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Original Research Article

Comparison of Physicochemical Properties and Hydroxymethylfurfural Content of Tualang Honey Treated Using Conventional Heating and Ultrasound Technique

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Abstract: Conventional heating is a common treatment applied to honey to preserve its quality and facilitate packaging. The treatment will reduce honey's viscosity and moisture level, destroy yeasts, liquefy crystals, and delay crystallisation. However, conventional heating may increase honey's hydroxymethylfurfural (HMF) content, which is a potential carcinogen to humans and leads to quality deterioration. Alternatively, the use of ultrasound to treat honey can minimize heat exposure. This study was conducted to determine the suitable process conditions to be applied to Tualang honey and to compare the quality of Tualang honey treated using two different techniques. The quality of honey was determined from physicochemical analysis (turbidity, colour, pH, water activity, and moisture content) and HMF analysis using the White method. Tualang honey samples were treated under different conditions: 50-90°C for 1-60 min using conventional heating and 50-90% amplitude for 10-30 min using ultrasound technique. The best situation for each treatment was determined at 70°C for 15 min using conventional heating and 70% amplitude for 10 min using ultrasound treatment. At these conditions, the pH, L value and HMF concentration of Tualang Honey differed significantly. The pH of conventionally heated and ultrasonically treated Tualang honey was 3.52 and 3.64, the L values were 58.0 and 55.7, and the HMF contents were 25.4 and 15.5 mg/kg, respectively. It can be concluded that honey producers can use ultrasound as an alternative processing method to retain the quality of honey.

Keywords: Tualang honey; conventional heating; ultrasound; physicochemical; hydroxymethylfurfural

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1. Introduction

Tualang honey is a multi-floral jungle honey produced by the type of Asian rock bee named Apis dorsata. The honey got its name after the bees build their hives high up in the branches of the Tualang tree (*Koompassia excelsa*) (Mohd Sairazi *et al*., 2017). Tualang honey is classified as light amber honey, and Gelam honey belongs as dark amber honey, whilst Kelulut honey is amber brown (Chua *et al.*, 2014). According to Nurul Afiqah and Mohd Hilmi (2021), tualang honey contains higher HMF content (0.75–8.08 mg/kg) when compared to kelulut honey (0.05–2.27 mg/kg) due to the different types of sugar content as well as its ratio of fructose and glucose.

Honey tends to become crystallized, ferment and degrade at certain times as it is significantly influenced by the storage time and heating (Al-Diab & Jarkas, 2015). Thus, retaining honey's liquid characteristic for consumers' preference requires processing, including heat exposure.

Commercial honey processing involves two essential stages, which are filtration and heating. Filtration aims to remove wax and any other foreign materials from honey. Honey is usually heated before packaging to destroy the microorganisms that can cause spoilage and reduce the moisture content to avoid fermentation (Subramaniam *et al.*, 2007). It is heated to 60°C or above for easier packing and delays crystallization (Eshete & Eshete, 2019). Moreover, sugar-tolerant osmophilic yeasts can be destroyed during heating to prolong the shelf life of honey (Guo *et al.*, 2011). Heating also retards the granulation as the sugar crystal nuclei are dissolved when exposed to heat (Eshete & Eshete, 2019).

However, conventional heating can reduce the quality and safety of honey if it is not applied correctly (Cozmuta *et al.*, 2011). It would reduce the enzymatic activity and increase the hydroxymethylfulfural (HMF) content (Chua *et al.*, 2014). HMF content and enzymatic activity are crucial to be analysed in honey samples as they are the indicators of honey quality (Ramly *et al.*, 2021). The enzymatic activity would significantly affect the quality of honey as it can affect its protein content, free amino acid profile and acidity. Furthermore, enzymatic activity can also affect the pH value and modify the flavour and aroma of honey after the fermentation process (Chua and Adnan, 2014). HMF is formed as a result of sugar degradation. There are two main ways to develop HMF: the caramelization of sugars under acid catalysis and heat and the Maillard reaction (a non-enzymatic browning reaction) (Choudhary *et al.*, 2020).

Consumers have recently chosen organic food to get healthier and more nutritious food. Food product which is less processed and has no preservative added with longer shelf life will get high demand from the consumer. Honey is a healthy food but tends to lose nutrients and quality when inappropriate heating is applied. Thus, it is necessary to use alternative methods to process the honey without affecting the quality.

Ultrasound is sound waves with a frequency more significant than the human hearing range. It produces high-frequency that destroys the yeast, enhances the appearance and prevents the granulation of the honey product (Kabbani *et al.*, 2011). Applying ultrasound to a liquid system causes acoustic cavitation, which is the phenomenon of generation, growth and eventual collapse of the bubbles. Acoustic cavitation leads to moisture removal as it involves rapid expansion and contraction of material, and eventually, cell rupture occurs. Using ultrasound in honey processing can destroy undesirable constituents, such as crystals and yeast cells, without reducing the quality of honey. The application of ultrasound can eliminate the existing crystals in honey, retard the crystallization process and remain honey in a liquid state for a much more extended period than heat-treated honey (Salazar *et al.*, 2010). Chaikam et al. (2016) found that conventional heating and ultrasonic processes eliminated the indicator microbes sufficiently. The results also showed that colour, browning index (BI), pH, diastase activity and HMF content were minimally altered. Kabbani *et al.* (2011) indicated that ultrasound treatment effectively inhibits the microorganisms, lowering and delaying microbial growth. This study was conducted to determine the suitable process conditions of ultrasound treatment for Tualang honey and compare its quality with conventional heating.

2. Materials and Methods

2.1. Preparation of Honey Samples

Fresh Tualang honey was purchased from a local honey manufacturer in Hulu Terengganu. After harvesting, it was stored in a 2-litre clear glass container and at room temperature (25^{o}C) for 2 days until analysis. The Tualang honey was stirred gently before measurement to obtain a homogeneous liquid honey.

2.2. Conventional Heating

Using a water bath, Tualang honey was heated at 50, 70, and 90±2.5°C (Kowalski et al., 2012) for 1, 15, 30, and 60 min. The Tualang honey was sealed properly into clear glass containers (5.6 x 8.9 cm). A thermocouple was put into the centre of a clear glass container to monitor sample temperature. The water bath temperature was adjusted to the process temperature before starting the conventional heating process. The glass containers were then placed in the water bath. The heating time was recorded when the temperature at the centre of the glass container had reached the desired temperature. The glass containers were removed from the water bath and immediately cooled to room temperature (25°C) in an ice bath for further analysis.

2.3. Ultrasound Treatment

The ultrasound treatment was conducted according to Chaikham *et al.* (2016) with some modifications. Approximately 15 ml of Tualang honey filled in a 25 ml glass beaker was used for each treatment. It was treated with ultrasound using the ultrasonic device (Biologics Inc. ultrasonic homogenizer, model 150 V/T) operating at a nominal frequency of 20 kHz (output power 50W). During this experiment, the ultrasonic was used at 50, 70 and 90% of maximum amplitudes for 10, 20, and 30 minutes.

2.4. Moisture Content Measurement

The moisture content of Tualang honey was determined based on the official analysis methods from the Association of Official Analytical Chemists (AOAC). 5 g of each Tualang honey sample was placed in a porcelain crucible and dried to constant weight in an oven at 105°C for 18 hours.

2.5. Water Activity Measurement

Water activity (aw) of Tualang honey was determined using the Aqualab CX2 water activity meter (Decagon Devices Inc., WA, USA). Distilled water was used to calibrate the equipment at an average of 0.9 to 1.0. Tualang honey was placed in the sample dishes and measured at room temperature (25°C).

2.6. pH Measurement

The pH of Tualang honey was measured using a pH meter Basic 20. The pH meter was calibrated with pH 4, 7, and 10 buffer solution. Tualang honey solution was prepared by dissolving 10 g of Tualang honey in 75 mL of distilled water. After homogenising the mixture, the pH electrode was immersed in the key, and the pH value was recorded.

2.7 Colour Measurement

The colour of Tualang honey was determined using a Konica Minolta chromameter (Minolta, Model CR-400, UK). Approximately 10 ml of Tualang honey sample was placed in a Petri dish. Each Petri dish was placed on a white tile, and three measurements were taken per treatment directly from a 1cm distance. The instrument was calibrated using a white tile (L* =94.52, a* = 0.36 and b* = 1.04) as a standard. The L* parameter (lightness index scale) ranges from 0 (black) to 100 (white). The a* parameter indicates the degree of red (+a*) or green (-a*) colours, whereas the b* parameter measures the degree of the yellow (+b*) or blue (-b*) colours.

2.8. Turbidity Measurement

The Tualang honey sample was put into a cuvette with a path length of 1 cm. The absorbance was measured at 660 nm with a spectrophotometer Beckman DU-650 (Lupano, 1997).

2.9. HMF Analysis

The 5-hydroxymethylfurfural (5-HMF) concentration of Tualang honey was determined using White's spectrophotometric method (Maryam, 2016; White, 1979). Five grams of honey were dissolved in 25 ml of water, transferred quantitatively into a 50 ml volumetric flask, added 0.5 ml of Carrez solution I and 0.5 ml of Carrez II and made up to 50 ml with water. The answer was filtered through 11 µm paper (Grade 1), rejecting the first 10 ml of the filtrate. Aliquots of 5 ml were put in two test tubes; to one tube was added 5 ml of distilled water (sample solution); to the second was added 5 ml of sodium bisulphite solution 0.2% (reference solution). The absorbance of the sample solution against the reference solution was measured at 284 and 336 nm in 10 mm quartz cells within one hour. The quantitative value of HMF was determined using Equation 1 (White, 1979).

$$\text{HMF in mg/kg} = (A_{284} - A_{336}) \times 149.7 \times 5 \times \frac{D}{W} \quad (1)$$

Where:
A284 = absorbance at 284 nm
A336 = absorbance at 336 nm
D = dilution factor, in case of dilution is necessary
W = weight in g of the honey sample

2.10. Statistical Analysis

Analysis of Variance (ANOVA) was used to analyse the study results. The collected data was evaluated with one-way ANOVA to compare the means, and the significance difference was defined at $p < 0.05$. Meanwhile, two-way ANOVA was used to analyse the impact of different treatments and times on honey. All statistical analysis was performed with Microsoft Excel 2010 and SPSS 20.

3. Results

3.1. Impact of Treatment Method on the Water Activity in Malaysian Tualang Honey

Figure 1 shows the effect of conventional heating with different times and temperatures on honey's water activity. It can be seen from the figure that the water activity of Malaysian Tualang honey increased significantly after 15 minutes of heating ($p < 0.05$). However, the water activity did not change significantly when more prolonged heating was applied. In addition, the temperature did not cause a significant change in water activity ($p > 0.05$). It can be seen that honey treated at 50°C for up to 60 minutes does not present a significant difference in water activity value.

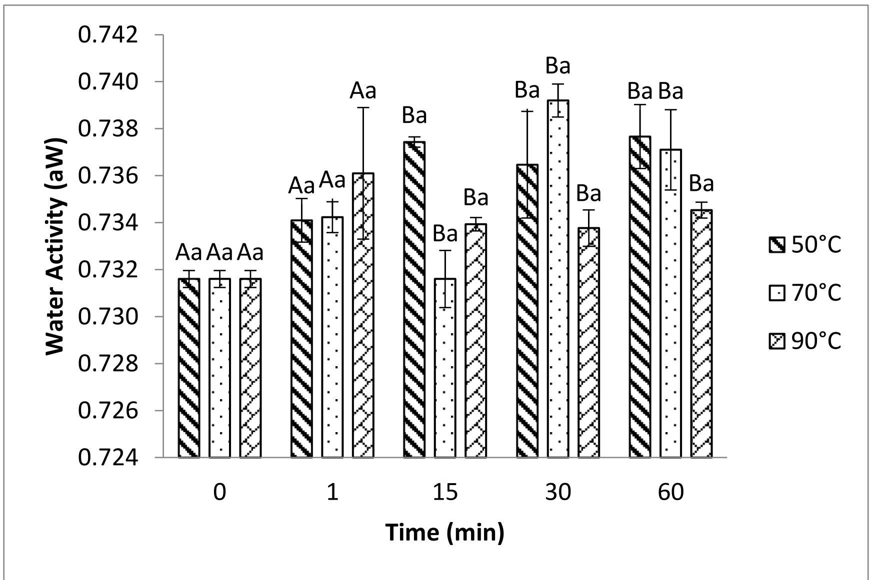

Figure 1. Effect of conventional heating treatment on water activity of Malaysian Tualang honey. Bars indicate ± SE. Small letters of the alphabet indicate statistical significance among temperature at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

Figure 2 shows the water activity of Tualang honey treated with ultrasound at different amplitudes and treatment times. The water activity of ultrasonically treated honey decreased significantly ($p < 0.05$) with the increasing amplitude for all the treatment times applied. Besides that, the water activity of Tualang honey did not change significantly after it was treated with ultrasound for 10 minutes. A significant reduction was only observed when treated for 20 minutes for all the amplitudes used. It was then further reduced when the treatment time was increased to 30 minutes. It can be concluded that the increase in ultrasound processing time and amplitude could decrease the water activity in honey. This indicates that the water activity is influenced by both amplitude and treatment time. Among all the combinations of amplitude and time of ultrasonic processing, Tualang honey treated at 90% amplitude for 30 minutes had the lowest water activity value (0.6285 ± 0.02).

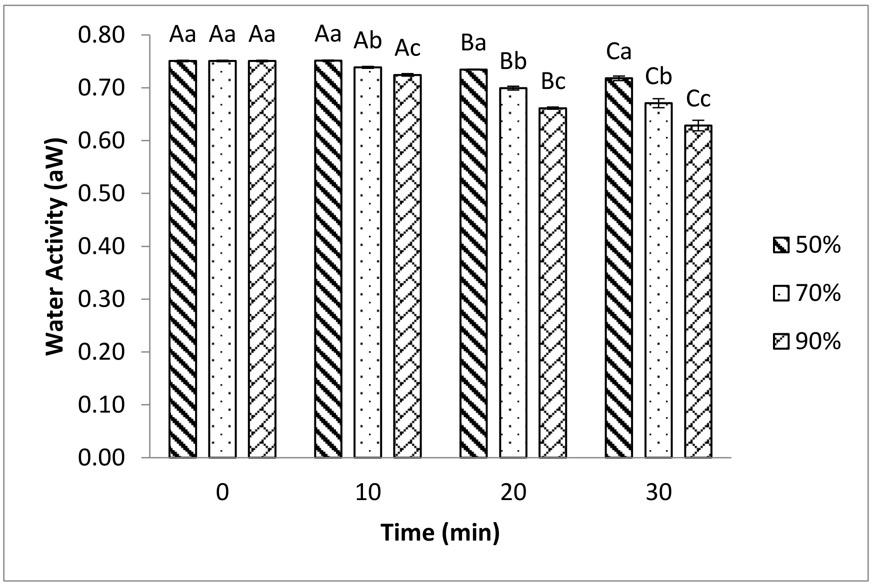

Figure 2. Effect of ultrasound treatment on water activity of Malaysian Tualang honey. Bars indicate ± SE. Small letters of the alphabet indicate statistically significant amplitude at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

3.2. Impact of Treatment Methods on the Moisture Content of Malaysian Tualang Honey

The moisture content of Tualang honey treated with conventional heating at different times and temperatures is illustrated in Figure 3. A quick glance at the graph in the figure shows that the moisture content decreased significantly when the heating time increased. Heating at 50°C for 30 minutes reduced the moisture content of Tualang honey from 28.9 to 28.5%, and it was further reduced to 28% after being heated for 60 minutes. When the Tualang honey was heated at 70°C, the moisture content reduced to 28 and 27.5% after 30 and 60 minutes of heating, respectively. However, the moisture content did not decrease significantly when the highest temperature was used.

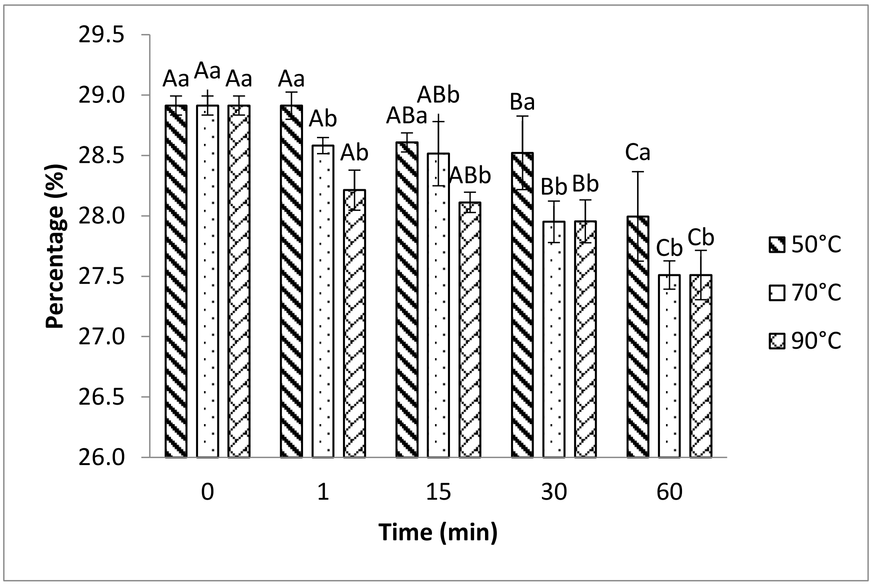

Figure 3. Effect of conventional heating treatment on the moisture content of Malaysian Tualang honey. Bars indicate ± SE. Small letters of the alphabet indicate statistical significance among temperature at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

Figure 4 displays the effect of ultrasound treatment using different times and amplitude on the moisture content of honey. It can be examined that the moisture content for all treatments decreased significantly ($p < 0.05$) as the percentage of amplitude was increased. The treated honey's moisture content decreased from 31.24% ± 0.22 to 23.05% ± 0.68. All amplitudes showed a significant decrease in moisture content; meanwhile, there is only a significant difference between 10 and 20 minutes for processing time. Prolonging the treatment time to 30 minutes did not cause any significant change in the moisture content of Tualang honey.

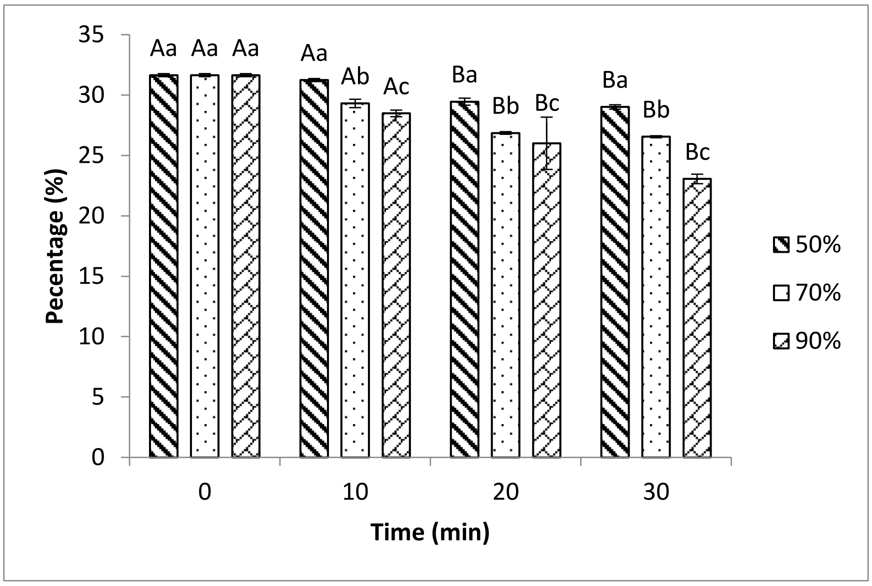

Figure 4. Effect of ultrasound treatment on the moisture content of Malaysian Tualang honey. Bars indicate ± SE. Small letters of the alphabet indicate statistically significant amplitude at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

3.3. Impact of Treatment Methods on the pH of Malaysian Tualang Honey

The pH values of Tualang honey for different conventional heating times and temperatures are depicted in Figure 5. It can be diagnosed that there was an increment in the pH value when the heating temperature was increased. Heating at 15 minutes for all temperatures significantly increased the pH of Tualang honey. Further increment in the heating time to 30 minutes did not affect the pH, while a significant increase was observed when the Tualang honey was heated for 60 minutes. Among all temperatures, 90^{o}C recorded the highest pH value of 3.6 at 60 minutes of heating. This indicates that increasing the temperature can significantly increase the pH value ($p < 0.05$). Annapoorani *et al*. (2010) found similar findings, which stated that the pH of honey heated at 140^{o}C increased compared to the unheated and 60^{o}C heated samples.

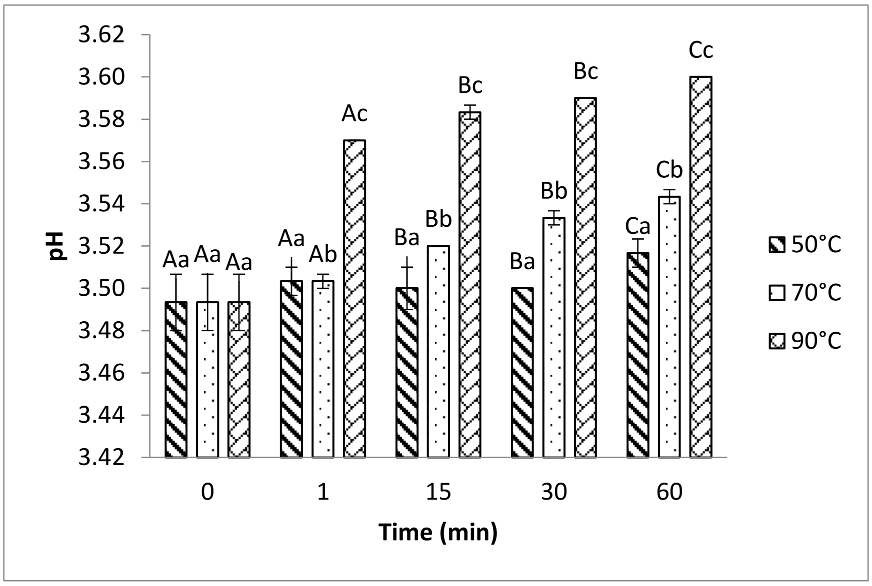

Figure 5. Effect of conventional heating treatment on pH of Malaysian Tualang honey. Bars indicate ± SE. Small letters of the alphabet indicate statistical significance among temperature at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

Figure 6 illustrates the pH values for ultrasound treatment at different amplitudes and times. From the figure, it is apparent that there is an increase in pH values after treatment. When the ultrasonic processing time and amplitude increased, the pH of treated honey also significantly increased ($p < 0.05$). In detail, honey samples treated at 90% amplitude had the highest pH, ranging from 3.64 ± 0.01 to 3.78 ± 0.01, compared to 50 and 70% amplitude. Moreover, the time variable was the most significant factor ($p < 0.05$) in increasing the pH value, as 30 minutes of heating time recorded the greatest pH increment for all the amplitudes. The interaction between processing time and amplitude shows a significant value ($p < 0.05$), indicating that the relationship between time and pH value also depends on the ultrasound amplitude. However, this finding did not agree with Chaikham *et al.* (2016), who found that increasing the ultrasound amplitude caused a significant decline in the pH value of lychee flower and wildflower honey after processing.

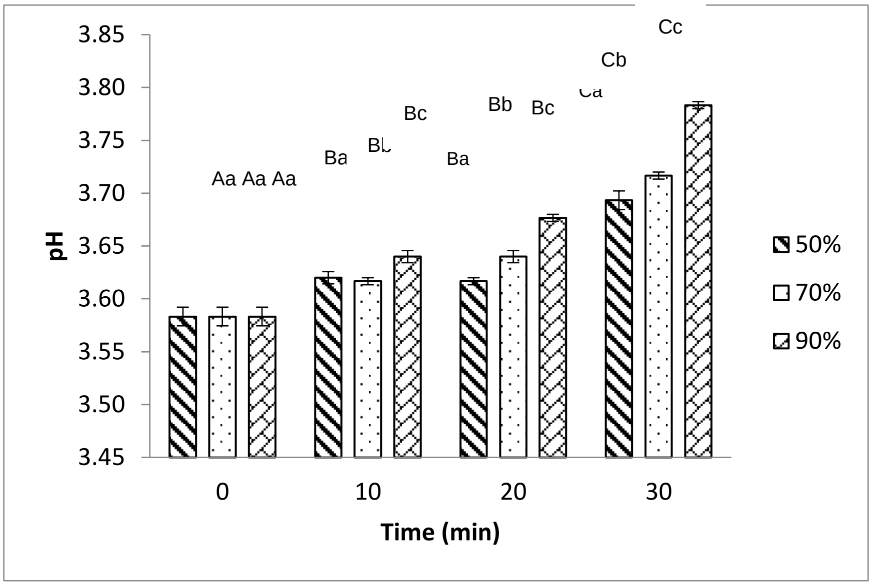

Figure 6. Effect of ultrasound treatment on pH of Malaysian Tualang honey. Bars indicate ± SE. Small letters of the alphabet indicate statistically significant amplitude at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

3.4. Impact of Treatment Methods on the Turbidity of Malaysian Tualang Honey

The turbidity level of honey after being treated with conventional treatment at different times and temperatures is presented in Figure 7. It was expressed in terms of its optimal density at 660 nm. A steady decrease in turbidity value can be seen, and it shows a significant difference ($p < 0.05$) in the turbidity value when the temperature was increased for all heating times applied. However, there was no significant difference ($p > 0.05$) in turbidity value in samples from 15 to 60 minutes when heated. Meanwhile, samples heated at all temperatures gave substantial results ($p < 0.05$) confirming that the turbidity of honey decreased when the heating temperature increased. It can be proved by referring to samples treated with 90^{o}C, which recorded the lowest absorbance value each time.

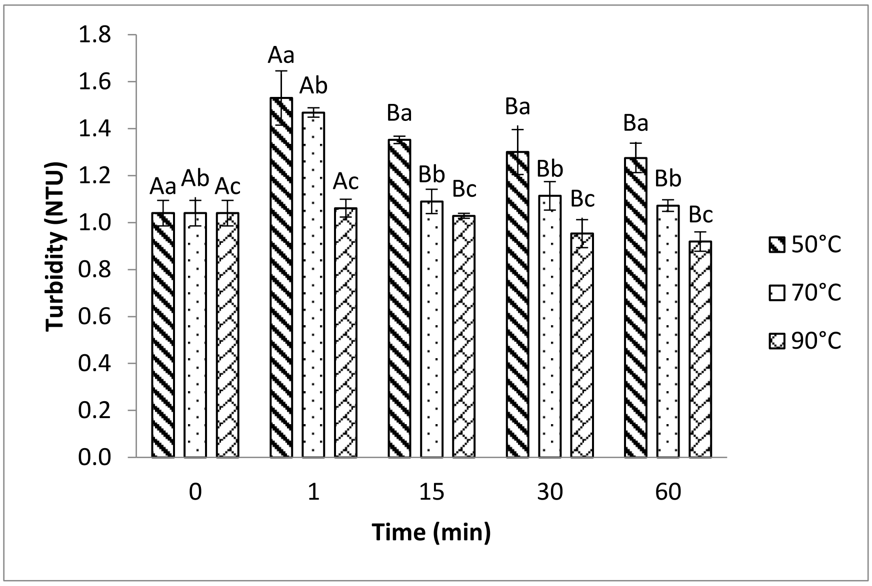

Figure 7. Effect of conventional heating treatment on the turbidity of Malaysian Tualang honey. Bars indicate ± SE. Small letters of the alphabet indicate statistical significance among temperature at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

Figure 8 shows the turbidity of honey treated at different treatment times and amplitudes. There was no significant effect ($p > 0.05$) of the amplitudes and treatment times of ultrasonic processing on the turbidity of honey.

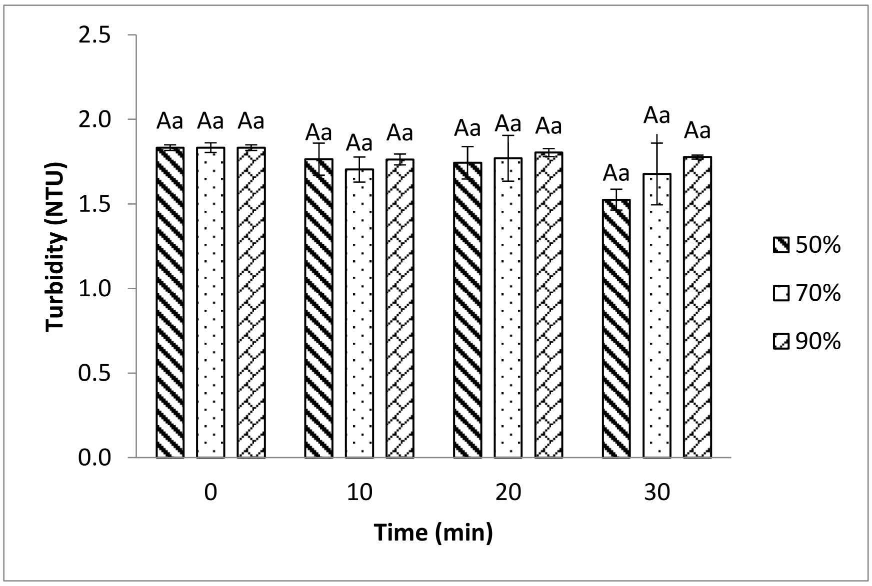

Figure 8. Effect of ultrasound treatment on the turbidity of Malaysian Tualang honey. Bars indicate ± SE. Small letters of the alphabet indicate statistically significant amplitude at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

3.5. *Impact of Treatment Methods on the Lightness (L*) of Malaysian Tualang Honey*

Based on Figure 9, the L* value increased when the temperature increased from 50°C to 70°C. However, when the temperature was further increased to 90°C, the L* value decreased significantly ($p < 0.05$). The heating temperature of 90°C seems to cause honey to darken in colour. This is because, at a higher temperature, honey tends to undergo a Maillard reaction and produces browning pigment. Ahmed *et al.* (2014) reported that the colour intensity of Sahara honey before and after heating ranged from 1.26 ± 0.09 to 1.44 ± 0.20 and 1.01 ± 0.56 to 1.88 ± 0.45 mAU, respectively. The increment values of colour intensity showed that the honey became dark due to the heat applied.

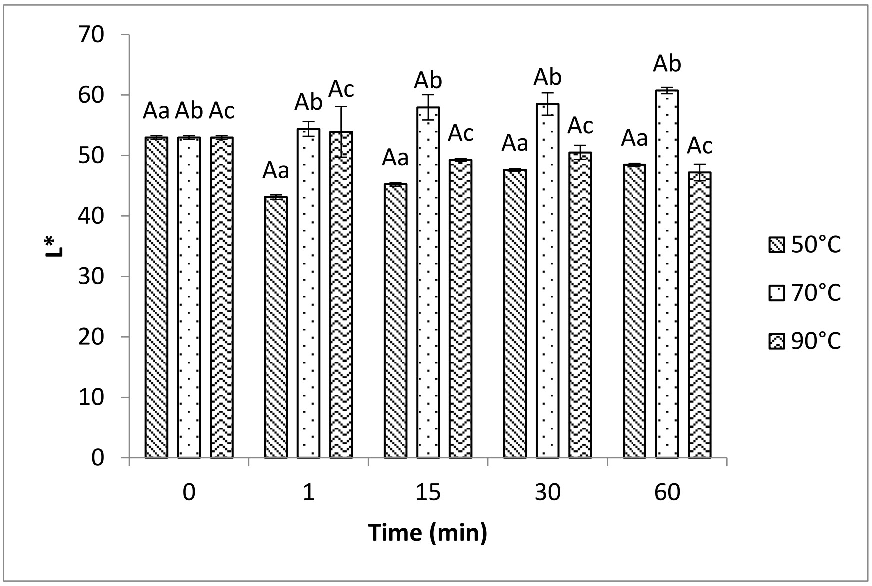

Figure 9. Effect of conventional heating treatment on L* value of Malaysian Tualang honey. Bars indicate ± SE. Small letters of the alphabet indicate statistical significance among temperature at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

Figure 10 shows the L* value of Tualang honey treated with ultrasound. L* value of honey was the highest when 50% amplitude of ultrasonic treatment was applied compared to 70% and 90% amplitude of ultrasonic treatment ($p < 0.05$). It indicates that increasing the amplitude can reduce the lightness of honey. All treatments showed a decrease in L* value when increasing process time and temperature, resulting in a darker colour of honey. Colour enhancement could be due to reduced moisture content and, consequently, the concentration of the components responsible for honey's colour, such as minerals.

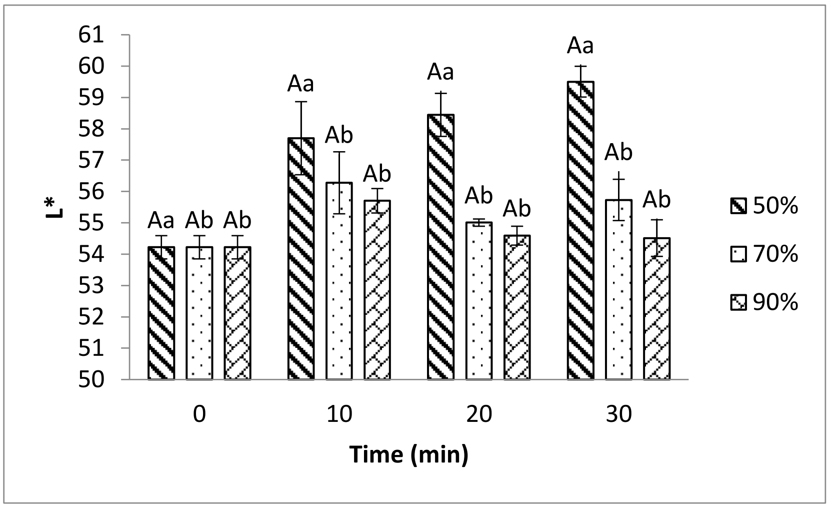

Figure 10. Effect of ultrasound treatment on L* value of Malaysian Tualang honey. Bars indicate ± SE. Small letters of the alphabet indicate statistically significant amplitude at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

3.6. Impact of Treatment Methods on the Hydroxymethylfurfural (HMF) Content of Malaysian Tualang Honey

Figure 11 shows the HMF concentration of honey treated with conventional heating for different treatment times at different temperatures. The figure shows that the HMF concentration increased gradually with the temperature increase from 50 to 90°C for all treatment times. The HMF concentration of honey at all temperatures differed significantly ($p < 0.05$) when the time increased to 60 minutes. HMF concentration for 90°C recorded the highest value for all heating times, rising from 26.66, 26.95, 33.40, and 63.82 mg/kg.

Prolonged heating time might also cause an increase in the HMF concentration. Ribeiro *et al.* (2012) reported that the time variable is the most significant factor in increasing HMF concentration. This coincides with the results obtained in this study, showing the increasing trend of HMF concentration when the heating time increased. Generally, it can be observed that honeys treated at 50, 70, and 90°C for 1 and 15 minutes had no significant difference ($p > 0.05$), indicating that short heating time resulted in a slight variation in HMF concentration. Further, increasing the heating time to 30 and 60 minutes significantly increased the HMF concentration ($p < 0.05$).

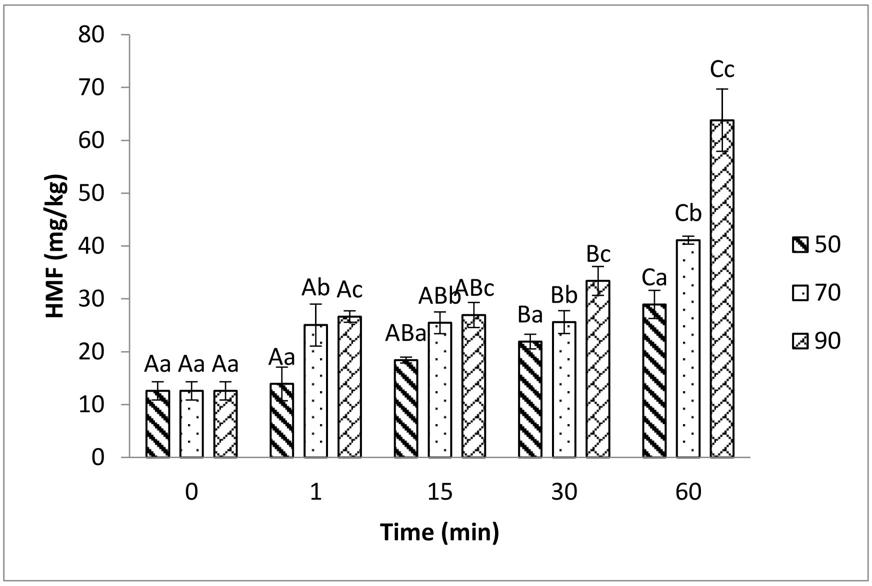

Figure 11. HMF concentration of honey treated with conventional heating. Bars indicate ± SE. Small letters of the alphabet indicate statistical significance among temperature at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

HMF concentration of Tualang honey treated with ultrasound for different treatment times at different amplitudes is presented in Figure 12. The figure shows that the HMF concentration increased gradually when time and amplitude increased. It can be noticed that the HMF concentration for 30 minutes of treatment time increased significantly ($p < 0.05$) for all amplitudes applied. A shorter treatment time did not substantially affect the HMF concentration. The effect of the amplitude also could not be seen at 50 and 70%, whereby the amplitude of 90% drastically increased the HMF concentration in Tualang honey. Besides that, the results also showed that the sample exposed to 90% amplitude for 30 minutes resulted in the highest HMF concentration (53.6 mg/kg), which had exceeded the maximum level of HMF permitted. The higher the amplitude applied might produce more heat in honey, thus increasing the HMF concentration.

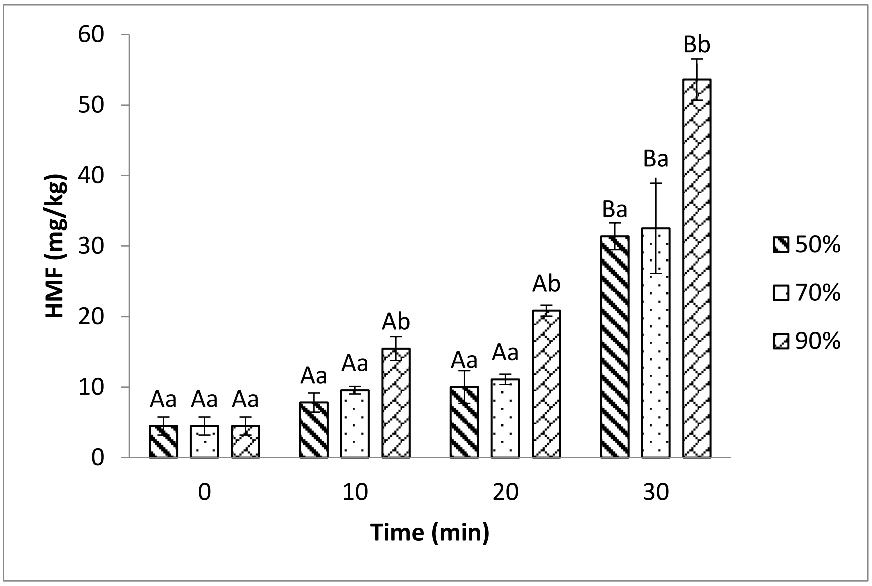

Figure 12. HMF concentration of honey treated with ultrasound method. Bars indicate ± SE. Small letters of the alphabet indicate statistically significant amplitude at level $p < 0.05$. According to Tukey's HSD-test, capital letters or the alphabet indicate statistical significance among time at level $p < 0.05$.

3.7. Comparison Between Treatments

Table 1 compares the physicochemical properties and HMF concentration of the treated Tualang honey at 70°C, 15 minutes (conventional heating) and 90%, 10 minutes (ultrasound). These process conditions were considered optimum conditions whereby the increment of HMF, one of the quality indicators of honey, was not drastic. There were significant differences in pH, L value and HMF concentration of Tualang honey. The pH of ultrasonically treated Tualang honey was higher than that of conventionally heated. At the same time, the L* value and HMF concentration were more elevated in Tualang honey that was heated conventionally.

Table 1. Comparison between conventional heating and ultrasound treatment at selected process conditions. Means with different letters within the same row represent significant differences.

Parameters	Conventional heating (70°C, 15 minutes)	Ultrasound (90%, 10 minutes)
Water activity	0.73^{a}	0.72 ^{a}
Moisture content (%)	28.50 ^{a}	28.50 ^{a}
pH	3.52 ^{a}	3.64 ^{b}
Turbidity (NTU)	1.09 ^{a}	1.19 ^{a}
L*	58.00 ^{a}	55.70 ^{b}
HMF (mg/kg)	25.40 ^{a}	15.50 ^{b}

4. Discussion

4.1 Impact of Treatment Methods on the Physicochemical Properties of Malaysian Tualang Honey

Water activity describes the moisture migration and microbial stability of food. It reported that the water activity of honey is linearly correlated with moisture content of honey. Prolonged heating time might reduce the water activity in honey. The findings indicate that conventional heating could reduce water activity in honey as it makes honey supersaturated. This result agrees with the research conducted by Shafiq *et al.* (2014), who stated that water activity will decrease if honey is heated. Pingret *et al.* (2011) studied the first investigation of sensory and physicochemical characteristics in ultrasound-assisted preparation of food products. The ultrasound treatment slightly reduced the water activity of chocolate mousse from 0.956 to 0.924.

Similarly, Shamaei *et al.* (2011) pointed out that the application of ultrasound decreased the water activity of cranberries. Stojkovic *et al.* (2020) also reported reduced water activity of honeydew honey subjected to ultrasound. This is due to the formation of ultrasonic waves responsible for creating microscopic channels, which reduce the diffusion boundary layer and increase mass transfer.

Treatment methods also affected the moisture content in Tualang honey. As heat was applied during the conventional heating, the honey surface underwent an evaporation process and subsequently reduced the moisture content. Thus, prolonged traditional heating could reduce the moisture content significantly as the evaporation rate increased. According to Subramaniam *et al.* (2007), heating duration had more impact than heating temperature. This statement is in alignment with the result presented in this study. The results obtained in this study are also similar to those reported by Chua *et al.* (2014), where conventional heating could reduce less than 20% of moisture content in honey samples after 30 minutes of heating at 90^{o}C. Bachirbey *et al.* (2017) illustrated that after the crystallized honey was treated with the ultrasound method, the moisture content was reduced from 15.40% (untreated honey) to 13.60%–15.20% in a range. In addition, Thrasyvoulou *et al.* (1994) also reported the reduction of moisture content of honey from 17.80% to 17.10% after being treated with ultrasound treatment.

Similarly, Stojkovic *et al.* (2020) found a decrease in the moisture content of honeydew honey after being treated with ultrasound. This results from the acoustic cavitation process, which increases the temperature of honey and the rates of mass transfer. Cavitation formed by the sonication produces bubbles in the honey and could explosively collapse and generate localized pressure. This process would remove the firmly attached moisture in honey, and an evaporation process will occur (Shamaei *et al.*, 2011). Furthermore, increasing the ultrasound amplitude may increase the thermal energy, which causes water evaporation in honey (Bachirbey *et al.*, 2017).

Conventionally heated Tualang honey values for turbidity decreased steadily when the temperature increased due to less molecular friction and reduced hydrodynamic forces (Mossel *et al.*, 2000; Akinwande & Oladapo, 2022). Hydrodynamic force is the force of water in motion. Thus, more light can penetrate the honey, resulting in a lower absorbance value. Tualang honey treated with ultrasound showed contradictory behaviour. Acoustic cavitation may break the molecules and produce bubbles in honey, which leads to the cloudiness of honey. Thus, less light transmission occurs through the honey, increasing the absorbance value. However, the results did not agree with the theory, as only small changes were detected in the absorbance value when the amplitude increased.

The reduction in the values of L* showed that the honey became dark due to the heat applied. This result was expected because the sample temperature rises during the exposure to the ultrasound wave. The results obtained were identical to the research done by Chaikham *et al.* (2016), which also reported that honey decreased brightness when treated with ultrasound from 40% to 80% amplitude. According to the Codex Alimentarius: International Food Standards (1981), the colour of honey varies from nearly colourless to dark brown. It is known that conventional heating accelerates the Maillard reaction. This reaction would be associated with the non-enzymatic chemical changes of browning (Moline *et al.*, 2015). Maillard reactions are the sugars that condense with free amino acids, producing various brown pigments. Browning rate can differ according to amino acid and reducing sugar in the sample (Lund & Ray, 2017; Turkmen *et al.*, 2006). Besides that, the caramelization of sugar or the presence of heat-sensible compounds during conventional heating might reduce the lightness of treated honey.

4.2. Impact of Treatment Methods on the Hydroxymethylfurfural (HMF) Content of Malaysian Tualang Honey

It was found that the higher the treatment temperature seemed to accelerate the HMF formation, and 60 minutes of treatment time was unsuitable for treating honey when treated at 90^{o}C as it rapidly increased the HMF concentration. This is because when honey is subjected to heat, decomposition of sugar occurs. The fructose will undergo three dehydration processes involving the formation of fructofuranosly oxocation, the release of the water molecules, and finally, breaking down the double bond in the furan ring, resulting in HMF formation (Kowalski *et al.*, 2013).

It is formed from the dehydration of hexoses or other sugars that produce hexoses after hydrolysis (Locas & Yaylayan, 2008) at a pH lower than 5 (Cozmuta *et al.*, 2011). Figure 13 shows a chemical reaction of HMF formation through Maillard reaction and acid-catalysed dehydration. At first, fructose undergoes dehydration and produces fructofuronose. Next, the enol of 2,5-anhydro-D-mannose was formed by eliminating a proton. Then, a water molecule was released from the enol, closing the first double bond in the furan ring. The carbonyl group at C5 was raised at this stage. The 5-hydroxymethyl-2-furfural structure was formed after the third and last dehydration process (Locas & Yaylayan, 2008).

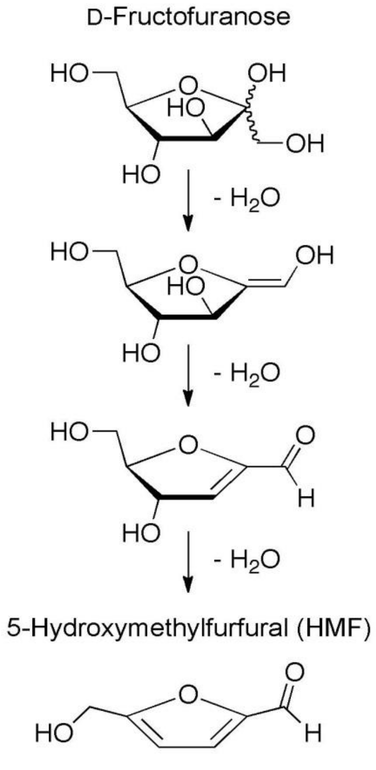

Figure 13. Model mechanism of HMF production (Shapla *et al.*, 2018)

Similarly, the drastic increment in the HMF content of the ultrasonically treated Tualang honey was possibly due to more heat generated by the ultrasound waves. The increasing heat was caused by the irregular oscillation of molecules in honey during the acoustic cavitation. At this point, the bubbles were formed in honey, which will grow, oscillate and then asymmetrically implode greatly (Peshkovsky & Peshkovsky, 2008). Acoustic cavitation involves the rapid expansion and contraction of nano/microbubbles of gas in honey, resulting in a sudden collapse of bubbles that generates exceptionally high temperatures. With the presence of heat, HMF was formed at a higher rate. This result was similar to Mahmoud and Owayss (2008) and Suhaela *et al.* (2016), who reported that HMF content would increase when exposed to higher temperatures for long periods.

4.3. Comparison of Physicochemical Characteristics and HMF Contents of Malaysian Tualang Honey Treated with Different Methods

The pH, L* value and HMF concentration of Tualang honey differed significantly between the two treatments. Conventional heating of Tualang honey at 70°C might have changed the morphology of the crystalline structure of honey and modified the optical properties, consequently causing the lightening of honey (Kedzierska-Matysek *et al.*, 2016). The lower value in pH of Tualang honey that was conventionally heated might be because of the increase in hydrogen ion concentration due to heating or because pollen releases organic acids during heating, which also affects the pH of the honey (Chaikham & Apichartsrangkoon, 2012).

In line with the L* value, the HMF content of conventionally heated Tualang honey was higher than that of the ultrasonically treated sample. The acceleration of Maillard reactions is one of the effects of conventional heating of honey. These reactions involve incorporating sugars and free amino acids or fructose caramelization. It would be associated with non-enzymatic chemical changes of browning, leading to the formation of various brown pigments and simultaneously the formation of intermediate products as HMF. Similarly, Chua *et al.* (2014) also reported that the HMF content appeared proportionally increased with the increase in heating time. The formation of HMF upon heating followed the zero-order kinetic model.

5. Conclusions

The best treatment combination was determined at 70°C for 15 minutes using conventional heating and 90% amplitude for 10 minutes using ultrasound treatment, as the increment of HMF was not drastic at these conditions. This coincides with the industry's mission, which prefers to shorten the processing time while producing the best product quality. Among all treatments, ultrasound treatment was found to potentially slow the increment of HMF content compared to conventional heating.

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