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Physicochemical Analysis of Several Natural Malaysian Honeys and Adulterated Honey

Puteri Nurul Syahirah Md Dan[1], Syafizal Omar[1], Wan Iryani Wan Ismail[1,2]

[1]Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) Selangor, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia
[2]School of Fundamental Science, Universiti Malaysia Terengganu (UMT), 21030 Kuala Nerus, Terengganu, Malaysia

Corresponding authors emails: waniryani@umt.edu.my

Abstract: Physicochemical analysis is one of the methods used to measure the quality and authenticity of honey. Quality and authenticity of honey are crucial to ensure only a high quality of natural honey is offered to consumers and to avoid any health complications in the future. However, relatively few physicochemical data is available on the Malaysian honey. In this study, selected physicochemical analyses were conducted on several local honey. Seven honey samples i.e. Tualang honey, Rubber honey, Acacia honey, Kelulut 1 honey, Kelulut 2 honey, adulterated honey 1 and adulterated honey 2 were used and their physicochemical properties measured. Natural honey samples exhibited varying physicochemical characteristics compared to adulterated honey. The adulterated honey 1 had the highest HMF level (164.55 ± 27.14mg/kg), with a low level of pH (3.2 ± 0.02), free acidity (20.8 ± 0.17) and electrical conductivity (0.003 ± 0.00) compared to the natural honey. These preliminary data could serve as a basis for the identification and characterisation of Malaysian honey based on the physicochemical analysis. Moreover, the data can support enforcement activities by the authorities in preventing adulterated honey production and trading in the future.

1. Introduction

Honey has been used in various applications of our daily lives since ancient times (Bogdanov & Martin, 2002), with the most common one being a natural sweetener. Other profound applications can be found in the medical field such as in wound healing, as an anti-inflammatory agent and as part of a healthy functional food in general (Khalil et al., 2012; Moniruzzaman et al., 2013). However, the beneficial properties can only be exerted by consuming a natural honey of good quality. Unfortunately, at present the supply of natural honey has been marred by the overabundance of adulterated honey in the market. According to Kamaruddin et al. (2006), over 77% of honey available in the Malaysian market was adulterated. Implementation of effective enforcement by local authority to counter the excess adulterated honey has proven to be a challenge. One of the issues that impede such enforcement is a lack of physicochemical data to identify and differentiate the adulterated honey from natural honey.

Malaysia is blessed with numerous natural resources including honey. Honey produced in Malaysia can be classified based on bee species or tribe such as *Apis* (i.e. *A. dorsata, A. mellifera* and *A. cerana*) (stinging bee) or Meliponine (stingless bee; locally known as Kelulut) (Ismail,

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2016). In addition, floral sources contribute to the variety of Malaysian honey such as Tualang honey, Gelam honey, Acacia honey and Rubber honey. Honey may also be influenced by the climate and its geographical origins, especially in tropical and temperate countries (Khalil et al., 2012). In order to gather the data on Malaysian honey, the study was conducted to analyse the physicochemical properties of several Malaysian honey. Concurrently, the properties of natural honey were compared with adulterated honey to observe and identify the differences between them.

2. Materials and Methods

2.1. Honey collection

Seven types of honey samples were used in this study. Five honey samples were freshly collected without any treatment from the hives of several bee species; Rubber and Acacia honey (*A. mellifera*), Tualang honey (*A. dorsata*) from Terengganu, Kelulut honey (*Heterotrigona itama*) from Kelantan (Kelulut honey 1) and Terengganu (Kelulut honey 2). Two adulterated honey samples were used in this study to compare their characteristics with the abovementioned natural honey. All samples were stored at 4°C throughout the experiment.

2.2. Physicochemical analyses

Physicochemical analyses including moisture, pH, free acidity, electrical conductivity and hydroxymethylfurfural (HMF) level were determined according to the protocols as detailed in the International Honey Commission (IHC) (2009) and Samat et al. (2017).

2.3 Moisture content

The moisture content of honey samples was determined at 20°C by using MISCO BKPR-2 digital refractometer (MISCO refractometer, United States) (IHC, 2009).

2.4 pH and free acidity

Delta 320 pH meter (Mettler Toledo, Switzerland) was used to measure the pH and free acidity of honey. Honey samples were titrated with 0.1M sodium hydroxide (NaOH) until pH 8.3 to measure their free acidity level, with the results expressed as milliequivalents acid/kilogram honey (meq/kg) (IHC, 2009).

2.5 Electrical conductivity

Electrical conductivity was carried out using HI-38311 electrical conductivity meter (Hanna Instruments, United States). A 20% (w/v) of each honey solution was prepared by dissolving 20g of honey in 100ml deionised distilled water prior to measuring the electrical conductivity using the meter (IHC, 2009).

2.6 Hydroxymethylfurfural (HMF)

5g of each honey was diluted with 25ml distilled water prior to transfer into a volumetric flask (White, 1979). 0.5ml Carrez solution I was added followed by 0.5ml Carrez II solution. Distilled water was then added up to the mark. The solution was filtered using a 0.45µm filter paper and the first 10ml filtrate was discarded. 5ml honey solution was mixed with 5ml water before its absorbance was measured against a reference solution of 5ml initial honey solution and 5ml 0.2% sodium bisulphite solution. The absorbance was measured at 284nm and 336nm, and the values were expressed in (mg/kg) according to the equation described by White (1979).

2.7 Data Analysis

Data were presented as mean ± standard error mean (SEM).

3. Results

Rubber honey displayed the highest moisture content, followed by Kelulut honey 2 and 1 (Table 1). Meanwhile, Acacia honey showed the lowest moisture content. Other honey samples including the adulterated honey demonstrated moisture content between 21.2 – 23.7%.

Table 1. Physicochemical analyses (moisture, pH, free acidity, electrical conductivity and HMF level) of several natural and adulterated honey samples. Data were measured duplicate and presented in average with SEM value.

Honey	Moisture (%)	pH	Free acidity (meq/kg)	Electrical conductivity (mS/cm)	HMF (mg/kg)
Tualang	22.5 ± 0.09	4.3 ± 0.01	16.8 ± 0.17	0.021 ± 0.00	10.5 ± 2.30
Rubber	29.3 ± 0.00	4.1 ± 0.01	18.5 ± 0.09	0.095 ± 0.00	0.60 ± 0.23
Acacia	20.2 ± 0.09	3.9 ± 0.01	18.0 ± 0.29	0.009 ± 0.00	11.62 ± 4.70
Kelulut 1	27.4 ± 0.03	3.3 ± 0.00	18.0 ± 0.29	0.068 ± 0.00	16.70 ± 2.68
Kelulut 2	28.2 ± 0.00	3.6 ± 0.01	83.0 ± 0.58	0.189 ± 0.00	0.57 ± 0.16
Adulterated 1	21.2 ± 0.03	3.2 ± 0.02	20.8 ± 0.17	0.003 ± 0.00	164.55± 27.14
Adulterated 2	23.7 ± 0.35	3.1 ± 0.06	53.2 ± 0.44	0.005 ± 0.00	126.94 ± 6.94

Adulterated honey samples had the lowest pH but are high in free acidity (Table 1). Interestingly, both kelulut honey are slightly acidic (pH of 3.3 – 3.6) compared to other natural honey but they have a wide range of free acidity (18.0 – 83.0 meq/kg). Tualang honey had the highest pH and lowest free acidity level compared to other honey. In terms of electrical conductivity, Kelulut honey 2 demonstrated the highest electrical conductivity while both adulterated honey samples showed the lowest conductivity. For HMF level, adulterated honey samples displayed very high HMF level. In contrast, the natural honey exhibited low HMF levels within the range of 0.57 – 16.70 mg/kg.

4. Discussion

Malaysian honey has a high commercial value because of its unique taste and aroma, particularly for the stingless bee (Kelulut) honey which is commonly found in the tropical countries. Based on the data obtained from the Department of Agriculture, Malaysia, the honey industry in Malaysia was estimated to be worth approximately RM17 million in 2017. Unfortunately, the quality and authenticity of the honey available in the market were unknown. A study conducted by our group revealed that long-term consumption of adulterated honey can cause various health complications on rats (Samat et al., 2018). Beneficial therapeutic effects can only be obtained from the natural honey consumption over a period of time (Samat et al., 2017). At present, enforcement by local authorities to prevent the adulterated honey from regulating in the market could not be done effectively as there is a lack of data on the local honey to identify, characterise and differentiate between natural and adulterated honey. Integration of physicochemical analysis for honey testing is seen as a good strategy as it is one of the methods which has been applied worldwide and is recommended by the International Honey Commission, a body which oversees world honey standard.

Physicochemical analysis is a set of tests to measure the quality and authenticity of honey. It includes moisture content, pH, free acidity, electrical conductivity, ash content, diastase, HMF and sugar content among others (Codex Alimentarius Commission, 2001). Multiple tests needed to be conducted on a single honey sample because honey is a complex mixture of various substances such as water, carbohydrates, proteins, enzymes, minerals and microorganisms (da Silva et al., 2016). However, selection of several parameters is adequate for the purpose of screening for a preliminary data on the differences between natural and adulterated honey. These include moisture content, pH, free acidity, electrical conductivity and HMF, which are chosen in this study.

From the results, only Acacia honey contains water (20.2 ± 0.09%) within the accepted limit set by

the Codex Standard for honey (20%). Both adulterated honey and Tualang honey have slightly exceeded the limit, while other honey samples contained higher water content up to 29.3 ± 0.00%. This high water content (<30%) is considered as acceptable for honey samples harvested from tropical countries such as Malaysia, which has relatively high levels of humidity throughout the year. In addition, honey has natural hygroscopic ability to absorb water (Mandal & Mandal, 2011) especially from this humid environment. The Codex Standard is developed mostly based on the data collected from the honey of temperate countries, which experience lower humidity.

Both kelulut honey samples have low pH but are high in free acidity. This is because Kelulut honey has a more acidic taste compared to other honey produced by *Apis* bee species. The kelulut honey samples also have high electrical conductivity, which could possibly be attributed to the presence of other contents in the honey such as minerals. In contrast, both adulterated honey samples had very low electrical conductivity (Codex: ≥0.8 mS/cm). This is acceptable because adulterated honey typically only contains water and sugar. The adulterated nature of the two honey samples was supported by very high level of HMF found compared to the natural honey (<80mg/kg set by the Codex). High HMF level can be found in samples subjected to either a prolonged storage (natural honey) or heating process (adulterated honey). In this study, all natural honey samples were freshly harvested from its hive. Thus, their HMF levels are low compared to the adulterated honey.

Data obtained from the study can be used as a stepping stone for more extensive characterisation of local honey and to differentiate local natural honey from adulterated honey. Because of limitation of sample used in this study, statistical analysis cannot be performed and projected for future investigation. Determination of honey's sugar composition can also be performed as the next step for further confirmation on the quality and authenticity of honey. The initiatives recommended above are opportune for more effective enforcement in the future to impede the trading of adulterated honey. Subsequently, it will increase the user's trust in the authenticity of local honey which could contribute to the increase of local honey's production and commercial value.

5. Conclusion

Natural and adulterated honey both exhibited different characteristics, which made it possible for early screening of its quality and authenticity. Thus, the physicochemical analysis including moisture content, pH, free acidity, electrical conductivity and HMF level can be used as a preliminary step to identify and characterise the quality of natural honey and the status of adulterated honey.

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