ ) reduced from 25.8% to 19.5% and from 0.79 to 0.70, respectively. Similar degrees of reduction were achieved at RT. However, moisture removal duration took 21 days and resulted in a loss of honey solids (21%) due to cross-wall diffusion. The pH and free acidity of honey were reduced, and the viscosity was increased at the end of storage. Interestingly, hydroxymethylfurfural (HMF) was not detected for all honey, indicating the quality of honey was preserved. The second part of the study was to investigate the effects of modern alternative

processing methods, high-pressure processing (HPP) and microwave pasteurization (MW), on the quality of the honey after storage for four weeks. From the results, HPP and MW treatments did not affect the moisture content of the honey compared to the honey without treatment. A similar trend was observed in total soluble solids (TSS), pH, free acidity, colour parameters and viscosity. Meanwhile, the colour intensity increased at the end of storage. The colour intensity of honey without treatment was the highest, followed by MW-treated honey and HPP-treated honey. Sugar profile analysis indicated that all sugar contents were significant reduced after MW and HPP treatments and after four weeks of storage, except the honey treated with HPP at a holding time of 5 minutes. However, neither HPP and MW did prevent the formation of HMF at the end of the storage study. To conclude, it is feasible to use a clay pot to reduce the moisture content in honey where the quality of honey was preserved and HMF formation was avoided. It is also was found to be the most effective method compared to conventional and both thermal and non-thermal alternative methods to increase stingless bee honey storage stability.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN KAEDAH KONVENSIONAL, PENYERAPAN DAN KAEDAH MODEN  
PENYINGKIRAN AIR TERHADAP KUALITI MADU KELULUT (*Heterotrigona  
itama* Cockerell)**

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Madu lebah tanpa sengat yang dihasilkan dari *Heterotrigona itama* adalah pemanis semula jadi yang popular yang mempunyai tinggi bioaktiviti dan faedah kesihatan. Walau bagaimanapun, madu itu menunjukkan kandungan lembapan yang tinggi (> 27.0%), menjadikannya sangat rentan terhadap penapaian mikroba yang tidak diingini. Kaedah pemprosesan konvensional yang diamalkan oleh peternak lebah di Malaysia dibandingkan, di mana madu lebah tanpa sengat itu disimpan pada penyimpanan suhu sejuk (CT), penyimpanan suhu bilik (RT), dididihkan berlapis (DB) dan pengeringan dulang terbuka (OT) selama lima hari. Menariknya, walaupun madu yang tersimpan di OT didapati dapat mengurangkan jumlah kelembapan yang lebih ketara (~38% dalam masa lima hari), peningkatan intensiti warna dan kelikatan pada madu OT tidak boleh diabaikan. Intensiti warna dan kelikatan meningkat dari 284 hingga 314 mm Pfund dan 0.13 hingga 6.80 Pa.s, masing-masing, dalam lima hari, menunjukkan madu berisiko pengoksidaan dan mungkin tidak mencapai penerimaan pengguna. Manakala, pengurangan tahap kelembapan ke bawah 20% tanpa merosakkan kualiti madu adalah kajian yang sangat penting. Oleh itu, kajian ini bertujuan untuk menilai kemungkinan kaedah pemprosesan alternatif dengan menggunakan periuk tanah liat yang direka khas untuk mengurangkan kandungan kelembapan madu lebah tanpa sengat dan menyiasat sifat fizikokimia dan kestabilan penyimpanan madu. Sebanyak 50 mL madu yang baru dituai dimasukkan ke dalam periuk tanah liat yang direka khas (diameter 5 cm x 6 cm tinggi), ditutup dan disimpan pada suhu  $25 \pm 1^\circ \text{C}$  (RT) (dikekalkan pada kelembapan relatif 60%) atau  $35 \pm 1^\circ \text{C}$  (ET) (dikekalkan pada kelembapan relatif 25%). Hasil kajian menunjukkan bahawa kandungan kelembapan madu dan aktiviti air setelah penyimpanan ET dalam periuk tanah liat secara signifikan ( $p \leq 0.05$ ) berkurang dari masing-masing 25.8% menjadi 19.5% dan dari 0.79 hingga 0.70. Tahap pengurangan yang serupa dicapai pada RT. Walau bagaimanapun, tempoh penyingkiran kelembapan mengambil masa 21 hari dan mengakibatkan kehilangan pepejal

madu (21%) kerana penyebaran dinding silang. pH dan keasidan madu bebas dikurangkan, dan kelikatannya meningkat pada akhir penyimpanan. Menariknya, hidroksimetilfurfural (HMF) tidak dikesan untuk semua madu, menunjukkan kualiti madu terpelihara. Bahagian kedua dari kajian ini adalah untuk mengkaji kesan kaedah pemprosesan alternatif moden, pemprosesan tekanan tinggi (HPP) dan pasteurisasi gelombang mikro (MW), terhadap kualiti madu setelah disimpan selama empat minggu. Dari hasilnya, rawatan HPP dan MW tidak mempengaruhi kandungan kelembapan madu berbanding madu tanpa rawatan. Trend yang serupa diperhatikan dalam jumlah pepejal larut (TSS), pH, keasidan bebas, parameter warna dan kelikatan. Sementara itu, intensiti warna meningkat pada akhir penyimpanan. Keamatan warna madu tanpa rawatan adalah yang tertinggi, diikuti dengan madu yang dirawat MW dan madu yang dirawat HPP. Analisis profil gula menunjukkan bahawa semua kandungan gula dikurangkan dengan ketara setelah rawatan MW dan HPP dan setelah penyimpanan selama empat minggu, kecuali madu yang dirawat dengan HPP pada waktu penahanan 5 minit. Walau bagaimanapun, HPP dan MW tidak menghalang pembentukan HMF pada akhir kajian penyimpanan. Sebagai kesimpulan, penggunaan periuk tanah liat terbukti berkesan untuk mengurangkan kandungan kelembapan dalam madu di mana kualiti madu terpelihara dan pembentukan HMF jugak dapat dielakkan. Ia juga merupakan kaedah yang paling berkesan berbanding dengan kaedah konvensional serta kaedah alternatif termal dan bukan termal untuk meningkatkan kestabilan penyimpan madu kelulut.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

AJS	Authorized Jewelers Scheme
AMOS	Analysis of Moments Structures
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Materials
$A_w$	Water Activity
BET	Brunauer-Emmet-Teller
CPWF	Clay pot water filters
CT	Chiller temperature
DB	Double-boiled
ET	Elevated temperature
F/G	Fructose/Glucose ratio
F+G	Fructose + Glucose
G/M	Glucose/Maltose ratio
HMF	Hydroxymethylfurfural
HPP	High-Pressure Processing
IHC	International Honey Commission
LED	Light-emitting diode
MARDI	Malaysia Agricultural Research and Development Institute
MW	Microwave
$N_2$	Nitrogen
OT	Open tray drying
RH	Relative humidity
RI	Refractive Index
RT	Room temperature
SEM	Scanning Electron Microscopy
SOPs	Standard Operations of Procedures
TCD	Total colour different

TPC	Total phenolic content
TSC	Total solids content
TSS	Total soluble solids
USA	United State of America



## CHAPTER 1

### INTRODUCTION

#### 1.1 Research Background

Stingless bees, also known as kelulut in Malaysia, originate from the Meliponini tribe and comprise three genera: *Melipona*, *Scaptotrigona*, and *Trigona* (Michener, 2013). Approximately 500 species of stingless bees have been identified in tropical and sub-tropical regions such as Central and South America, Africa, northern Australia and Asia, including Malaysia (Chuttong et al., 2016). To date, only the *Trigona* spp. can be found in Malaysia (Salim et al., 2012), in which *Heterotrigona itama*, a subgenus of *Trigona*, is often reared by local beekeepers. Several scientific reports and anecdotes (Halcroft et al., 2013; Abu Bakar et al., 2017; Ismail, 2016; Ismail & Ismail, 2018) have reported that stingless bee honey has the potential to generate a comfortable income for the beekeepers and add value to the functional food industry due to its high nutritional content (Vit et al., 2004), good antioxidant properties (Biluca et al., 2017; da Silva et al., 2013; Harif Fadzilah et al., 2017, Kek et al., 2014), and antimicrobial activities (Abd Jalil et al., 2017; Boorn et al., 2010; Choudari et al., 2012; Torres et al., 2004; Zainol et al., 2013). Garedew et al. (2004), Vit et al. (2004), and Rosales (2013) reported that stingless bee honey could potentially be used as a remedy for many illnesses such as stomach and intestinal ulcers, mouth diseases, sore throats, and wound dressing in Ethiopia and other countries. Recently, two reviews were performed by Abd Jalil et al. (2017) and Rao et al. (2016) on the wound healing properties of honey and the biological and therapeutic values of the honeybee and stingless bee honey respectively.

Due to the tropical climate, stingless bee honey from South East Asia has been shown to contain a very high moisture content (approximately 31%) (Chuttong et al., 2016) compared to the honey from *Apis mellifera*, the common honeybee (17.2 to 20.2%). Likewise, honey produced in Malaysia was also shown to have a high moisture content, ranging between 23 and 35% (Souza et al., 2006; Malaysian Standard MS 2683, 2017). Akbulut et al. (2009) indicated that the water content was recognized as a critical factor in honey fermentation. Therefore, a cause of concern for the honey industry as fermented honey is usually regarded as spoilt honey. Hence, if the honey is not correctly processed, it will lead to fermentation and the subsequent deterioration of its quality. Özcan & Al Juhaimi (2011) also reported that the close contact with the metal wires used in the *Apis mellifera* beekeeping process to keep the honeycomb together could affect honey's quality. Therefore, the lack of a proper standard of operation procedures (SOPs) in the stingless bee honey industry is a serious concern that needs to be addressed. To prevent the onset of fermentation, stingless bee beekeepers in Malaysia will either place the honey in a chiller directly after harvest, or heat the honey at 40°C for a few

minutes to reduce the moisture content before bottling, or leave it at room temperature for a few days for natural evaporation to occur.



**Figure 1.1: Stingless beehive box containing a colony of *Heterotrigona itama***

Several techniques have been practised to improve the quality of stingless bee honey, including low-temperature storage, heat treatment, and moisture removal (Kowalski et al., 2013; Tosi et al., 2002, Turhan et al., 2008). Among these techniques, pasteurization of the honey by heat treatment of up to 60-70 °C and subsequent storage at refrigerator temperatures before consumption was shown to be effective (Contrera et al., 2011). However, changes in the physicochemical properties during the heating process, such as the formation of 5-hydroxymethylfurfural, are inevitable (Khalil et al., 2010). Besides pasteurization and low-temperature storage, Contrera et al. (2011) proposed a fourth alternative method known as the 'maturation process.' Although regarded as a deteriorative phenomenon, the fermentation process was allowed to occur naturally in a closed container at room temperature. A few conventional methods were applied in stingless bee honey processing from the conversation with the local beekeepers in Malaysia. Some of the stingless bee entrepreneurs dried the honey in a shallow tray and let the honey evaporate to the surrounding. However, if it is conducted in unhygienic conditions, the honey cleanliness was at stake because the insects or other possible contaminants' possible sources will happen. The other producers heat the honey by using the double-boiled method without direct heating towards the honey, and the temperature was controlled to be below 40°C. Through visits and conversations with local beekeepers, a few have been using clay pots in reducing the moisture content of the stingless bee honey. However, the scientific knowledge of that method is still unknown because no further research has been done. Most of the stingless beekeepers admitted that they usually did not perform any

prior treatment or processing of their products where the honey will be stored in the chiller or storage at room temperature and directly sold to the customers.

To conclude, the processing methods of stingless bee honey are rather scarce. No standard processing technique was investigated to see the effectiveness of increasing the stingless bee honey storage stability. As stingless beekeeping in Malaysia is still on a small scale, a new cost-effective, comfortable and natural processing method is demanded to occupy the gap in the stingless bee honey industry and help the beekeepers grow and sustain their business. The present work investigates the application of conventional methods and moisture reduction by means of clay pots of stingless bee honey and its effect on honey storage stability. This work also investigates the effect of modern treatment (microwave pasteurization and high-pressure processing (HPP)) on stingless bee honey storage stability

## **1.2 Problem Statement**

Due to readily high moisture content, stingless bee honey undergoes rapid deterioration and darker in colour due to oxidization and fermentation. Customers always considered dark honey to be rotten, unpleasant, or adulterated with sugars. In the meantime, the processing techniques of stingless bee honey are scarce. Beekeepers practised a few conventional methods, but no scientific study has been done on their honey products that make the quality of the honey retailed to the customers is unknown. Furthermore, the effects of applying modern treatment (thermal and non-thermal) on stingless bee honey have yet to be discovered.

## **1.3 The Main Objective of the Study**

The main objective of the study is to investigate the effects of conventional and modern processing techniques in increasing the stingless bee honey stability

### **1.3.1 Specific Objectives of the Study**

This study is designed to achieve the following specific objectives: -

1. To investigate the application of conventional methods and its effect on honey storage stability.
2. To investigate the application of moisture reduction by means of clay pots of stingless bee honey and its effect on honey storage stability.



3. To investigate the effect of modern treatment (microwave pasteurization and high-pressure processing (HPP)) on stingless bee honey storage stability.

### 1.3.2 Research Hypotheses

The hypotheses of this study are as follow: -

1. Clay pot treated stingless bee honey with low moisture content has higher storage stability.
2. Deterioration of stingless bee honey will not occur after the moisture content is reduced below 20%.
3. High-Pressure Processing (HPP) is an alternative method for mass production of stingless bee honey industry better than microwave treatment.

### 1.4 Significance of the Study

Stingless bee honey is in high demand in Malaysia and many other countries. It is nutritionally high in carbohydrates and possesses many functional properties such as antioxidant and antimicrobials properties. However, the honey is susceptible to fermentation as its moisture content is often much higher than that necessary for microbial stability. Traditionally, the moisture content of stingless bee honey is reduced by drying the honey in shallow trays at room temperatures, but this can lead to contamination if done under unhygienic conditions. It is expected to reduce the moisture content and retain most of the properties of the honey when the filtration process is conducted by means of clay pots diffusion method under appropriate conditions. Meanwhile, modern treatments, microwave pasteurization and high-pressure processing (HPP) are suitable for the big production of stingless bee honey. The outcomes of this study are targeted to the new and former beekeepers to enhance the quality of their products, sustain the industry and established a standard method to process stingless bee honey.

#### 1.4.1 Scope of the Study

This study only covered stingless bee honey (*Trigona* spp.). *Heterotrigona itama* is chosen as it is widely reared and consumed in Malaysia. Three processing methods are introduced to the stingless bee honey: conventional methods, moisture reduction method using clay pots, modern treatments, High-Pressure Processing (HPP) method and microwave pasteurization method. Honey was analysed for its physicochemical properties to find the best alternative method for stingless bee honey processing.

## 1.5 Structure of Thesis

This thesis consists of five chapters. Chapter 1 is an introduction that begins with the overview of the stingless bee honey, conventional honey processing methods, properties of raw stingless bee honey, current problems faced by the beekeepers and potential applications of a few methods adopted as an alternative to stingless bee honey processing. Problem statements regarding the use of clay pots, High-Pressure Processing (HPP) and microwave pasteurization as an alternative method to increase storage stability of the stingless bee honey are highlighted by the main and specific objectives, research hypotheses, significance and scope of the study.

Chapter 2 covers the literature review that consists of a critical review of previous works related to stingless beekeeping, uses and market demands of stingless bee honey and conventional and alternative methods for stingless bee honey processing. Physicochemical properties of stingless bee honey include moisture content, total sugar content, water activity, pH, free acidity, electrical conductivity, ash content, viscosity, colour analyses, sugar profiling, and HMF content of the stingless bee honey are also explained in this chapter.

Chapter 3 reports a detailed description of sample preparation and treatments, the method to determine the surface area, pore size, porosity, and microstructure of clay pots. Preliminary studies are also included in this chapter. Detailed materials and methods were discussed for each physicochemical analysis conducted in this study.

Chapter 4 presents the results and discussion on the main findings of the present work, including the analysis and interpretation of the data collected. The results and discussion presented in Chapter 4 are presented in the following objectives manner of the present study.

Chapter 5 presents the overall conclusion from the results discussed in Chapter 4 and some recommendations for potential future work of the research field.



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## BIODATA OF STUDENT

Syahrul Anis Hazwani Binti Mohd Baroyi was born on 5<sup>th</sup> April 1993 in Kota Bharu, Kelantan. She received her primary education at Sekolah Kebangsaan Seberang Pasir Mas, Kota Bharu, Kelantan. Later, she continued her secondary education at Sekolah Menengah Kebangsaan Kubang Bemban, Pasir Mas, Kelantan and completed her Sijil Pelajaran Malaysia (SPM) in 2010. After completing her secondary school years, she further her studies in Universiti Teknologi Malaysia (UiTM) Puncak Alam to complete her foundation level for one year before accepted to pursue her degree at Universiti Putra Malaysia (UPM) in Bachelor of Food and Process Engineering. Throughout her study at UPM, she was actively participated and handled many activities in her faculty and residential college. Due to her active participation in both residential college and faculty, she was rewarded *Anugerah Ikon Wibawa* (2014) from Eleventh College and *Anugerah Kecemerlangan Akademik Kokurikulum* (2016) by the Department of Food and Process Engineering. She graduated in 2016 and was rewarded another two awards, which are *Anugerah Graduan Terbaik Keseluruhan bagi Bacelor Kejuruteraan Proses dan Makanan* and *Anugerah Pelajar Bumiputera Terbaik*. Following her graduation, she pursued her Master of Science (Food Engineering) study at the Department of Process and Food Engineering, UPM. During her postgraduate study, she experienced working as a demonstrator for undergraduate laboratory class in at early semester of her studies. She was also actively joined several conferences and seminars related to her research project.



## LIST OF PUBLICATIONS

### Journals

- Baroyi, S. A., Yusof, Y. A., Ghazali, H. M., Chin, N. L., Othman, S. H., Chang, L. S., & Ghazali, N. S. (2019). A novel method based on passive diffusion that reduces the moisture content of stingless bee (*Heterotrigona itama*) honey. *Journal of Food Process Engineering*, 42(6).
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