Volume 2991, Issue 1
7 June 2024

PROCEEDINGS OF THE INTERNATIONAL CONFERENCE ON GREEN ENGINEERING &

RESEARCH ARTICLE | JUNE 07 2024

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AIP Conf. Proc. 2991, 020051 (2024)
https://doi.org/10.1063/5.0199035

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Investigating The Effect of Thermal Treatment on Nutritional Properties of Kelulut Honey

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Abstract. The natural antioxidants flavonoids and phenolic acids make Kelulut honey a healthy alternative, but heating it over 90 degrees Celsius can cause it to lose some of its flavour and nutritional value. The aim of this study was to investigate how thermal treatment affects the antioxidant activity, total phenolic content (TPC), and brown pigment production of Kelulut honey. The samples were heated at three different temperatures (50, 60, and 70 degrees Celsius) for various durations (5, 10, 15, and 30 minutes). Antioxidant activity and TPC were assessed using the DPPH radical scavenging method and the Folin-Ciocalteau reagent, respectively. The findings revealed that the honey heated to 70 degrees Celsius for 15 minutes had the highest antioxidant activity compared to the unheated honey. The TPC was at its peak after 5 minutes of heating at 50 degrees Celsius, but it decreased significantly after 30 minutes. The optimal nutritional value of honey was achieved by treating it at 70 degrees Celsius for 15 minutes. Fourier transform infrared spectroscopy was used to identify the phenolic compounds, and the IR spectra peaks were found to be located in the hydroxyl group (-OH) in all samples. Therefore, it can be inferred that Kelulut honey's nutritional value was not impaired by thermal treatment at 50-70 degrees Celsius for 5-30 minutes.

INTRODUCTION

Because of its nutritional and medicinal benefits, honey has been valued as a significant natural food product since ancient times. Honey is made from liquid plant exudates, which are collected, processed, and stored by various species of bees. The wonderful health benefits honey provides have led to a dramatic increase in the import and export of honey in recent years. Like other nutritional supplements, the efficacy of honey for medicinal purposes depends on its purity. Honey quality is often determined by its sensory, chemical, physical, and microbiological characteristics [1]. Honey is a naturally occurring substance that consists of a highly concentrated solution of various sugars. Minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes, and other phytochemicals can be found in minute quantities as well [2].

Kelulut honey, also known as KH, is produced by stingless bees of the Trigona and Meliponine species, both of which are native to the Malaysian rainforest and can be harvested for their honey. Its traditional use in the treatment of conditions like as coughs, sore throats, acne, and burns has contributed to its rise in popularity. This honey has several beneficial pharmacological qualities, including antibacterial, anti-inflammatory, and antioxidant benefits, and it contains many of these properties. According to the findings of the earlier research [3], the level of antioxidant activity present in KH is twice as high as that found in Tualang honey. According to the findings of a large number of studies, the antioxidant capacity of honey varies widely depending on the floral source. The antioxidant activity of honey is primarily determined by the plants from which it is derived, while factors such as processing, handling, and storage have only a marginal bearing on the phenomenon [2].

The process of oxidation generates free radicals such as superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide. These radicals are formed due to various factors such as exposure to sunlight, pollution, processed

food, stress, and environmental contaminants. These free radicals can trigger a chain reaction in the body that damages cells in the affected area. Antioxidants are chemicals that prevent molecules from undergoing oxidation and becoming reactive. They can donate an electron to free radicals, which helps to boost the immune system and protect cells from harm [4]. Eating foods rich in antioxidants has been shown to have beneficial effects on health. Kelulut honey, with its high phenolic content, may be a potential source of antioxidants and have superior antioxidant effects compared to honey with a lower phenolic content [5].

In addition to yeast, honey contains a number of undesirable components that, in order to enhance the product's quality and extend its shelf life, need to be removed. Heating up such substances to a predetermined temperature is the quickest and most efficient approach to get rid of them [6]. Therefore, thermal processing can be utilised in the same manner that it is typically utilised in the production of food. This process requires a significant amount of energy and consists of preheating the honey to a temperature of 40 degrees Celsius, straining it, filtering it, and then heating it indirectly at a temperature of 60–65 degrees Celsius for 25–30 minutes in a tubular heat exchanger. This is done in order to preserve the honey's natural colour, flavour, enzyme content, and other biological components [7]. Caramelization, lipid oxidation, and the Maillard reaction are all processes that could take place along the way; if they do, the result will be unwanted byproducts and alterations. Raw honey has a low sensory impact since it is prone to crystallisation, it is contaminated with germs and impurities such as bee wax, bee nests, and bee pupae, and it contains bacteria.

Thermal treatment, that is commonly used in raw honey processing, is a technique that a manufacturer could use to dissolve crystals, lower viscosity and eradicate bacteria all at the same time [8]. However, honey's quality is known to be affected by heat treatment due to unstable and thermolabile components, vitamin breakdown, and enzyme integrity degradation. Honey should not be heated beyond 90°C since it destroys bioactive components and antioxidants, lowering product quality [9]. When pasteurizing and stabilizing honey, the temperature and time of treatment must be kept to a minimum since thermal treatment might raise the 5-hydroxymethylfurfural (HMF) concentration [7]. HMF concentration is generally acknowledged as a factor influencing honey freshness, and a prior study found that honey with a low HMF concentration has better quality than honey with a high HMF concentration [10].

The conventional method of applying heat to complex food systems often results in a decline in their sensory, physical, and nutritional attributes. The non-uniformity of the process, as well as overcooking in the region with the slowest heat, contributes to this. Therefore, the primary aim of this investigation is to examine the changes that occur in the nutritional properties of KH when it is thermally processed at different temperatures (50, 60, and 70 °C) and heating durations (5, 10, 15, and 30 minutes). This research will also assess the total polyphenolic content (TPC), total antioxidant activity, and brown pigment production of KH. Additionally, the composition of the phenolic compound present in KH was further investigated using FTIR. Finally, based on the findings, recommendations were proposed regarding the optimal conditions for thermal processing of KH samples.

MATERIALS AND METHODS

Honey Samples Preparation

The untreated stingless bee (Kelulut) honey was collected from the local breeder from Nyaleh Trigona Farm, Kg Nyalas, Melaka. The honey samples were stored in a glass bottle at about 10℃. Next, 6 g of the honey weighed with analytical balance in 12 test tubes to make it as triplicate samples. The samples were then being kept in a cold storage at temperature of 4 ℃ in order to keep them from contamination.

Thermal Treatment

In the thermal treatment process, the temperature was set at 50, 60 and 70 ℃ for 5, 10, 15 and 30 minutes. The equipment involved in this process was a thermostatic water bath. This setting was chosen based on the previous studies which have produced high amounts of antioxidant after treatment [11]. The time for the experiment to start was when the sample reaches the temperature at 50, 60 and 70 ℃. The samples were immediately put into an ice-cooled water in order to stop the heat treatment.

Determination of Total Antioxidant Activity

The DPPH test [12] was used to assess the samples' antioxidant activity, which was the focus of this research. To do this, ten minutes were spent centrifuging a mixture of two grammes of honey and ten millilitres of distilled water at a speed of 10,000 revolutions per minute. A digital pocket refractometer was used to filter and dilute the resulting solution to 4 °Brix. Then, 1.5 millilitres of honey was combined with 3.5 millilitres of 0.1 millimetre DPPH radial and suspended in methanol. In the dark at 25 degrees Celsius for an hour, the absorbance at 517 nm was measured using a UV-VIS spectrophotometer; a blank reading was taken from methanol, and a control reading was taken from a solution of distilled water and DPPH in methanol. Equation 1 was used to calculate the antioxidant activity, where AA (percent) is the antioxidant activity, Abscontrol is the absorbance reading from the control, and Abssample is the absorbance reading from the sample. This procedure was based on a reference [13], and triple analyses were performed on the samples [12].

$$AA(\%)=(Abs_{control}-Abs_{sample})/Abs_{control}\times 100 \quad (1)$$

Determination of Brown Pigment Formation

The technique developed by Turkmen et al. [12] for analysing the formation of brown pigments in Kelulut honey was applied and modified [14]. Honey samples weighing 2 grammes were mixed with 10 millilitres of distilled water using a vortex mixer; the resulting solution was centrifuged for 10 minutes at 10,000 rpm; the filtrate was then filtered through a Whatman No. 1 filter; and the final concentration was determined to be 4 degrees Brix using a digital refractometer model RX-5000a. Using an absorbance of 420 nm, a spectrophotometer determined the exact amount of brown pigment formed in the honey. On a regular basis, this process was repeated three times. The outcomes met expectations.

Determination of Total Phenolic Content

The Folin-Ciocalteu method [15] was used to calculate the total phenolic content (TPC) of each honey sample. Each sample was filtered using Whatman No. 1 paper after being diluted to a level of 20 mL with distilled water. After that, 75 mg/l sodium carbonate (in 0.4 ml) and 0.2 N Folin-Ciocalteu reagents (in 0.5 ml) were added and stirred for 5 minutes. Following a 2-hour room-temperature incubation, the solution's absorbance at 760 nm was measured against a blank of methanol. The total phenolic content (TPC) of the honey samples was expressed as GAE per 100 grammes of honey using a standard calibration curve produced using gallic acid [15].

Fourier-Transform Infrared (FTIR) Spectroscopy Analysis

FTIR was being used to determine the phenolic compound of Kelulut honey sample [16]. The IR absorption spectra were acquired in the range of 4000 to 400 cm^{-1} with a resolution of one data point per 1 cm^{-1} [17]. The data collected was displayed on a computer which was connected to the FTIR. The crystal surface on FTIR was cleaned with Kimwipe and distilled water before the sample was being analyzed. The cleansing was to prevent any error that could affect the results. One drop of honey was then placed on the surface of the crystal and the reading was measured. 39 samples of honey were being measured which includes untreated and treated honey samples at different temperatures and time.

Data Analysis

The analysis of data for total phenolic content, total antioxidant activity, and brown pigment production was conducted using Microsoft Excel (version 2016). The data were collected in two sets and averaged, with mean and standard deviation being used to summarize the information. To evaluate whether the untreated and treated honey samples differed significantly, the collected data were subjected to statistical analysis (one-way ANOVA).

RESULTS AND DISCUSSION

Effect of Total Antioxidant Activity

To assess the overall antioxidant activity of the honey sample, we employed a technique that involves converting the purple-colored DPPH free radical to the yellow-colored diphenylpicrylhydrazine [18]. A higher DPPH scavenging activity leads to a greater total antioxidant activity [19]. As indicated in Table 1, the unprocessed Kelulut honey sample exhibited an antioxidant activity value of (74.44 5.94%), which is consistent with research on honey produced by stingless bees. Previous studies on untreated Heterotrigona itama honey revealed DPPH scavenging activities ranging from 50 to 70% [20] and 50 to 60% [21].

TABLE 1. Antioxidant Activity (%) of untreated and treated Kelulut Honey samples at different treatment temperature and time.

Honey Samples	Time (min)	Antioxidant Activity (%)
Untreated		74.44 ± 5.94a
50	5	74.41 ± 12.22a
60		81.40 ± 3.71a
70		85.23 ± 2.45a
50	10	75.82 ± 11.94a
60		83.62 ± 3.10a
70		86.40 ± 0.443a
50	15	76.17 ± 9.00a
60		85.32 ± 1.710a
70		86.76 ± 2.35a
50	30	80.54 ± 4.66a
60		85.61 ± 2.14a
70		85.77 ± 2.63a

[a,b] Mean values (means ± standard deviation), where n=3 and the different letters in each column indicate a significant difference ($p<0.05$) based on Tukey's HSD test (Minitab v18, Statistical Software)

Due to the fact that most bioactive molecules are very unstable at higher temperatures, nutritional characteristics of food can be lost to a large degree during thermal processing. Heat treatment, on the other hand, might either have no effect or improve the antioxidant activity of certain foods [18]. In general, the antioxidant activity of treated Kelulut honey showed an increment with increased temperature. In particular, thermal treatment at 70℃ for 15 minutes had the highest antioxidant activity (86.76 ± 2.35%) compared to treatment at 50℃ for the same heating period (76.17 ± 9.00%). Thermal treatment seems to give positive influence on antioxidant activity of Kelulut honey samples as depicted by the increment of antioxidant activity along with increment of temperature. However, the antioxidant activity dropped after being treated at 70℃ for 30 minutes (85.77 ± 2.63%). This may happen due to the effect of prolonged treatment time which can cause the antioxidant activity to decrease. Earlier research has also suggested that depending on the treatment time, thermal pasteurisation might boost or reduce antioxidant extractability [22].

Figure 1 illustrates the total antioxidant activity of treated Kelulut honey samples whereby it shows an increment value with increase temperature and time. According to the findings, the increase in overall antioxidant activity could be related to the synthesis of molecules with varying antioxidant activity at different phases of Maillard reactions, depending on treatment temperatures [16]. Nevertheless, at 70 ℃ for 30 minutes the antioxidant showed a dropped value of antioxidant activity. The degradation of flavonoid molecules may contribute to a reduction in total antioxidant activity since the radical scavenging activity also depends on the flavonoid compounds [20]. From Table 1, there was no significant difference for its antioxidant activity between the samples as it has higher p-value of 0.082. From this study, it concludes that thermal treatment of 50, 60 and 70℃ do not have a significant effect on antioxidant activity ($p>0.05$).

However, the antioxidant activity still has an increasing value followed with increased temperature. Previous research showed that all their honey samples have an increase of total antioxidant activity with increasing temperature. The temperatures used were 45, 55 and 65℃ whereby it is similar to this study and the highest amount of antioxidant activity level was at 65℃ [23]. As compared to this study, the highest antioxidant level was at 70℃ for 15 minutes and this indicates that the results obtained are plausible. Previous study [24] has reported that their honey samples showed no significant difference after being treated at 50 and 70℃ for 5 minutes which is also similar to results

obtained for this study. However, when the samples were being heated at 100°C for 5 minutes, it showed a significantly lowest level of antioxidant activity ($p<0.05$). The result also decreased its quality of honey due to the degradation by high temperature and prolonged heating process.

As shown in Figure 1, at 70°C the antioxidant activity decreased (85.77 ± 2.63%) when being heated for 30 minutes. From the result obtained in this study, thermal treatment has affected the total antioxidant activity whereby it increases the antioxidant activity with increasing temperature. Hence, it can be deduced that the best temperature for treating Kelulut honey samples in order to retain a high antioxidant level is at 70°C for 15 minutes even though there is no significant difference was recorded for 60°C for same heating period.

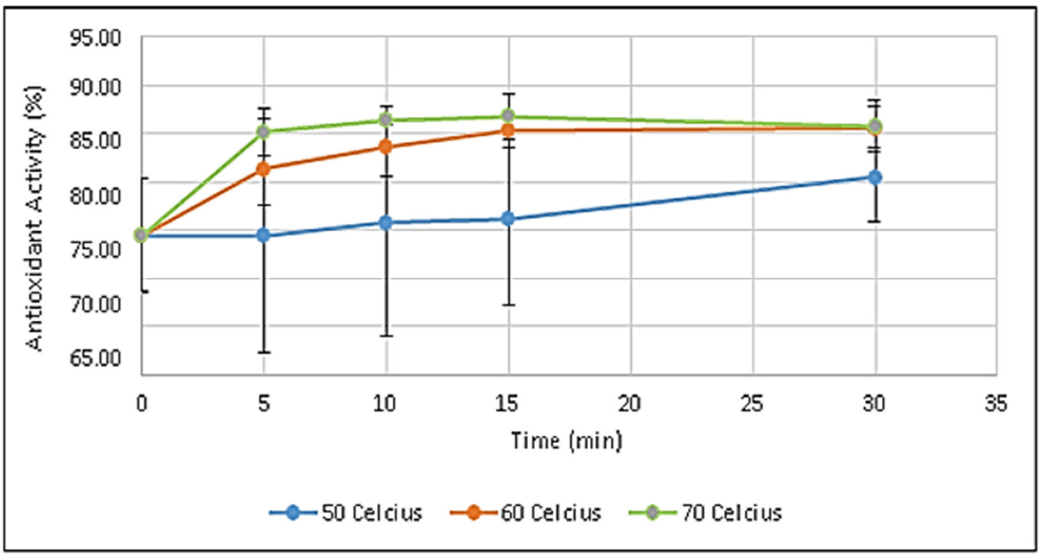

FIGURE 1. The effect of different temperature (50,60 and 70 °C) and time (5, 10, 15 and 30 minutes) on antioxidant activity of Kelulut Honey samples

Effect of Brown Pigment Formation

Browning is the progressive change in colour of foods from white to brown or dark brown over time, which can have a beneficial or bad effect on food quality. It has the potential to give processed foods a distinctive colour and flavour [25]. The brown pigment formation (BPF) showed (in Table 2) that untreated Kelulut honey samples surprisingly has a quite high value compared to other studies (0.186 ± 0.0307). A study by Fauzi & Farid [25] stated the untreated honey sample has smaller value of brown pigment formation (0.108 ± 0.010). In addition, another study by Starowicz et al. [26] has also stated that acacia honey, one of the stingless bee honeys, has BPF of 15-fold lower than buckwheat honey (3.73 – 3.51 AU420). Therefore, high BPF value (0.186 ± 0.0307) for this study may be due to the higher formation of melanoidin which is a Maillard reaction product. Concentrations of catalytic sugars and a pool of free amino acids were found to encourage and facilitate the reaction's final stage [27].

The BPF value shows an increment value along with an increment of temperature after 5 minutes of treatment. From the result obtained (in Figure 2), there is an uneven change throughout the treatment whereby it decreases after 5 minutes and continues to increase at 15 minutes for temperature at 60 and 70 °C. This may happen due to the processing of honey, storage area and thermal sensitivity [28]. The highest BPF was recorded at the temperature of 50 °C with treatment for only 5 minutes (0.255 ± 0.1396) as shown in Table 2. However, at temperature 50 °C with a heating period of 30 minutes, it showed the lowest value of BPF (0.168 ± 0.0215) and this may be due to the decrease of browning reaction and reduction of MRPs during the process. Heat treatment of honey elevated Maillard reaction products (MRPs), and the development of MRPs corresponded to improved antioxidant activity [29]. In addition, prolonged heating of Kelulut honey samples can also cause the browning reaction to decrease due to its correlation of total antioxidant activity at various temperatures [22,23].

From this study, analysis has been made in order to investigate the significant difference between untreated and treated samples. The result showed that there are no significant difference between samples ($p<0.05$). Previous study by Brudzynski & Miotto [28] and Molaveisi et al. [22], shows a similar result whereby there is no significant difference

despite having increasing value of total antioxidant activity and brown pigment formation. Except for one sample from two separate stingless bee species, another investigation found no significant inhibitory action ($p>0.05$) in either Malaysian or Australian stingless bee honey samples after being treated at 45, 55 and 65 ℃ [20]. However, a study from Jahan et al. [29] and Turkmen et al. [12] reported that there is significant difference between honey samples when high temperature and long heating period was applied. This implies that temperature and time are responsible for its influence on brown pigment formation.

From Figure 2, the best condition for treating honey can be decided. The most suitable temperature to minimize the formation of MRPs would be at 70℃ for 30 minutes. This is because the browning reaction indicates the formation of MRPs which have both positive and negative effects toward the nutritional properties of honey [30-31]. The increase of MRPs will cause structural changes in proteins that inhibit digestive peptidases from acting in the vicinity of crosslinks. The digestive enzymes' functionality will be harmed if there is a high concentration of MRPs [32]. On the other hand, with an increased amount of MRPs and total antioxidant activity, it can protect human lymphocytes against DNA damage [32].

TABLE 2. Brown pigment formation of untreated and treated Kelulut Honey samples at different treatment temperature and time.

Honey Samples	Time (min)	Brown Pigment Formation (AU420nm)
Untreated		0.186 ± 0.0307[a]
50	5	0.255 ± 0.1396[a]
60		0.249 ± 0.0609[a]
70		0.203 ± 0.0261[a]
50	10	0.200 ± 0.01332[a]
60		0.216 ± 0.0357[a]
70		0.207 ± 0.0372[a]
50	15	0.195 ± 0.00624[a]
60		0.210 ± 0.0289[a]
70		0.171 ± 0.01537[a]
50	30	0.168 ± 0.0215[a]
60		0.241 ± 0.0613[a]
70		0.182 ± 0.01457[a]

[a,b] Mean values (means ± standard deviation), where n=3 and the different letters in each column indicate a significant difference ($p<0.05$) based on Tukey's HSD test (Minitab v18, Statistical Software)

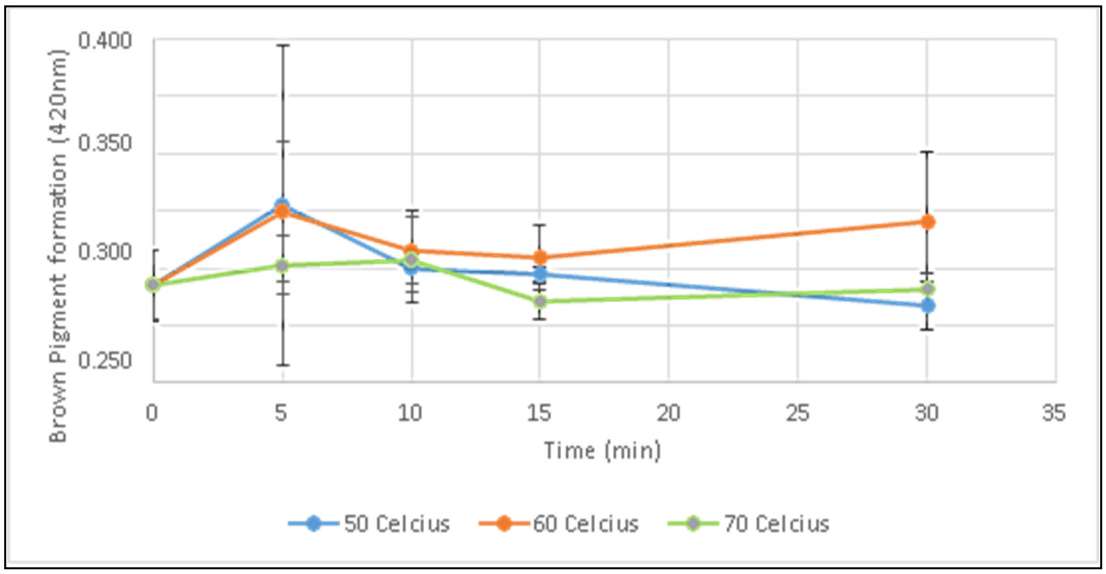

FIGURE 2. The effect of different temperature (50, 60 and 70 ℃) and time (5, 10, 15 and 30 minutes) on brown pigment formation (BPF) of Kelulut honey samples.

Phenolic molecules are linked to a wide range of biological activities, with antioxidant capacity being one of the most interesting [33]. The radical-scavenging and metal-chelating activity of phenolic compounds is related to their structure. A variety of tests can be used to determine the ability of phenolic compounds to scavenge free radicals [34].

Folin-Ciocalteu method was used in this study to determine the total phenolic content (TPC) throughout the treatment. The value of TPC for untreated honey sample in this study was 39.18 mgGAE/100g. Several studies showed a similar value of TPC for stingless bee honey which includes study from Shamsudin et al. [34] with value of 33.12 mgGAE/100g (Acacia honey), 40.44 mgGAE/100g (Starfruit honey) and 52.25 mgGAE/100g (Gelam honey) and a study from Kowalski [35] with value of 38.29 mgGAE/100g (Acacia honey). The previous study showed that data obtained for the current study to be within range of accepted value. It can also be explained that the inconsistent value of TPC for various types of stingless bee honey may be due to its geographical location, composition and source of food for honey bee [36]. The samples of thermal treated Kelulut honey had the lowest value of TPC (29.73 mgGAE/100g ± 3.38) at temperature 50 ℃ with heating period of 10 minutes (as shown in Table 3). However, the value of TPC for the untreated sample was higher (39.18 mgGAE/100g ± 0.955) and there is a significant difference ($p<0.05$) between both of the samples.

Effect of Total Phenolic Content

Table 3 shows the TPC of untreated and treated Kelulut honey samples. The highest value of TPC was at temperature 60℃ with heating period of only 5 minutes (40.26 mgGAE/100g ± 1.095) but it does not show significant difference to untreated samples ($p>0.05$). There is also an uneven trend from the results obtained which has affected the TPC for most of the treated samples. In theory, TPC of food samples will increase as the temperature increases with time [12].

TABLE 3. Total phenolic content of untreated and treated Kelulut Honey samples at different treatment temperature and time.

Honey Samples	Time (min)	TPC (mg GAE/100 g)
Untreated		39.18 ± 0.955[a]
50	5	29.73 ± 3.38[ab]
60		40.26 ± 1.095[a]
70		30.86 ± 1.53[ab]
50	10	25.60 ± 11.29[b]
60		38.95 ± 2.10[a]
70		34.59 ± 3.87[ab]
50	15	29.07 ± 3.80[ab]
60		39.44 ± 4.02[a]
70		38.56 ± 5.96[a]
50	30	28.27 ± 0.991[ab]
60		31.49 ± 1.86[ab]
70		34.27 ± 1.77[ab]

[a,b]Mean values (means ± standard deviation), where n=3 and the different letters in each column indicate a significant difference ($p<0.05$) based on Tukey's HSD test (Minitab v18, Statistical Software)

From Figure 3, it shows a decreasing value of TPC for all temperatures (50, 60 and 70℃) except at temperature 60℃ for heating time of 5 minutes. This may happen due to the composition and the condition that has affected the honey. Honey's geographical and botanical origins are thought to play a major role in determining the best heating conditions. Honey's biochemical content varies depending on where it comes from. A study has reported that individual samples showed unequal changes in antioxidant activity and total phenolic content, with some showing a drop-in antioxidant activity after heating and others showing an increase [12]. The total phenolic content behaves the same way.

Using three techniques of investigation (TPC analysis, DPPH radical scavenging assay and FRAP assay), statistical analysis of the results (t-test) revealed no statistically significant differences between the results obtained in different varieties of honey at two different temperatures, 23 and 95℃ ($P > 0.05$) [12]. Similar to this study, there are no

significant differences between the untreated sample (39.18 mgGAE/100g ± 0.955) and treated sample at temperature 60°C for 5 minutes (40.26 mgGAE/100g ± 1.095) despite having an increment of TPC. Figure 3 tells that a prolonged heat treatment may give a negative impact to the TPC of honey samples. Furthermore, the way honey is processed, handled, and stored has an impact on its TPC and antioxidant activity [19]. As a result, the therapeutic impact of honey may be diminished due to the absence of high phenolic substances such as flavonoids and phenolic acids [37-40].

From the result obtained for treated honey samples, the best temperature in order to retain or improve its TPC value would be at 70°C for 15 minutes treatment. A previous study has also supported that heating honey at a temperature of 70°C for 24 minutes has increased the TPC of honey (*Tetragonisca angustula*) sample [41]. The increase of TPC has resulted to an increase in its bioavailability and also total antioxidant activity. Several studies have found a strong and favorable correlation between phenolic components in fruits and vegetables and their antioxidant activity. A high concentration of both qualities not only preserves the quality of the food, but also lowers the chance of contracting certain diseases [42].

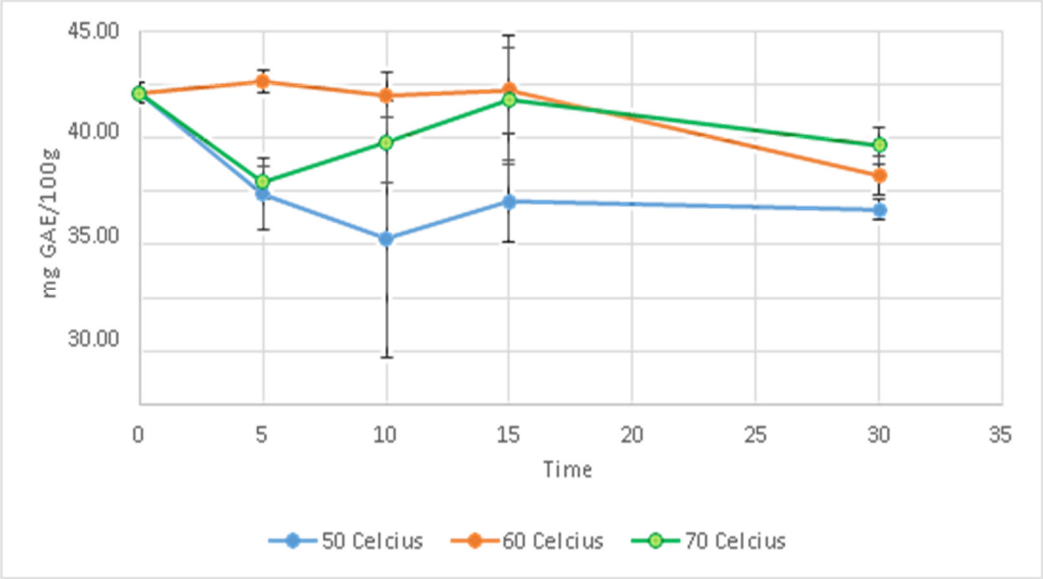

FIGURE 3. The effect of different temperature (50, 60 and 70 °C) and treatment time (5, 10, 15 and 30 minutes) on total phenolic Content (TPC) of Kelulut honey samples.

FTIR-Spectroscopy Analysis

Infrared (IR) light is employed in the Fourier transform infrared (FTIR) analysis [43] to assess the organic composition and components, such as chemical bonds. This study so characterised the functional class of Kelulut honey. Absorption bands at 3300 cm^{-1} (O-H stretching) and 1640 cm^{-1} (O-H deformation) are characteristic of water and can be seen in the untreated sample in Figure 4 [44]. Carboxylic acid's functional group was traced to a modest absorption between 2900 and 3000 cm^{-1} [45], indicating C-H stretching. The presence of carbohydrates, free amino acids, and phenolics may account for the detection of ammonia (NH_3) at the desired absorption (2800 - 3000 cm^{-1}) [16]. The existence of phenolic molecules was indicated by the presence of a substantial band from 1700 cm-1 to 1600 cm^{-1}, which is thought to be caused by the stretching of the carbonyl group C=O and C=C. Deformation of O-H, C-O, C-H, and C=C functional groups, which are associated with flavanol and phenol molecules, is shown in the area between 1500 and 1100 cm^{-1}.

In addition, a previous study by Nandiyanto et al. [42] stated that the functional group for phenol or tertiary alcohol (OH bend) is within range of 1410 to 1310 cm^{-1} and primary amine (NH bend) is within range of 1590 to 1650 cm^{-1}. This proves that composition for untreated honey in this study can be accepted and in line with previous study as it also showed a similar result for pure honey [43-45]. From this study, phenolic compound was presented within various range of IR-spectrum absorption but most of the phenolic group was within range of 1410 cm^{-1} to 1310 cm^{-1} [43,46,47].

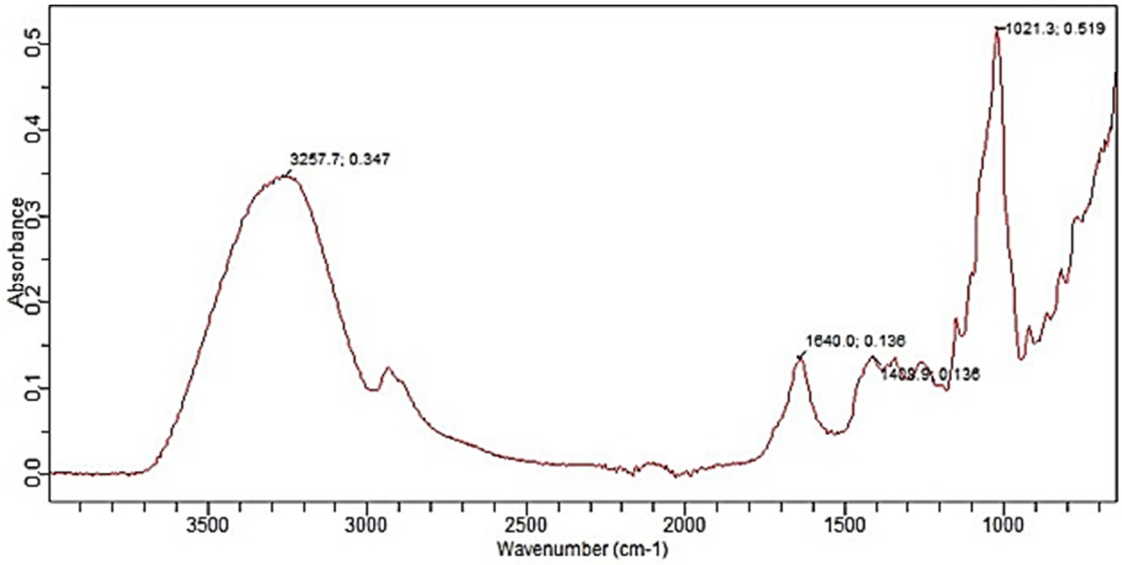

FIGURE 4. The absorbance value of untreated sample

Thermal treatment may result in deterioration of Kelulut honey samples at certain temperature and time. From Figure 4(b), (c) and (d), the absorption band from FTIR-spectra showed a similar absorption band for both untreated and treated honey samples at temperatures of 50, 60 and 70°C for 5, 10, 15 and 30 minutes.

Therefore, from this observation it can be deduced that thermal treatment gives no effect to the chemical composition of honey.

However, chemical composition can be further analyzed especially for its phenolic compound. This can be done by conducting analysis of honey with the help of Gas Chromatography – Mass Spectrometer (GC-MS). Previous study shown that the analysis can be done through ethanolic extraction of honey sample and its structural composition for phenolic component can be identified [47].

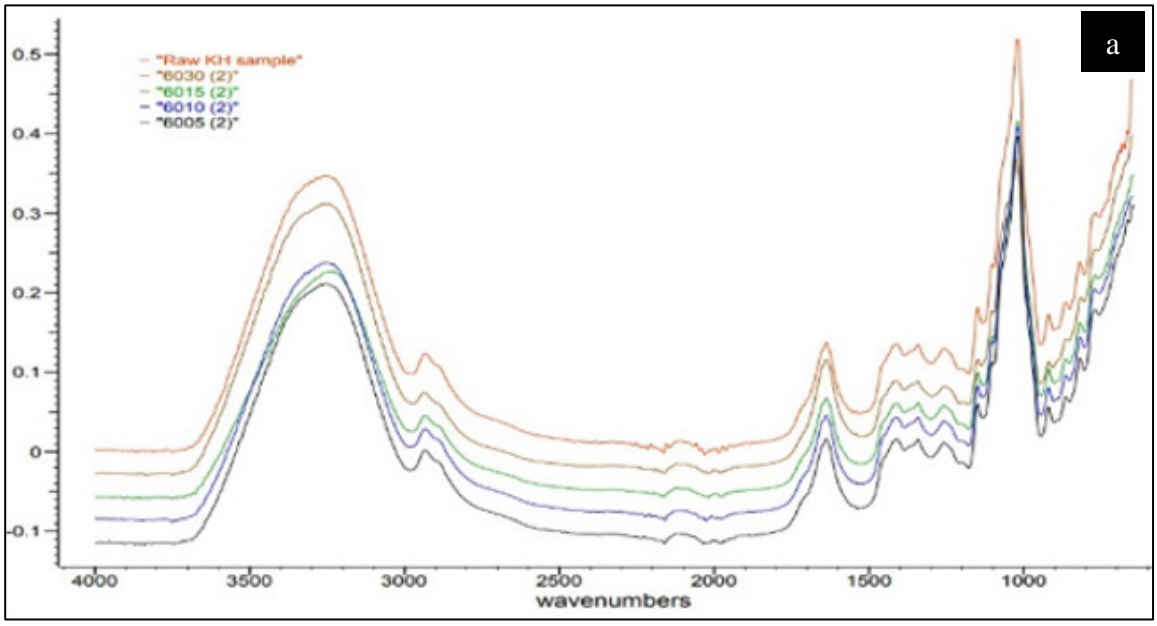

FIGURE 5(a). FTIR-spectra of untreated honey (a) and thermal treated honey at temperature of 50°C

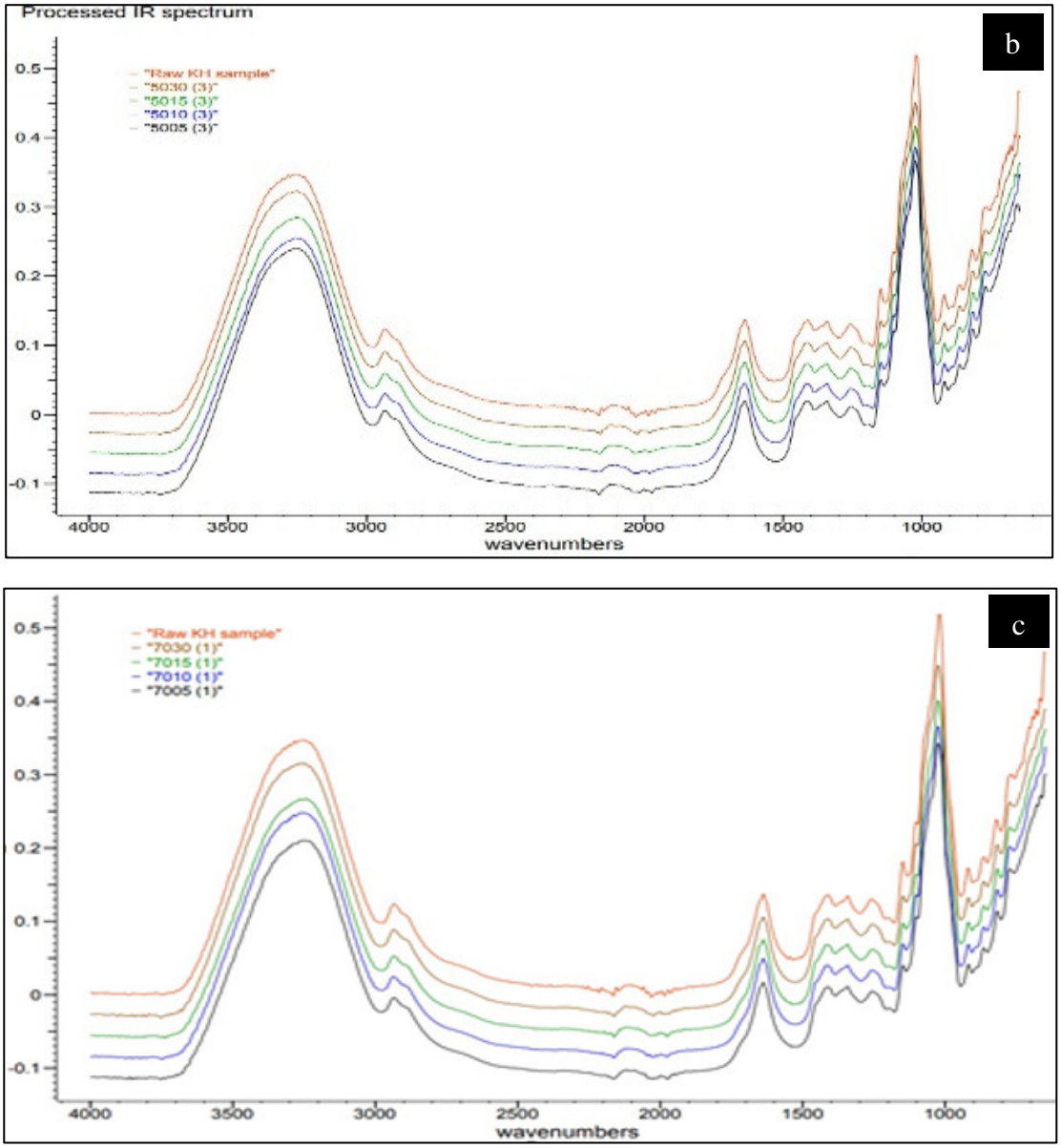

FIGURE 5(b) & (c). FTIR-spectra of untreated honey (a) and thermal treated honey at temperature of 60℃ (b) and 70℃ (c)

CONCLUSION

In a conclusion, thermal treatment within the temperature range of 50-60℃ has the ability to improve nutritional properties of honey. The best temperature would be at 70℃ for 15 minutes heating time as it increases the total antioxidant activity and phenolic compound for Kelulut honey. Therefore, thermal treatment can still be a good food processing method of Kelulut honey as it can remain its nutritional properties as untreated one.

ACKNOWLEDGEMENTS

The first author is grateful to the Fundamental Research Grant Scheme (K302) of the Malaysian Ministry of Higher Education. Nyaleh Trigona Farm's Kelulut honey samples are likewise very much appreciated. For their technical

assistance, we are extremely grateful to the Department of Chemical Engineering Technology in the Faculty of Engineering Technology.

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