Evaluating Compliance of Commercial Stingless Bee Honey According to Malaysian Standard 2683:2017 – Physicochemical Properties

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project entitled "**Evaluating Compliance of Commercial Stingless Bee Honey According to Malaysian Standard 2683:2017- Physicochemical Properties**" by **Nur Rahiiqin Maktuum Binti Baharuddin** matric number **F15A0157** has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Product Development Technology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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LIST OF SYMBOLS/ ABBREVIATION/ NOMENCLATURE

MS-2863:2017	Malaysian Standard 2683- Stingless Bee Honey Specification
HMF	Hydroxymethylfurfural
H. itama	*Heterotrigona itama*
G. thoracica	*Geniotrigona thoracica*
SD	Standard deviation
ANOVA	One-way Analysis of Variance
mL	Mililiter
cm	Centimeter
g	Gram
kg	Kilogram
mg	Miligram
℃	Degree Celsius
%	Percentage
µ	Mean
Mg/kg	Milligram per kilogram

Penilaian Pematuhan Madu Lebah Kelulut Komersial Mengikut Standard Malaysia 2683:2017- Sifat Fizikokimia.

ABSTRAK

Madu kelulut semakin mendapat tumpuan kerana nilai pemakanan dan pendebungaan yang lebih baik daripada lebah madu. Madu kelulut mempunyai komposisi yang berbeza daripada madu lebah oleh itu, perbezaan sifat fizikokimia dibentangkan mengikut spesis kelulut. Penubuhan Standard Malaysia-2683 Spesifikasi Madu Kelulut (MS-2863: 2017) pada tahun 2017 dibentuk sebagai garis panduan penilaian kualiti madu kelulut. Kajian ini tertumpu kepada pematuhan madu kelulut komersial terhadap standard ini. Dengan menggunakan prosedur dan kaedah yang dicadangkan dalam MS 2863: 2017, semua sifat fizikokimia yang diperolehi dianalisis menggambarkan kualitinya. Tiga belas sampel madu kelulut di kumpulkan sekitar Kawasan Kelantan yang terdiri daripada dua sampel madu lebah campuran, dua dari spesis *G. thoracica* dan sembilan dari spesis *H. itama.* Sampel juga termasuk satu sampel madu yang melalui rawatan haba dan dua belas madu mentah telah menjalani analisis fizikokimia. Analisis fizikokimia dalam madu termasuk kelembapan, kandungan mineral, hydroxymethylfurfural (HMF) dan nilai pH dengan hasil 27.05% kepada 32.61%, 0.08 g kepada 0.14 g, 8.75 mg / kg kepada 218.66 mg / kg dan 2.32 hingga 3.22. Ini menggambarkan kandungan kelembapan dan mineral dalam madu kelulut lebih tinggi daripada *Apis spp.* dengan nilai pH dan kandungan HMF terendah. Sesetengah madu kelulut mematuhi MS-2863: 2017 yang berdasarkan kepada jenis asal geografi, spesies lebah, sumber bunga dan bentuk madu.

Kata kunci: Madu kelulut, Standard Malaysia -2683 Pengkhuhusan Madu Kelulut, ciri-ciri fizikokimia.

Evaluating Compliance of Commercial Stingless Bee Honey According to Malaysian Standard 2683: 2017- Physicochemical Properties.

ABSTRACT

Stingless bee honey is gaining its popularity due to nutritional value and better pollinators than honey bee. Since stingless bee honey has different composition than *Apis spp.* honey, further physicochemical parameters are presented according to stingless bee species. Hence, an establishment of Malaysian Standard- 2683 Stingless Bee Honey Specification (MS-2863:2017) in year 2017 was formed as guideline towards stingless bee honey quality. This current study focusing on the compliance of commercial stingless bee honey towards this standard. By using procedure and method proposed in MS 2863:2017, all physicochemical properties honey was analysed portrays its quality. Thirteen samples of stingless bee honey around Kelantan was collected, two from blended species of stingless bees, two from *G. thoracica* and nine from *H. itama* species. Samples also including a single heat treatment honey sample and twelve raw honey which undergo physicochemical analysis. Physicochemical analysis in honey includes moisture, ash, hydroxymethylfurfural (HMF) and pH value with results 27.05 % to 32.61 %, 0.08 g to 0.14 g, 8.75 mg/kg to 218.66 mg/kg and 2.32 to 3.22. It represents, moisture and ash content in stingless bee honey is higher than *Apis spp.* with lowest pH value and HMF content. Some of the stingless bee honey were complies with MS-2863:2017 which based on varieties of geographical origin, bee species, flower sources and honey form.

Keywords: Stingless bee honey, Malaysian Standard 2683-Stingless Bee Honey Specification, physicochemical properties

CHAPTER 1

INTRODUCTION

1.0 Research Background

Honey is defined as sweet, dense, crystallized, viscous product that produced by honeybee from nectar of honey plant flowers or from secretion of living parts (conifer or hardwood species), which bees collect and transform by combining with specific substances of their own and deposited in honeycomb to mature according to the Regulation (Official Gazette of SCG, No.45/2003). According to Vit, Bogdanov, & Kilchenmann (1993) study, it stated that *Apidae* is the family to which honey bees and stingless bees belong that assigned them into two separate subfamilies, *Apidae* and *Meliponinae. Meliponinae* or stingless bees have approximately 500 species within the stingless bee genus, with the majority of these species being located in Latin America, the mainland of Australia, Africa and Eastern and Southern Asia (Rasmussen et al., 2010 ; Abd Jalil, Kasmuri, & Hadi, 2017).

Furthermore, stingless bee is the one of important pollinators that play a major roles in spreading the pollen and widely known to be efficient also effective pollinators of many plant species (Azmi et al., 2017; Mohd et al., 2010). This also been supported by Azmi et al. (2017) which reported that the advantage of stingless bee honey which are more efficient pollinators compare with honey bee. Studied by Mohd et al., (2010) found

that insect, including bees is the highest pollinators of flowers plants that contribute 80% to our ecosystem. This means the important role of bees that will conserve our ecosystem (Ismail, 2016).

On the other hands, honey can be producing by both types of bees either honey bees or stingless bees and has two types of beekeeping which the commercial kind with *Apis mellifera* and meliponiculture that use stingless bees. Meliponiculture is known as dealing with the stingless bees, obtaining the honey as the primary product (Almeida-Muradian, 2013).

In addition, stingless bee honey is known as nectar collect in flowers that contain the mixture and let it maturing in the pots of the colonies (Nascimento et al., 2015). Flowers visited by bees and surrounding climate area where the honey was harvested will affect the variation of honey composition and properties (Nazmul et al., 2013). Based on Muruke (2014) stated that stingless bee honey has darker colours compare to the honey bee. Darker colour honey has high in phenolic compound with contribution of polyphenols (Khalil et al., 2012). This shows, stingless bee honey contains more minerals than honey bee likes iron, copper and manganese regarding to its colour. Hence, all these elements make it more valuable to be use as for medical treatment. Study from Silva et al., (2013) stated that, stingless bees contain composition which associated with antiseptic, antimicrobial, anticancer, anti-inflammatory and wound-healing properties and able provides cell defence and promote cell functions in erythrocytes. The texture of stingless bee honey is more fluidity due to high water content and undergo slow crystallization process (Biluca et al., 2016).

Other than that, stingless bee does not sting their enemies. However, they defend themselves by producing resin called propolis or commonly recognized as bee glue. The sticky materials content in propolis able to trap ants or other pests and at once protect their colony and hive from other threats. Propolis contain natural insect repellent that produces by stingless bee saliva that mix with other component from flowers (Ibrahim et al., 2016; Rintos, 2016). Besides, chemical component content in propolis is very complex that mixed up more than 200 types of compound that already been identified by many countries. Such as, Brazilian, Europe and also Canary Islands. Propolis also contain important healing properties like antibacterial, antiulcer and antioxidant (Ibrahim et al., 2016). Therefore, research by Ibrahim et al., (2016) showed that the reason why stingless bee honey become famous and well known honey is not due to the high honey production but because of large quantity of propolis produce by them.

Honey is not only use as the natural sweeteners but also traditionally used as medicine that heals many diseases and as energy booster. According to Biluca et al. (2016), stingless bee are abundant in Brazil and become common use as medical properties likes antiseptic, antimicrobial, anticancer also wound healing properties. This have been supports by a study from Al-waili, Salom, & Al-ghamdi (2011) that honey can be used for wound treatment. Study founds, honey showed less edema and better wound contraction that promotes the formation of granulation tissue and epithelialization of wounds. It able to stimulate tissue growth, synthesis of collagen, and development of new blood vessels in the bed of wounds.

Hence, physicochemical is the criteria that indicate overall honey quality. Thus, physicochemical analysis was determined based on the Malaysian Standard 2683-Stingless Bee Honey Specification that become the guideline for all the manufacturer and producer of stingless bee honey in Malaysia that been establish in 2017. It covers all

aspect from the raw material, processing until the testing of honey. One of the examples for physicochemical of honey is colour. Every types and different honey sources contain different physicochemical properties which may differ such as in colour range. The colour will range from pale yellow up to amber and darkish red amber hence nearly to black in colour (Adenekan et al., 2010).

1.1 Problems Statement

In the present time, honey become more crucial traditional supplement that can heal many diseases that make increasing in demand. The shortage of honey products became one of the reason honey adulteration with cheaper price and inconsistency in quality honey production. This cause toxic effect on human health in worse case may cause liver and kidney failure. This was supported with study by Ismail (2016) that conduct a test on rats that revealed the long-term effect of consuming adulterated honey.

Secondly, now a days, stingless bee honey that has high abundant sources due to increasing bee keepers make people try to search about it more specifically. However, due to lack of a monitoring and enforcement of Malaysian honey quality among beekeeper's, it becomes a major challenge and problem to the honey production to identify the best honey and honey without adulteration. Hence, this make the limitation to the community to make as reference before Malaysian Standard of Stingless bee (*kelulut* honey) specification being introduced in 2017. However, do the bee keepers comply with this standard? Not only that, honey also can contain toxic compound if kept in long storage period. Thus, it is important to evaluate either honey in market comply with Malaysian standard or not. Hence, this study aimed to study the physicochemical properties of stingless bee honey around Malaysia using Malaysia Standard 2683-

Stingless Bee Honey Specification as guideline. The physicochemical properties of honeys will represent the criteria of honey quality.

1.2 Hypothesis

Hypothesis one:

H_0: All the physicochemical properties of stingless bee honey in market complies with Malaysian Standard 2863:2017.

H_a: Some of physicochemical properties of stingless bee honey in market complies with Malaysian Standard 2683:2017.

1.3 Objective

1. To evaluate physicochemical analysis of pH value, HMF content, total ash content and moisture content on stingless bee honey products existing in the market based on Malaysian Standard 2863:2017-Stingless Bee Honey Specification.

1.4 Scope of Study

This research focuses on quality assessment of stingless bee honey by determining its physicochemical properties including pH, hydroxymethylfurfural (HMF), ash and moisture content determination following Malaysian Standard regulation of stingless bee honey. Samples majorly obtain from Kelantan state including Kota Bharu, Wakaf Bharu, Pengkalan Chepa, Ketereh and Jeli. It comes in different form including raw and process

honey either blended or single species of stingless bees. This study was portrayed the maximum quality of stingless bee honey in market.

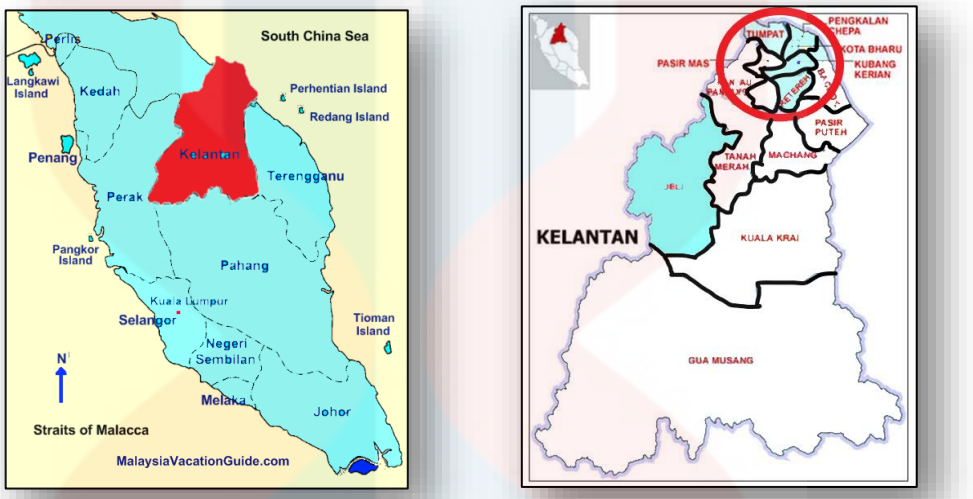

Figure 1.1: Origin of stingless bee honey collected from Kelantan state area

1.5 Significance of Study

This main contribution of this study is towards researcher, manufacturer also community. The data collected from research work were contributed for the further research of stingless bee honey. Hence, the manufacturer also being more strictly followed the Malaysian Standard in other to maintain the honey quality for consumer purchasing. Besides, consumer and community were able to increase their knowledge about stingless bee honey regarding it benefits and useful.

1.6 Limitation of Study

This research did not has similar number for process and raw honey including species of stingless bees, *H.itama* or *G.thoracica.* Hence, it limits the comparison between samples and results. Other than that, getting a fresh stingless bee honey from beekeepers were limited in Kelantan because of smaller industry from stingless bees. Furthermore, the aged, origin and flowers sources of honey samples were becoming the limitation because of an estimation done by beekeepers.

CHAPTER 2

LITERATURE REVIEW

2.1 The History of Stingless bees

'Stingless bees are highly spreading social insects that live in a colony with organized system. Some species have clusters of as many as 80,000 individuals and other less than 100. Based on Michener (2013), *Meliponini* belongs to a monophyletic group of four tribes which are *Apini, Meliponini, Bombini* and *Euglossini. Meliponini* bees able to differentiate from all other bees due to the lack or weak sting produced. The workers or the females possess weak stingers that unable to inflict pain while the male bees are completely stingless. Hence, the term "stingless" is being used to designate the species. Some species have mandibles sufficiently strong to inflict a moderate bite or may able to crawl into ears or nostrils of the attackers. (Michener, 2013; Rahman et al., 2015).

Even though stingless bees has limited dispersal ability due to their slow progress for colony establishment and also has small flight range (Rasmussen & Cameron, 2010). However based on Rasmussen (2008), stingless bees have been spreading out to the Indo-Malay/ Australasian and has greater abundance in Thailand also Malaysia including all of Borneo region from India to the Solomon Islands and China which includes Yunnan,

Hainan and Taiwan. The distribution happen is may due to the resin-secreting trees and humid tropical climate of Asian region.

2.1.1 Bee Species

In Malaysia, there are several types of honey that been produced in this tropical country such as *Tualang* honey, Pineapple honey, *Gelam* honey and *Acacia* honey. Recently, stingless bee honey getting more attention due to its unique sour taste and distinct flavour besides their health benefits (Keng et al., 2017). As for examples, the *Tualang* honey is derived from Giant honey bee colonies which is called as *Apis dorsata* from bee trees in the tropical rainforest (Ismail, 2014).

The stingless bees was enclosed more than 600 species that in approximately has 61 genera comparing with *Apini* which only has 11 species in single genus with major widespread *Apis mellifera* species (Kek et al., 2017; Rasmussen & Cameron, 2010). Study also found that almost 250 species has been identified throughout the South America temperate zones and Indo-Burma- Malayan and Australian region around the world (Rahman et al., 2015). In addition, species found around Indo-Malayan/Australasian stingless bees that locality from Australian, Indonesia, Singapore, Thailand and Malaysia are commonly name as *Austroplebeia* for example *A. australis, A. cassia, A. cincta and A. cockerelli* while for Indonesia, Thailand, Singapore and Malaysia are known as *Geniotrigona* and *Heterotrigona. G. thoracica and H. itama* is one of the stingless bees that can be found all around Asian country Singapore, Malaysia, Myanmar, Thailand, Indonesia and Brunei (Rasmussen, 2008).

Figure 2.1: *Geniotrigona thoracica* species of stingless bee

In Malaysia, the stingless bees that commonly been found are from species from *H. itama* and *G. thoracica* that become the main pollinators for wild and tropical plant which produce honey, pollen and cerumen that use for human consumption (Abd Jalil et al., 2017; Fadzelly et al., 2017).

In addition, study by Kelly et al., (2014) reported that five stingless bee species were found in bee farm at Kelantan. The most famous stingless bees in Malaysia are from *H. itama* and *G. thoracica* and the major honey produce from stingless bee is by species *H. itama* or also known as *Trigona itama*. *H. itama* can easily distinguish by coloration and size. It is the major colony from Singapore and more preferred by consumer and bee farmers in Malaysia that contributes almost 83.2 % in the farm due to the benefit that able to treat eye cataract also can control digestive system due to high flavonoids and polyphenols content. Besides, less defend of bees make it more flexible to foster towards seasoning change and become more pricy (Biluca et al., 2016; Kelly et al., 2014). Other than that, study by Fonseca in 2012 stated that each species of stingless bee has their own requirement for nest building depends on their colonies size and habitat quality.

As results, *G. thoracica* preferred tree trunk circumferences that range between 82 cm to 129 cm while *H. itama* preferred smaller tree trunk that range between 71 cm to 164 cm.

Honey bee and stingless bee also have different beehive structure. Honey bees build hexagonal-shaped combs with wax in the nests and the honey produced is known as comb honey while stingless bees construct horizontal pots made of cerumen, a mixture of propolis and wax for their nests to store honey and honey produced is known as pot honey (Kek et al., 2017). Sommeijer (1999) stated that, stingless bees have more elaborate and complex nest compare to *Apis spp*. Stingless bees' nest being built within protective area and environment such as at hallow trees or in the ground. It also has narrow nest entrance for defends purpose.

2.1.2 Benefits of Stingless Bee Honey

Study by Sousa et al. (2016) stated that, honey from stingless bee contain antimicrobial, while study by Silva et al. (2013) stated that antioxidant activity in honey relates to the quantity of plant phenolic compound. The amount of phenolic are definitely different in every variety of honey due to the bee species, region, season also type of floral sources. Furthermore, study by Yaacob et al., (2017) showed plant phenolic content in stingless bee from *Plebeia* spp. is higher than *Apis* spp. Hence, honey sample which high in plant phenolic content higher ABTS+ cation radical scavenging capacity due to breakdown of free radical chain that cause harmful effect to injured area or wound on skin (Abd Jalil et al., 2017). This indicates a correlation between phenolic content and antioxidant activity in the stingless bee's honey (Silva et al., 2013). Generally, phenolic content also can reduce the disease that associated with oxidative stress like neurological damage likes Alzheimer's disease and Parkinson's disease.

In addition, antimicrobial activity in honey also depends on total phenolic presence (Silva et al., 2013). This has been showed by Abd Jalil et al. (2017) that antimicrobial properties also can control the bacterial contamination hence improve the healing rate. Antimicrobial activity is important in other to prevent infection especially during injury period. Honey that been stored in the cerumen pots made from wax and propolis contributes to the antimicrobial properties of honey. It also been stated effective on inhibition of both gram-positive and gram-negative bacteria and yeast (Abd Jalil et al., 2017). In the other hands, the antimicrobial properties also been contributing by presence of hydrogen peroxide. Hydrogen peroxide has been produced from oxidation of glucose that been found in natural honey (Marshall, Gu, & Schneider, 2015).

Another benefit produce by stingless bee are known as propolis and cerumen. Propolis is produce by combination of plant resin and beeswax while cerumen contains one additional compound which is mandibular that been secrets by stingless bee during construction. Cerumen is use in honey storage in pots and control sterile environment in honey hive while propolis is used to internally coating around the hive. Hence, with the presence of these by-product of stingless bee, it effect the honey production quality which contain higher phytochemical properties and active compound (Abd Jalil et al., 2017).

2.1.3 Propolis and Cerumen

Propolis is known as sticky resin that flows from the buds and bark of some trees. Bees gather propolis, sometimes called bee glue, and carry it home in their pollen baskets. They mixed it with wax flakes secreted from special glands on their abdomens (Pino et al., 2006). In addition, propolis contain in very complex compound and majorly found were flavonoid, phenolics and aromatic compound. Furthermore, the composition of

propolis were different according to the plants in a specific region. Propolis contain many health benefits such as able to inhibit bacterial and fungus infection due to high presence of flavonoids contain (Farnesi et al., 2009). Propolis is product from both type of bees, honey bee and stingless bee. Propolis in stingless bee is called as cerumen to avoid further confusion. Propolis in honey bee is form of mixing beeswax and resin collected from varies flora, however cerumen consists similar mixture of propolis with addition of mandibular secretion of stingless bee during its construction. Cerumen is used as a storage pot for the honey and to mummify trespassers as well as to ensure that the environment in the hive is sterile. Furthermore, propolis is used as an internal layer and for sealing the extra space surrounding the hexagon-shaped of honey combs. Hence, due to the storage of honey in the cerumen pots, the quality of the stingless bee honey is influenced by the infiltration of phytochemicals from the cerumen (Azri et al., 2017).

Based on the Farnesi et al. (2009), propolis produce by stingless bees was more better in inhibition of *Pseudomonas aeruginosa* bacteria than propolis produce by honey bee. Apart from that, study also showed propolis extraction from stingless bee species either in *H. itama* or *G. thoracica* that able to inhibit microbial growth likes *Staphylococcus aureus*. However, propolis produce by *H. itama* species are more effective in inhibition of Gram-positive and Gram-negative with concentration 5 mg/mL and 10 mg/mL compare to the *G. thoracica* (Ibrahim et al., 2016). On the other hands, propolis also act as defend materials for bee hive from predators like flies, ants and spiders. It is important to make sure that all access into hives are completely sealed to avoid insects entering and lay eggs that may cause colonies damaged (Halcroff et al., 2013).

Figure 2.2: Propolis and honey pots of *Heterotrigona itama* species.

2.1.4 Honey Composition

Honey commonly contain sugars including fructose, glucose, sucrose or maltose. Glucose one of the simple sugars that act as primary sources in human body while fructose that found naturally with glucose. Both of them, either glucose and fructose are influence the sweeteners to the food and beverages. In the other hands, sucrose that usually known as table sugar that composed with ten units of glucose and one fructose. Lastly, maltose that built with two glucose units that usually found in fermentation process (Schorin et al., 2012).

Usually, the composition of honey by stingless bees content more than 60 % of simple reducing sugar which are fructose and glucose also non-reducing sugar mainly sucrose and maltose, water and ash. However, in Malaysia, honey may content lower than 60 % of sugar due to high humidity climax (Fuenmayor et al., 2013). In high quality of honey, its contents low level of sucrose which only 5 % compare to other reducing sugar. Another study by Sousa et al. (2016) also supports the previous finding by Biluca et al.

(2014) that fructose contain was much higher in stingless bee honey. The fructose and glucose ratio are important in influencing the sweet taste of honey hence the high sucrose content that reflected the low quality of honey due to additional sugar which content high sucrose compound (Biluca et al., 2014). Furthermore, another factor that contribute to the high sucrose level is because of premature harvesting of honey. Sucrose content in premature honey do not fully or completely converted into glucose or fructose by involving of invertase enzyme (Nascimento et al., 2015). In addition, higher fructose sugar contributed to the sweet taste and hygroscopicity which maintain the liquidity of honey (Biluca et al., 2014).

Based on the study using swift scheme method, the total sugar content in stingless bee honey by *H. itama* species for every 100 g of honey content 9.74 to 54.3 g of maltose, 5.37 to 19.9 g fructose and 3.91 to 27.2 g glucose (Se et al., 2018). Furthermore, study by Se et al. (2018) on *H. itama* in Malaysia also content maltose sugar other than fructose, glucose and sucrose that slightly different from Brazilian stingless bee that only content three types of sugar. There also found that *H. itama* species contain less fructose and glucose that bellow the Codex Alimentarius 2001 which less than 60 g/100 g and content high level of sucrose. In addition, studied by Sousa et al. (2016) found that honey from similar floral source produced by different species of bee were different in sugar detected. Moreover, the taste of stingless bee honey was influenced by the low sugar content and the acidic pH.

2.2 Physicochemical Studies

The physicochemical properties of stingless bee honey are diverse according to origin of geographical or botanical and fruit or flower season. Hence, it differs in pH value, ash content, colour and also water content even through similar species of bees (Fatima et al., 2018).

2.2.1 Moisture Content

Water is one of the major element content in stingless bee honey that recorded up to 31 % and make it differ from honey bee species (Biluca et al., 2016). Study by Almeida-Muradian (2013) stated that, honey having high water content is easily undergo fermentation which making the preservation and storage difficult. However, despite the higher moisture content in stingless bee honey, it fairly resistant to spoilage by unwanted fermentation due to other physicochemical factors. This is because, resin presents in the cerumen used to build up the storage pots could be present in honey and may serve as biocidal agents that prevents the fermentation to occur (Vit et al., 1993).

Furthermore, major factor that contributes to high moisture content in stingless bee honey is because, honey from *Apis spp.* is easily undergo evaporation process with development of some behaviour mechanisms while stingless bee honey does not. Other than that, due to different flower origins and sources behaviour of stingless bees which prefers high moisture matured fruits and flowers substrates to be collected able to increase water content in honey (Suntiparapop, Prapaipong, & Chantawannakul, 2015).

On the other hands, among *Apis spp.* honey, honey produce by *A mellifera* shows the lowest moisture content which only 17.68 g/ 100 g while *A. dorsata* indicates the highest moisture content comparing to other species, *A. cerana* and *A. florea* (Farida, Asif, & Waghchoure, 2011). However, comparing honey from *A dorsata*, *H. itama* and *G. thoracica*, still has the lowest value of moisture content (Fatima et al., 2018).

In addition, study shows, moisture content are depends on the type of flowers, climate condition, size of the colony, collection period also the way of honey being process (Bijlsma et al., 2006; Sousa et al., 2016). In Malaysia, Keng et al. (2017) found the moisture content of stingless bees is much more higher than honey bees due to the seasonal weather conditions or regional humidity of area collection. Hence, the drying process is suggesting to extend the honey shelf life. However, the drying temperature need to be set accordingly low to avoid denaturing of phenolics content in honey.

According to the Malaysian Standard MS-2683, Stingless bee Honey Specification, the allowed water content in honey must be less than 22 %. However, the moisture content in honey are slightly different between raw and process honey. Processing honey that already undergo thermal process will preserve the nutrition content and medical properties but it will produce HMF and lowering the sugar content in honey (Biluca et al., 2014).

There are lot of study regarding moisture content in stingless bee honey that slightly similar to the Malaysian Standard requirement. Firstly, study by Biluca et al. (2016) shows 23.1 to 43.5 % moisture content in Brazil stingless bees which similar to study by Sousa et al. (2016) stated that water content in honey samples found in Brazil was in between 23.9 to 28.9 %. Hence this shows moisture content in stingless bee honey from Brazil is between 23.1 % to 43.5 %. Last but not least, study by Fatima et al. (2018) indicates common stingless bees from Malaysia, *H. itama* and *G. thoracica* has moisture

content ranging from 28.3 to 33.7 %. Therefore, moisture content in stingless bee honey are between 23.1 % up to 43.5 % that slightly exceed the allowed value.

In conclusion, the moisture content in stingless bee honey is higher than *Apis spp*. Bijlsma et al. (2006) found that this is because the amount of honey collected by stingless bees is in small amount. In addition, due to the stingless bee honey storage using cerumen resin pots which has been made from wax combined with propolis and differ from honey bee that stored in brood combs made only with wax also contributes to the high moisture content in honey (Kek et al., 2017).

2.2.2 Ash Content

Ash refers to the inorganic residue remaining after complete oxidation of organic matter in a food sample (Ismail, 2017). Ash determination is important to detect the quality of food that depends on the concentration of minerals, reflect the taste, appearance, texture and stability of honey. Ash content also can be use as for floral origin determination (Boussaid et al., 2018). The residue left may content many types of minerals concentration like potassium, magnesium, zinc and else.

Study by Sousa et al., (2016) showed, honey comes from similar origin has similar mineral content but according to the study by Prica et al., (2015) found that some honey exceed the limitation of ash content stated by regulation (Official Gazette of SCG, No. 45/2003) that limit the ash content only up to 0.50 %. This may due to the different environment, geographical and botanical species also soil condition (Sousa et al., 2016).

On the other hands, study found by Keng et al., (2017) that stingless bee honey in Malaysia content ash within the range between 0.22 % to 0.41 % which still in the limit allowed. This also been supported by Fuenmayor et al., (2013) which declared the Colombian stingless bee honey meet the standard that bellow the 0.5 g/100 g ash content however contain lower mineral than stingless bees found in Malaysia which are *H. itama* and *G. thoracica.* Colombian honey has higher level of magnesium and zinc however *H. itama* and *G. thoracica* has higher level of potassium and calcium minerals (Fadzelly et al., 2017). Furthermore, study by Biluca et al., (2014) is become the first study about mineral abundant in stingless bee that can be compare towards *Apis mellifera.* In both honeys, it majorly contains potassium, followed by calcium. However, in stingless bee honey it contains more level of calcium, than honey bees.

2.2.3 Hydroxymethylfurfural (HMF) Content

Normally, HMF is generated by the decomposition of fructose in acid conditions and increase upon the condition. HMF or 5-hydroxymethyl2-furaldehyde, is a water-soluble heterocyclic organic compound derived from sugars. Basically, HMF also can be found in fresh sugar containing in food like milk, juice, bread and honey (Basumallick & Rohrer, 2013). It occurs naturally in most honey and increase rapidly according to heat treatment (Elmer, 2015). However, honey also content several toxic compounds such as 2-furaldehyde, furan-2-carboxylic acid or 2-furoic acid and also HMF. HMF becomes the bigger concern in honey because it has high toxic potential and widespread (Nazmul et al., 2013). Study by Martysiak-Zurowska & Borowicz (2009) stated that, HMF is a substances or compound that has mutagenic activity and consider cytotoxic, genotoxic and carcinogenic effect on human health.

Good quality of honey has a lower amount of HMF. HMF is not consider as harmful chemical but according to the National Institute of Environmental Health Sciences, HMF that has high potential distribution through the food, being consider as carcinogen to the human that will bring negative affect to the human health (Basumallick et al., 2013). Furthermore, HMF also can be produce either from furanic compound form by Maillard reaction or from hexose dehydration in acid media. HMF is a product form by Maillard reaction through reducing sugar reaction with amino acid during heat treatment and cause increasing of HMF rate formation will increase when enolization rate also increase as well as increase in acyclic and furanose forms of fructose. This also reflect why adulteration honey contain high HMF due to addiction of high concentration fructose syrup in honey or by feeding the bees during winter season (Zirbes et al., 2013).

Been exposure to HMF will give negative effect to human health that has been prove studied by Zirbes et al. (2013) which stated that HMF can contribute to the mutagenic and genotoxic on bacterial and human cells which promotes colon and liver cancer study on rat and mice. However, study on honey bees shows 50 % mortality due to HMF occurs within 16 days after feeding started and 30 mg/kg of HMF in honey is maximum value to harmless occurring.

In addition, HMF level also can be used as an indicator of heating and poor storage, excessive heat-treatment, spoilage, and possible adulteration with other sugars or syrups that suitable to text quality of product (Basumallick et al., 2013; Khalil et al., 2010). On the other hands, study by Biluca et al. (2014) shows HMF production are depends on many factors but the main contributes to HMF formation is only the storage period, acidity of honey and free acid content. This also being discover by Maria et al. (2011) that formation of HMF in foods is dependent on sugar type, pH value and also water activity content. Study of *Tualang* honey from Malaysia that been stored for one

year supported the fact that long period of storage will lead to high HMF level production and recommended the best honey storage to be consume is within 6 months to one year only (Khalil et al., 2010). Furthermore, it also shows the HMF content in stingless bee honey has lower value than *Apis mellifera* when undergo thermal treatment (Biluca et al., 2014). Sousa et al. (2016) also found that, in raw honey which not undergo any thermal processing are absent detection of HMF and almost nearly to zero (Martysiak-Zurowska & Borowicz, 2009).

Basically, HMF formation also can also be justified by type of carbohydrates contain. Based on Biluca et.al (2014) discovered that, stingless bee honey contains fructose as the major contain compare to the honey bees that has glucose sugar as it major contain. The increasing the value of glucose will trigger the HMF formation hence, HMF contain in stingless bee honey is lower than honey bees. In addition, the acidity and water activity of honey also important in HMF formation. The higher the acidity level will lead to inhibitory of HMF content due to lowering the Maillard reaction take place. Furthermore, this also been supported by Fuenmayor et al. (2013) that found HMF content in Colombian stingless bee honey were much lower than maximum accepted content in *Apis mellifera.*

2.2.4 pH Value

Based on the recent study by Boussaid et al. (2018), they conclude that almost stingless bee honey contains acidic properties and has been supported by Fuenmayor et al., (2013) that free acid content in *Meliponini* honey is usually significantly higher than *Apis mellifera* that reflect their pH and flavour. The higher in pH will contributes to the extra flavour of honey (Keng et al., 2017).

In addition, pH value also can be used to determine the honey texture, stability and their shelf life. This is because, the lower the value of pH will inhibit the microorganism growth that will longer the shelf life of a honey because bacteria could only growth in neutral and slightly alkaline medium (Boussaid et al., 2018; Keng et al., 2017). According to the Sousa et al., (2016) stated that pH value can be relates to the geographical also botanical origin of the bee and mineral content in honey. The acidity value of honey also corresponds to the organic acid that already content in honey.

The pH values studies by Biluca et al., (2016); Sousa et al., (2016) shows the value between 3.33 to 6.56 and 3.1 to 5.3 of variety species of stingless bee honey. Different flower sources, geographical and botanical origin such as mineral content lead to different value of honey pH (Sousa et al., 2016). On the other hands, Keng et al., (2017) stated that, stingless bee honey locally in Malaysia content 3.29 to 3.71 pH range. This is match with another study that mention about stingless bee's pH are 4.0 ± 0.1. The variety value of pH is influence by different extraction method and storage condition.

Other than that, honey produced from *Apis mellifera* also normally has acidic pH due to presence of organic acid but slightly higher than stingless bee honey that been supported by study of Algerian honey contain acidic pH between 3.7 to 4.0 (Khalil et al., 2012). This also being supported by Sohaimy, Masry, & Shehata (2015) founds honey bee has pH in the range of 4.11 to 4.64. This also been prove by Fadzelly et al., (2017) that found stingless bees honey either from *G. thoracica* or *H. itama* contain low pH compare with another honey from *Apis dorsata* and *Apis mellifera.*

2.3 Refractometer

Refractometer is used to measure the concentration of aqueous solution that only require small quantity of samples drop and undergo refraction by UV light. The light changes direction is called angles of refraction and are correlates with refractive index (nD) values that has been established. By using those values, the concentration of solution can be determined (Cole-parmer, 2018).

Study by Khalil et al., (2012) stated the use of refractometer in determining the moisture content in honey is simple and a consistence method (Bijlsma et al., 2006). The refractive index that use to determine the water content in samples are depending to the quantity of solid content in samples (Khalil et al., 2012).

2.4 Spectrophotometer

Based on The International Honey Commission stated that, there are three methods can be used for HMF analysis content in honey which are two spectrophotometric methods from White and Winter methods and also using RP-HPLC method. The best method use for HMF detection is by using spectrophotometer by White and Winter methods due to high specification and sensitivity. RP-HPLC is slower but it can give accurate results (Zirbes et al., 2013). Spectrophotometer by White involves the use of UV absorbance in aqueous honey solution with or without bisulphate. However, spectrophotometer by Winkler also involves of UV absorbance of honey solution but it uses barbituric acid and p-toluidine. Furthermore, for HPLC method only involves dissolving of honey samples in water and determination of HMF using reverse phase of HPLC column using water and methanol as mobile phase (Zappalà et al., 2005).

The use of UV- visible Array spectrophotometer in identification of HMF level in honey has been studied by Keppy & Allen (2009) shows different HMF level content in different type of honey. Furthermore, study by Martysiak-Zurowska et al., (2009) that compared the HMF content in honey using different method by HPLC and spectrophotometer of Winker method. The results show HMF determined by the Winkler method is higher than the using by HPLC technique due to detection of aldehydes hence HPLC is the superior method that can be used among those methods for HMF determination in natural honey. This may due to HPLC method that can separate HMF from other components and avoid interference (Zappalà et al., 2005).

Study by Zappala et al. (2005) also shows the HMF content in honey that definitely different from each other. HMF content using White method shows 27.7 to 31.3 mg/kg while by Winkler method shows much higher which is 52.4 and 45.4 mg/kg while HPLC shows the least that no measurable detected. This shows that White method and HPLC were almost the same. In addition, Zappala et al. (2005) also been suggested to not use Winkler method in determine HMF content in honey due to carcinogen of –toluidine and less accuracy of results.

2.5 Moisture Analyser

Moisture analyser is a compact instrument that depend on high heat or temperature which able to detect moisture using sample weights of 150 mg to 40 g. In addition, it also be used for quick and reliable determination of moisture content in liquid, pasty, and solid substances by using the thermogravimetric method (Sartorius Lab Instruments Gmbh, 2017). Furthermore, by using a digital balance, the test sample is placed on an aluminium pan or glass fiber sheet or filter paper and by supply heat to control program with a heating

range between 25 ℃ to 275 ℃. As the moisture is driven from the sample, the instrument automatically weighs and calculates the percentage moisture or solids content. In addition, it also commonly applied and used in food-based industry due to accurate and quick results. However, moisture analysis method also has the risk to gain the accuracy which contain some factors that must be controlled such as the sample collection and handling procedure that need to be extra cautions (Bradley, 2010).

2.6 AOAC and IHC methods

Due to the less procedure elaboration in Malaysia Standard and method guideline to perform the experiment and testing, Association of Official Analytical Chemistry (AOAC) International was used as for the reference. AOAC is the leader which provides valid methods, proficiency test samples, accreditation criteria and academic institution towards industry, government agencies and academic institution. International AOAC also initiated the procedure of validation through interlaboratory studies from single sample methods until random sample of laboratories (Horwitz Wil liam, 2006).

Furthermore, Malaysia Standard 2683- Stingless Bee Honey Specification was mainly adopted from International Honey Commission (IHC) methods. This is maybe due to the IHC methods that has been formed for a decade ago in 1990 which present the selected methods in determination of honey quality. IHC methods includes the old and modern methods in determining honey quality (Bogdanov, 1997).

2.7 Malaysian Standard (2683)- Stingless Bee Honey- Specification

Malaysian Standard (2683) Stingless Bee Honey- Specification (MS-2683), being establish in 2017 after issues rise due to existence adulteration stingless bee honey in market. This regulation be a requirement for all stingless beekeeper's in Malaysia which rise from time to time to be used as reference in other to determine honey quality, hence ensuring that local product of honey is safe, high quality and meet the needs of consumers.

MS-2683 was developed with full set of quality requirements including sampling, preparation of test samples, testing methods, hygiene, packaging and labelling for *Meliponinae* beekeepers (Malaysia Standard, 2017).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

The material used in this research were Olive oil, Carrez solution 1 ($K_4Fe(CN)_6$**.** $3H_2O$), Carrez solution 2 ($Zn(CH_3COO)_2$**.** H2O, buffer solution, distilled water, Sodium carbonate, Sodium dioxide, alcohol (ethanol).

3.1.2 Equipment

The equipment used for this research were glass rod, volumetric flask (100 mL), syringe, membrane filter (pore size 0.45 µm), refrigerator(4 ℃), vials, pipette (2 mL), pre- filter holder, platinum or quartz or porcelain ash dish, appliance for preliminary ashing (infra-red heater/ gas burner/ hot plate), electric furnace, desiccator, analytical balance, beaker, spectrophotometer UV, pH meter, magnetic stirrer, beaker (250 mL), test tube (18 mm×150 mm), centrifugal tubes, vortex mixer, moisture analyser, forceps, glass fiber sheet (78 mm).

3.2 Methods

3.2.1 Honey Sampling

Thirteen honey samples were collected around Kelantan state. All the honey samples were identified either raw honey or process honey and also differentiate between species of stingless bees. Samples were harvested from matured honey pots and representative of the identified beekeeping area where the samples were taken. The minimum volume of samples that was collected is 200 g to 250 g and they were placed in clean, dry and sealed suitable food grade container which in sterilize glass bottles. The samples also be separated according to their species and kept in a chiller at temperature between 0 ℃ to 4 ℃. Analysis of phytochemical parameters were carried out within four to eight weeks after collection of samples were done.

Figure 3.1: Thirteen stingless bee honey samples collected

During samples collection, the survey was performed by interviewing the beekeeper's regarding honey age, flower sources, honey form either process or raw honey and type of honey which comes from single or blended species either *H. itama* or *G. thoracica* of stingless bees.

3.2.2 Experimental Design

In this research study, all the methods used were from Malaysia Standard (MS:2683) due to comparison assessment of honey samples in other to know the complying of standard guideline in market that reflect its quality. All the reading of quality assessment on stingless bee honeys based on physicochemical parameters were taken immediately after sample collection. All the samples results test was performed in triplicate to ensure its accuracy. Samples were collected from local bee keeping that contain blended and singles species that was determined and separated before undergo quality analysis. Besides, analysis also was conducted on single species of *Geniotrigona thoracica* stingless bees and *Heterotrigona itama* that being personally harvested in bee's farm hence, become one of control standard. Samples were identified to has its natural characteristic flavour and aroma, free from foreign matter, not contain any food additives and either raw or process honey should be harvested and processed in accordance to MS-2683 before physicochemical analysis being done. Data and results were evaluated according to Malaysian Standard Stingless Bee-2683 Specification.

3.2.3 Data Analysis

Data analysis was performs using IBM SPSS that was employed for statistical analysis. One-way ANOVA with Tukey's Honest Significant Different (HSD) test was used for physicochemical analysis of stingless bee honey. The significant level was set as $\alpha = 0.05$.

3.3 Physicochemical Analysis

Moisture content, ash content, HMF determination also pH value of honey were including as physicochemical parameters. The moisture content was determined by another method that being used in another supported journals as for replacement the original method used in MS-2683.

Moisture analyser was performed which getting more accurate results comparing from other method in determination moisture content. In addition, it only needs a small quantity of honey sample which only 1 g per sample and will be heated at 120°C for at least 30 minutes. This temperature and weight for every samples have been provided in their moisture analyser book guideline for honey.

Furthermore, ash content in honeys was determined using this formula:

$$\%\ Ash\ Content = \frac{\mathrm{m1} - \mathrm{m2}}{\mathrm{m0}} \tag{3.1}$$

Next, the hydroxylmethylfurfural (HMF) was determined using spectrophotometer UV. The determination is based on the UV absorbance of HMF at 284 nm. The HMF content is calculated after subtraction of the background absorbance at 336 nm. This method is based on the original work of White. HMF content in samples was calculated and expressed in mg/kg using the following formula:

$$\text{HMF Content} = \frac{(A284 - A336) \times 149.7 \times 5 \times D}{W} \tag{3.2}$$

Lastly, the pH was measured after samples dissolving in water using pH meter. The calibration of pH meter will be conducted at pH 3.7 (or pH 4.0), pH 7.0 and pH 9.0 (Malaysia Standard, 2017).

3.3.1 Moisture Content Analysis

Moisture content in honey samples was determined using moisture analyser as a replacement for refractometer analysis.

Moisture determination is by using moisture analyser was firstly set up on a stable surface which not exposed to vibrations. Furthermore, to deliver exact results, the moisture analyser was warm up and switch on for at least 30 minutes before use. The moisture analyser was setting upon the parameter used for honey samples. The samples were prepared and 1 g for each sample was weight using analytical balance provided by moisture analyser at 120 ℃ for at least 30 minutes. Then, the sample was spread evenly using glass rod on glass fiber sheet that specialized used for liquid samples. It must be major concern which uneven spread of samples will results in non-uniformly distribution of heat hence the sample will not be dried completely or the sample will burn hence form a crust on its surface layer. The crust makes it difficult for moisture to escape during drying process. After that, the chamber lid was closed tightly and left for several minutes. The result was recorded (Sartorius Lab Instruments Gmbh, 2017).

3.3.2 Ash Content Analysis

The ash dish or the crucibles were dried and sterilised free from foreign particles in the electrical furnace at ashing temperature (≤ 600 ℃) which is 550 ℃ and subsequently cooled to room temperature and were weighed to 0.001 g. 5 g of each samples were weighed to the nearest 0.001 g into the dried ash dish. Two drops of olive oil were added to the sample. The preliminary ashing was commence without loss at low heat rising to 350 ℃ to 400 ℃ until turn black and no loss by foaming. Next, the all ash dish was placed in the preheated furnace and heating at least for 1 hour at 550 ℃ (Tesfaye, Begna, & Eshetu, 2016). The dish was cooled in room temperature and weighed. The ashing was continuing until constant weight is reaching. The total ash content in honey is calculated using formula (Malaysia Standard, 2017).

3.3.3 Hydroxymethylfurfural (HMF) Analysis

HMF concentration was measured using UV spectrophotometer by White method. 5 g honey was weighed in small beaker and 25 mL of water transferred into 50 mL volumetric flask. 0.50 mL Carrez solution 1 was added and mixed with 0.50 mL Carrez solution 2. The solution further was diluted with water until reach the marked. Two to three drops of alcohol of 80 % ethanol were added to suppress foam. The solution was filtered through filter paper (90 mm) and 10 mL first filtrate is degrading. 5 mL filtrate is pipetting into each two 18 mm x 150 mm test tubes. 5.0 mL of water was added to one tube(sample) and 5.0 mL NaHSO3 solution was added to another test tube(reference). The samples will be mixing well using vortex mixer. The sample A was against the reference at 284 nm and 336 nm in 1 cm cells. The Carrez solution 1 was

prepared by dissolving 15 g K_4 Fe $(CN)_6.3H._2O$ while the Carrez solution 2 was dissolved with 30 g Zn $(CH_3COO)_2.2H_20$. Both solutions were further diluted to 100 mL with H20. Furthermore, to avoid the interference of other component when detection of HMF in honey samples, the different between the absorbance of a clear aqueous honey solution and honey solution that been added with bisulphate were determined (Malaysia Standard, 2017).

3.3.4 pH Determination

10 g of sample was dissolved in 75 mL of carbon dioxide-free water in 250 mL beaker. The solution was stirring using magnetic stirrer. The pH electrode was immersing in the solution. The pH is recording. Before starting the analysis, the pH meter was calibrated using buffer solution at pH 3.7, pH 7.0 and pH 9.0. Calibration of pH meter is important to ensure the functionality of instrument that will give accurate readings (Malaysia Standard, 2017).

CHAPTER 4

RESULTS & DISCUSSION

In this research, four analysis of physicochemical properties of stingless bee honey were performs including determination of pH value, hyroxymethylfurfural (HMF), total ash and moisture. Among 13 samples, there was a sample of processing honey, honey sample 2 and two control samples, honey 7 and 13. Stingless bee honey 7 was raw harvested from *Heterotigona itama (H.itama)* species while sample 13 was from *Geniotrigona thoracica (G. thoracica).* All samples were randomly collected from varies origin including blended species of *H. itama, G. thoracica, Tetragonula laeviceps (T. laeviceps)* and *Lepidotrigona terminata (L.terminata)* also a single species either *H.itama or G.thoracica.*

Sohaimy et al., (2015) stated on their study, properties and compositions of honey depend on its variety floral origin, climate conditions located in its geographical region in its production, environmental factors, different treatment and processing technology of beekeepers and also storage conditions of honey possesses a unique combination of components and properties (Moo-huchin et al., 2015).

The distribution of stingless bee honeys in the world market is limited in comparison with honeys from honey bee. This is consequence of their limited industrial production, shorter shelf life and the lack of international quality standard, which in turn is due to a relatively limited knowledge of the product (Moo-huchin et al., 2015).

Table 4.1 represents overall physicochemical analysis undergoes on thirteen stingless bee honey samples. All twelve samples were complying with Malaysian Standard-2683 for pH, ash, moisture and HMF content except for honey sample 2.

Table 4.1: All the physicochemical analysis of stingless bee honey

Test	pH value	Ash content(g)	Moisture Analyser (%)	Hydroxymethylfurfral (mg/kg)
Sample 1	2.79 ± 0.00*	0.13 ± 0.00*	29.41 ± 0.51*	25.75 ± 1.90*
Sample 2	2.34 ± 0.01	0.01 ± 0.01*	27.05 ± 1.39	218.66 ± 27.70
Sample 3	2.70 ± 0.01*	0.13 ± 0.00*	28.04 ± 1.88*	19.34 ± 0.47*
Sample 4	2.92 ± 0.01*	0.04 ± 0.01*	27.20 ± 0.22*	22.90 ± 2.16*
Sample 5	2.92 ± 0.01*	0.12 ± 0.01*	29.21 ± 2.708*	16.42 ± 2.50*
Sample 6	2.97 ± 0.00*	0.12 ± 0.00*	29.99 ± 0.90*	16.42 ± 1.53*
Sample 7	3.22 ± 0.02*	0.10 ± 0.00*	29.59 ± 0.36*	12.38 ± 1.77*
Sample 8	2.91 ± 0.00*	0.08 ± 0.01*	27.96 ± 2.39*	23.40 ± 1.41*
Sample 9	2.87 ± 0.01*	0.11 ± 0.00*	32.61 ± 2.79*	19.41 ± 3.83*
Sample 10	2.82 ± 0.00*	0.09 ± 0.02*	32.19 ± 0.34*	15.22 ± 1.37*
Sample 11	2.81 ± 0.00*	0.10 ± 0.00*	29.24 ± 0.14*	10.98 ± 2.91*
Sample 12	2.83 ± 0.01*	0.11 ± 0.01*	27.61 ± 3.20*	8.78 ± 0.92*
Sample 13	2.87 ± 0.01*	0.14 ± 0.00*	29.78 ± 1.81*	16.17 ± 1.30*

*Represents compliance with MS-2683

According to Table 4.2, stingless bee honey samples were randomly collected in market through beekeepers around Kelantan. There were eight difference places including Kubang Kerian, Kg. Sering, Pengkalan Chepa, Koklanas, Ketereh, Wakaf Bharu, Jedok and Jeli. All honey samples were representing major species that commonly being found in Malaysia and Kelantan which is *H. itama, G. thoracica, T. laeviceps* and *L.terminata* species. Furthermore, all the information gather in Table 4.2 was obtained from beekeepers for each sample in other to relates the physicochemical properties of honey and its physical characteristics.

Table 4.2: Data collection of stingless bee honey samples

Honey sample	Honey type	Bees species	Honey aged (days)	Flower sources	Geographical origin
1	Raw	Blended	7	Multiflora	Kubang Kerian
2	Process	Blended	28	Multiflora	Kg. Sering
3	Raw	*H. itama*	112	Multiflora	Pengkalan Chepa
4	Raw	*H. itama*	84	Multiflora	Koklanas
5	Raw	*H. itama*	84	Multiflora	Ketereh
6	Raw	*H. itama*	140	Multiflora	Wakaf Bharu
7	Raw	*H. itama*	0	Multiflora	Wakaf Bharu
8	Raw	*H. itama*	84	Multiflora	Wakaf Bharu
9	Raw	*H. itama*	14	Multiflora	Wakaf Bharu
10	Raw	*H. itama*	1	Multiflora	Wakaf Bharu
11	Raw	*H. iItama*	28	Multiflora	Jedok
12	Raw	*G. thoracica*	28	Multiflora	Jeli
13	Raw	*G. thoracica*	1	Multiflora	Jeli

4.1 pH Value Determination in Honey from Stingless Bees

Based on Figure 4.1, pH values of the stingless bee honey were ranged between 2.34 to 3.22. The highest acidity content with smaller pH value was sample number 2 and the lowest acidity value was sample number 7. 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 was almost within the same range. The *p* value for pH is below than 0.05 ($p < 0.05$) which shows significant and the null hypothesis was rejected. Hence, it means all samples has significant different which indicates difference of honey contain different pH value.

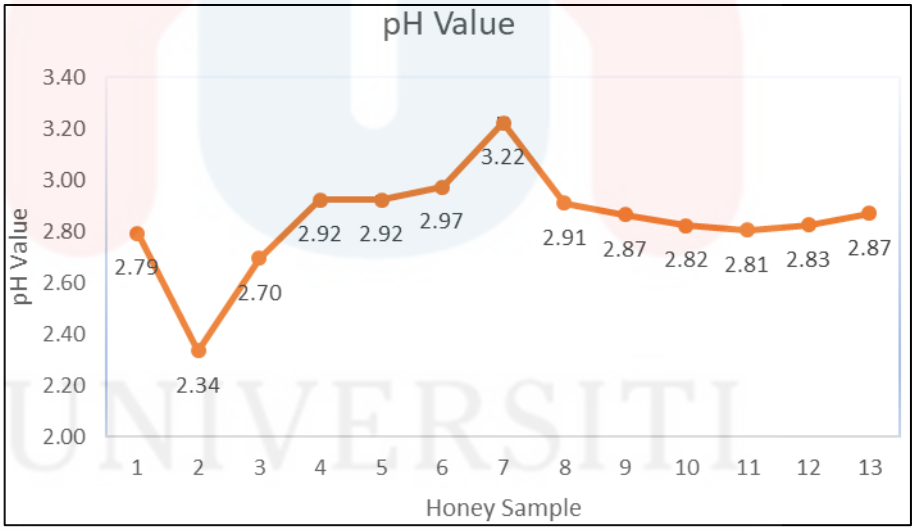

Figure 4.1: pH value of stingless bee honey samples

The value of pH obtained from this study were almost similar with the findings by Fatima et al. (2017) which indicates, pH value from common domestic Malaysian stingless bee were range in between 2.79 ± 0.04 to 2.95 ± 0.02 and being supported by research from Suntiparapop et al., (2015) that stingless bee honey from Thailand which is *Tetragonula leaviceps* species that were range between 3.48 ± 0.01 to 3.76 ± 0.01.

Study indicates that, sample 7 as control sample of *H. itama* species has lower acidity compared to single species of control sample *G. thoracica* which 3.22 and 2.87. This similarly been found by Nizam et al., (2017) that *H. itama* has lower acidity content then *G. thoracica* stingless bee honey. However, study by Fadzelly et al., (2017) found *H. itama* has lower pH value with higher acidity than *G. thoracica* but surely high acidity than honey bees. The differences finding of stingless bee honey pH were directly influenced by organic acids and mineral substances concentration (Nizam et al., 2017).

By comparing honey from *Meliponinae* and *Apis mellifera*, high acidity honey of *Meliponinae* gives importance implication regarding antibacterial activity (Vit et al., 1993). Low pH value usually indicates more acidity and more hydrogen ion also organic acid, that involved in the formation of other compounds in stingless bee honey and sugar fermentation in honey which able to lowering the microbial content. High acidity will able to inhibit the growth of microorganisms (Fatima et al., 2018;Fadzelly et al., 2017). This being support from Moniruzzaman et al., (2013) that honey bees pH commonly ranging between 3.53 to 4.03 that slightly higher than stingless bee honey even comes in different honey bees; acacia honeys, pineapple honeys, tualang honeys and borneo honey. However, the pH still falls within the limit that indicates freshness, (pH 3.4 to 6.1). Among the honey of *Apis*, the highest acidic content was acacia honey which from *Apis mellifera*, hence prove it has more antimicrobial activity than tualang and borneo honey from *Apis dorsata* and *Apis cerana.* However, comparing honey from stingless bee and honey bee, stingless bee has the most acidic content and low pH value (Fatima et al., 2017; Kek et al., 2017).

Figure 4.1 shows honey sample 7 has the highest pH value may due to the aged of honey. This is because, storage period honey which cause increasing in total acid content due to unproper storage temperature that increase the pH value. According to Rebiai et al. (2015), low acidic content in honey was due to less presence or absence of organic acid that shows honey do not perform any undesirable fermentation process that might change alcohol to organic acid. Organic acid than commonly been found in stingless bee honey was nonaromatic which being identified more than thirty types of it including predominance nonaromatic gluconic acid and other malic, citric and many more. This nonaromatic was responsible used as antimicrobial in honey pot against the microbial spoilage (Sancho et al., 2013). This been proved in study by Azri et al. (2017) that free phenolic acids content in stingless bee honey consist of protocatechuic acid (PCA) that is strong contributors to antioxidant properties which improves the cell proliferation through wound healing process. Another study by Garedew, Schmolz, & Lamprecht (2003) stated that due to its strong sour taste of stingless bee honey, it becomes suitable use for medicinal purpose to treat cough, stomach disturbance, sore throat, tonsillitis, stomach and intestinal ulcers, cold, disease of the mouth and mucus membrane also as wound dressing.

In general, stingless bee honey has pH ranging from 2.00 to 4.7 which indicates to honey fermentation that content free acid however this not indicates the honey spoilage and it was used as common parameter in honey pot (Sancho et al., 2013). Hence, this shows the quality of honey itself. Another study by Nizam et al., (2017) also stated that the stability and shelf life of honey is also influenced by pH value during the extraction from the hives and storage. Other than that, despite of low pH and high acidity content, stingless bee honey was been used to treats gastrointestinal tract diseases, dyspepsia and periodontal diseases. In addition, used of stingless bee honey in children with

gastroenteritis able to reduce the rate of diarrhea (Rao et al., 2016). Another finding was supported this statement by Vit, Medina, & Enríquez (2004) stated, fermented stingless bee honey which content more acidity was more effective in treating respiratory disease however in controlling production of end products.

Table 4.3: Homogenous test of pH value in honey sample

Honey Sample	Value of pH
Honey 1	2.80^{e}
Honey 2	2.34^{g}
Honey 3	2.70^{f}
Honey 4	2.92^{c}
Honey 5	2.92^{c}
Honey 6	2.97^{b}
Honey 7	3.22^{a}
Honey 8	2.91^{c}
Honey 9	2.87^{d}
Honey 10	2.82^{e}
Honey 11	2.81^{e}
Honey 12	2.83^{e}
Honey 13	2.87^{d}

'a,b,c,d,e,f', and 'g' indicates the significant level for each honey samples,

Sample with similar alphabets indicates; not significant to each other

Table 4.3 indicates honey sample that has similar alphabet label was not significant to each other hence contain smaller significant different of each other. As for honey sample 4,5 and 8, they do not significant while honey sample 9 and 13 also honey sample 1, 10 to 12 were not significant to each other. Rather than that, it shows all samples were significant to control honey 7 and 13 from single *H.itama* (sample 3-11) and *G.thoracica* (sample 12). This represent that, there was significant different in each sample. This can be said; all honey quality was differed in term of pH value.

Based on Malaysia Standard-2683, limitation for pH value is between 2.5 to 3.8, hence recent study on all twelve samples shows majority of stingless bee follow the limitation set by Malaysia regulation except sample number 2 was below the allowance limit set by regulation with pH value 2.34 ± 0.012 which too acidic for stingless bee honey. Thus, this different pH value may due to the heat treatment process that has been done to remove yeast and mould at temperature below than 40 ℃ for at least 30 minutes. This being supported by study from Atikah, Akhmazillah & Faiz (2018) that processing honey affected on stingless bee honey antioxidant activity which highly influence the physical properties of honey like pH value, colour and electrical conductivity. However, study by Fuad et al. (2017) presents that, stingless bee honey was influence by low temperature which drop the rate of enzyme reaction lead to lowering pH value. This may be one of the reasons why honey sample 2 has low pH with highest acidity because of storage temperature at chilling degree.

In addition, colour also influence pH value of honey. According to the Prica et al. (2014) stated that, honey with darker colour will be appear to has high acidity content. This similarly with study findings that, most sample with darker colour were shows low value of pH compare towards sample 7 which has lightest colour.

4.2 Total Ash Content

Ash is one of the indicator amount of mineral collected by the bees during the flora foraging (Suntiparapop et al., 2015). Based on the results Table 4.4, all the honey samples content ash below the allowance limit specified by MS-2863:2017 which is below 1.0 g/100 g. The range of ash content is between 0.08 to 0.14 g/100 g. This is identically similar with finding by Fatima et al. (2018) and Fadzelly et al. (2017) which stated that ash content in unprocessed stingless bee honey from Malaysia were range between 0.15 ± 0.01 to 0.67 ± 0.00 g/100 g and 0.13 ± 0.03 to 0.53 ± 0.83 g/100 g which also below than 1.0 g/100 g. The *p* value for ash content is below than 0.05 ($p < 0.05$) which means significant and there is no chance of findings the differences to occur while reject the null hypothesis. The value for all samples has significant different to each other.

Table 4.4: Total ash content in honey sample

Honey Sample	Mean, μ (g/100g)
1	0.13 ± 0.00*
2	0.11 ± 0.01*
3	0.13 ± 0.00*
4	0.14 ± 0.01*
5	0.12 ± 0.01*
6	0.12 ± 0.00*
7	0.10 ± 0.00*
8	0.08 ± 0.01*
9	0.11 ± 0.00*
10	0.10 ± 0.02*
11	0.10 ± 0.00*
12	0.11 ± 0.01*
13	0.14 ± 0.00*

*complies with MS-2853:2017 (Ash content< 1.00g/100g)

The different botanical and geographical origin of honey may influence the variability of mineral content (Fadzelly et al., 2017; Suntiparapop et al., 2015). This statement was supporting this study that indicates by Figure 4.2, the highest value of ash content was sample 4 while the lowest value of ash was sample 8. Both samples were harvested from *H. itama* species and has similar honey age about 3 months. Hence, this show, species and honey age do not affect ash content at all. The different of ash in honey are strongly depends on honey sources (Vit et al., 1993).

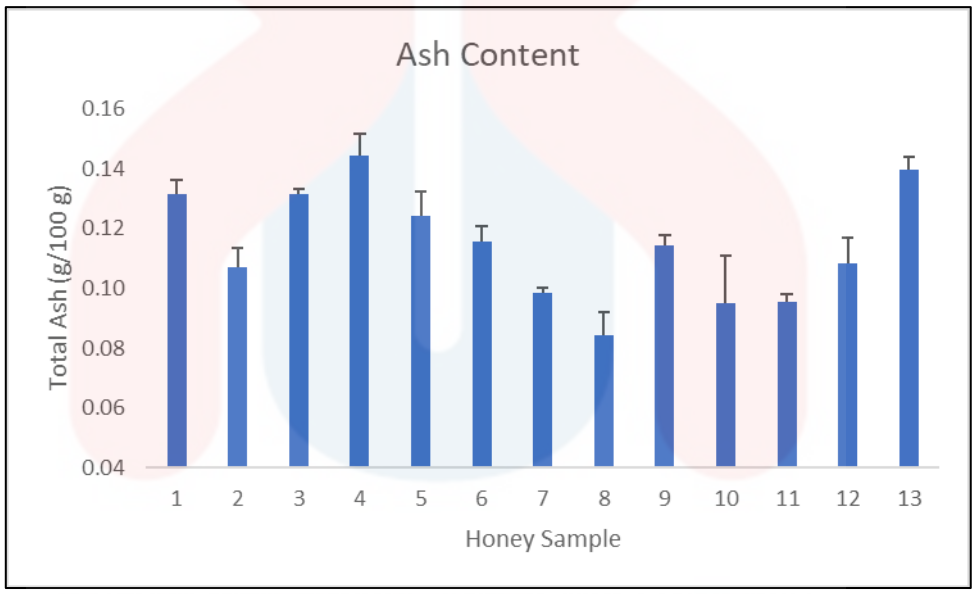

Figure 4.2: Total ash content in stingless bee honey

In addition, based on the study, the control samples, *H. itama* (sample 7) content less mineral compared to *G.thoracica* (sample 13) which 0.10 and 0.14 and it is similar to the finding by Fadzelly et al. (2017) which also found the lowest ash content was in *H. itama* species.

Moreover, Table 4.5 represents, honey sample 2,5,6,8,9 to 11 were not significant to the control honey sample 7 that means the samples were not has significant difference. All samples that not significant to the control honey 7 were contain mineral and quality similarly to the control honey. Furthermore, for honey control 13 from *G. thoracica*, there

is no significant honey sample comparing with it. Hence, no sample from *G. thoracica* was similarly to the control.

Table 4.5: Homogenous test for ash content

Honey Sample	Ash Content (g/100g)
Honey 1	0.13^{abc}
Honey 2	0.11^{cdef}
Honey 3	0.13^{abc}
Honey 4	0.15^{a}
Honey 5	0.13^{abcd}
Honey 6	0.11^{bcde}
Honey 7	0.10^{def}
Honey 8	0.08^{f}
Honey 9	0.12^{bcde}
Honey 10	0.09^{ef}
Honey 11	0.09^{ef}
Honey 12	0.11^{cdef}
Honey 13	0.14^{ab}

'a,b,c,d,e,f' represent the significant level for each sample
Sample with similar alphabets shows; not significant to each other

Finding from Muruke (2014) stated that, minerals content in honey also based on the colour and flower sources. The darker the colour of honey, the higher the minerals content. Hence, this no different with data collected that, sample 13 has darker colour than sample 7 and was harvested from multiflora sources, hence it contents high ash value. It also been supports by Fatima et al. (2018) which mention, honey from multiflora sources content high ash between 0.4 ± 0.03 to 0.67 ± 0.28 while monoflora stingless bee content less between 0.13 ± 0.03 to 0.27 ± 0.21 g/100g.

On the other hands, another research performed by Vit et. al (1993) found, *Apis mellifera* honey content less value of ash comparing with stingless bee honey. This also due to the botanical preference factor. Study shows, fruit juice feeding was only gives stingless bees attraction which normally contain high ash value compare to nectar collected (Vit et al., 1993). However, another study comparing honey from both species

shows, *Apis mellifera* honey content higher ash than stingless bee honey but still below limitation (Almeida-Muradian, 2013).

4.3 HMF Determination

According to MS-2863:2017 HMF determination in honey was performs using White method (White, 1979), 0.2 % sodium bisulphate was used as reference. It means that, sample contain 0.2 % sodium bisulphate supposedly content lower HMF concentration or do not have any HMF detection at all. This is because, sodium bisulphate that being added will destroyed the HMF content (Keppy et al., 2009; Ng & Reuter, 2015; Ummay et al., 2018).Regarding those, result in Table 4.6 was representing the values of absorbance for both wavelength, wavelength at 284 nm as absorbance for HMF detection while wavelength at 336 nm act as background absorbance. In other to avoid any interferences of other component, the different between clear aqueous honey solution and similar solution with addition of bisulphite was determined. Hence, from this study conducted, all sample solution versus reference solution were slightly higher in HMF content. Furthermore, negative value not indicate zero HMF content however it shows that the HMF value was extremely below than 5 mg/kg using White method. In addition, this similarly same with finding mention by Truzzi et al. (2012) that HMF content detection for every methods were different. For HMF content ranging between 1 to 4 mg/kg, the most appropriate and has greater precision is by detection using high performance liquid chromatography (HPLC) method. However, White method is convenient to be used on HMF content limit at 0.67 mg/kg while HPLC limit at 0.83 mg/kg. After all, for HMF content less than 1 mg/kg, both methods are inaccurate to be used (Truzzi et al., 2012).

Table 4.6: Value of absorbance at wavelengths 284 and 336 (nm)

Honey Sample	Mean (μ)	
	284 nm	336 nm
1	0.300	0.128
2	0.497	0.080
3	0.214	0.085
4	0.257	0.104
5	0.206	0.096
6	0.230	0.120
7	0.123	0.040
8	0.297	0.141
9	0.308	0.178
10	0.208	0.106
11	0.210	0.137
12	0.139	0.080
13	0.189	0.081

Table 4.7 shows the actual HMF content in thirteen stingless bee honey samples after subtraction of background absorbance in aqueous solution. It indicates that, twelve out of thirteen samples content small amount of HMF which below than 30.0 mg/kg and complies with MS-2683, however it may be differ as time changing.

Table 4.7: Value HMF of stingless bee honey sample

Honey Sample	HMF Value (mg/kg)
1	25.75 ± 1.90*
2	218.66 ± 27.70
3	19.34 ± 0.47*
4	22.90 ± 2.16*
5	16.42 ± 2.50*
6	16.42 ± 1.53*
7	12.38 ± 1.77*
8	23.40 ± 1.41*
9	19.41 ± 3.83*
10	15.22 ± 1.37*
11	10.98 ± 2.91*
12	8.78 ±0.92*
13	16.17 ± 1.30*

*Complies with MS-2863:2017: HMF content<30.0 mg/kg

Based on Malaysia standard, HMF concentration content in stingless bee honey should not exceed 30.0 mg//kg which nearly similar to the Codex Alimentarius Commission and the European Union established that its concentration in honey should not exceed 80 or 40 mg/kg, respectively (Zappalà et al., 2005). The *p* value for HMF content is below than 0.05 ($p < 0.05$) which means there is no chance of findings the differences to occur and the null hypothesis is rejected.

Figure 4.3 shows that, honey sample 2 has the highest and exceed the limitation allow by MS 2863: 2017 with HMF content 218.66. Besides, all 12 samples collected were analysed in the allowance limit set by guideline including the standard and control samples 7 and 13 with low HMF content, 12.38 and 16.17. Hence, the HMF content in this samples were ranged between 8.78 mg/kg to 218.66 mg/kg.

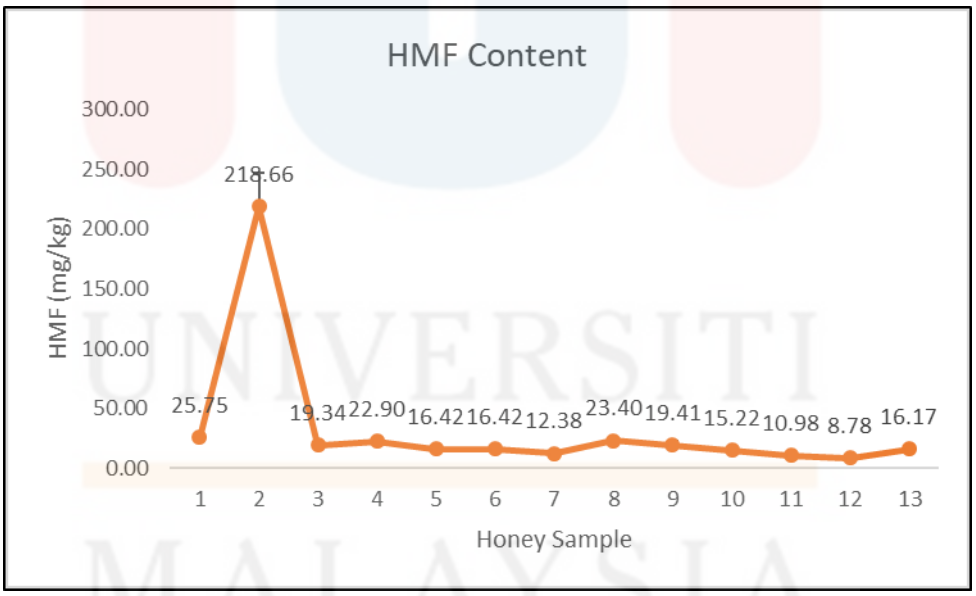

Figure 4.3: HMF content in stingless bee honey

This being supported with study by Nascimento et al. (2015) that similarly founds all the stingless bee honey content lower HMF value allow by current legislation which below 40 mg/kg^{-1} to 80 mg/kg^{-1}. Recently, study by Biluca et al. and Sousa et al. (2016) indicates that all stingless bee honey samples collected did not detect any HMF content

and was below the limit quantification which 0.31 mg L^{-1}. This may be due to the correct storage and age of honey samples either fresh honey or old storage honey. It defined that, stingless bee honey that has high moisture, acidity and fructose as predominance sugar lead to presents HMF formation resistance even after subjected to high temperatures. The variation of HMF content in honey was influence by time and temperature condition of honey stored in hive prior to harvesting (Chuttong et al., 2016).

Other than that, Table 4.8 indicates, among 13 samples of stingless bee honey, only honey sample 2 was significant different to other samples. It also represents, honey sample 12 has the lowest HMF content while the highest was honey sample 2. Higher value of HMF content may be due to the heat treatment applied on honey sample 2 before being sold to market that trigger the formation of HMF. Study by Nazmul et al. (2013) also stated, formation of HMF was triggered by heating or preservation processes of honey. Heat treatment was used to remove yeast at temperature below than 40 ℃ for at least 30 minutes. This being supported by Yuan et al., (2009) and Zappalà et al. (2005) in their study that shows concentration of HMF in honey highly depends due to the overheating or heat, poor storage conditions and the age of honey itself. They found that any sources of heat such as storage of finish product that expose directly to the sunlight or surrounding temperature of honey product in the warehouse or either honey has been subjected to heat treatment during processing or packaging and longer storage time trigger HMF formation. Other than that, high acidic content in honey also one of the factors that helps in HMF formation. High acidic content that also lead to high water content that could increase the HMF content to a greater level than honey bees (Chuttong et al., 2016; Mouhoubi-Tafinine et al., 2017). This also being support by Fuenmayor et al. (2013) that high moisture content could lead to HMF production. Coincidently, honey sample 2 has the highest acidity and moisture content that similarly to the finding. This form higher

HMF content and low honey quality. Hence, HMF or 5-hydroxymethylfurfural is usually use as the indicator to evaluate honey in other to knowing their safety and its quality.

Table 4.8: Homogenous test of HMF content in honey sample

Honey Sample	HMF Content (mg/kg)
Honey 1	25.75[b]
Honey 2	218.66[a]
Honey 3	19.34[b]
Honey 4	22.90[b]
Honey 5	16.42[b]
Honey 6	16.42[b]
Honey 7	12.35[b]
Honey 8	23.90[b]
Honey 9	19.41[b]
Honey 10	15.22[b]
Honey 11	10.98[b]
Honey 12	8.78[b]
Honey 13	16.17[b]

*a significant with b

Furthermore, Table 4.8 also represents honey sample 3 to 11 were not significant to the control honey 7 while honey 12 not significant with honey control 13. Hence, this can be conclude, all honey samples either *H.itama* or *G.thoracica* contain HMF level similarly which not has significant difference to each other and has similar toxicity level.

Sample data collected represent in Table 4.2, honey sample number 6 being stored for at least 5 months and is the oldest age of honey harvested among samples however its content low HMF which is 16. 42 mg/kg that similar to others samples. This may be due to proper handling and correct storage of honey by retailer. This also being study by Khalil et al., (2010) that honey bee stored more than one year produce greater HMF value that exceed the limit of International Honey Commission (IHC). The HMF content in fresh honey was extremely lower which was range in 2.80 to 24.87 mg/kg that increase rapidly due to storage up to 128.19 to 1131.76 mg/kg. Hence, this prove that storage

period effects on honey bee quality, however, there is no study regarding HMF content in stingless bee honey yet.

By comparing honey 7 and 13 as control, *H.itama* and *G.thoracica* shows different HMF value. *H. itama* has lower HMF compare to *G.thoracica* which 12.38 mg/kg and 16.17 mg/kg. Research by Tuksitha et al., (2018) indicates that *H. itama* content high fructose compared *to G. thoracica* that helps in lowering the HMF content. This also can be reconnected towards honey bee. Honey bee content less fructose while high glucose that able to increase the Maillard reaction. Maillard reaction will produce more HMF and increase its level compared to stingless bee honey (Biluca et al., 2014). Fructose was more reactive sugar and unstable at pH 4.6 (Khalil et al., 2010). This found similarly in study by Almeida-muradian et al. (2013) found that *A. mellifera* has greater HMF value than *Meliponinae* bees which has mean 10.82 and 7.56 mg/kg. In addition, among the *A. mellifera* species, tualang honey has the greater HMF value when stored for 24 months compared to another honey bees from other species of *Apis* like gelam, borneo and manuka honeys. However, honey bees that expose under radiation shows decreasing value of HMF content which means, it was possible that radiation able to reduced HMF formation by reducing the number of microorganisms that may accelerate its formation (Khalil et al., 2010).

Other than that, HMF formation in stingless bee honey or honey bees could lead to many side effects. One of effect been reported in Nazmul et al. (2013) that HMF content ranging 0.08 mg/kg to 500 mg/kg may be more rapidly absorbs by gastrointestinal tract which may converted into furfuryl alcohol by bacteria strains. However, it will be secreted out via urine but some of covalent bonding occurs attached to kidneys, bladder and liver and cause organ damage. This been proved by testing on mice which cause tumors when consumed more than 200 mg/kg of HMF. In addition, in worse cases of

consuming high concentrated HMF, the conversion HMF to SMF, sulfoxymethyfurfural. SMF is highly reactive that can react with DNA which release toxic and mutagenic effects (Nazmul et al., 2013).

Malaysia Standard 2863- Stingless Bee Honey Specification is use as the guideline to determine the HMF content in stingless bee honey samples. However, it been found that some mistakes regarding the procedure. The procedure from MS-2863:2017 shows, when the absorbance reading for the sample solution is less than 0.6, further dilution needs to performs. Otherwise, it being found, all samples were less than 0.6 when performs the absorbance reading using spectrophotometer except for one sample was higher than 0.6 absorbance. Hence, further research had been done. Some reference from International Honey Commission(IHC) by Bogdanov (2002) and also Department of Molecular Medicine, Faculty of Medicine (2013) of University of Malaya stated that when absorbance of sample solution is more than 0.6 would need to undergo further dilution. Hence, all samples solution was below 0.6 except for sample number 2 that perform further dilution using distill water for sample test tube while added of 0.1 % sodium bisulphate for reference test tube with dilution factor 3.5. In addition, this shows MS-28563 :2017 was less inspection regarding the procedure specified towards HMF determination procedure using original White method and need to further develop for further research about stingless bee honey.

4.4 Moisture Content using moisture analyser

According to original method of MS-2863:2017, moisture content in stingless bee honey is determining by using refractometer based on refractive index principle. However, due to the constrains of equipment, replacement method to further identify moisture content was performed using moisture analyser. Moisture analyser was performed accordingly to get more accurate result by following the provided procedure that already has its own guideline use for honey testing.

Based on the Figure 4.4, the highest and lowest moisture content identified was honey sample 9 and 2. Honey sample 9 has highest moisture with 32.61 % while sample number 2 has lowest moisture with 27.05 %. High moisture can easily lead to honey fermentation caused by osmotolerant yeasts, thus affects the quality of honey by resulting in the formation of ethyl alcohol and carbon dioxide. The ethanol produce may break down into acetic acid and water, giving the honey a distinctly sour and a runny texture with small bubbles, surface heaving or foaming (Moo-huchin et al., 2015). However, due to high acidity of stingless bee honey, it may inhibits the presence and growth of microorganisms and prolong honey shelf life (Kek et al., 2017). Other than that, honey sample 9 was harvested earlier than sample 2 aged for about 14 days and has different origin which from Wakaf Bharu and Pengkalan Chepa. However, study by Chuttong et al. (2016) found storage temperature and storage period of stingless bee honey do not affect honey moisture. This being support with finding by Sohaimy et al. (2015) that stated moisture content depends on the temperature and relative humidity in the geographical origin during honey producing in honey colonies.

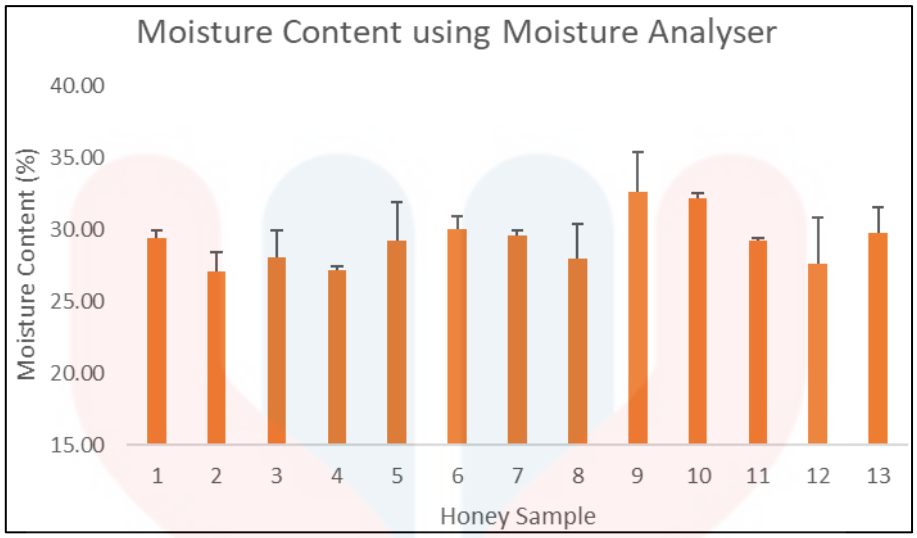

Figure 4.4: Moisture content in stingless bee honey

Other than that, it also shows moisture content was ranged in between 27.05 to 32.61 %. This similar to the finding by Biluca et al. (2016) that found *Melliponinae* stingless bee content moisture range from 23.1 % to 43.5 % which below limitation set by regulation. Even through moisture content in stingless bee honey was below the limit and comply with Malaysia Standard 2863:2017, however, there was an exception for honey sample 2. Honey sample 2 not complies with MS-2863:2017 that content moisture up to 27.05 % (>22.0 %) for processing honey. This is because, study by Chua et al. (2014) stated that heat process able to reduce honey viscosity and minimize honey water content in other to prevent fermentation by dissolving nuclei of sugar crystal to retard granulation and destroys the sugar tolerant osmophilic yeasts to prolong the shelf life of honey. This being proves by heating treatment conducted on tualang, gelam and acacia honey. Increasing temperature use for heat treatment will reduce the moisture content in honey (Chua et al., 2014). This accuracy being proved by the bee keeper of honey sample 2 that stated 27 % moisture content on its packaging.

Based on survey form of sample collection shows by Table 4.2, honey sample 12 and 13 were single species *of G. thoracica* and has lower moisture content ranged from 27.61 to 29.78 % compared to the sample number 3 to 11 which were single species of *H. itama* ranged from 27.20 to 32.61 %. Study by Nurhamizah et al. (2015) stated that even the origin and geographical of honey is same, it can be different of physicochemical content due to bees species. This been more assured with findings that *H.itama* content high moisture, 23.72 ± 0.621 than *G.thoracica*, 9.90 ± 11.82. However, *G.thoracica* can highly prevent bacterial, yeast and fungal growth through storage. Hence, moisture content of stingless bee honey is varying according to the species and the area where it is produce (Bijlsma et al., 2006).

In addition, indicating by result shows, all stingless bee honey samples either from single *H. itama*, single *G. thoracica* or blended species honey were complying with Malaysian standard except honey sample 2.

Moisture content is the major different between stingless bee honey and honey bee. Honey bees content less moisture and more viscous texture compared to stingless bee honey (Muruke, 2014). Furthermore, high moisture content of honey from stingless bee may also be affected by the different honey storage by the stingless bee honey using cerumen resin pots made of wax combined with propolis as compared to brood combs made from wax only by *Apis spp*. bees. These moisture content of honey itself may tell the difference in honey source by its bee origin (Kek et al., 2017). Biluca et al. (2016) also mention in their study that, the occurrence of watery honey from stingless bees may be related to the humid tropical environment, in which it is difficult to extract nectar with low water content and nectar collections of the undergrowth flowers and ripe fruits rich in water or even different species of bees. Other than that, moisture content also relates to the degree of maturity of honey (Moo-huchin et al., 2015). Hence, this high of moisture

content in stingless bee honey make it has stronger bacteriostatic effect than honey bee. High water content will not able fermentation to occur in nest probably due to the abundant resin chemicals that contributes to dark colour of it (Garedew et al., 2003).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

As the conclusion, the physicochemical analysis on stingless bee honey were different based on species, origin, aged of harvest and flower sources. Result in this study indicates 7.69 % of stingless bee honey from samples collected were not complies according to MS-2863:2017. This may be due to some factors which majorly due to honey form itself. Some of the samples were collected as raw and some was been undergo heat treatment. Even though heating is a great important to be used however, there is no guideline available for suitable heating temperature and time used specifically for particular type of honey (Chua et al., 2014). This also can be said; 92.3 % physicochemical properties of stingless bee honey samples were follows accordingly to Malaysia standard. However, for total ash content, all samples comply towards MS-2863:2017. In addition, all physicochemical analysis was significant ($p < 0.05$) that shows chance for difference to occur except for moisture content. There also no significant different between samples and control which portrays its quality following Malaysian standard. Hence, 92.31 % of stingless bee honey samples were in good quality and can be said without any adulteration from other species of bees or sugar syrup addition at the moment. However, these values might differ as the storage time increase.

5.2 Recommendation

Future studies could be done by addition of process honey sample. Among 13 samples of stingless bee honey, there is only one sample of processing honey while others are raw honey collected. Hence, there is some constrains because the result getting from experiment cannot be compare among process honey and this shows significant different among them. Other than that, HMF content in honey can be more accurate analyse using HPLC method even though HPLC and White method do not have big difference in results. This being support by Truzzi et al. (2012) that HPLC method is more appropriate being used for HMF content range between 1 to 4 mg/kg due to greater precision. However, for HMF content below than 1 mg/kg, both methods are not appropriate to be conducted.

A part from that, research on storage period of stingless bee honey that may affect the HMF content and others physicochemical can also be done. This is because, they are only study of storage period conducted on honey bee. Other than that, further research on minerals content in stingless be can be perform in other to get more specified mineral types which domination.

Last but not least, more inspection towards Malaysian Standard 2863- Stingless Bee Honey Specification especially on the procedure compartment. There is some error regarding the HMF determination procedure that need to be more taking care for future development.

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APPENDIX A

i) ANOVA analysis for all physicochemical parameters

		Sum of Squares	df	Mean Square	F	Sig.
pH value	Between Groups	1.39	12	.12	738.47	.00
	Within Groups	.00	26	.00		
	Total	1.39	38			
HMF mg/kg	Between Groups	113202.82	12	9433.57	100.08	.00
	Within Groups	2450.80	26	94.26		
	Total	115653.62	38			
Ash g/100g	Between Groups	.01	12	.00	13.85	.00
	Within Groups	.00	26	.00		
	Total	.02	38			
Moisture analyser (%)	Between Groups	107.20	12	8.93	1.89	.09
	Within Groups	123.24	26	4.74		
	Total	230.45	38			

ii) Paired sample test for moisture content using moisture analyser and oven drying methods.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Moisture_analyser - Moisture_oven	6.36	2.50	.40	5.55	7.17	15.88	38	.00

A paired-sample t-test was conducted to evaluate the significant between two common methods in moisture determination by using moisture analyser and moisture open air-drying oven. Based on table above, result shows significant differences between both methods. The p value was below than 0.05 with t (15.88) and degree of freedom, 38.

iii) Paired sample statistic of both methods for moisture content

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Moisture_analyser	29.22	39	2.46	.39
	Moisture_oven	22.86	39	2.02	.32

The different between mean of each group, moisture analyser and moisture oven were decrease with 6.36 with a 95 % confidence interval ranging from 5.55 to 7.17. Therefore, it can be concluded that there was significant decrease in moisture analyser method to moisture oven method used. This may due to some error that arise when conducting the experiment. Open air or environment content moisture that may lead to effect honey moisture when weighing or after completely drying while transfer samples in desiccator.

APPENDIX B

Raw data of physicochemical analysis for pH and HMF value

Test	pH value					HMF Value A				
	1	2.00	3.00	Ave	Std	1	2	3	Ave	Std
Sample 1	2.79	2.80	2.79	2.79	0.00	23.35	25.90	27.99	25.75	1.90
Sample 2	2.35	2.32	2.34	2.34	0.01	255.16	212.72	188.10	218.66	27.70
Sample 3	2.68	2.70	2.71	2.70	0.01	19.58	18.69	19.76	19.34	0.47
Sample 4	2.91	2.93	2.93	2.92	0.01	25.30	20.06	23.35	22.90	2.16
Sample 5	2.94	2.92	2.91	2.92	0.01	14.22	19.91	15.12	16.42	2.50
Sample 6	2.98	2.97	2.97	2.97	0.00	18.56	15.12	15.57	16.42	1.53
Sample 7	3.2	3.22	3.25	3.22	0.02	12.43	10.18	14.52	12.38	1.77
Sample 8	2.91	2.91	2.91	2.91	0.00	21.41	24.55	24.25	23.40	1.41
Sample 9	2.88	2.85	2.87	2.87	0.01	14.07	22.90	21.26	19.41	3.83
Sample 10	2.82	2.83	2.82	2.82	0.00	15.87	13.32	16.47	15.22	1.37
Sample 11	2.81	2.80	2.81	2.81	0.00	6.89	13.47	12.57	10.98	2.91
Sample 12	2.84	2.82	2.82	2.83	0.01	9.88	8.84	7.63	8.78	0.92
Sample 13	2.88	2.86	2.87	2.87	0.01	14.37	16.77	17.37	16.17	1.30

Raw data for physicochemical analysis of ash and moisture content

Test	Ash content (g)					Moisture Content (%)				
	1	2	3	AVE	STD	1	2	3	AVE	STD
Sample 1	0.14	0.13	0.13	0.13	0.00	29.98	28.74	29.52	29.41	0.51
Sample 2	0.12	0.10	0.11	0.11	0.01	28.77	27.00	25.37	27.05	1.39
Sample 3	0.13	0.13	0.13	0.13	0.00	26.99	30.68	26.46	28.04	1.88
Sample 4	0.15	0.14	0.15	0.14	0.01	27.13	27.50	26.98	27.20	0.22
Sample 5	0.12	0.12	0.14	0.12	0.01	31.02	31.21	25.40	29.21	2.70
Sample 6	0.12	0.11	0.11	0.12	0.00	31.24	29.56	29.18	29.99	0.90
Sample 7	0.10	0.10	0.10	0.10	0.00	29.90	29.77	29.09	29.59	0.36
Sample 8	0.08	0.08	0.09	0.08	0.01	30.18	29.05	24.65	27.96	2.39
Sample 9	0.12	0.11	0.12	0.11	0.00	29.58	31.93	36.31	32.61	2.79
Sample 10	0.07	0.11	0.10	0.09	0.02	31.74	32.57	32.27	32.19	0.34
Sample 11	0.09	0.09	0.10	0.10	0.00	29.25	29.06	29.41	29.24	0.14
Sample 12	0.11	0.10	0.12	0.11	0.01	29.88	23.09	29.87	27.61	3.20
Sample 13	0.14	0.13	0.15	0.14	0.00	30.33	31.67	27.33	29.78	1.81

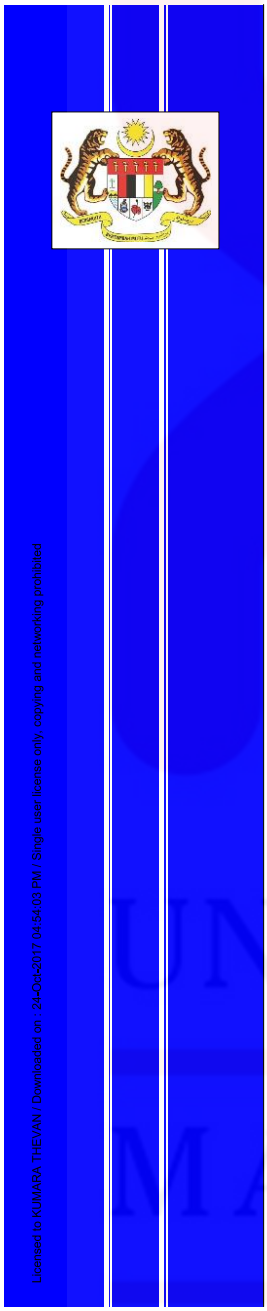
APPENDIX C

Collection form of stingless bee honey samples

Samples no.	Honey location	Types of Honey		Honey type		Pollen sources / Plant sources	Date of harvesting	Age of honey	Location (company/ individual/ distributor)
		Raw	Process (°C)	Blended species	Single species				
1	Kubang Kerian	✓		*T. thoracica, H. itama, Lepidotrigona terminate, T. laeviceps*		Multiflora	4/7/2018	7 days	Company
2	Kg. Sering		✓	*T. thoracica, H. itama, T. laeviceps*		Multiflora	10/6/2018	1 month	Distributors
3	Pengkalan Chepa	✓			*H. itama*	Multiflora	April 2018	4 months	Individual
4	Koklanas	✓			*H. itama*	Multiflora	May 2018	3 months	Individual
5	Ketereh	✓			*H. itama*	Multiflora	May 2018	3 months	Individual
6	Wakaf Bharu	✓			*H. itama*	Multiflora	March 2018	5 months	Individual
7	Wakaf Bharu	✓			*H. itama*	Multiflora	15/7/2018	0 day	Individual
8	Wakaf Bharu	✓			*H. itama*	Multiflora	May 2018	3 months	Individual
9	Wakaf Bharu	✓			*H. itama*	Multiflora	1/7/2018	14 days	Individual
10	Wakaf Bharu	✓			*H. itama*	Multiflora	14/7/2018	1 days	Individual
11	Jedok	✓			*H. itama*	Multiflora	Jun 2018	1 month	Individual
12	Jeli	✓			*G. thoracica*	Multiflora	Jun 2018	1 months	Individual
13	Jeli	✓			*G. thoracica*	Multiflora	21/7/2018	I days	Individual

APPENDIX D

Malaysian Standard 2863- Stingless Bee Honey Specification



MS

MALAYSIAN STANDARD

Kelulut (Stingless bee) honey- Specification

ICS: 67.180.10

Descriptors: *Kelulut* (Stingless bee), honey, specification,sampling, test methods

DEPARTMENT OF STANDARDS MALAYSIA

Kelulut (Stingless bee) honey - Specification

1 Scope

This Malaysian Standard specifies the quality requirements, sampling, preparation of test sample, test methods, hygiene, packaging and labelling for *kelulut* honey produced by stingless bee of Meliponini tribe intended for direct human consumption.

It is applicable to both raw and processed *kelulut* honey.

2 Normative references

The following normative references are indispensable for the application of this standard. For dated references, only the edition cited applies. For undated references, the latest edition of the normative reference (including any amendments) applies.

MS 1514, *General principles of food hygiene*

MS 2679, *Amalan Pertanian Baik (APB) - Pemeliharaan lebah (tribus Apini) dan kelulut (tribus Meliponini)*

Food Act 1983

Food Regulations 1985

Food Hygiene Regulations 2009

3 Terms and definitions

For the purposes of this standard the following terms and definitions apply.

3.1 *kelulut* (stingless bee) honey

A natural sweet with certain acidity substance produced by stingless bees of Meliponini tribe from the nectar of plants or from secretions of living parts of plants, which the stingless bees collect, transform by combining with the specific substances of their own, deposit, dehydrate, store and leave in the natural honey pots to ripen and mature.

In this standard it is referred to as *kelulut* honey.

3.2 processed *kelulut* honey

Raw *kelulut* honey which undergoes drying process at a temperature not more than 40 °C to reduce moisture content.

3.3 raw *kelulut* honey

Kelulut honey that is collected from natural sealed honey pots.

4 Requirements

4.1 General

4.1.1 *Kelulut* honey shall has its natural characteristic flavour and aroma.

4.1.2 *Kelulut* honey shall free from foreign matter.

4.1.3 *Kelulut* honey shall not contain any food additives.

4.1.4 Both raw and processed *kelulut* honey should be harvested and processed in accordance with MS 2679.

4.2 Quality requirements

4.2.1 *Kelulut* honey shall comply with the requirements given in Table 1.

Table 1. Quality requirements for *kelulut* honey

Characteristics	Requirements		Test method[a]
	Raw honey	Processed honey	
Moisture, %	Not more than 35.0	Not more than 22.0	Annex A
Sucrose, g/100 g	Not more than 7.5	Not more than 8.0	Annex B
Fructose and glucose (sum), g/100 g	Not more than 85.0	Not more than 90.0	
Maltose, g/100 g	Not more than 9.5	Not more than 10.0	
Ash, g/100 g	Not more than 1.0	Not more than 1.0	Annex C
Hydroxymethylfurfural, mg/kg	Not more than 30.0	Not more than 30.0	Annex D
pH	2.5 to 3.8	2.5 to 3.8	Annex E
Plant phenolics[b]	Present	Present	Annex F

[a] Other equivalent and recognised test methods can be used.

[b] *Kelulut* honey shall contain naturally occurring plant phenolic. A typical HPLC pattern of plant phenolics in *kelulut* honey is shown in Figure F.1. of Annex F.

5 Sampling

5.1 General

5.1.1 Different sampling methods are employed for raw *kelulut* honey (5.2) and processed *kelulut* honey (5.3).

5.1.2 The analysis of physicochemical parameters shall be carried out within two to four weeks after collection of samples.

NOTE. Prescribed period indicates the freshness level of the samples for analysis.

5.2 Raw *kelulut* honey

5.2.1 Sampling shall be carried out as the following.

a) Species of the *kelulut* honey shall be identified.

b) The *kelulut* honey shall be harvested from matured honey pots i.e. sealed honey pots by piercing the upper part of the pots using sharp tool followed by suction using clean syringe or specific honey pump.

c) The *kelulut* honey harvested shall be representative of the identified beekeeping area where the sample is taken.

d) The minimum amount of samples to be collected is 350 g.

e) The *kelulut* honey shall be placed in clean, dry and sealed suitable food grade container. It shall be separated according to the specific species.

a) The *kelulut* honey collected shall be kept in a chiller at the temperature between 0 °C to 4 °C.

5.2.2 The samples shall be labelled to include the following information:

a) name of contributors, organisation/company together with contact person and phone number;

b) *kelulut* (stingless bee) species;

c) date of sampling;

d) beekeeping area; and

e) major plant species surrounding the beekeeping area.

5.3 Processed *kelulut* honey

5.3.1 Sampling shall be carried out as the following.

a) Species of the *kelulut* honey and producer of the honey shall be identified.

b) The minimum amount of samples to be collected is 350 g.

c) The *kelulut* honey shall be placed in clean, dry and sealed suitable food grade container. It shall be separated according to the specific species and producer.

5.3.2 The samples shall be labelled to include the following information:

a) name and address of company/manufacturer; and

b) a statement to indicate the samples are processed *kelulut* honey.

6 Preparation of test sample

Crystallised honey should be left at room temperature to allow the crystal to dissolve. Alternatively, the crystallised honey should be heated up to less than 40 °C. Homogenise the sample at room temperature.

7 Test methods

Testing should be carried out as specified in Annexes A to J.

NOTE. Alphabet "I" is not used for labelling of annexes.

8 Packaging and labelling

8.1 Packaging

The product shall be packed in suitable, hygienic, food grade packaging materials which are able to withstand the acidity of *kelulut* honey so as to protect the safety and quality of the product in accordance with MS 2679.

8.2 Labelling

8.2.1 The product shall be labelled in accordance with *Food Act 1983* and *Food Regulations 1985*.

8.2.2 Each container/bottle of product shall be legibly and indelibly labelled with the following information:

a) name of product;

b) batch or code number;

c) date of packing and expiry date;

d) name, and address of the packer;

e) net weight;

f) country of origin;

g) storage instruction; and

h) packer registered trade mark, if any.

8.2.3 The containers may also be marked with the producer specified branding, place of origin and associated plant source.

9 Hygiene

Kelulut honey shall be processed under the good processing and harvesting of *kelulut* honey in accordance with MS 2679, MS 1514 and *Food Hygiene Regulations 2009*.

10 Legal requirements

The product shall in all other aspects comply with the requirements of the legislation currently in force in the country.

Annex A
(normative)

Determination of moisture content by refractometric method

A.1 Scope

This annex describes the procedure to measure the water content of *kelulut* honey.

A.2 Principle

The method is based on the principle that refractive index increases with solids content. The table was constructed from a plot of the logarithm of the refractive index minus unity plotted against the water content as determined by vacuum drying, a technique which requires much greater manipulative skill.

A.3 Apparatus

Use the usual laboratory apparatus and, in particular, the following.

A.3.1 Flasks, 50 ml.

A.3.2 Water bath.

A.3.3 Reichert refractometer or equivalent refractometer, regularly calibrated with distilled water or with another certified reference material. The refractive index for water (n_D) at 20 °C is 1.333 0.

A.4 Procedure

A.4.1 Sample preparation

See Clause 6.

A.4.2 Dissolution

Homogenise the prepared sample again and put in a flask. Close the flask and place in a water bath at 50 °C (± 0.2 °C) until all the sugar crystals are dissolved. Cool the solution to room temperature and stir again. Ensure that the flask is air tight.

A.4.3 Determination

Ensure that the prism of the refractometer is clean and dry. Directly after homogenisation, cover the surface of the prism evenly with the sample. After 2 min, read the refractive index by using

the refractometer. Measure each honey thrice and take the average value. Read the corresponding moisture content from Table A.1. Carefully clean the prism after use.

NOTE. The method refers only to the use of the Reichert refractometer, not to digital instruments.

Table A.1. Relationship of water content of *kelulut* honey to refractive index

Water content (g/100 g)	Refractive index at 20 °C	Water content (g/100 g)	Refractive index at 20 °C	Water content (g/100 g)	Refractive index at 20 °C
13.0	1.504 4	17.2	1.493 5	21.4	1.483 0
13.2	1.503 8	17.4	0.493 0	21.6	1.482 5
13.4	1.503 3	17.6	1.492 5	21.8	1.482 0
13.6	1.502 8	17.8	1.492 0	22.0	1.481 5
13.8	1.502 3	18.0	1.491 5	22.2	1.481 0
14.0	1.501 8	18.2	1.491 0	22.4	1.480 5
14.2	1.501 2	18.4	1.490 5	22.6	1.480 0
14.4	1.500 7	18.6	1.490 0	22.8	1.479 5
14.6	1.500 2	18.8	1.489 5	23.0	1.479 0
14.8	1.499 7	19.0	1.489 0	23.2	1.478 5
15.0	1.499 2	19.2	1.488 5	23.4	1.478 0
15.2	1.498 7	19.4	1.488 0	23.6	1.477 5
15.4	1.498 2	19.6	1.487 5	23.8	1.477 0
15.6	1.497 6	19.8	1.487 0	24.0	1.476 5
15.8	1.497 1	20.0	1.486 5	24.2	1.476 0
16.0	1.496 6	20.2	1.486 0	24.4	1.475 5
16.2	1.496 1	20.4	1.485 5	24.6	1.475 0
16.4	1.495 6	20.6	1.485 0	24.8	1.474 5
16.6	1.495 1	20.8	1.484 5	25.0	1.474 0
16.8	1.494 6	21.0	1.484 0		
17.0	1.494 0	21.2	1.483 5		

NOTES:

1. Value for 20 °C are Wedmore's calculation, Bee World 36. 197-206 (1955).

2. If refractive index is measured at temperatures above 20 °C, add 0.000 23 per degree and if measured below 20 °C, substract 0.000 23 per degree before using table.

The table is derived from a formula developed by Wedmore from the data of Chataway and others as the following equation:

$$W = 1.731 \quad \frac{90 - \log (R.I - 1)}{0.002\,243}$$

where

W is the water content in g per 100 g honey; and

$R.I$ is the refractive index.

Annex B
(normative)

Determination of sugar content

B.1 Scope

This annex describes the procedure to determine fructose, glucose, sucrose and maltose, in *kelulut* honey, for which precision data are acquired.

B.2 Principle

After filtration of the solution, the sugar content is determined by HPLC (High Performance Liquid Chromatography) with refractive index (R.I) detection. Peaks are identified on the basis of their retention times. Quantification is performed according to the external standard method on peak areas or peak heights.

B.3 Reagents

Unless otherwise specified, chemicals shall be of recognised analytical reagent quality and distilled water should be used in the test.

B.3.1 Methanol, HPLC grade.

B.3.2 Acetonitrile, HPLC grade.

B.3.3 Eluent solution for the HPLC, mix 80 volumes of acetonitrile with 20 volumes of water. Degas prior to use.

B.3.4 HPLC water or of equivalent purity, for example double distilled deionised water.

B.3.5 The standard solution, fructose, glucose, sucrose and maltose.

Annex C
(normative)

Determination of ash content

C.1 Scope

This annex prescribes the procedure to determine the ash content in *kelulut* honey.

C.2 Principle

The honey is ashed at a temperature of less than 600 °C and the residue is weighed out.

C.3 Reagents

Unless otherwise specified, chemicals shall be of recognised analytical reagent quality and distilled water should be used in the test.

C.3.1 Olive oil, free from ash.

C.4 Apparatus

Use usual laboratory apparatus and, in particular, the following.

C.4.1 Platinum or quartz or porcelain ash dish, of suitable size.

C.4.2 Appliance for preliminary ashing, such as an infra-red heater, a gas burner or a hot plate.

C.4.3 Electric furnace, adjustable to 600 °C (± 25 °C).

C.4.4 Desiccator, containing an efficient desiccant.

C.4.5 Analytical balance, accurate to 0.1 mg.

C.5 Procedure

C.5.1 Preparation of the ash dish

Dry the ash dish in the electrical furnace at ashing temperature, subsequently cool in a desiccator to room temperature and weigh to 0.001 g.

C.5.2 Sample preparation

See Clause 6.

C.5.3 Determination

C.5.3.1 Weigh 5 g to 10 g of the sample to the nearest 0.001 g into the dried ash dish. Add two drops of olive oil. Commence preliminary ashing without loss at a low heat rising to 350 °C to 400 °C by using one of the appliances, until the sample is black and there is no loss by foaming.

C.5.3.2 After the preliminary ashing, place the dish in the preheated furnace and heat for at least 1 h. Cool the ash dish in the desiccator and weigh. Continue the ashing procedure until constant weight is reached.

C.6 Calculation and expression of results

The ash content in honey, expressed as percentage by weight is calculated using the following formula:

$$\frac{m_1 - m_2}{m_0} \times 100$$

where

m_0 is the weight of honey taken; m_1 is the weight of

dish and ash; and m_2 is the weight of dish.

Round the result to two decimal places.

Annex D
(normative)

Determination of hydroxymethylfurfural by spectrophotometric method

D.1 Scope

This annex describes the procedure to determine hydroxymethylfurfural in *kelulut* honey using spectrophotometric method.

D.2 Principle

The determination of hydroxymethylfurfural (HMF) content is based on the determination of UV absorbance of HMF at 284 nm. In order to avoid the interference of other components at this wavelength the difference between the absorbances of a clear aqueous honey solution and the same solution after addition of bisulphite is determined. The HMF content is calculated after subtraction of the background absorbance at 336 nm. This method is based on the original work of White.

D.3 Reagents

Unless otherwise specified, chemicals shall be of recognised analytical reagent quality and distilled water should be used in the test.

D.3.1 Carrez solution I, dissolve 15 g $K_4Fe(CN)_6.3H_2O$ and dilute to 100 ml with H_2O.

D.3.2 Carrez solution II, dissolve 30 g $Zn(CH_3COO)_2.2H_2O$ and dilute to 100 ml with H_2O.

D.3.3 Sodium bisulphite solution, 0.20 %, dissolve 0.20 g $NaHSO_3$ (technical grade is satisfactory) and dilute to 100 ml with H_2O. Dilute 1 + 1 for dilution of reference solution if necessary. Prepare fresh daily.

D.4 Apparatus

Use usual laboratory apparatus and, in particular, the following.

D.4.1 Spectrophotometer UV, to measure A at 284 nm and 336 nm.

D.5 Procedure

D.5.1 Sample preparation
See Clause 6.

D.5.2 Determination

D.5.2.1 Accurately weigh 5 g honey in small beaker and transfer with total of 25 ml H_2O to 50 ml volumetric flask. Add 0.50 ml Carrez solution I, mix, add 0.50 ml Carrez solution II, mix, and dilute to volume with H_2O. Drop of alcohol may be added to suppress foam. Filter through paper, discarding first 10 ml filtrate.

D.5.2.2 Pipet 5 ml filtrate into each of two 18 mm x 150 mm test tubes. Add 5.0 ml H_2O to one tube (sample) and 5.0 ml $NaHSO_3$ solution to other (reference). Mix well.

D.5.2.3 Determine A of sample against reference at 284 nm and 336 nm in 1 cm cells. If A is less than 0.6, dilute sample solution with H_2O and reference solution with 0.1 % $NaHSO_3$ solution to same extent and correct A for dilution.

D.6 Calculation and expression of results

The HMF content, expressed in mg/kg, of the sample is calculated using the following formula:

$$\frac{(A_{284} - A_{336}) \times 149.7 \times 5 \times D}{W}$$

where

A_{284} is the absorbance at 284 nm;

A_{336} is the absorbance at 336 nm;

149.7 is the factor $= \frac{126 \times 1\,000 \times 1\,000}{16\,830 \times 10 \times 5}$;

126 is the molecular weight of HMF;

16 830 is the molar absorptivity of HMF, λ at 284 nm;

1 000 is the conversion g into mg;

10 is the conversion 5 into 50 ml;

1 000 is the conversion g of honey into kg;

5 is the theoretical nominal sample weight;

D is the dilution factor, in case dilution is necessary; and

W is the weight of honey taken.

Results are expressed in mg/kg to one decimal place.

Annex E
(normative)

Determination of pH

E.1 Scope

This annex describes the procedure to determine the pH of *kelulut* honey.

E.2 Principle

The sample is dissolved in water and the pH is measured.

E.3 Reagents

Unless otherwise specified, chemicals shall be of recognised analytical reagent quality and distilled water should be used in the test.

E.3.1 Distilled water, carbon dioxide-free.

E.3.2 Buffer solutions, for calibration of the pH meter at pH 3.7 (or pH 4.0), pH 7.0 and pH 9.0.

E.4 Apparatus

Use the usual laboratory apparatus and, in particular, the following.

E.4.1 pH meter, accurate to 0.01 units.

E.4.2 Magnetic stirrer.

E.4.3 Beaker, 250 ml.

E.5 Procedure

E.5.1 Calibration of pH meter

E.5.1.1 The meter should be calibrated at pH 7 (or pH 4.0), pH 7.0 and pH 9.0.

E.5.2 Sample preparation

See Clause 6.

E.5.3 Determination

Dissolve 10 g sample in 75 ml of carbon dioxide-free water in a 250 ml beaker. Stir with the magnetic stirrer, immerse the pH electrodes in the solution and record the pH.

E.6 Expression of results

Report the pH to two decimal places.