

## EFFECT OF HIGH-PRESSURE PROCESSING (HPP) ON ANTIOXIDANT, DIASTASE ACTIVITY AND COLOUR FOR KELULUT (STINGLESS BEE) HONEY

Muhammad Faiz Razali<sup>a</sup>, Noor Akhmazillah Mohd Fauzi<sup>a\*</sup>, Alifdalino Sulaiman<sup>b</sup>, Nur Atikah A Rahman<sup>a</sup>

<sup>a</sup>Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh Education Hub, KM1, Jalan Panchor, 86400 Panchor, Muar Johor, Malaysia

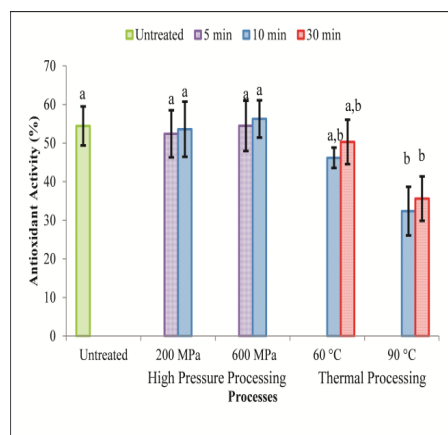
<sup>b</sup>Department of Food Engineering, Faculty of Engineering, Universiti Putra Malaysia (UPM), 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

### Article history

Received  
30 August 2018  
Received in revised form  
19 February 2019  
Accepted  
21 February 2019  
Published online  
18 April 2019

\*Corresponding author  
akhma@uthm.edu.my

### Graphical abstract



### Abstract

The quality of Kelulut honey is heavily affected by conventional thermal processing due to the existence of thermolabile compounds. Hence, high-pressure processing (HPP) was employed as an alternative method to minimize any quality deterioration during processing. HPP was carried out at two different pressures (200 and 600 MPa) with two holding times (5 and 10 minutes) respectively. Thermal processing was also carried out (60 and 90 °C for 10 and 30 minutes) for comparison. After treatment, the changes in antioxidant activity, diastase activity and colour were determined. Increment of antioxidant activity by 3 % was recorded by HPP-treated (600 MPa/10 minutes) compared to thermally-treated. Diastase activity which was used as freshness indicator showed non-significant changes ( $p>0.05$ ), whereas colour evaluated based on total colour different (TCD) and browning index (BI) showed decrement after HPP. Fortunately, the TCD recorded for HPP was below the range noticeable by human eyes, ranging from 1.5 to 3.0. In conclusion, HPP is better than conventional thermal processing in producing minimally-processed Kelulut honey as evident by the retention of diastase activity, antioxidant activity with increment at 600 MPa/10 minutes, and unnoticeable changes in colour. This hence has an implication on post-harvest processing of Kelulut honey.

**Keywords:** HPP, stingless bee honey, antioxidant, diastase, colour

### Abstrak

Kualiti madu Kelulut amat terjejas apabila diproses menggunakan rawatan haba secara konvensional disebabkan oleh kewujudan komponen sensitif haba. Oleh itu, pemrosesan tekanan tinggi (HPP) digunakan sebagai rawatan alternatif bagi mengurangkan penurunan kualiti semasa pemrosesan. HPP dijalankan dengan dua tahap tekanan (200 and 600 MPa) dengan dua tempoh masa berbeza (5 and 10 minit). Rawatan haba turut dijalankan (60 and 90 °C/ 10 dan 30 minit) untuk perbandingan. Selepas rawatan, perubahan aktiviti antioksidan, aktiviti diastase dan warna ditentukan. Peningkatan aktiviti antioksidan sebanyak 3 % direkodkan oleh madu rawatan HPP (600 MPa/10 min) berbanding rawatan haba. Aktiviti enzim diastase yang mana digunakan sebagai penanda kesegaran menunjukkan perubahan tidak ketara ( $p>0.05$ ) manakala warna yang dinilai berdasarkan perbezaan warna total (TCD) dan indeks pemerangan (BI)

menunjukkan perubahan selepas HPP. Walau bagaimanapun, nilai TCD yang direkodkan untuk HPP adalah dibawah aras perubahan yang ketara untuk penglihatan manusia, antara 1.5 – 3.0. Kesimpulannya, HPP adalah lebih baik berbanding rawatan haba dalam menghasilkan madu Kelulut yang diproses secara minima berdasarkan pengekal aktiviti diastase, aktiviti antioksidan dengan penigkatan di 600 MPa/ 10 min dan perubahan warna yang tidak ketara. Maka ianya mempunyai implikasi untuk pemprosesan pascatuai madu Kelulut.

*Kata kunci:* HPP, madu kelulut, antioksidan, enzim diastase, warna

© 2019 Penerbit UTM Press. All rights reserved

## 1.0 INTRODUCTION

Modern society is more aware of the quality of food that they purchase and consume. Driven by the notion of a healthy life style, many people are prone to find minimally-processed food, and healthier substitutes for their daily food. One of the food products is honey which has always been used as a substitute for natural sugar. In recent years, stingless bee (Kelulut) honey has been gaining popularity as it contains high antioxidant activity, flavonoid, and polyphenol [1]. Kelulut honey is a stingless bee honey produced commonly by bees from tribe *Trigona* spp. In general, stingless bee honey is different from the honey produced by bees from *Apis* sp in which it possesses more fluid in the texture, and undergoes slow crystallization [2]. However, the distribution of this honey in the world market is limited due to the lack of institutional standard, lower shelf-life, and limited industrial production [3]. The lack of knowledge in the compositions of stingless bee honey [2] is one of the reasons why there is no specific institutional standard like the International honey standard for honey bee honey, Codex Alimentarius Commission [4].

In line with consumer demand for fresh and minimally-processed food products, high pressure processing (HPP) is a novel processing technique that utilises pressure to pasteurise food. HPP is one of the most developed food-processing technologies that is able to destroy food-borne pathogens and spoilage organisms which improves product safety and allowing longer shelf life [5]. The absence of significant heating makes HPP to not break covalent bonds, hence eliminate undesirable effects that are produced at high temperatures such as texture defects, off-flavours, nutrient destruction, and colour changes [6]. Principally based on le Chatelier and isostatic pressure, HPP can utilise pressure up to 900 MPa to kill many of the microorganisms in food without degrading low molecular weight food components. On the other hand, high molecular weight components such as enzymes, proteins, lipids, and cell membranes are pressure-sensitive [7, 8]. This hence makes the end-product of HPP microbiologically safe, and retains the fresh characteristics.

Due to the benefits offered, HPP can be a better alternative for Kelulut honey processing as opposed to the conventional thermal processing method. Thermal processing usually uses conduction and convection heat transfer from the heat generating device [9] which is detrimental to many of the composition of honey. The damage brought about by heat is more apparent due to the existence of many unstable and thermolabile components in honey [10]. This will eventually damage the biological properties as well as mask its originality [11]. Many studies have demonstrated the damage done by heat processing to the antioxidant activity, phenolic and biochemical compound, proline and threonine [12-16]. Therefore, HPP was chosen to be studied as the processing method for Kelulut honey where the changes in colour, antioxidant activity, and diastase activity were evaluated.

The changes in antioxidant activity due to processing need to be evaluated as honey has been demonstrated to exhibit health promoting properties, contributed by the existence of antioxidant compounds. Examples of compounds that exhibit this property are phenol compounds, flavonoids, carotenoids, catalase, vitamins C, peroxides and glucose oxidase enzymes [17]. The antioxidant property is imperative as the compound will bind to any free radical that has been proven to be harmful towards human where it could cause cellular damage, and over time will cause age-related diseases. Besides that, the expectation in food is literally generated by its total appearance [18] in which colour was one of the attributes that influenced the perception or acceptance of consumers. It is often used as the initial criterion for judgment as it portrays quality or variation resulting from geographical origin or processing [19]. Since honey is generally amorphous, colour plays an important role in determining the initial acceptance by consumers. Owing to its geographical origin, honey exists in a variety of colours such as light yellow to amber, dark amber, and even black [20]. There is a perception that the darker the honey, the more nutritious it is. This is mainly due to the hypothesis that dark honey contains more antioxidant capacity than its lighter counterpart. Kelulut honey that was used in this research had a dark blackish colour.

Enzyme activity, on the other hand, makes up the quality indication of honey. Diastase (amylase), glucose oxidase, invertase ( $\alpha$ -glucosidase), acid phosphate, and catalase are examples of enzymes that exist in small amounts in honey. Diastase in particular can turn  $\frac{3}{4}$  of sucrose in honey. Hence the older the honey, the less sucrose it contains. Moreover, the activity of diastase enzyme is reduced upon heat encounter thus making it an indicator of possible heating, and quality reduction [12]. Therefore, the changes in these parameters need to be evaluated after HPP and thermal treatment.

## 2.0 METHODOLOGY

### 2.1 Sample Honey Preparation

Unprocessed Kelulut honey was freshly collected from the local breeder; MAEPS, Serdang. The fresh honey was stored in a glass bottle at about 10 °C. For HPP treatments, Kelulut honey was stirred manually and 6g was packed in 5 cm x 5 cm transparent plastic film pouches, and thermosealed under vacuum. The plastic film has an excellent transparency and heat sealing qualities, and is able to withstand temperature up to 125°C as it was made of cast polypropylene [21].

### 2.2 High Pressure Processing (HPP)

HPP was carried out with HPP unit (Avure Technologies, Ohio, USA) where distilled water served as the medium in the chamber. Figure 1 shows the schematic diagram of the HPP system. 6 g vacuum-packed honey samples were subjected to HPP with pressure of 200 MPa and 600 MPa at close to ambient temperature for 5 and 10 minutes, respectively. Pressure holding time was the treatment time, and did not include the pressure come up and the decompression times. Thermocouples (located at mid and top of the vessel) which were immersed in the pressure medium (distilled water) were used to monitor the temperature inside the pressure chamber. The compression time, decompression time, and average temperature were on the cycle report which was directly obtained from a control system operated through a computer with software. After processing, the packed honey samples were immediately placed in ice water before analyses. All honey samples were taken from the same honey batch and the experiment were carried out with triplicate samples [22].

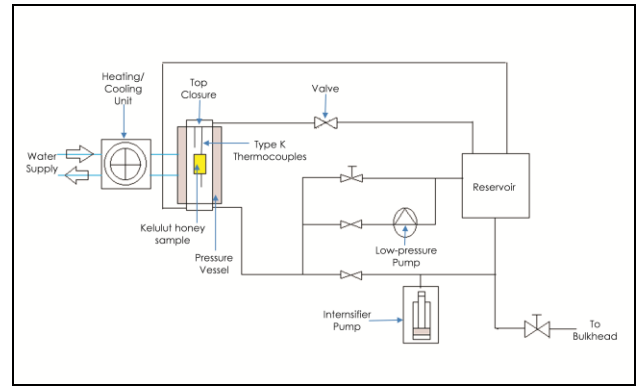


Figure 1 Schematic diagram of the HPP system

### 2.3 Thermal Treatment

Based on preliminary studies (data not shown), 6g of honey was placed in the test tube after being manually stirred, and thermally treated at 60 and 90 °C for 10 and 30 minutes, respectively using a Digital Water bath (KNK, Korea). The treatment time started when the control sample with a thermometer inside achieved the intended temperatures where it took  $5 \pm 2$  minutes for it to reach 60 °C and  $7 \pm 3$  minutes for it to reach 90°C. The samples were immediately placed on an ice-cooled water to stop the heat treatment. All honey samples were from the same honey batch, and the experiment was carried out with triplicate samples.

### 2.4 Determination of Antioxidant Activity

Antioxidant activity was determined according to the methods used by Turkmen *et al.* [10] using the 2, 2'-diphenyl-2-picryl-hydrazyl (DPPH). One gram of Kelulut honey was dissolved in 5 mL of distilled water using a vortex mixer and centrifuged for 15 minutes at 5000 rpm (Hermle Labortechnik, Germany). Next, the solution was filtered through Whatman No. 1 and diluted precisely to 4 °Brix with distilled water using a digital pocket refractometer (Atago, Japan). A 0.5 mL of honey extract was then mixed with an aliquot of 1.5 mL of 0.1 mM DPPH radical (Sigma) in methanol. The reaction mixture was then vortex-mixed and left to stand at 25°C in the dark for 60 minutes. Absorbance at 517 nm was measured using a spectrophotometer (Labomed, Inc., USA) with methanol as blank and a mixture of distilled water/aliquot of DPPH in methanol as a control. Antioxidant activity was expressed as percentage inhibition of DPPH radical and was determined by Equation 1:

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

Where AA (%) is the antioxidant activity in percentage,  $Abs_{\text{control}}$  is the absorbance reading of the control and  $Abs_{\text{sample}}$  is the absorbance reading of the sample.

## 2.5 Determination of Colour

A colourimeter (model Colour Quest XE, Hunter Lab, Reston, VA) was used to measure the colour parameters including  $L^*$  (0 black, 100 white),  $a^*$  ( $-a^*$  greenness,  $+a^*$  redness), and  $b^*$  ( $b^*$  blueness,  $bb^*$  yellowness) for all honey samples. Honey samples with thickness of 0.2 cm were placed in a transparent plastic container and measured with the Hunter Lab equipment.  $L^*$ ,  $a^*$ ,  $b^*$  values were directly obtained from the equipment. Consequently,  $a^*$  and  $b^*$  values were used to calculate the browning index (BI) [20] [23], and Total colour difference (TCD) (3.0) based on Equations 2 and 3, respectively.

$$BI = 100((x - 0.31)) / 0.172,$$

Where  $x = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$  (2)

$$TCD = \sqrt{[(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]} \quad (3)$$

## 2.6 Determination of Diastase Activity

The diastase activity (DN) of honey samples was determined based on the method described in the International Honey Commission (IHC, 2009) by using Phadebas tablets (Pharmacia Diagnostics, Sweden). Results were the mean value of triplicate absorbance measurement at 620 nm spectrophotometrically ( $A_{620}$ ). One DN was expressed as a diastase unit per gram of honey as presented in Equation 4.

$$DN \text{ (units/g)} = 35.2 \times A_{620} - 0.46 \quad (4)$$

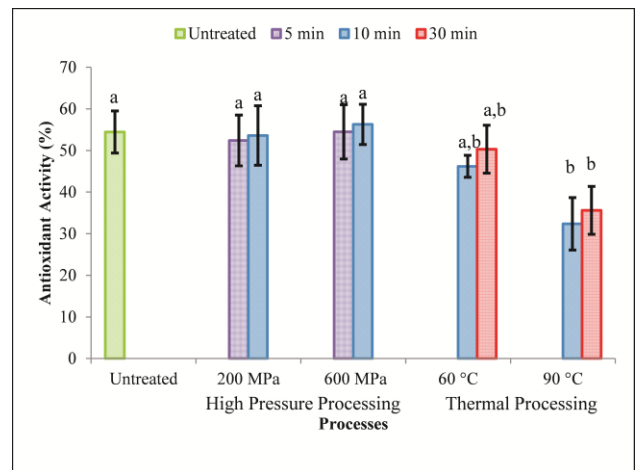
## 2.7 Statistical Analysis

Results were expressed as mean  $\pm$  standard deviation of triplicate samples. One-way analysis of variance (ANOVA) was used to compare the means where differences were considered significant at  $p < 0.05$ . Tukey's honestly significant difference (HSD) test was also carried out to determine where the significant different lies on the mean group. All statistical analyses were performed with Minitab version 16 (Minitab Inc., USA) and Microsoft Excel (Microsoft, Los Angeles, CA, USA).

## 3.0 RESULTS AND DISCUSSION

### 3.1 Effect on Antioxidant Activity (AA)

Kelulut honey in this study exhibited outstanding antioxidant prowess where the untreated sample registered  $54.43 \pm 5.06\%$  scavenging activities. After processing, the changes in antioxidant activity of HPP-treated are shown in Figure 2 with comparison to the thermally-treated.



**Figure 2** Changes in antioxidant activity (AA) of Kelulut honey. Means that do not share a letter are significantly different ( $P < 0.05$ ) as determined by Tukey HSD test in Minitab 16 (Pennsylvania, USA)

In general, the antioxidant activity of HPP-treated honey showed a small decrement at 200 MPa, yet at 600 MPa, it increased from the untreated particularly with 10 minutes holding time. However, these changes on all pressure, and time parameters were not significant ( $p < 0.05$ ). This proves that HPP can actually maintain, and even slightly enhance the antioxidant properties of Kelulut honey.

This particular finding at 600MPa/10 minutes showed a similar trend with HPP-treated Manuka honey where the antioxidant activities of HPP-treated Manuka honey with 10 minutes holding time increased significantly ( $p < 0.05$ ) from the untreated [24]. Fauzi *et al.* [24] also concluded that HPP of Manuka honey at 600 MPa with 10 minutes holding time is favourable in retaining and enhancing the antioxidant activities by 30%. Their finding is similar with HPP-treated Kelulut honey in this research in which HPP-treated Kelulut honey at 600 MPa with 10 minutes increased the antioxidant activity (see Figure 2). Moreover, HPP-treated Longan flower honey also showed significant increase ( $p < 0.05$ ) in antioxidant activities which is more apparent with increasing pressure and holding time particularly at HPP (500 MPa/20 minutes) [13]. The improvement of antioxidant activity was most probably due to pollen disintegration under pressure [13, 24]. Pollen, known to possess a variety of antioxidant compounds [13], disintegrates under pressure and releases these compounds in honey thus increasing the antioxidant activity.

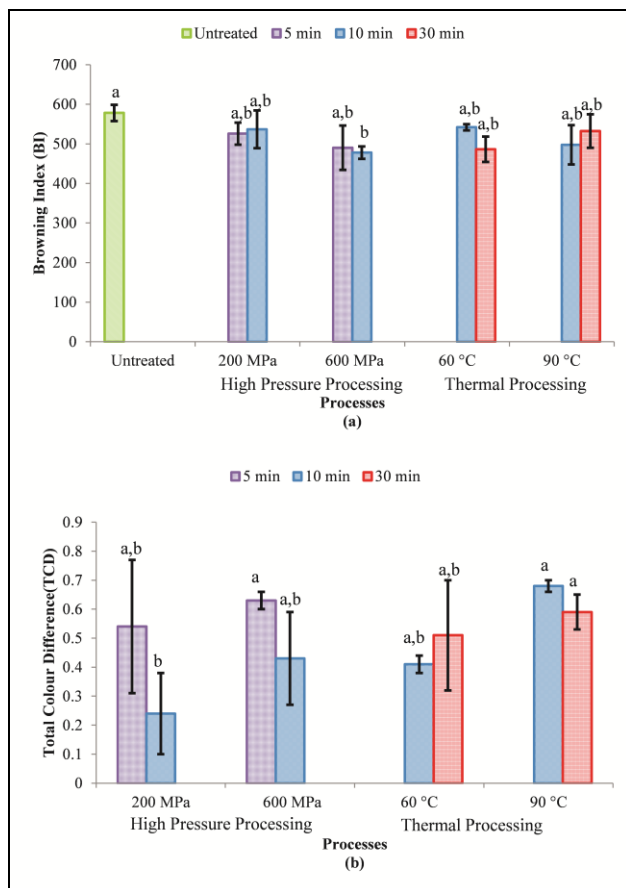
In comparison, thermal processing was shown to diminish antioxidant powers where the lowest antioxidant activity was registered by thermally-treated at 90°C, 10-minute time with only  $32.38 \pm 6.31\%$ . It is interesting to note that for thermal treatments, longer treatment time (30 minutes) seemed to increase the antioxidant activity from 10-minute treatment time which was postulated to be



contributed by Maillard reaction product [10]. However, the scavenging activities were still lower compared to untreated and thermally-treated. Looking at the common processing time; HPP-treated illustrated the positive effect of pressure whereas thermally-treated illustrated the negative effect towards the scavenging activities respectively. Based on the antioxidant activity parameter, HPP was shown to be far superior in terms of retaining and enhancing powers as pressure could add more antioxidant compound from disintegration of pollen [13] whereas heat could further degrade this compound and reduce the antioxidant prowess.

### 3.2 Changes in Colour

The changes in colour were evaluated based on total colour different (TCD) and browning index (BI). Figure 3 shows the TCD and BI of HPP-treated in comparison to thermally-treated. The Browning Index (BI) which gives an indication of browning development in honey was shown to be decreasing in HPP-treated compared to the untreated. The decrease in BI were significant ( $p < 0.05$ ) at HPP-treated 600 MPa with 10 minutes holding time, with it being the lowest.



**Figure 3** Changes in Colour parameter (a) Browning Index, (b) total colour different. Means that do not share a letter are significantly different ( $P < 0.05$ ) as determined by Tukey HSD test in Minitab 16 (Pennsylvania, USA)

In terms of TCD, HPP at 600 MPa with 5 minutes holding time had the highest impact on TCD value whereas the lowest impact on TCD value was recorded by HPP at 200 MPa with 10 minutes holding time. Both impact on TCD were significantly different ( $p < 0.05$ ) from each other. Fortunately, TCD values of HPP-treated were below the range that can be classified as noticeable which range from 1.5 to 3.0 [25] in which the threshold for noticeable change by human eye is above 3 [25]. The results of colour parameter and TCD of HPP-treated Kelulut honey were different from HPP-treated Manuka Honey [24] which had a low TCD value ( $< 1.0$ ). It can be postulated that Kelulut honey contained different type and concentration of colour pigments.

According to the harvester/beekeeper of Kelulut honey used in this project, the geographical area where the bee was reared was mostly surrounded with *M. malabathricum* which is locally known as 'pokok senduduk'. One of the colour pigments of *M. malabathricum* that gives out the dark blue-red or purple colour is anthocyanins [26]. Since honey content is heavily dependent on the food sources, it is probable that a high amount of anthocyanins was present in this Kelulut honey. Past literature has supported the existence of anthocyanins in honey where Jordanian honey exhibited a high content of anthocyanins during summer [27]. Su et al. [28] demonstrated that anthocyanin pigment of Chinese Bayberry juice degraded when it was treated to high pressure processing at 400, 500 and 600 MPa with 5 to 10 minutes holding time. The authors also concluded that anthocyanin is pressure sensitive. Hence it is probable that the anthocyanins pigments in Kelulut honey were degraded by high pressure processing (HPP) therefore clarifying the contradicting result between it and HPP-treated Manuka honey [24]. More in-depth studies should be done to further prove this theory.

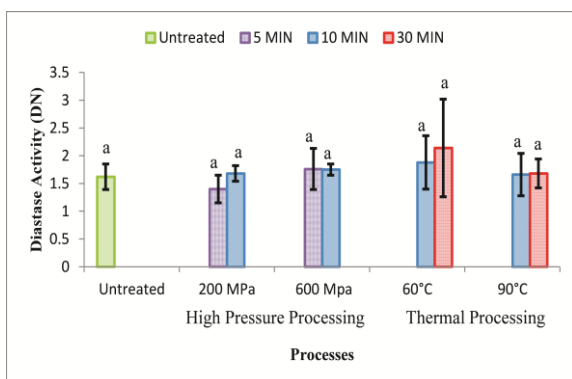
In comparison to thermal processing, the BI of both HPP-treated and thermally-treated were all less than the untreated with 600 MPa/10 minutes being the lowest and significantly different ( $p < 0.05$ ). The results indicate that processing reduced the browning in Kelulut honey. In reference to TCD, the highest impact was recorded by thermally-treated at 90 °C with 10-minute treatment time whereas the least impacted were HPP-treated at 200 MPa. Although both treatments caused changes in the colour of Kelulut honey, thermal treatment at 90 °C showed the largest difference from the untreated. At this point, HPP showed less impact towards the overall colour of Kelulut honey. It was earlier postulated that Kelulut honey contained colour pigments anthocyanin which are pressure-sensitive [28]. Moreover, anthocyanin was also easily degraded when subjected to thermal treatments [29]. Hence, this could explain the resulting decrement in BI and high TCD from the untreated.

Narrowing down to common processing time (10 minutes), HPP-treated at 600 MPa showed less browning index followed by thermally-treated at 90

°C. The brown pigment in honey was due to Maillard reaction in honey which was postulated to be affected by both processes. Hence, this explains the reduction in browning index in all treated samples. In terms of TCD, thermally-treated at 90°C showed the highest colour difference from the untreated, and the lowest impact was recorded by HPP-treated at 200 MPa. These results showed that at 10 minutes holding time, the effect of pressure on overall colour was less than heat on thermal treatment thus giving HPP one plus point than thermal treatment. However, both treatments did not exceed the threshold of colour change detectable by human eyes, ranging from 1.5 to 3.0 [25]. As evident by the results, it can be concluded that HPP caused less changes in the overall colour of Kelulut honey compared to thermally-treated.

### 3.3 Effect on Diastase Activity

Diastase activity is often used to evaluate the freshness of honey in industries because it gives an indication of possible heating or poor storage temperatures. Untreated Kelulut honey has a diastase number of  $1.62 \pm 0.23$  DN which is similar with stingless bee honey from Thailand with diastase activity of 1.5 Gothe [30]. Moreover, the DN was within the range of 0.9–23.0 DN [3]; the same as other stingless bee honey. After being treated with HPP, the changes of DN (see Figure 4) for all pressure and holding time were not notable ( $p > 0.05$ ). The DN of all HPP-treated increased except for 200 MPa with 5 minutes holding time. The results demonstrated that HPP, in general, was able to maintain the diastase activity of Kelulut honey hence verifying the capability of HPP to produce a fresh like quality of food product. To solidify this fact, Al-Habsi and Niranjana [31] had demonstrated that high hydrostatic pressure (HHP) maintained the diastase activity of Manuka honey with no significant difference ( $p > 0.05$ ) between untreated and treated samples.



**Figure 4** Changes in diastase activity of Kelulut honey. Means that do not share a letter are significantly different ( $P < 0.05$ ) as determined by Tukey HSD test in Minitab 16 (Pennsylvania, USA)

In comparison to thermally-treated, the changes in diastase activity was also not significant ( $p > 0.05$ ) (see Figure 4). It is interesting to note that although the changes in diastase activity were not statistically significant, the diastase activity of thermally-treated Kelulut honey showed a slight increment with thermally-treated at 60°C/ 30 minutes being the highest. These results contradicted previous results that showed a decrement in diastase activity in honey after thermal treatment [14, 32] and defeats the purpose of diastase activity as a freshness indicator. However, there were also other studies that showed a possible recovery of activity after thermal treatments. Tosi *et al.* [33] demonstrated activity recovery of diastase in honey treated at medium temperature treatments with longer times during the isothermal heating. This finding is similar to our finding as the Kelulut honey was heated isothermally and the sample at 60°C/30 minutes showed the highest DN among the other thermal-treated samples. The recovery of enzyme activity was probable due to the changes in the structure of enzyme that eventually affected the enzymatic activity. Based on the Eyring transition state theory, an irreversible denaturation would occur when free energy of the molecules exceeds the energy barrier, promoted by the heat during transient heating. However, in an isothermal system the temperature is maintained; in our study it was at 60°C and 90°C; the recovery of enzymatic activity was probable due to a low number of activated molecule that could exceed the energy barrier of transition state [33]. This eventually caused less complete denaturation and all the activated molecules that did not exceed the energy barrier would return back to its native-like state once the heating stop. As a consequence, enzymatic activity would recover [33, 34]. This postulation is in line with that reported by Kuwajima [35] where during transition stage; only the tertiary structure of protein was disrupted while the secondary structure was maintained in native-like structure. Accordingly, reversible inactivation of enzyme promoted by treatment condition occurred [34, 36]. Tosi *et al.* [33] also concluded that variable behaviour of diastase activity made it unreliable to be a heating indicator. Nevertheless, the effect of HPP on diastase activity of Kelulut honey was not severe and comparable to the effect demonstrated by conventional thermal processing.

## 4.0 CONCLUSION

The application of novel high pressure processing (HPP) on Kelulut honey (stingless bee honey) showed better retention of antioxidant activity than conventional thermal processing with a slight increment of 3% at 600 MPa/ 10 minutes. Moreover, the changes in colour parameter by HPP were not as severe as thermal processing with the changes being insignificant to the human eye. Furthermore, HPP also

showed retention of diastase activity thus demonstrating its ability to maintain the natural enzymatic activity of Kelulut honey. In conclusion, HPP showed better performance than thermal processing as an alternative method to cater to consumer demand of minimally-processed and fresh-like quality Kelulut honey.

### Acknowledgement

The first author would like to thank The Ministry of Higher Education of Malaysia for the Research Acculturation Grant Scheme (RAGS) R062. Geran Penyelidikan Pascasiswazah (GPPS) U937 from Research Management Center (RMC), UTHM is also greatly acknowledged. The supply of honey samples from Ladang Mini Kelulut MAEPS, Serdang, Malaysia is much appreciated. Special thanks to staff from Department of Chemical Engineering Technology, Faculty of Engineering Technology for their technical support.

### References

- [1] Kek, S. P., Chin, N. L., Yusof, Y. A., Tan, S. W. and Chua, L. S. 2014. Total Phenolic Contents and Colour Intensity of Malaysian Honeys from the *Apis* spp. and *Trigona* spp. Bees. *Agriculture and Agricultural Science Procedia*. 2:150-155.
- [2] Biluca, F. C., Della Betta, F., De Oliveira, G. P., Pereira, L. M., Gonzaga, L. V., Costa, A. C. O., and Fett, R. 2014. 5-HMF and Carbohydrates Content in Stingless Bee Honey by CE Before and After Thermal Treatment. *Food Chemistry*. 159: 244-249. <https://doi.org/10.1016/j.foodchem.2014.03.016>.
- [3] Souza, B., Roubik, D., Barth, O., Heard, T., Enríquez, E., Carvalho, C., Villas-Bôas, J., Marchini, L., Locatelli, J., Persano-Oddo, L., Almeida-Muradian, L., Bigdanov, S., and Vit, P. 2006. Composition of Stingless Bee Honey: Setting Quality Standards. *Interciencia*. 31(12): 867-875. <https://doi.org/10.1007/s00464-010-1064-4>.
- [4] Codex Alimentarius Commission. 2001. Codex Alimentarius Commission Standards. *Codex Stan 12-1981*. 1-8.
- [5] Rastogi, N. K., and Knorr, D. 2013. *Recent Developments in High Pressure Processing of Foods*. 1a edição. Nova Iorque: Springer. <https://doi.org/10.1007/978-1-4614-7055-7>.
- [6] Gupta, S. 2012. *High Pressure Processing of New Zealand Mussels (Perna Canaliculus)*. University of Auckland: Ph.D. Thesis.
- [7] Patterson, M. F. 2005. Microbiology of Pressure-treated Foods. *Journal of Applied Microbiology*. 98(6): 1400-1409.
- [8] Yaldagard, M., Mortazavi, S., and Tabatabaie, F. 2008. The Principles of Ultra-high Pressure Technology and Its Application in Food Processing/Preservation: A Review of Microbiological and Quality Aspects. *African Journal of Biotechnology*. 7(16): 2739-2767. <https://doi.org/10.5897/AJB07.923>.
- [9] Pereira, R. N., and Vicente, A. A. 2010. Environmental Impact of Novel Thermal and Non-thermal Technologies in Food Processing. *Food Research International*. 43(7): 1936-1943. <https://doi.org/10.1016/j.foodres.2009.09.013>.
- [10] Turkmen, N., Sari, F., Poyrazoglu, E. S., and Velioglu, Y. S. 2006. Effects of Prolonged Heating on Antioxidant Activity and Colour of Honey. *Food Chemistry*. 95(4): 653-657. <https://doi.org/10.1016/j.foodchem.2005.02.004>.
- [11] Vaikousi, H., Koutsoumanis, K., and Biliaderis, C. G. 2009. Kinetic Modelling of Non-enzymatic Browning in Honey and Diluted Honey Systems Subjected to Isothermal and Dynamic Heating Protocols. *Journal of Food Engineering*. 95(4): 541-550.
- [12] Chaikham, P., Kemsawasd, V., and Apichartsrangkoon, A. 2016. Effects of Conventional and Ultrasound Treatments on Physicochemical Properties and Antioxidant Capacity of Floral Honeys from Northern Thailand. *Food Bioscience*. 15:19-26. <https://doi.org/10.1016/j.fbio.2016.04.002>.
- [13] Chaikham, P., and Prangthip, P. 2015. Alteration of Antioxidative Properties of Longan Flower-honey after High Pressure, Ultra-sonic and Thermal Processing. *Food Bioscience*. 10: 1-7. <https://doi.org/10.1016/j.fbio.2015.01.002>.
- [14] Chua, L. S., Adnan, N. A., Abdul-Rahaman, N. I., and Sarmidi, M. R. 2014. Effect of Thermal Treatment on the Biochemical Composition of Tropical Honey Samples. *International Food Research Journal*. 21(2): 773-778.
- [15] Adnan, N. A., Chua, L. S., and Sarmidi, M. R. 2014. Thermal Treatment Effect on Free Amino Acids in Honey Samples. *Jurnal Teknologi*. 69(4): 29-33.
- [16] Kowalski, S. 2013. Changes of Antioxidant Activity and Formation of 5-Hydroxymethylfurfural in Honey during Thermal and Microwave Processing. *Food Chemistry*. 141(2):1378-1382. <https://doi.org/10.1016/j.foodchem.2013.04.025>.
- [17] Khalil, M. I., and Sulaiman, S. A. 2010. The Potential Role of Honey and Its Polyphenols in Preventing Heart Diseases: A Review. *African Journal of Traditional, Complementary and Alternative Medicines*. 7(4): 315-321. <https://doi.org/10.4314/ajtcam.v7i4>.
- [18] Hutchings, J. 1999. The Perception and Sensory Assessment of Colour. *Colour in Food: Improving Quality*. MacDougall, D. *Colour in Food*. United Kingdom: Woodhead Publishing Limited. 9-32. <https://doi.org/10.1533/9781855736672.1.9>.
- [19] Hutchings, J. B., Luo, M. R., and Ji, W. 2012. Food Appearance and Expectations in Color. In J. L. Caivano & M. del Pilar Buera (Eds.). *Food: Technological and Psychophysical Aspects*. Boca Raton, Florida, USA: CRC Press. pp. 3-10. DOI: <http://dx.doi.org/10.11113/jt.v79.9987>.
- [20] Bogdanov, S., Jurendic, T., Sieber, R., and Gallmann, P. 2008. Honey for Nutrition and Health: A Review. *Journal of the American College of Nutrition*. 27(6): 677-689. <https://doi.org/10.1080/07315724.2008.10719745>.
- [21] Fauzi, N. A., and Farid, M. M. 2015. High-pressure Processing of Manuka Honey: Brown Pigment Formation, Improvement of Antibacterial Activity and Hydroxymethylfurfural Content. *International Journal of Food Science and Technology*. 50(1): 178-185. <https://doi.org/10.1111/ijfs.12630>.
- [22] Fauzi, N. A. 2014. *Quality Improvement of Manuka Honey through the Application of High Pressure Processing*. University of Auckland: Ph.D. Thesis.
- [23] Ferrari, G., Maresca, P., and Ciccarone, R. 2010. The Application of High Hydrostatic Pressure for the Stabilization of Functional Foods: Pomegranate Juice. *Journal of Food Engineering*. 100(2): 245-253.
- [24] Fauzi, N. A., Farid, M. M., and Silva, F. V. M. 2014. High-Pressure Processing of Manuka Honey: Improvement of Antioxidant Activity, Preservation of Colour and Flow Behaviour. *Food and Bioprocess Technology*. 7(8): 2299-2307. <https://doi.org/10.1007/s11947-013-1204-7>.
- [25] Almeida, F. D. L., Gomes, W. F., Cavalcante, R. S., Tiwari, B. K., Cullen, P. J., Frias, J. M., Bourke, P., Fernandes, F. A. N., and Rodrigues, S. 2017. Fructooligosaccharides Integrity after Atmospheric Cold Plasma and High-pressure Processing of a Functional Orange Juice. *Food Research International*. 102: 282-290. <https://doi.org/10.1016/j.foodres.2017.09.072>.
- [26] Aishah, B., Nursabrina, M., Noriham, A., Norizzah, A. R., and Mohamad Shahrimi, H. 2013. Anthocyanins from *Hibiscus sabdariffa*, *melastoma Malabathricum* and *Ipomoea*

- batatas and Its Colour Properties. *International Food Research Journal*. 20(2): 827-834.
- [27] Rababah, T. M., Al-Omoush, M., Brewer, S., Alhamad, M., Yang, W., Alrababah, M., Ghzawi, A. A., U'datt, M., Ereifej, K., Alsheyab, F., Esoh, R., and Almajwal, A. 2014. Total Phenol, Antioxidant Activity, Flavonoids, Anthocyanins and Color of Honey as Affected by Floral Origin Found in the Arid and Semiarid Mediterranean Areas. *Journal of Food Processing and Preservation*. 38(3): 1119-1128. <https://doi.org/10.1111/jfpp.12071>.
- [28] Su, G., Zhu, S., Xu, M., Ramaswamy, H. S., Lin, Y., and Yu, Y. 2016. Pressure Degradation Kinetics of Anthocyanin Pigment and Visual Colour of Chinese Bayberry Juice. *International Journal of Food Properties*. 19(2): 443-453. <https://doi.org/10.1080/10942912.2015.1038562>.
- [29] Patras, A., Brunton, N. P., O'Donnell, C., and Tiwari, B. K. 2010. Effect of Thermal Processing on Anthocyanin Stability in Foods: Mechanisms and Kinetics of Degradation. *Trends in Food Science and Technology*. 21(1): 3-11. <https://doi.org/10.1016/j.tifs.2009.07.004>.
- [30] Chuttong, B., Chanbang, Y., Sringarm, K., and Burgett, M. 2016. Physicochemical Profiles of Stingless Bee (Apidae: Meliponini) Honey from South East Asia (Thailand). *Food Chemistry*. 192: 149-155. <https://doi.org/10.1016/j.foodchem.2015.06.089>.
- [31] Al-Habsi, N. A., and Niranjana, K. 2012. Effect of High Hydrostatic Pressure on Antimicrobial Activity and Quality of Manuka Honey. *Food Chemistry*. 135(3): 1448-1454. <https://doi.org/10.1016/j.foodchem.2012.06.012>.
- [32] Kowalski, S., Lukasiewicz, M., Bednars, S., and Panus, M. 2012. Diastase Number Changes During Thermal and Microwave Processing of Honey. *Czech Journal of Food Sciences*. 30(1): 21-26.
- [33] Tosi, E., Martinet, R., Ortega, M., Lucero, H., and Ré, E. 2008. Honey Diastase Activity Modified by Heating. *Food Chemistry*. 106(3): 883-887. <https://doi.org/10.1016/j.foodchem.2007.04.025>.
- [34] Cozmuta, A. M., Cozmuta, L. M., Varga, C., Marian, M., and Peter, A. 2011. Effect of Thermal Processing on Quality of Polyfloral Honey. *Romania Journal of Food Science*. 1(1): 45-52.
- [35] Kuwajima K. 1989. The Molten Globule State as a Clue for Understanding the Folding and Cooperativity on Globular-Protein Structure. *Proteins*. 6(2): 87-103.
- [36] Richardson T., and Hyslop D. 1992. Enzimas. In: Fennema O. (Ed.). *Química de los Alimentos*. Publicaciones Acribia SA. Zaragoza, Espana. 451-456.