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Heat impact on total phenolic content and antioxidant activity of Malaysian Tualang and Kelulut honey

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Abstract

The effect of temperature at 50°C, 70°C and 100°C for up to 120 min on the content of phenolic compounds and antioxidant activity of Tualang honey and Kelulut honey was studied. Unheated honey samples for both varieties were used as control. The total phenolic content and antioxidant activity were examined using Folin-Ciocalteau and DPPH assays, respectively. Both honeys vary widely in the total phenolic content as well as antioxidant activity upon heating and time of heating. The total phenolic content in both honeys showed fluctuations at all temperatures throughout 120 min. In comparison to controls for both honeys, heating at 100°C for 10 min demonstrated a drop in total phenolic content in Tualang honey (13%) and Kelulut honey (29%), but the antioxidant activity increased 29% and 57% in Tualang and Kelulut honey, respectively. The study generates information on the characteristics of components in both honeys which react differently to heat and time of heating, therefore this will help the honey manufacturers or public to optimize the processing protocols and later to preserve the quality of honey.

1. INTRODUCTION

Honey has been well documented as having antioxidant activity (Ahmed *et al.,* 2018). Various organic compounds with antioxidative properties such as polyphenolics, ascorbic acids, amino acids, protein and Maillard reaction products could act as antioxidant agent. Among these substances, various studies have shown a positive correlation between phenolic compounds presence in honey and the antioxidant activity (Di Marco *et al.,* 2018; Vasić *et al.,* 2019). However, some authors found a relation between proline (Meda *et al.,* 2005), amino acids (Perez *et al.,* 2007) and Maillard reaction products (MRPs) (Brudzynski and Miotto, 2011a) with antioxidant activity in certain types of honey.

Generally, honey is consumed raw or added to foods. Nowadays, honey is one of the natural products that widely being used as preservative in processed food and beverages. Other than that, honey is also commonly been added in barbeque and confectionary products to enhance the taste and colour of the foods. At the manufacturing site, the thermal treatment is subjected to honey for delaying crystallisation process, destroying yeasts, and facilitating filling by viscosity reduction (Turhan *et al.,* 2008). The temperature used for baking and industrial thermal treatment varies. Industrial heating is usually carried out in two ways, either in air-ventilated chambers at 45–50°C for 4-7 days or by immersion of honey containers in hot water (Fallico *et al.,* 2004), while baking temperature is commonly above 100°C.

In Malaysia, Tualang honey is produced by *Apis dorsata*, a wild jungle honeybee, whereas Kelulut honey is commonly produced by stingless bees such as *Geniotrigona thoracica*, *Tetragonula laeviceps* and *Heterotrigona itama* which have been successfully domesticated for the honey. Tualang and Kelulut honey are commonly used for medicinal purposes in Malaysia. The most general way of consuming these honeys is to dissolve them in plain hot water or mix the honey in hot beverages like tea, coffee, or chocolate. Studies showed that the heating process could jeopardise the antioxidant property of honey by suppressing its natural antioxidants (Jahan *et al.,* 2015; Nayik and Nanda, 2015). Furthermore, the thermal treatment of honey also favour the formation of 5-hydroxymethylfurfural (5-HMF), an intermediate product from Maillard reaction that has been a concern for public health (Önür *et al.,* 2018; Shapla *et al.,* 2018). This

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compound can exert various toxicity effects in human (Han *et al.,* 2017; Severin *et al.,* 2010). The antioxidant compounds and activity in honey are expected to differ in their stability once subjected to temperature. Therefore, the aim of this study was to investigate the effect of heat treatment on the total phenolic content and antioxidant activity of the Malaysian multifloral types of honeys; Tualang and Kelulut honey.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid were purchased from Sigma-Aldrich (St., Mo., USA). Sodium carbonate was purchased from Merck (Darmstadt, Germany).

2.2. Honey samples

Tualang honey and Kelulut honey were harvested in October 2017. They were obtained from local apiarist in Kota Bharu, Kelantan. The Kelulut honey was produced by the stingless bee species (*H. itama*). Both types of honey were packed in glass bottles and kept at 4°C until use. The duration between honey collection and the assays was within one month.

2.3. Heat treatment of honey

Samples of honey (20 g) were individually placed in 25 ml glass bottles and incubated in the water bath (Julabo PC, Life Technologies) at 50°C, 70°C and 100°C. Samples (2 ml) of each of these were removed after 10, 30 and 60 min and quickly chilled in a container filled with ice cubes. Then, the samples were subjected to the assays immediately. Temperature parameter was chosen since it is one of the vital factors during cooking process and industrial scale. Control samples were prepared in the same way without temperature treatment (left at room temperature, 25°C). All samples were prepared in triplicate.

2.4. Determination of total phenolic content

The total phenolic content (TPC) in honey samples were determined by using the Folin-Ciocalteu method as described by Piljac-Zegarac *et al.,* (2009) with slight modifications. From the 2 ml of each of the heated honey samples, a concentration of 1 mg/mL of honey solution was prepared. The honey solution (0.5 mL) was mixed with 2.5 mL of 0.2 N solution of Folin-Ciocalteu reagent for 5 minutes. Then, 2 mL of sodium carbonate solution (75 g/L) was added to the test mixture. After that, the mixture was incubated for 2 hours in the dark at room temperature. By using a UV-VIS spectrophotometer (CARY 50), the absorbance of reaction mixture was determined at 760 nm. Gallic acid solution in different concentrations between 0 to 250 mg/L was used to construct a calibration curve. The TPC was expressed as mg of gallic acid equivalents of 1 g honey (GAE/g). All samples were performed in triplicate.

2.5. DPPH antioxidant activity

The radical scavenging capacity of each types of honey was estimated using the DPPH assay as described by Jimenez *et al.,* (2016). Briefly, 0.75 ml of honey sample (1 mg/mL) was mixed with 0.75 ml of DPPH reagent in methanol (0.02 mg/mL). The mixture then was kept in the dark for 30 min. The absorbance of the reaction was measured at 517 nm in a UV/VIS spectrophotometer against a methanol as blank. The radical scavenging activity was calculated as the percentage of DPPH discoloration using the equation:

$$\% \text{ RSA} = (A_{DPPH} - [A_{sample}]/A_{DPPH}] \times 100$$, where A_{DPPH} is the absorbance of DPPH solution and A_{sample} is the absorbance of the mixture honey solution and DPPH.

2.6 Statistical analysis

All assays were carried out in triplicate. The data were expressed as mean ± standard deviation. A one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test was carried out to determine any significant different in TPC and RSA in each types of honey once subjected to temperature comparing to control. Data were entered in Microsoft Excel® (2010) statistical package and analysed using SPSS for Windows, Version 21; SPSS Inc., (Chicago, IL, USA). A p value less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Effect of heat on total phenolic content (TPC)

Figure 1 shows the changes in the TPC values of honey samples exposed to different temperature for 60 min. TPC in Tualang honey showed reduction at 100°C (Figure 1a), whereas Kelulut honey decreased at all temperature throughout 60 min (Figure 1b) as compared to the controls. In comparison to controls for both types of honey, heating at 100°C for 10 min clearly showed a 29% reduction of TPC in Kelulut honey and 13% reduction in Tualang honey. Heating the Kelulut honey for 10 min at 50°C and 70°C has also triggered a decreased of TPC by 22% and 25%, respectively. It could be concluded that phenolics in Kelulut honey were not stable to heat even the heat was introduced for a short time. In Tualang honey, the TPC values remained stable at 50°C and 70°C throughout 60 min. It is interesting to note that Tualang honey exhibited higher TPC value indicating higher concentration of phenolics than Kelulut honey as shown in controls (Figure 1a and Figure 1b). High TPC indicates high concentration of phenolics. Similar result was reported by Zainol (2016) who noticed that Tualang honey had more phenolic (1.961 ± 0.014 µg GAE/mg extract) than Kelulut honey (1.667 ± 0.019 µg GAE/mg extract).

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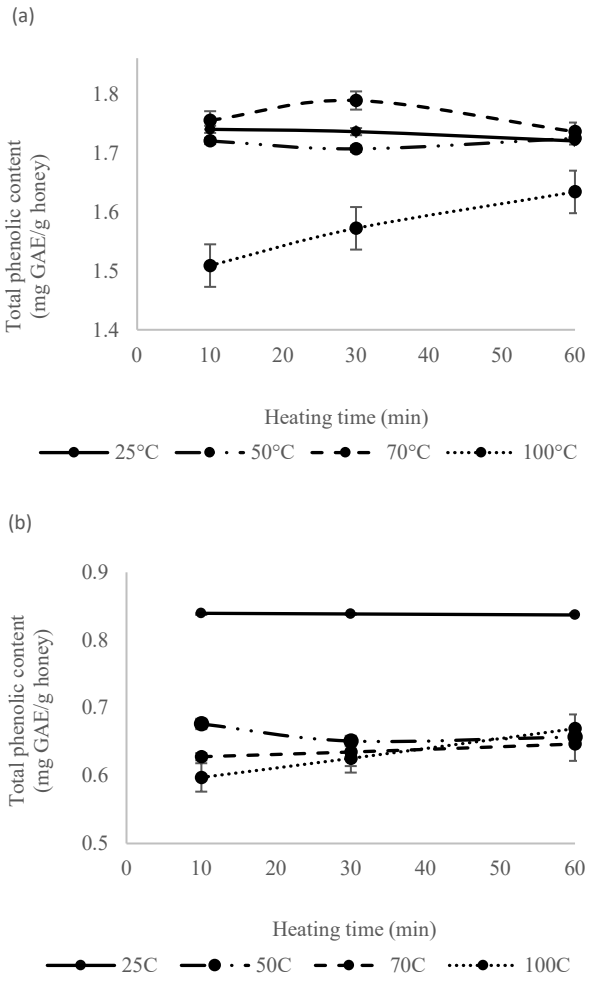

Figure 1: Effect of temperature on total phenolic content in (a) Tualang honey, (b) Kelulut honey. Unheated honey of both honey types was used as control.

The variability in TPC values between unheated Tualang and Kelulut honey in this study was expected and could be due to several factors. Both types of honey are multiflora and the content of phenolics, including its concentrations, mainly depends on the botanical origin, collection region and harvesting season (Kavanagh *et al.,* 2019; Mahmoodi-Khaledi *et al.,* 2017). The wild honeybee, *A. dorsata*, builds its comb on Tualang (*Koompassia excels*) trees, collect nectar from various flowers in the deep jungle, whereas the Kelulut honeybees are commonly domesticated and collect nectar from nearby fruit trees. Ismail *et al.,* (2016) had made an assumption that the foraging activities of *Apis* and *Trigona* species affected the TPC in honey. In addition, Strenzeir *et al.,* (2016) observed that the body size of stingless bee species gave effect to the foraging behaviour of the bees, which could influence the content and type of phenolic compounds in honey.

From our results, it showed that Tualang and Kelulut honey may contain different phenolic compounds which response differently to the temperature. This result was in agreement with the suggestion of Del Pino-Garcia *et al.,* (2017) who suggested that each types of phenolic compounds did not react in the same way to heat. It was evidenced in this study that phenolics in Kelulut honey were more vulnerable to heat where they significantly degraded at all temperatures (p<0.05) as compared to control (Figure 1b). On the other hand, there was no significant degradation (p>0.05) of TPC in Tualang honey at 50°C and 70°C in 60 min of heating, but significant degradation (p<0.05) at 100°C throughout 30 min was observed. The possible explanation to the results is that in general, phenolic compounds are known as very unstable and thermolabile (Bakowska *et al.,* 2003; Decker *et al.,* 2005). However, recent findings showed that certain phenolic compounds are stable upon heating. Lindquist and Yang (2011) carried out the stability study for benzoic acid derivatives at temperatures ranging from 50-350°C for 10-630 min. They found that some of the benzoic acid derivatives showed very mild degradation at 150°C. Carciochi *et al.,* (2016) reported that quercetin and kaempferol are stable at 100-190°C while hydroxybenzoic acids showed more stability at 190°C than hydroxycinnamic acids counterparts in malted quinoa seeds. The data collected by Chaaban *et al.,* (2017), who studied the stability of flavonoids showed that glycosylated flavonoids are more resistant than aglycon flavonoids to heat treatment. Since honey contains various type of phenolic compounds (i.e; phenolic acids and flavonoids) the possibility of each phenolic compounds in Tualang and Kelulut honey reacted to heat is varied.

3.2 Effect of heat on antioxidant activity

Figure 2 shows the influence of temperature on the antioxidant activity of honeys. By comparing to controls, the antioxidant activity in Tualang honey increased significantly (p<0.05) at all temperatures within 60 min as compared to control (Figure 2a). The similar trend has also been observed in Kelulut honey (Figure 2b). Both types of honey demonstrated the highest antioxidant activity at 100°C, followed by 70°C and 50°C, respectively. These results are consistent with many works which reported the heat treatment can enhance the antioxidant activity in honey (Jahan *et al.,* 2015; Nayik and Nanda, 2015; Saric *et al.,* 2013). It is interesting to note that heating at 100°C for 10 minutes have increased the antioxidant activity by 29% and 57% in Tualang and Kelulut honey. This condition might be due to the formation of MRPs which was likely to have occurred in the solution by the fact that the honey solution turned to brown in colour (Tamanna and Mahmood, 2015). MRPs are reported to form when honey is treated at higher temperature (Antony *et al.,* 2000). MRPs such as melanoidins, which exist in honey are known to have antioxidant activity (Brudzynski and Miotto, 2011b). MRPs could also have occurred at 50°C and 70°C, but different type of compounds might have involved in the

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antioxidant activity due to the fact that the antioxidant activity of honey at 50°C and 70°C were lower than at 100°C. This is possible since the formation of MRPs involves three major stages (i.e; early, advanced and final stages) which different types of MRPs are formed at each stage (Tamanna and Mahmood, 2015).

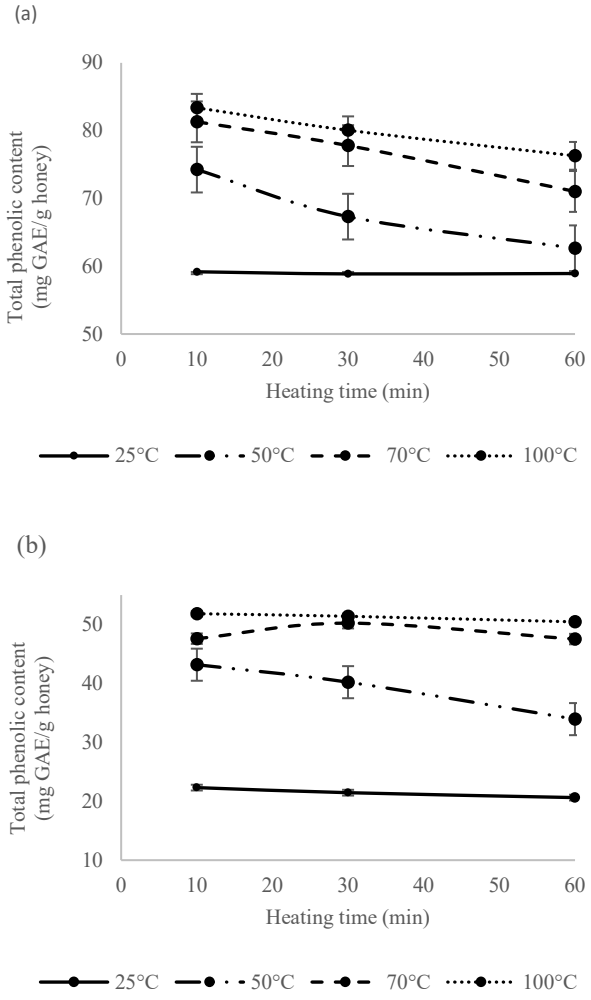

Figure 2: Effect of temperature on radical scavenging activity in (a) Tualang honey, (b) Kelulut honey. Unheated honey of both honey types was used as control.

High increment of antioxidant activity in Kelulut honey (57%) might be due to different phenolic compounds that react with MRPs to gain the activity. Brudzynski and Miotto (2011a) suggested that phenolic compounds in honey may exert their antioxidant activity through their interaction and incorporation with other molecules, specifically MRPs, rather than acting alone. However, it is difficult to confirm the radical scavenging activity recovered after the heat treatment is originally from either phenolics or MRPs. Another type of analysis such as liquid chromatography-mass spectrometry (LC-MS) needs to be carried out to determine the compounds in the honey solution. In spite of that, the drawback of DPPH assay is that the DPPH radical does not react with flavonoids that lack of hydroxyl groups in B ring and with monohdroxylated aromatic acids (Deng *et al.,* 2011). It is because in this assay, the DPPH radical received an electron or hydrogen from an antioxidant compound to become a stable diamagnetic molecule which results in the decrease in absorbance (Amorati and Valgimigli, 2015; Kedare and Singh, 2011). It is also worth noting that molecule with weak hydrogen bond or reductants (with no antioxidant activity, e.g. H_2O_2) prefer to react with DPPH radical (Amorati and Valgimigli, 2015). Furthermore, a steric hindrance effect in this assay which limits for heavier molecules to react with DPPH radicals (Schaich *et al.,* 2015; Xie and Schaich, 2014). Therefore, high molecular weight of MRPs such as melanoidins (Brudzynski and Miotto, 2011b; Wang *et al.,* 2011) could possibly skipped the reaction. Due to the drawbacks of this assay, it is reasonable to assume that certain group of compounds present contribute to the overall antioxidant capacity in these honeys, rather than on single compound.

Currently, there is a major concern related to public health relevant regarding the changes in bioactive components especially in the formation of MRPs or other compounds that are not naturally present in honey when honey is subjected to heat. MRPs such as carboxymethyl lysine promotes diabetes and cardiovascular diseases, with acrylamide acts as a carcinogen (Capuano and Fogliano, 2011). Besides, furan derivatives, such as 5-(hydroxyl-methyl)-2-furaldehyde (5-HMF), 5-chloromethyl and 5-sulfidemethylfurfural are significantly manifested their toxicity effects (Janzowski *et al.,* 2000; Severin *et al.,* 2010). Apart from moisture content, conductivity and total acidity of honey, 5-HMF also is used as an indicator for the freshness of honey and its quality (Islam *et al.,* 2014). The high value of 5-HMF indicates overheated or long stored honey (Khalil *et al.,* 2010; Kowalski, 2013), which influence the quality of honey. The European Union (2002) has set up a lower limit of 40 mg/kg for honey with an exception; 80 mg/kg is allowed only for honey that originates from countries or regions with tropical temperatures. However, 5-HMF is usually absent (Escriche *et al.,* 2008), or occur in a very low concentration in freshly raw honey (Truzzi *et al.,* 2012). Kowalski (2013) showed that there is a relative increase in the content of 5-HMF in honey during conventional heating (90°C up to 60 min) and microwave processing (power level 1.26 W/g up to 6 min).

Our study has clearly demonstrated that heat treatment to different types of honey affected their bioactive compounds, either in degradation of the naturally present compounds or the formation of new compound(s) in honey during heating. This has been shown by the TPC and DPPH results for both types of honey. However, the effect of high temperature on honey enzymes should not be neglected. For instance, glucose oxidase, which facilitates the formation of hydrogen peroxide that contributes to the antibacterial activity is thermolabile. Kretavicius *et al.,* (2010) showed that at 70°C, the activity of glucose oxidase

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was reduced by 10%. The existence of naturally occurrence sugar in honey without doubt stimulates the formation of MRPs. Even though the exact new formation compounds are unknown in the present study, the information given by other studies should be considered. It also should be borne in mind that MRPs that change during food processing might contribute for either disease progression or combating diseases (Tamanna and Mahmood, 2015). Nevertheless, it is worth noting that many honey producers will process honey by heating at mild temperatures below 100°C or pasteurization prior to packaging. This practice is more obvious if honeys are catered for exportation. Therefore, at this stage, the introduction of heat to honey is unpreventable.

4. CONCLUSION

The present results describe the effect of temperature to TPC and antioxidant activity in Malaysian Tualang honey and Kelulut honey. The results showed that temperature affects the TPC and antioxidant activity of the studied types of honey. High phenolics content and antioxidant activity in Tualang honey before or after heat treatment may relate to its floral sources. It is known that tropical jungles are highly loaded with floral diversity. Hence, it is a good reason to sustain such ecosystem by preventing the human activity such as logging in order to preserve the quality of Tualang honey. This finding also helps in understanding the behaviour of components in honey during heat treatment. However, due to limitations in colourimetric assay, a more accurate and reliable methods for quantification is required. Hence, more accurate identification method such as high-pressure liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) techniques would be necessary to be used to avoid overestimated of results given by colourimetric methods. However, the use of the Folin-Ciocalteu and DPPH assays with consideration of potential interferences in honey samples could lead to very informative results.

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