



**PHYSICOCHEMICAL AND ANTIOXIDANT
PROPERTIES OF MALAYSIAN TRIGONA AND
TUALANG BEE HONEY**

BY

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**A thesis submitted in fulfilment of the requirement for the
degree of master of health sciences (Nutrition Sciences)**

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ABSTRACT

This research was performed to explore the physicochemical and antioxidant properties of selected Malaysian honeys namely Trigona bee honey from Kedah (TB1), Trigona bee honey from Kelantan (TB2), wild Tualang honey from Pahang (TU1) and farmed Tualang honey from Pahang (TU2). Proximate compositions were determined according to AOAC methods. Sugar contents were quantified using HPLC. Minerals were determined by using ICP-MS. Honeys were analyzed for their physical and antioxidant properties in vitro. Findings showed that TB1 had significantly the highest dietary fiber content (0.612 ± 0.027 g/100g ; $p < 0.05$). TB2 showed significantly the higher moisture (30.586 ± 0.109 g/100g ; $p < 0.05$) and ash (0.766 ± 0.010 g/100g ; $p < 0.05$) contents. TU1 recorded the highest value protein content (1.776 ± 0.010 g/100g ; $p < 0.05$). TU2 had significantly the highest total carbohydrate (79.980 ± 0.289 g/100g ; $p < 0.05$) and gross energy (322.780 ± 1.074 Kcal/100g ; $p < 0.05$). Total fat content was significantly the lowest in TB2 (0.025 ± 0.003 g/100g ; $p < 0.05$). For sugar contents, TU1 had higher value of fructose content (33.500 ± 3.473 g/100g ; $p < 0.05$) compared to TB2 and TU2 while TB1 showed significantly the highest value of glucose (40.983 ± 0.941 g/100g ; $p < 0.05$). Furthermore, TB2 possessed significantly ($p < 0.05$) the highest values of potassium, calcium, magnesium, manganese, cobalt and barium with means of 5162.93 ± 23.17 μ g/g, 214.80 ± 2.76 μ g/g, 364.23 ± 2.8 μ g/g, 14.967 ± 0.153 μ g/g, 0.328 ± 0.009 μ g/g and 0.845 ± 0.003 μ g/g, respectively. TU2 recorded significantly ($p < 0.05$) the highest values of iron, chromium, vanadium, silver and nickel with means of 30.233 ± 0.321 μ g/g, 0.079 ± 0.012 μ g/g, 0.09 ± 0.001 μ g/g, 0.599 ± 0.074 μ g/g and 0.309 ± 0.003 μ g/g. Results from heavy metals showed that TB2 possessed the highest values of lead (1.967 ± 0.058 μ g/g ; $p < 0.05$) and cadmium (0.050 ± 0.002 μ g/g ; $p < 0.05$) while arsenic was found only in TU2 (0.003 ± 0.001 μ g/g). However, the amount of heavy metals existed is considered safe for consumption. In term of physical properties, TB1 had significantly the lowest value of pH (3.08 ± 0.020 ; $p < 0.05$). TB2 exhibited significantly the highest values of electrical conductivity (EC) (1.473 ± 0.018 mS/cm ; $p < 0.05$) and mm Pfund (672.883 ± 13.64 ; $p < 0.05$). TU1 recorded significantly the lowest value of water activity (a_w) (0.675 ± 0.002 ; $p < 0.05$). TU2 possessed significantly the highest value of TSS (76.267 ± 0.058 °Brix ; $p < 0.05$). For antioxidant contents, TB2 showed significantly the highest values of TPC (33.711 ± 0.590 mg GAE/100g ; $p < 0.05$) and TFC (2.217 ± 0.126 mg QE/100g ; $p < 0.05$). Antioxidant activities assays showed that TB2 possessed significantly the highest values in DPPH assay (84.521 ± 1.859 % ; $p < 0.05$) and the highest value in FRAP assay (368.472 ± 0.939 μ M TE/100g ; $p < 0.05$). It can be concluded that, honey have many beneficial properties with tremendous potential to be used as a nutritional supplements particularly with a preference for TB2 due to its higher mineral contents, antioxidant contents and activities.

خلاصة البحث

تم إجراء هذا البحث لاستكشاف الخصائص الفيزيوكيميائية و نشاطات مضادات الأكسدة لعينات العسل الماليزي. لهذه الدراسة تم اختيار أربع عينات مختلفة وهي عينة تريغونا من ولاية قدح و تم ترميزها ب TB1 , عينة تريغونا من ولاية كلانتان TB2, عينة توالانغ البري من ولاية باهانج TU1 و عينة توالانغ المنتج في باهانج TU2. كما تم تقدير تراكيز السكر بجهاز HPLC. تم تحديد و قياس تراكيز المعادن المختلفة باستخدام جهاز ICP-MS. تم اختبار الخواص الفيزيائية وخواص مضادات الأكسدة لعينات العسل المختلفة مخبرياً. أظهرت النتائج أن عينة TB1 سجلت المحتوى الأعلى من الألياف الخام. كما و أظهرت النتائج أن عينة TB2 تحتوي على مستوى أعلى من الرطوبة و الرماد الخام. وأظهرت عينة TU1 القيمة الأعلى من البروتين الخام. وسجلت عينة TU2 القيمة الأعلى من الكربوهيدرات والطاقة الكلية. وكما أظهرت النتائج أن عينة TB2 احتوت أقل تركيز من الدهن الخام. وبالنسبة لاختبار السكريات فقد سجلت عينة TU1 المحتوى الأعلى من الفركتوز مقارنة ب TB2 و TU2، بينما سجلت عينة TB1 التركيز الأعلى من الجلوكوز. بالإضافة لذلك فقد سجلت عينة TB2 المحتوى الأعلى من البوتاسيوم، الكالسيوم، المنغنيزيوم، المنغنيز، الكوبالت، والباريوم. وكما سجلت عينة TU2 المحتوى الأعلى من الحديد، الكروميوم، الفاديوم، الفضة، والنيكل. وأما بالنسبة للعناصر المعدنية السامة فقد سجلت عينة TB2 التركيز الأعلى من الرصاص و الكاديوم بينما وجد الزرنيخ فقط في عينة TU2. على أي حال فإن كل عينات العسل في هذه الدراسة تعتبر صالحة للتناول بدون أي مخاطر. فيما يتعلق بالخواص الفيزيائية لعينات العسل المدروسة فقد سجلت عينة TB1 المستوى الأدنى من الحموضة pH. أما TB2 فقد سجلت القيمة الأعلى من حيث الناقلية الكهربائية و الكثافة اللونية. وفيما يخص فعالية الماء فقد سجلت عينة TU1 القيمة الأدنى. أما عينة TU2 فقد سجلت القيمة الأعلى من المكونات الذائبة الكلية. بالنسبة لمحتوى مضادات الأكسدة، فقد أظهرت النتائج أن TB2 احتوت التركيز الأعلى من الفينولات والفلافونيدات الكلية. فيما يخص النشاطات المضادة للأكسدة فقد سجلت عينة TB2 القيم الأعلى في اختباري FRAP , DPPH. وفي الخاتمة أظهرت الدراسة أن كل عينات العسل تتمتع بخواص صحية مفيدة، مع احتمال استخدامها كمكملات غذائية، مع الأفضلية لعينة TB2 وفقاً لامتلاكها قيم أعلى من المعادن الأساسية ومركبات مضادات الأكسدة.

APPROVAL PAGE

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences (Nutrition Sciences).



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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
WHO	World Health Organization
FRAP	Ferric reducing antioxidant power
TB1	Trigona Bee Honey from Kedah
TB2	Trigona Bee Honey from Kelantan
TU1	Wild Tualang Bee Honey from Pahang
TU2	Farm Tualang Bee Honey Bee Honey from Pahang
HPLC	High Performance Liquid Chromatography
a_w	Water Activity
TSS	Total soluble solids
EC	Electrical conductivity
TPC	Total Phenolic content
TFC	Total flavonoid content
TCC	Total carotenoid content
DPPH	Diphenyl-picrylhydrazyl
SPSS	Statistical Packages for Social Science
PTWI	Provisional Tolerable Weekly Intake
PTMI	Provisional Tolerable Monthly Intake
SD	standard deviation
GAE	Gallic acid equivalents
QE	Quercetin equivalents
BC	β carotene equivalents

TE	Trolox equivalent
ICP-MS	Inductively Coupled Plasma Mass Spectrometer

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

According to Codex Alimentarius Commission,. (2001), honey is defined as “a natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature”. The main content of honey is carbohydrate mainly fructose and glucose. In addition, there are some other minor substances such as enzymes, proteins, minerals, vitamins, phenolic acids, flavonoids and carotenoids. The content of natural honey depends mostly on the floral source of nectar which bees collect from, while other factors such as environment and geographical conditions, processing, beekeeping expertise and storage also play important roles (Ajao, Idowu, & Obembe, 2013; Karimov et al, 2014).

The inverse influences on human health of chemicals and synthetic medications in the stage of technology have induced the usage of more traditional and natural products (Can et al., 2015). For many civilizations, honey is considered a natural foodstuff of great importance, with many useful applications. In addition to being a source of sweet, honey is known as nourishment with important nutritional properties and as a natural product which has a worthy therapeutic applications (Guerrini et al., 2009). For example, bee products can be used to protect and strengthen the immune system (Can et al., 2015). Furthermore, additional therapeutic properties of honey have been discovered. Modern science has applied honey to be

used for healing wounds and burns oncology care as well as its antioxidant and antimicrobial properties (Gomes, Feas, Iglesias, & Estevinho, 2011).

In Malaysia, Tualang honey is a wild multifloral honey collected by Asian rock bees (*Apis dorsata*). In addition, Tualang honey is produced in farm by beekeepers. Recently, there has been a huge interest in the use Tualang honey as a supplement and medicinal product according to its ability to cure and decrease many diseases (Ahmed & Othman, 2013; Tan et al., 2009).

Unlike Tualang honey, Trigona honey (stingless bee honey) produces by Stingless bees. Trigona honey has led scientists to discover food quality standard parameters, presenting the high compositional and functional variability largely due to ethno- logical and floral sources, environmental conditions and harvesting season (Guerrini et al., 2009; Souza et al., 2006). Numerous of bioactive constituents have already been discovered in stingless honeys from many *Melipona* species (Mc Cabe, Hrcncir, & Farina, 2015).

The major focus of this study was to evaluate the physicochemical properties (including proximate composition, sugar, minerals, and physical characteristics) as well as the antioxidant properties of different type of honey for determination of honey quality. Four different honey samples from Tualang and Triona bee honey obtained from three different areas (Pahang, Kedah and Kelantan) were used in this research.

1.2 STATEMENT OF THE PROBLEM

Even though honey is widely used and consumed in Malaysia, there is very limited data available to support using it in medical claims. Currently there are very limited studies conducted upon the composition and antioxidant properties of Trigona and

Tualang bee honey in Malaysia. Therefore, it is necessary to evaluate the chemical compositions, physical characteristics and the antioxidant properties of the tropical honey found in Malaysia.

1.3 SIGNIFICANCE OF THE STUDY

Honey is known to possess minerals, vitamins, proteins, carbohydrate, polyphenols and other beneficial micronutrients. Honey has become an important part of daily human diets and offers a plenty amount of antioxidants which provide protective effects of against many diseases (Boussaid et al., 2014; Guerrini et al., 2009; Schramm et al., 2003). In addition, several scientific studies have proven that honey contains a numerous of therapeutic compounds and micronutrients which have potential health benefits (Stefan Bogdanov, Tomislav, Sieber, & Gallmann, 2008; Can et al., 2015; Shehu, Adzim, Rohin, Aziz, & Ismail, 2013)

The current study was conducted to explore the potential of Malaysian Trigona and Tualang bee honey. This investigation is significant in order to find the physicochemical and antioxidant properties that these honeys could possess. Besides that, the nutrient compositions that were explored can act as a guideline in producing health supplement products from Tualang and Trigona bee honey.

The present study produced additional information on nutrients and antioxidant properties present in Trigona and Tualang bee honey. Thus, this research can provide information regarding physicochemical and antioxidant properties of Trigona and Tualang honey to be included in Malaysian food composition database. Also, obtained results could be used to prepare ingredients that could be beneficial and healthy for human consumption.

1.4 RESEARCH OBJECTIVES

1.4.1 General Objective

To determine the physicochemical and antioxidant properties of Trigona and Tualang bee honey.

1.4.2 Specific Objectives

1. To determine the proximate, mineral, sugar compositions and physical characteristics of Trigona and Tualang bee honey.
2. To evaluate the antioxidant contents and activities of Trigona and Tualang bee honey.

1.5 HYPOTHESIS

Tualang and Trigona bee honey are rich source of antioxidants, energy source and macrominerals.

CHAPTER TWO

LITERATURE REVIEW

2.1 HONEY

Honey is a natural sweet viscous fluid produced by honeybees from the nectar of flowering plants or from the nectar of blossoms which honeybees collect and transform by combining with their salivary secretions, and deposit, dehydrate and store in the honey comb to ripen (A. Rebiai & Lanez, 2014; Adenekan, Amusa, Lawal, & Okpeze, 2010).

In general, honeys are classified as monofloral or polyfloral. Monofloral honey is produced by one plant species containing predominantly its nectar with minor nectar contributions from other botanical origins. On the other hand, polyfloral honey has several plant sources, none of which is predominant. In practical terms it can be considered as a blend of several monofloral honeys with significant nectar or honeydew contributions from different plants (Gašić et al., 2014).

Several reports mentioned that honey contains more than 200 elements. Some of honey elements are essential for human life such as sugars (complex mixture of sugars, in which fructose and glucose are the main ingredients and these two account for nearly 85–95% of the honey carbohydrates with low quantities of disaccharides (sucrose) and polysaccharides), proteins, vitamins, organic acids and minerals (Alqarni, Owayss, Mahmoud, & et al, 2012; Alqarni, Owayss, Mahmoud, & Hannan, 2014; Sulieman, Abdelhmied, & Salih, 2013). Although the major constituents of honey are nearly the same in all honey samples, the precise chemical composition and physical properties of natural honeys differ according to the plant species on which the bees forage, i.e., Tualang, Trigona, Gelam, Rubber, Sourwood honeys, etc. (Buba,

Gidado, & Shugaba, 2013). However, the quality of honeys depends on the climate and environmental conditions around the foraging area of bees. Further processing and improper storage condition also indirectly influence the quality of honeys (Chua, Abdul-Rahaman, Sarmidi, & Aziz, 2012).

2.1.1 Stingless Bee Honey

Stingless bee honey is a precious bee product of the stingless bee. Despite the low production of stingless bee honeys at any level, when compared to the production of *A. mellifera* honeys, stingless bee honeys reach a higher price in the market (Ramón-Sierra, Ruiz-Ruiz, & de la Luz Ortiz-Vázquez, 2015). However, the distribution of stingless bee honeys in the world market is limited as compared to honeys from the *Apis mellifera* owing to a limited supply, a lower shelf life, and also lack of an institutional quality standard due to less knowledge available on the product. Due to insufficient information about stingless bee products, stingless bee honey is not involved in international standards for honey and it is not regulated by food control authorities (Kek, Chin, Yusof, Tan, & Chua, 2014).

In fact, stingless bee honey is different from that produced by the bees of the genus *Apis* (i.e., the honey bee) in terms of its color, taste and viscosity. Moreover, it is more acidic than *Apis mellifera* honeys (Almeida-Muradian et al., 2014; Guerrini et al., 2009). Stingless honey could be distinguished from *Apis spp* honeys by its higher average of moisture content. Nevertheless, despite the high moisture content of stingless bee honeys, they are resistant to spoilage by undesirable fermentation because of the existence of polyphenolic compounds derived from the plundered flora and because of cerumen enzymatic processing by bees in their hives (Guerrini et al., 2009). To some extent it was found that the composition of sugar content in stingless

honey quite different from *Apis mellifera* honey (Oddo et al., 2008). For instance, Oddo et al., (2008) was found that the sugar spectrum in *Trigona carbonaria* honey from Australia was quite different from that of *Apis mellifera* honey, with a mean of 20.3 ± 2.9 g of maltose/100 g of honey. Also, a higher level for maltose in some neotropical stingless bee honey has been reported by Bogdanov, Vit and Kilchenmann.,(1996) with a range of 2.5–32 g/100 g. According to Chuttong et al., (2016), the results from 28 stingless honey from Thailand produced by 11 stingless bee species, showed that maltose was the predominant sugar with a mean of $(41 \pm 15$ g/100 g).

Stingless beekeeping is known as meliponiculture. Stingless bees are considered as a group of eusocial insects, from a family of *Apidae* and a tribe of *Meliponini*. They belong to five different genera, viz. *Melipona*, *Trigona*, *Meliponula*, *Dectylurina* and *Lestrimelitta*. They are important pollinators which play a significant role in pollination. These eusocial insects are pan-equatorial and can be found in tropical and subtropical areas of the world such as, Southeast Asia, South America, Africa and Australia. Universal there are above 500 defined species in 32 genera with around 100 new species to be described (Chuttong et al. 2016;Guerrini et al. 2009). The largest genus of stingless bees is *Trigona* which was found exclusively in the Neotropics, from Mexico to Argentina and in the Indo- Australian region from India– Sri Lanka to Taiwan, the Solomon Islands, South Indonesia, Australia and New Guinea (Choudhari et al. 2012;Barth, Hrcir, and Jarau 2008). However, the number of stingless bee species in Malaysia varies between 17 to 32 species (Kelly, Farisya, Kumara, & Marcela, 2014). In the reality, the colony has a nice cohesive unit where the only queen lays the eggs and the worker forage, clean, and protects the colony (Koedam, Contrera, Fidalgo, & Imperatriz-Fonseca, 2005).



Figure 2.1 Internal and external shape of stingless bee nests

2.1.2 Tualang Bee Honey

Malaysian Tualang honey is a wild multifloral honey collected from the combs of Asian rock bees (*Apis dorsata*). It has a dark brown appearance (Ahmed & Othman, 2013). Tualang bees collect nectar from plants and blossoms in the tropical rain forest and construct their hives on branches of tall Tualang tree (*Koompassia excelsa*). The *Koompassia excelsa* tree is amongst the tallest trees in the world, and also one of the most prominent trees in the tropical rainforests of the Sunda Shelf. It can reach up to 250 feet (roughly 80 m) in height. The north western region of Peninsular Malaysia is known as the main source of Tualang tree. It is also found in the tropical rain forests

of Sumatra, Borneo and South Thailand. Tualang tree was named by different names according to the regions of existence. For instance, Mengaris in Brunei and Sabah, Tualang in Peninsular Malaysia, Sialang in Indonesia and Tapang in Sarawak. The name Tualang originates from the Malay words tua which means old, and helang which means eagle. The combs are up to 6 feet across and each hive may have an average of 30,000 bees. One tualang tree can contain more than 100 nests and may yield about 450 kg of honey (Devasvaran & Yong, 2016 ;Khalil et al. 2011; Ahmed & Othman ,2013; Othman et al., 2015). However, Tualang bee honey possesses many potential health benefits which will be discussed in section 2.8.2.



Figure 2.2 Tualang tree contains bee combs
(Retrieved from <http://mymaa-madutualang.blogspot.my/2008/10/english-nutrition-of-tualangs-black.html>)

2.2 PRODUCTION AND CONSUMPTION OF HONEY

There is an increasing trend in total honey production worldwide. The European Union (EU) is the world's largest consumer of honey, with 22% of global production. Honey is an important commodity in the international market; serving as foreign

exchange earner for many countries (Buba et al., 2013). In fact, the major honey exporting countries include China and Argentina (Mogni, Palau, Tresoldi, Senesi, & Vilella, 2007; Vanhanen, Emmertz, & Savage, 2011). At present the annual world honey production is about 1.2-1.3 million tons; of which 350 thousand are sold in the global market. This production is less than 1% of the total sugar production. However, Ismail, (2014) reported that total Malaysian production of honey in 2010 was 8548 metric ton. Also, the value of exports of Malaysian honey increased from 0.74 RM million in 2005 until 47.65 RM million in 2010.

The main uses of honey can be classified as directly consumed honey (table honey) or industrial honey, honey used in food manufacturing or as a base ingredient (Mogni et al., 2007; Vanhanen et al., 2011). The consumption of honey differs strongly from country to country. However, honey consumption is higher in developed countries, where the home production does not always cover the market demand (Stefan Bogdanov et al. 2008; Mogni et al. 2007). Generally, Malaysians consume local natural honey for medicinal purposes. This means that honey intake may be once or twice a year during the sickness period of two or three days. That is why the average consumption per capita is only 100 g which is about 5 table spoons (Ismail, 2014).

In fact, honey was used by human since around 8000 years ago. The ancient Egyptians, Greeks, Chinese, Assyrians and Romans applied bee products for wounds and cure some diseases (Eteraf-Oskouei & Najafi, 2013). Indeed, medicinal importance of honey has been reported in the world's oldest medical literatures (Castaldo & Capasso, 2002; Kaškonien et al., 2009; Marcucci, 1995; Tumin et al., 2005). Also, honey is considered to be an important part of traditional and folk medicine. Moreover, it has been used in ethnomedicine since ancient eras, and in more