Published: 31st July 2021

The Physicochemical Properties of Stingless Bee Honey: A Review

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Abstract:

Honey is a saturated sugary solution derived from the nectar of flowers collected by worker bees. The main constituents of honey are water, sugars, proteins, minerals, and organic acids. Malaysia has a wide distribution of stingless bees, especially the *Heterotrigona itama* species. The composition of stingless bee honey is greatly dependent on its geographical origins and the flora sources. This leads to huge variations in stingless bee honey obtained from different regions. In Malaysia, stingless bee honey is being sold as a health-promoting product, although its composition and therapeutic effects have never been studied in detail. Thus, the need to study the properties of stingless bee honey is presented here. The main parameters reviewed are moisture, pH, hydroxymethylfurfural, ash, electrical conductivity, antioxidant activity, total phenolic content, and total flavonoid content.

Keywords: Honey, Stingless bee, Physicochemical properties

INTRODUCTION

Honey is a saturated sugary solution derived from the nectar of flowers collected by worker bees. Honey is mainly composed of water, carbohydrates, proteins, sugars, phenolic acid, flavonoids, enzymes, and organic acids [1]. Honey has been used as a traditional medicine due to its chemical and physical composition. For instance, it has been used for centuries for wound healing as well as curing certain diseases [2]. The most remarkable functions of honey are their antioxidant and antibacterial activities [3].

The most common and popular honeybee species in the world is Apis mellifera, which is a type of western honeybee. The stingless bee is less common compared to A. mellifera. There are about 500 different species of stingless bees discovered in the world. A total of sixty species can be found in Asia [3]. According to Azri et al. [4], the most common stingless bees that exist in Malaysia are the Trigona species and Melipona species. The Melipona species is widely distributed throughout the world where its population could rival that of the A. mellifera species. Unlike western honeybees, stingless bees are smaller in size. Stingless bees do not cause harm to humans as their stings have undergone evolutionary decline. Stingless bees produce and store less honey compared to A. mellifera. Moreover, the colour and taste of stingless bee honey are different from those of A.

mellifera honey [5]. The production of honey by stingless bees is limited and there is a lack of international and institutional standards as well as research knowledge [6]. Today, researchers are interested in studying the various properties of stingless bee honey as it has high functional variability [5]. The physicochemical properties of stingless bee honey are mainly influenced by flora type, climate, and geographical region [7].

Although the characteristics of stingless bee honey are not yet fully studied, it has gained popularity as a health-promoting natural product [8]. The nutrients of stingless bee honey could improve and maintain the metabolism rate, circulatory system, and physiological reactions [9]. Rao *et al.* [7] stated that stingless bee honey can lower cardiovascular risk.

The main purpose of this paper is to review the physicochemical properties of stingless bee honey. The main physicochemical properties reviewed are moisture content, pH, sugar content, hydroxymethylfurfural content, ash, electrical conductivity, antioxidant activity, total phenolic content, and total flavonoid content.

Malaysian Standard MS 2683: 2017

Malaysian Standard MS 2683 governs the quality of stingless bee honey in Malaysia [10]. The standard states the minimum quality requirements for some of the

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principal parameters, such as moisture, sucrose content, fructose and glucose content, maltose content, of stingless bee honey as depicted in Table 1.

Moisture

Moisture content is the quantity of water in honey. According to MS 2683, the moisture content of stingless bee honey should not be more than 35% [10]. According to Baroni et al. [9], the climatic conditions as well as maturity of honey played a significant role in determining the moisture content of the honey. Vit et al. [11] concluded that the moisture content of stingless bee honey was higher compared to A. mellifera honey. They further suggested that the maximum moisture content of stingless bee honey should be 30 g/100 g of sample. Research conducted by Chuttong et al. [12] showed that the highest moisture content for twenty eight samples of Thailand stingless bee honey was 47 g/100 g while the lowest was 25 g/100 g. Suntiparapop et al. [13] also analysed the moisture content of nineteen Thai stingless bee honey of Tetragonula leaviceps species from two different locations, i.e., Chantaburi (n = 13) and Trat (n= 6). The moisture contents were found to be 27.46 \pm 0.23% and 26.50 \pm 0.24% respectively. The stingless bee honey from Australia possessed an average moisture content of 25.4 \pm 1.0% [14]. Nascimento *et al.* [15] reported that the range of moisture content for Brazilian stingless bee honey was from 26.0% to 35%. Lower moisture content could prolong the shelf life of stingless bee honey. This is due to the inhibition of microbial growth as well as the prevention of the fermentation process.

pН

pH is a value that expresses the acidity or alkalinity of a solution. As reported by Vit *et al.* [6], stingless bee honey has a lower pH value that leads to greater acidity and sourness compared to *A. mellifera* honey. This property might affect consumers' preferences of stingless bee honey. The lower pH value of stingless bee honey is due to the presence of organic acids such as citric acid, acetic acid, gluconic acid, and benzoic acid. As fermentation takes place during storage, the acidity of stingless bee honey become higher. Furthermore, the pH of the nectar consumed by the bees also affects the acidity of the stingless bee honey produced [15].

The pH of thirty three stingless bee honey samples from Brazil ranged from 3.33 to 6.56 [16]. Another research by Nascimento *et al.* [15] showed that the average value of pH of thirty Brazilian stingless bee samples varied from 2.93 to 4.08. Besides that, the pH for stingless bee honey from Thailand reported an average value of 3.6 ± 0.198 [12]. The average pH value for Australian stingless bee honey was slightly higher, i.e., pH 3.85 ± 2.6 [14]. Study performed by Oddo *et al.* [17] on Australian *Trigona carbonaria* honey also reported similar mean pH value of 4.0 ± 0.1 .

The Malaysian *H. itama* stingless bee honey recorded a mean pH value of 3.26 ± 0.17 [18], which complied with MS 2683. A low pH value could prevent the growth of bacteria and thus enhance the honey's antibacterial properties [19].

Sugars

Honey is a solution of sugary liquid that contains not only the monosaccharides glucose and fructose, it also contains disaccharides such as sucrose and maltose. Majority of the stingless bee honey has low content of oligosaccharides [20]. The sweetness of stingless bee honey is mainly attributed to fructose [2].

Nweze *et al.* [21] reported that the mean value of reducing sugar for Nigerian stingless bee *Melipona* species was 75.64 \pm 1.99% with sucrose content of 5.06 \pm 0.75%. For the *Hypotrigona* species, the average reducing sugar was 60.49 \pm 5.21% and the sucrose content was 1.83 \pm 0.14%. For the twenty eight stingless bee honey from Thailand, the average total sugars content was 51 \pm 21 g/100g (fructose, 17 \pm 9.7%; glucose, 14 \pm 8.6%; maltose, 41 \pm 15%; sucrose, 1.2 \pm 2.7%) [12]. According to Chuttong *et al.* [12], maltose was only detected in three stingless bee species in Thailand – *Lepidotrigona terminate, Lepidotrigona flavibasis* and *Tetragonula laeviceps-pagdeni* complex.

Suntiparapop *et al.* [13] reported that the total reducing sugars for nineteen stingless bee samples (*T. laeviceps*) from Thailand had a range of 45.5% to 50.28%. Relatively higher sucrose content was detected from these samples, ranging from 18.9% to 19.5%. Sugars analysis by Guerrini *et al.* [5] on Ecuadorian stingless bee honey reported a fructose content of $25.2 \pm 3.37 \text{ g}/100\text{g}$, glucose content of $25.5 \pm 3.41 \text{ g}/100\text{g}$ and sucrose content of 3.72 ± 0.49 %. There was, however, no maltose detected in these honey samples. According to Azeredo *et al.* [22], early harvest of honey from bee hives could be one of the reasons causing high sucrose content in the sample, in which the sucrose has not yet fully been converted into glucose and fructose by the enzyme invertase.

For the stingless bee honey from Sarawak, Malaysia, the reported range were: $12.01 \pm 1.1\%$ to $19.5 \pm 1.3\%$ for fructose, $14.9 \pm 0.7\%$ to $21.0 \pm 1.7\%$ for glucose and $33.7 \pm 2.1\%$ to $45.2 \pm 1.1\%$ for maltose, with no sucrose detected [3]. Nevertheless, the Malaysian Standard MS 2683 stated that maltose content for Malaysian stingless bee honey should not be more than 9.5 g/100g.

As reported by Vit *et al.* [11], the reducing sugar content for Venezuelan stingless bee honey of 48.18% to 79.94% were much lower than the *Apis mellifera* honey with sugar content ranging from 68.4% to 77.5%.

Characteristics	Requirements
Moisture, %	Not more than 35.0
Sucrose, g/100g	Not more than 7.5
Fructose and glucose (sum), g/100g	Not more than 85.0
Maltose, g/100g	Not more than 9.5
Hydroxymethylfurfural, mg/kg	Not more than 30.0
pH	2.5 to 3.8

Table 1: Malaysian Standard MS 2683: 2017 for raw stingless bee honey

Meanwhile, the sucrose content reported were from 1.09% to 12.30%. On the other hand, the sugar content of Australian stingless bee (*T. carbonaria*) honey as reported by Oddo *et al.* [23] were as follows: fructose content, $24.5 \pm 1.9\%$; glucose content, $17.5 \pm 2.8\%$; maltose content, $20.3 \pm 2.9\%$; and, sucrose content, $1.8 \pm 0.4\%$. Biluca *et al.* [16] reported the total sugar content of thirty three Brazil stingless bee honeys as 48.6% to 70.5%, with an average value of $62.1 \pm 5.36\%$. The sucrose content for all the samples were below the quantitation limit of 0.074 mg/L.

Nascimento *et al.* [15] reported that the average sugar contents of thirty stingless bee honey from Brazil ranged from 66.12% to 75.21% with 1.61% of sucrose content. The authors explained that lower reducing sugars and high moisture content in stingless bee honey were mainly due to rapid fermentation. The fermentation process could be minimised with an appropriate storage of honey samples.

5-Hydroxymethylfurfural (HMF)

HMF is a type of Maillard reaction product generated due to the reaction of reducing sugars with amino acids at high temperatures [24]. The production of HMF is mainly due to the fructose, acids, high temperature, pH, water content as well as the minerals [25]. The HMF level could be used to determine the honey quality [12]. The lower the HMF contents in the honey, the better the honey's quality is [21]. The concentration of HMF in freshly harvested honey is relatively low, but the concentration increases with longer storage period [26]. Oddo et al. [17] investigated the quality and freshness of honey through HMF determination and honey with HMF content exceeding 10 mg/kg were excluded from the study. Moo-huchin et al. [27] claimed that HMF could not be detected in fresh honey. In addition, honey produced in tropical regions with higher environmental temperature could cause the production of HMF [12]; 13].

The highest HMF content for the twenty eight stingless bee honey samples from Thailand was 46 mg/kg while some of the honey samples did not contain HMF [12], with the mean HMF of 8.7 ± 12 mg/kg recorded. Another research involved Thailand stingless bee honey reported the HMF range of 0.25 mg/kg to 1.89

mg/kg [13]. Honey from Australian stingless bee (*T. carbonaria*) also had an average HMF content of 1.2 ± 0.6 mg/kg [23]. On the other hand, significant high HMF content (average value of 23.42 ± 17.79 mg/kg) was found in Malaysian stingless bee (*H. itama*) [18].

Ash

Ash content is the amount of mineral collected by stingless bee from honey sources [28]. It represents the minerals richness of honey [29]. The average ash content for Thai stingless bee honey was 0.531 ± 0.632 g/100 g [12] and 0.48 ± 0.06 g/100g of ash was detected for the Australian stingless bee (*T. carbonaria*) honey [23]. Ash content of Brazilian stingless bee honey was reported to be in the range of 0.1% to 0.3% [15]. The ash content of Ecuadorian stingless bee (*Meliponinae*) honey was 0.28 ± 0.04 g/100g [5].

Electrical Conductivity

Electrical conductivity represents the honeys' mineral content [15]. The electrical conductivity of honey depends on the content of ash and acid. The higher the acid and ashes in honey, the higher the electrical conductivity [30]. There is a relationship between electrical conductivity and ash, however, different parameters were used. For example, electrical conductivity measures the inorganic and ionisable organic compounds while ash only measures inorganic residues after carbonization process of the honey [31].

The electrical conductivity of Ecuadorian stingless bee (*Meliponinae*) honey was 0.48 ± 0.06 mS/cm [5]. Almost similar range of electrical conductivity (0.53 to 0.71 mS/cm) was detected for Thai stingless bee honey [13]. The average electrical conductivity reported by Chuttong *et al.* [12] was slightly higher with an average value of 1.1 ± 0.78 mS/cm. For one of the Brazilian stingless bee honey, the average value of electrical conductivity was from 514.78 to 728.34 µS/cm [15]. However, significant low level of electrical conductivity (0.150 to 1.34 mS/cm) was detected for another reported Brazilian stingless bee honey [16]. Australian stingless bee (*T. carbonaria*) species recorded electrical conductivity with a range of 1.33 mS/ cm to 169 mS/cm [23].

Antioxidant Activity

Antioxidant activity is the capability of honey to decrease the oxidative reactions in human body that could affect our health [32]. Excessive generation of free radicals results in oxidative stress [33] and a variety of chronic diseases such as cancer, aging, and heart diseases [34]. Wang *et al.* [35] claimed that antioxidant activity of honey will not be affected by high temperature. In contrast, the elevated temperature could help to improve the antioxidant activity [36].

The antioxidant activity of stingless bee honey can be determined by three major methods namely DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay, ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) assay and FRAP (Ferric Reducing Antioxidant Power) assay. As stated by Otto *et al.* [23], it was not recommended to measure the antioxidant activity by using a single antioxidant test only. Two or more antioxidant tests should be used to compare the results.

The DPPH radical scavenging effect from thirty three Brazilian stingless bee honeys were within the range from 1.41 mg equivalent ascorbic acid (EAA)/100 g to 18.5 mg EAA/100 g. The wide variation of results could be due to the different geographical origins of honey samples [16]. The DPPH radical scavenging effect of Brazilian stingless bee honey analysed by Silva *et al.* [37] were ranging from 10.6 mg EAA/100 g to 12.9 mg EAA/100 g. For stingless bee honey from Sarawak, the DPPH assay values were ranging from 17.0 \pm 7.5 to 47.5 \pm 3.2% [12].

Oddo *et al.* [23] analysed the antioxidant activity of Australian stingless bee using both DPPH and ABTS assay methods. The mean ABTS assay value reported for stingless bee (*T. carbonaria*) honey was 233.96 \pm 50.95 μ M Trolox equivalents while the mean DPPH assay value was 48.03 \pm 12.58 % ascorbic acid equivalent. By using the FRAP assay, a minimum value of 61.1 μ mol Fe II/100 g and a maximum value of 624 μ mol Fe II/100 g for the stingless bee honey were reported by Biluca *et al.* [16].

By using ABTS cation radical-scavenging tests, the EC_{50} value for seven stingless bee honeys from Brazil were varied from 210 ± 0.25 to 337 ± 3.17 mg/mL [37]. There was a high correlation between total phenolic content and antioxidant activity [38, 39]. Saric *et al.* [40] also stated that the total phenolic content and total flavonoid content will affect the antioxidant activity in honey. The antioxidant activity of stingless bee honey is greatly dependent on the geographical region as well as the species of stingless bee [41].

Total Phenolic Content (TPC)

As reported by Tuksitha *et al.* [3], the phenolic content for the three Sarawakian stingless bee honey samples ranged from 44.72 ± 6.50 to 99.04 ± 5.14 mg/mL. From their results, the stingless bee honey produced by *H. itama* has the highest phenolic content. According to Kek *et al.* [42], the total phenolic content present in stingless bee honey (*Trigona species*) was 33% higher than that of *Apis mellifera* honey.

Silva et al. [37] investigated the total phenolic content of Brazilian stingless bee honey and the values obtained varied from 1.30 to 66.0 mg GAE/100g. Biluca *et al.* [16] reported the range of 10.3 to 98 mg GAE/100g for the total phenolic compounds found in stingless bee honey (*Meliponinae*). The total phenolic content of *T. carbonaria* stingless bee honey from Australia had an average value of 55.74 ± 6.11 mg EGA/100 g [23]. The wide variation of the value could be due to different geographical area.

Ranneh *et al.* [43] analysed the total phenolic content of Malaysian stingless bees (*Trigona species*) honey harvested from Kedah and Johor Bahru region. The stingless bee honey from Kedah had an average total phenolic content of 228.09 ± 7.9 mg GAE/kg and 235.28 ± 0.6 mg GAE/kg for stingless bee honey from Johor Bahru. The authors also compared stingless bee honey with Tualang honey, and the result showed that stingless bee honey contains greater amount of total phenolic content.

Total Flavonoid Content (TFC)

Flavonoids are a type of phenolic compounds with low molecular weight. Flavonoids also play an important role in the antioxidant activity of honeys [11]. The flavonoid content for three Sarawakian stingless bee honey samples varied from 12.41 ± 0.62 to 17.67 ± 0.75 mg/mL [3]. The highest flavonoid content was detected in the *G. thoracica* species. Malaysian stingless bee honey from Kedah had a total flavonoid content of 97.88 ± 10.1 mg CE/kg while stingless bee honey from Johor Bahru had a total flavonoid content of 101.5 ± 11.4 mg CE/kg [43]. The flavonoid content of Australian (*T. carbonaria*) stingless bee honey had an average value of 10.02 ± 1.59 mg Quercetin Equivalents (EQ)/100g, which was higher than Czech floral honey (6.59 mg EQ/100g) and honeydew honey (7.25 mg EQ/100g) [23].

CONCLUSION

Stingless bees are distinctive because they produce a rare kind of honey that has gained attention due to its particular characteristics and exotic flavour. However, studies conducted on stingless bee honey are limited compared to those of *A. mellifera* honey. Therefore, the value of research on Malaysian stingless bee honey is remarkably high. This review paper shows that the physicochemical properties of stingless bee honey are very much affected by the flora sources as well as the geographical regions in which the samples are collected.

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