Effect of Six Month Storage on Physicochemical Analysis and Antioxidant Activity of Several Types of Honey

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Abstract. Honey composition could change during storage, which might affect the quality of honey. Subsequently, it could reduce the honey commercial value. However, data on the effect of the storage on several types of local honey is still lacking. Thus, in this study, moisture content, pH and antioxidant level of honey stored at 4°C were analysed after six-month of the storage. Initially, physicochemical properties (moisture, electrical conductivity, ash content, pH, free acidity and hydroxymethylfurfural (HMF) level) and antioxidant levels of five local honey samples (Tualang, Gelam, Pineapple, Acacia and Kelulut honey) and Manuka honey as a control were determined. Afterwards, honey samples were stored at 4°C for six months and their moisture content, pH and antioxidant levels were monitored monthly. Physicochemical properties were measured based on the standard method set by the International Honey Commission (IHC). Antioxidant activity was determined based on previous studies. Most of the honey samples exhibited physicochemical properties within the range set by the Codex Standard for Honey but displayed lower antioxidant level compared to other studies. On average, honey moisture content increased by 0.1 to 0.4% while their antioxidant level increased by 2.6 mg GAE/kg to 6.3 mg GAE/kg monthly. In contrast, pH level of honey decreased by 0.03 to 0.09 monthly. Storage of honey at low temperature increases its moisture and antioxidant level while decreases its pH level. However, further study needs to be carried out to observe whether the pattern will continue even after a lengthy storage period exceeding six months.

1. Introduction

Honey consists of a mixture of carbohydrates, mainly simple sugars such as glucose and fructose and other important constituents such as amino acids, enzymes, minerals, vitamins, organic acids, phenolic acids and volatile chemicals [1], [2]. It is unique as its characteristic differs based on its geographical origin, floral sources and bee species. Additionally, the quality of honey is also affected by the weather conditions, packaging, processing and storage time [3].
Honey can be categorized as food and is susceptible to spoilage or degradation. It can deteriorate rapidly without proper care and under average condition it can be considered as a readily perishable product [4]. Proper storage is crucial to preserve the quality of honey since honey composition could change during storage through chemical reactions such as oxidation and fermentation [5].

Changes in honey composition always reflect to low quality of honey and will affect its commercial value. Temperature is one of the factors that plays an important role during prolong storage of foods. Storage at low temperature could slow down food degradation by lowering the activity of enzymes and chemical reactions and preventing the growth of microorganism.

Several studies have been conducted to investigate the effect of low temperature on honey [6], [7]. However, most of them are interested on the crystallization of honey and very few studies analyse the changes in the physicochemical properties and stability of compounds in the form of antioxidant level throughout storage at low temperature. Thus, in this study two physicochemical properties of honey; moisture content and pH as well as antioxidant level of several types honey stored at low temperature of 4°C were observed monthly for 6 months period.

2. Materials and Methods

2.1. Honey collection

Six types of honey samples, namely Tualang, Gelam, Pineapple, Acacia, Kelulut and Manuka honey were used in this study. Tualang honey was harvested from Kedah, Gelam honey was collected from Terengganu, Pineapple, Acacia and Kelulut were obtained from Johor. Two honey samples (from two different hives) were collected from each of the location and were harvested as mentioned by Ismail [8]. Manuka honey was purchased from the local Guardian pharmacy. Tualang and Manuka honey samples were used as controls in the study. All honey samples except of Kelulut honey were produced by Apis species of bee. Meanwhile, Kelulut honey was produced by Meliponini bee, i.e. Heterotrigona itama. All samples were kept in glass jars, wrapped with aluminium foil and stored in a refrigerator at 4°C throughout the experiment for six months.

2.2. Physicochemical analyses

Right after sample collection and prior to store as mentioned above, physicochemical analyses were carried out according to the methods outlined in the International Honey Commission (IHC) [10] and Samat et al. [9]. These include moisture, electrical conductivity, ash content, pH, free acidity and hydroxymethylfurfural (HMF) level.

2.2.1 Moisture content

The moisture content of honey was measured using MISCO BKPR-2 digital refractometer (MISCO refractometer, United States) at 20°C.

2.2.2 Electrical conductivity

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

2.2.3 Ashcontent

Ash content was indirectly determined using the measured electrical conductivity as stated in Bogdanov et al. [10]. The following equation was used:

$$X1 = \frac{(X2 - 0.143)}{1.743}$$

(1)

Where X1 = ash value and X2 = EC (µS/cm) at 20 °C. The result is expressed as ash content in g/100g honey.
2.2.4 pH and free acidity
pH and acidity were measured using a Delta 320 pH meter (Mettler Toledo, Switzerland). 10g of honey was dissolved in 75ml of distilled water before the pH was measured. The dissolved honey solution was then titrated with 0.1M sodium hydroxide (NaOH) until pH 8.3 to obtain the free acidity value of honey.

2.2.5 Hydroxymethylfurfural (HMF)
HMF was determined as per method outlined by White [11]. 5g honey was diluted with 25ml distilled water and transferred into a 50ml volumetric flask. 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 then filtered using a 0.45µm filter paper, discarding the first 10ml filtrate. 5ml honey solution was mixed with 5ml water before measuring its absorbance against 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).

2.3 Antioxidant level
Total phenolic content (TPC) was estimated using Folin-Ciocalteu based on the method described by Khalil et al. [14] and Salleh et al. [15]. 0.2g honey was diluted in 1ml distilled water before the honey solution was mixed with 1ml Folin-Ciocalteu. 1 ml of sodium bicarbonate (10%) was added after 3 minutes. The solution was mixed and distilled water was added until 10 ml. The sample was left in the dark for 90 minutes. After incubation, the absorbance was read at 725 nm, with gallic acid used as a standard.

2.4 Storage condition
Evaluation on the effect of storage condition was conducted where moisture content, pH and TPC of honey samples were monitored monthly for six months. Samples were kept in glass jar, wrapped with aluminium foil and stored under low temperature of 4°C.

3. Results
3.1 Physicochemical analyses and antioxidant level
Physicochemical analyses were conducted to characterize the obtained samples prior to store as mentioned above. Honey samples displayed a diverse range of values, depending on the physicochemical analysis conducted, as observed in Table 1. All honey exhibited a small range of pH and ash content, while huge variations were observed in other analyses, in particular HMF content and free acidity. In several physicochemical analyses, variations were also observed for honey samples from the same type, as evident in the HMF analysis of Gelam honey and electrical conductivity of Tualang honey. Kelulut and manuka honey exhibited high antioxidant level compared to other honey samples.

3.2 Storage condition
The moisture content of honey increases while their pH level decreases the longer it is stored at low temperature (4°C), as shown in Figure 1. The moisture content of honey samples does not increase dramatically, except for Pineapple 2 honey which exhibited a 3.1% increase in moisture after six months. In general, the increase in monthly moisture content of honey at 4°C is very low (0.1 to 0.4%). pH of honey does not drop drastically at low temperature with the exception of Pineapple 2, Acacia 1 and Acacia 2 honey, which have shown a pH decrease of 0.6, 0.63 and 0.63 respectively after six months. On average, the pH level of honey decreases by 0.03 to 0.09 monthly.

TPC of honey samples increases over time when stored at low temperature (4°C), as observed in Figure 2. A remarkable increase in TPC was observed for both Kelulut 1 (40.81 mg GAE/kg) and
Gelam 2 (57.52 mg GAE/kg) honey after storage for six months. On average, the TPC of honey increases by 2.6 mg GAE/kg to 6.3 mg GAE/kg monthly.

Table 1. Physicochemical analyses and antioxidant level of honey samples from various origin before storage procedure

| Honey   | Moisture (%) | pH  | Free acidity (meq/kg) | Ash (g/100g) | Electrical conductivity (mS/cm) | HMF (mg/kg) | TPC (mg GAE/kg) |
|---------|--------------|-----|-----------------------|--------------|---------------------------------|-------------|-----------------|
| Manuka  | 19.4         | 4.30| 6.5                   | 0.332        | 0.721                           | 63.42       | 71.20           |
| Kelulut 1 | 32.5       | 3.67| 24.0                  | 0.600        | 1.187                           | 14.34       | 89.25           |
| Kelulut 2 | 34.0       | 3.63| 26.0                  | 0.594        | 1.178                           | 25.60       | 51.94           |
| Acacia 1 | 21.0         | 4.03| 7.5                   | 0.438        | 0.906                           | 2.09        | 43.75           |
| Acacia 2 | 20.4         | 3.99| 7.5                   | 0.430        | 0.893                           | 3.73        | 40.21           |
| Pineapple 1 | 24.0      | 3.57| 5.0                   | 0.118        | 0.348                           | 25.13       | 33.96           |
| Pineapple 2 | 23.2      | 3.99| 4.0                   | 0.090        | 0.299                           | 4.94        | 26.60           |
| Gelam 1  | 27.0         | 3.66| 6.0                   | 0.350        | 0.753                           | 5.82        | 30.64           |
| Gelam 2  | 23.5         | 3.76| 7.0                   | 0.418        | 0.872                           | 114.81      | 60.85           |
| Tualang 1 | 23.3       | 4.21| 11.0                  | 0.248        | 0.576                           | 13.46       | 68.09           |
| Tualang 2 | 26.6         | 4.30| 6.0                   | 1.067        | 2.003                           | 7.42        | 52.67           |

Figure 1. Selected physicochemical analyses on several types of honey stored at 4°C for six months. (A) Moisture content and (B) pH
4. Discussion
Most of the physicochemical properties (moisture, electrical conductivity, ash content, pH and free acidity) of honey samples used in the study are in accordance with the values set by the Codex Standard for Honey [12]. In terms of HMF level, all the honey samples show below than level set by the Codex (80mg/kg) except for the Gelam and Manuka honey. Both honey collected/purchased at least six months earlier than the other honey. Thus, the honey samples displayed higher level of HMF. It is difficult to obtain fresh Manuka honey since the honey is originally produced in New Zealand. Meanwhile, six month-Gelam honey was used because of lacking the honey sample during the sampling activity and can be used to compared with the Manuka honey. The finding is parallel to a current study by Shapla et al. [20], which storage can increase HMF level.

The antioxidant level of the honey, in the form of total phenolic content are lower compared to other studies on Malaysian honey [13], [14], [16]. Differences in the physicochemical properties and antioxidant levels between different honeys could be attributed to different floral sources and geographical origins. Differences in the physicochemical properties are common even for honey of the same type and at similar geographical origins [17].

The effects of storing honey at low temperature (4°C) for long periods of time were demonstrated by the changes observed in the moisture, pH and antioxidant level of honey. Contrary to honey stored at freezing temperature which experience reducing moisture content [18], honey stored at low temperature of 4°C has a slight increase in moisture. This could be due to the hygroscopic property of honey combined with the humidity present in the freezer at low temperature. Increase in moisture content above permitted level will increase the chances of honey spoilage through fermentation [18],

![Figure 2. Antioxidant level of honey samples stored at 4°C for six months](image-url)
[19], which lowers its quality. Thus, to avoid the increase for prolong storage at low temperature, it is recommended that the storage apparatus is sealed from air, not exposed to multiple freeze-thaw cycles and presence of air in the container reduced to a minimum.

Honey pH levels decreased slowly throughout time when stored at low temperature. This might be correlated to the increasing honey moisture content. However, some honey have shown dramatic decrease in pH level (Acacia 1, Acacia 2, Pineapple 2) but only experience slight increase in moisture content. This indicated that there might be other factors that influenced the pH of honey such as the reactions between various components of honey [17] and concentrations of ions present in honey solution.

It is expected that honey’s antioxidant level will decrease slowly at low temperature over time as a food is susceptible to degradation process during long storage period. Phenolics could degrade to other volatile compounds, a by-product of degradation. Floral sources and different sampling area, may affect the antioxidant level as well. However, surprisingly the antioxidant level of honey samples was found to increase throughout the six months storage period. Particularly, Gelam honey showed the highest level of antioxidant. This is in agreement with a study by Batumalaie et al. [21] that Gelam honey contained high level of antioxidant properties particularly phenolics. This may indicate that complex interactions between myriads of components in honey could affect its chemical composition which in turn affects its antioxidant level [22]. Through the reaction of enzymes or other secondary reactions, honey may degrade into new phenolic compounds or other by-products. Ultimately, it is important to note that the increase in honey antioxidant level would not last indefinitely. The antioxidant level might stop or decrease at certain time point after the six months period, which warrants further investigation in the future.

5. Conclusion
Storage of honey at low temperature above freezing point for six months will increase its moisture and antioxidant level and decrease its pH level. However, a long term study needs to be conducted to observe whether this pattern will continue indefinitely or cease to exist at a certain point in time.

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