

Original Research Article

Impact of UV-C Assisted Drying Treatment on the Quality of Malaysian Stingless Bee Honey

Nurul Alissa Afendi¹, Nor Nadiyah Abdul Karim Shah^{1,2*}

¹Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, alissafndi@gmail.com

²Halal Products Research Institute, Universiti Putra Malaysia, Putra Infoport, 43400 Serdang Selangor, nadiyahkarim@upm.edu.my

*Corresponding author: Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43300 Serdang, Selangor, nadiyahkarim@upm.edu.my

Abstract: Stingless bee honey (SBH) has been the focus of various drying studies aimed at lowering the moisture content to an acceptable limit of less than 20%. The low moisture level of SBH has been found to slow yeast development and hinder the fermentation process, thereby prolonging its shelf-life. Conventionally, SBH is usually treated using thermal treatment to lower its moisture content. Due to issues with the quality degradation of thermal-treated SBH, other alternatives are being explored. Non-thermal treatment, namely ultraviolet (UV-C) assisted drying, has been proposed in this study with the expectation of replacing the conventional heat treatment. The UV-C closed system, when properly employed, may provide enough radiation energy (below 40°C) to evaporate the moisture bound in the honey. Hence, this study was aimed at determining and correlating the effects of the UV-C assisted drying process on the quality of UV-C treated SBH. The experiment was carried out on SBH (*Heterotrigona itama*) under UV-C treatment with the stated parameters; wavelength: 254 nm, power: 7 W, UV-C dose: 8 mJ/cm², thickness of SBH: 3 mm for 0, 30, 50, 75, and 120 min in a controlled environment (35 ± 5% relative humidity and 25 ± 5 °C temperature). The results of this study showed that the moisture level of SBH was below the critical moisture content of 20%, with the lowest moisture content recorded at 17.42% after 120 min of UV-C treatment time (moisture loss: 3.5%) and the highest moisture content of 18.40% after 30 min of treatment time (moisture loss: 2.21%). However, the value of 5-hydroxymethylfurfural (5-HMF) obtained in this study was significantly high (above 80 mg/kg), which might be due to the high content of fructose to glucose ratio in SBH. Nevertheless, while it has been demonstrated that UV-C assisted drying was able to lower the moisture content of SBH, further study is necessary to evaluate its effectiveness without compromising on the quality of SBH.

Keywords: Stingless bee honey; drying; moisture content; ultraviolet light (UV-C); 5-HMF

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1. Introduction

Stingless bees honey (SBH) can be found in tropical and subtropical regions worldwide, including Africa, Southeast Asia, Australia, and South America. There are 32 genera with more than 500 species that have been identified, and more than 100 new species are still to be identified (Rafie *et al.*, 2018). *Heterotrigona itama* is one of the most common stingless bee species reared for commercial purposes in Malaysia. It is locally known as *Kelulut* bee honey and can be found in all states of Malaysia with a distinct taste described as a mix of acidic and sour tastes (Yegge *et al.*, 2021). Abu Bakar *et al.* (2017) determined that the average Malaysian SBH contained moisture (26.5 ± 0.00 to 31.8 ± 0.00 %), ash (0.15 ± 0.01 to 0.67 ± 0.00 g/100 g), protein (0.016 to 0.54 g/100 g), fat (0.02 to 0.15 g/100 g), and carbohydrate (67.12 to 73.26 g/100 g). A study by Ávila *et al.* (2018) also revealed that SBH has higher acidity, slightly lower total carbohydrate content, and higher moisture content than *Apis mellifera* with significant fructose content followed by glucose and sucrose content. SBH is getting popular and has been found to benefit human health as it has antiseptic, anti-microbial, anti-cancer, anti-inflammatory, and wound-healing properties (Braghini *et al.*, 2019). It can be used for direct consumption or for medicinal purposes, including alleviating tumours, eye infections, wound healing treatments, and sore throat (Ávila *et al.*, 2018).

Chuttong *et al.* (2016) discovered in two studies that SBH in Australia and Thailand have higher moisture content when compared to *Apis mellifera* honey with moisture content of SBH ranged from 22.0% to 25.6%. High moisture content in SBH leads to spoilage due to its natural fermentation process, resulting in quality degradation, acidic taste, and unpalatable appearance (Lim, 2019). With its high moisture content, SBH also has the limited capability to be distributed globally due to the difficulty of preserving the quality of SBH despite the high demand. The current process to preserve and produce shelf-stable SBH involves the use of heat treatment. Yap *et al.* (2019) iterated in their study that the current practice to process SBH is: Pre-heating at 40°C, straining, filtering, indirect heating at 60–65°C for 25–30 min on honey and followed by rapid cooling to stop the heat treatment. Similarly, in Thailand, the current practice of floral honey industries is generally to heat the honey to a temperature above 70 °C (Chaikham *et al.*, 2016). Since there is no standard operating procedure and lack of knowledge of drying honey, most honey producers dry honey using heat treatment due to its low cost and readily available equipment.

However, the conventional technique, which perused high-temperature thermal treatment, causes other detrimental effects on the physicochemical properties of SBH (Razali *et al.*, 2019). The application of heat alters the water activity, humidity, colour intensity, viscosity, and 5-hydroxymethylfurfural (5-HMF) content in SBH when treated between 45 and 90 °C for 30 – 120 min as reported by Braghini *et al.* (2019). Previous studies have also found reductions in antioxidant activity, phenolic and biochemical compounds, diastase activity, proline, and threonine in thermal-treated SBH (Razali *et al.*, 2019). The reduction is

due to many unstable and thermolabile components present in honey, which will eventually degrade the quality of honey and lose its health-beneficial compounds after undergoing thermal treatment (Razali *et al.*, 2019). Meanwhile, a detrimental increment of 5-HMF formation was also observed to be caused by longer thermal treatment (24 h), which had an inversely-proportional relationship with the water activity and acidity of SBH (Biluca *et al.*, 2014). The combination effect of storage length and heat exposure on SBH has been proven to increase the 5-HMF content (Yap *et al.*, 2019). An increase in 5-HMF content has been negatively correlated to the customer's acceptance (Chaikham *et al.*, 2016). Furthermore, the presence of HMF and its derivatives have been proven to confer genotoxic, mutagenic, carcinogenic, DNA-damaging, organotoxic and enzyme inhibitory effects towards humans (Shapla *et al.*, 2018). Additionally, it was also confirmed that high temperatures (>40 °C) and long treatment time were able to reduce the bioactive compounds in honey (Halim *et al.*, 2020), altering the essential composition and thus, not recommended to be continued as a common practice. Hence, limited heating to 40 °C can help preserve the nutrition and quality of the SBH.

Various drying methods to reduce the moisture content in honey have been actively investigated in the past decade namely the Dehydrator, microwave, vacuum-based methods (Singh & Singh, 2018), high-pressure processing (HPP) (Razali *et al.*, 2019) and passive diffusion (Baroyi *et al.*, 2019), among others. On the other hand, UV-C is a popular non-thermal method that has yet to be explored. At wavelengths between 200 to 280 nm, germicidal activity can be produced by the conversion of electrical energy into UV radiation form, where it is becoming more popular in the food industries for the preservation and shelf-life extension of various food (Choudhary & Bandla, 2012; Koutchma, 2009). UV-C light is able to preserve food products without raising the process temperature (less than 40°C), thus, removing the drawbacks of high-temperature processing (Ros-Polski *et al.*, 2016). Studies performed by Ros-Polski *et al.* (2016) on the use of UV-C technology as an alternative for drying treatment for high fructose corn syrup (HFCS) and stuffed pasta have proven that UV-C could be used as a drying technique for processing low-temperature food to produce high-quality products. However, no research on UV-C drying treatment on honey had been published at the time of writing. Hence, this study was proposed to determine the effects of the drying method utilising a novel non-thermal preservation technique, UV-C, to reduce the moisture content of SBH. Subsequently, the UV-C impact on the quality of SBH, including changes in antioxidant activity, 5-HMF content and physicochemical properties, was analyzed.

2. Materials and Methods

2.1 Materials

The untreated samples of SBH produced by *Heterotrigena itama* were procured from Hulu Langat, Selangor. All other chemicals and reagents used in this work were of analytical grade.

2.2 Preparation of UV-C Assisted Drying of SBH

2.2.1 Preparation of SBH

The raw SBH was stored in a glass bottle in a chiller (Protech Cooler SD-700, Malaysia) at the temperature of ± 10 °C prior to UV-C treatment. SBH samples were weighed (Mettler Toledo NewClassic MF JP303G, Switzerland) and placed in a glass Petri dish of 8.5 cm internal diameter. Approximately 10 gram of SBH was poured into the petri dish, giving a height/thickness of 1 cm and the sample was treated with UV-C treatment. The sample was then analysed directly after UV-C treatment.

2.2.2 Preparation of UV-C treatment

The UV-C treatments were carried out in batch mode using a UV-C lamp with a wavelength of 254 nm (power of 7W; UV-C dose of 8 mJ/cm²). SBH underwent a drying process in a UV-C controlled chamber (Figure 1) with an ambient room temperature of 25 ± 5 °C for 0, 30, 50, 75, and 120 min and relative humidity of 35 ± 5 %. The humidity is continuously monitored using a humidity sensor and adjusted with silica gel. The samples were distributed equally inside the chamber to maintain the controlled environment when the relative humidity was approximately 30–40%. The temperature was maintained at 25 ± 5 °C and was monitored using a temperature sensor and ventilator. For each treatment, the two Petri dishes (limited by the size of the UV-C controlled chamber) were placed under the UV-C lamp at a pre-determined position with the highest irradiance (visual observation). The following tests were carried out in triplicate in samples before (control) and after (treated) UV-C exposure.

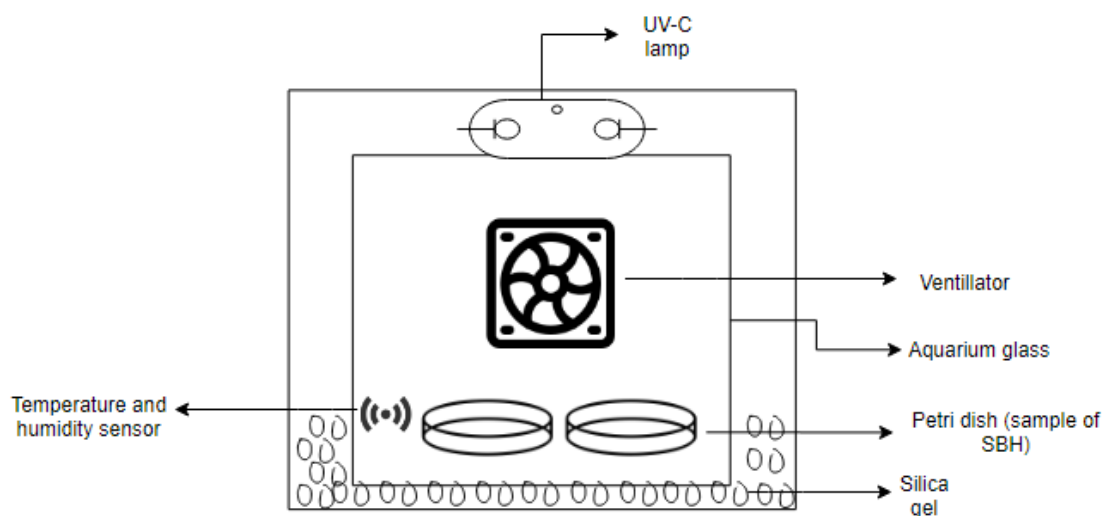


Figure 1. Schematic diagram of UV-C controlled chamber

2.3 Analysis

2.3.1 Moisture content, moisture loss and dehydration rate of SBH

The moisture content was measured using a RX-5000a digital refractometer (Atago, Japan) at 20°C. The moisture content was indirectly assessed by measuring the refractive index of the sample. The water content corresponding to the refractive index was calculated using Wedmore's table from International Honey Commission (2009). All measurements were carried out in triplicate. Moisture loss of SBH was calculated using Equation (1).

$$\text{Moisture}_{(\text{before treatment})} - \text{Moisture}_{(\text{after treatment})} = \text{Moisture loss of SBH} \quad (1)$$

The mass of SBH was measured before and after the UV-C treatment using the analytical scale (Mettler Toledo New Classic MF JP303G, Switzerland). The dehydration process yield of SBH was calculated using Equation (2) from the method by Yap *et al.* (2019). All measurements were carried out in triplicate.

$$\frac{\text{Mass of SBH after treatment (g)}}{\text{Mass of SBH before treatment (g)}} \times 100 = \text{yield of SBH} \quad (2)$$

2.3.2 5-Hydroxymethylfurfural (5-HMF) analysis

The 5-HMF analysis of treated and untreated honey was done using the method proposed by Yang *et al.* (2019). Honey samples (0.1 g) were dissolved in 10 mL water. The solution (10 g/L) was then filtered with a Millex-HN nylon clarification kit (0.45 µm pore

size) for analysis by an HPLC–PDA system (LC-20A, Shimadzu, Japan) with a WondaSilC18-WR column (5 μm , 4.6 \times 250 mm, Shimadzu-GL) and PDA detector (Shimadzu). The injection volume was 20 μL . The mobile phase was 100% water for 0–10 min, then a linear gradient of 100% water to 100% methanol, from 10 to 55 min, and 100% methanol from 55 to 65 min at a flow rate of 0.5 mL/min. The column temperature was set at 25 $^{\circ}\text{C}$. Spectral data from all peaks were accumulated in the range of 200–800 nm, and chromatograms were recorded at 284 nm for 5-HMF.

2.3.3 Brown pigment analysis

The brown pigment of SBH was determined by adopting Yegge *et al.* (2021). Using a vortex mixer (WiseMix VM-10 Vortex, Germany), one gram of SBH was dissolved in 5 ml of distilled water. The solution was centrifuged using a centrifuge (Thermo Scientific MicroCL 21 microcentrifuge, USA) for 15 minutes at 5000 rpm. Whatman No.1 filter paper has been used to filter through the solution. Then, the filtered solution was diluted to 4 $^{\circ}$ Brix with distilled water using a digital refractometer (Atago A733114, Japan). The absorbance of diluted SBH was measured using a UV–VIS spectrophotometer (SQ2810, USA) at 420 nm.

2.3.4 Antioxidant activity analysis

Antioxidant activity in the sample was determined using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method (Yegge *et al.* 2021). One gram of each SBH sample was dissolved in 5 mL of distilled water. The solution was then centrifuged (Hermle Larbotechnik, Germany) for 15 min at 5000 rpm and filtered through Whatman No.1 filter paper before being diluted to 4 $^{\circ}$ Brix with distilled water using a digital refractometer (Atago A733114, Japan). A 0.5 mL of honey extract was mixed with an aliquot of 1.5 mL of 0.1 m DPPH radical (Sigma-Aldrich, USA) in methanol. The reaction mixture was vortex-mixed (WiseMix VM-10 Vortex, Germany) and left to stand a 25 $^{\circ}\text{C}$ in the dark for 60 min. Absorbance at 517 nm was measured using a UV–VIS spectrophotometer (SQ2810, USA) using methanol as a blank, whereas distilled water was mixed with DPPH solution using a ratio of 1:3 as a control. The experiment was carried out three times, each time with triplicate samples. Antioxidant activity (DPPH scavenging activity) is expressed as percentage inhibition of the DPPH radical determined by Equation (3).

$$\text{Antioxidant (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (3)$$

Where; $\text{Abs}_{\text{control}}$ was the absorbance reading of the control and $\text{Abs}_{\text{sample}}$ was the absorbance reading of the sample.

2.4 Statistical Analysis

All data were presented as the mean of triplicates. The data were analysed using Excel software for analysis of variance (one-way ANOVA), where all observations were independent. The results were expressed as mean value \pm standard deviation and level for statistical significance value of 0.05. Tukey's test was used to determine the significant difference ($P < 0.05$) in the data. Pearson correlated coefficient.

3. Results

Figure 2 shows the moisture loss and yield of SBH during the dehydration process. The moisture content of UV-C dried SBH showed significant decreasing ($P < 0.05$) trend. The changes appeared to be linearly proportional ($R^2 = 0.83$; $P < 0.05$; Table 1), which means that as the UV-C treatment time increases, the moisture loss of SBH increases. The moisture content of UV-C dried SBH was below the crucial moisture content of 20%, with the highest moisture loss recorded at 16.7% at 120 min of treatment time, and the lowest moisture loss recorded at 30 min of treatment time at 10.7%. It is worth noting that the moisture content of UV-C treated SBH was lower than 20%, which was well within Codex's guidelines (Codex Alimentarius Commission, 2001).

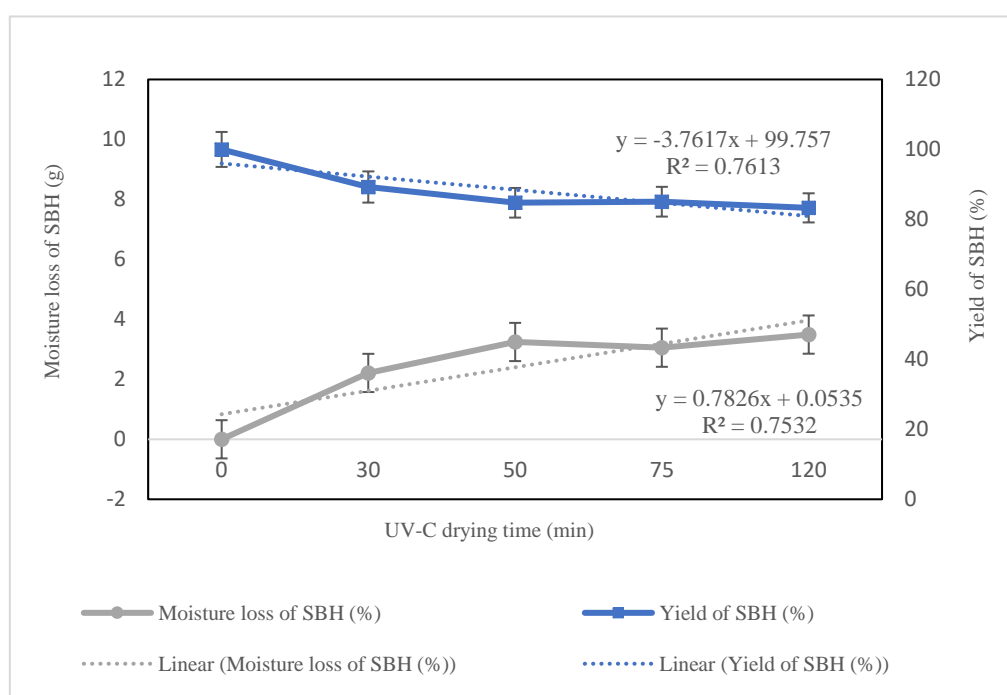


Figure 2. Effects of UV-C drying time on moisture loss and yield of SBH

Table 1. Matrix of Pearson correlation coefficients

	Temperature	Time	Moisture Content	5-HMF	Brown Pigment	Antioxidant Activity
Temperature	1					
Time	0.137 0.244*	1				
Moisture Content	-0.506 0.000	0.833 0.057	1			
5-HMF	0.899 0.144*	0.108 0.762*	0.638 0.039	1		
Brown Pigment	0.038 0.000	-0.953 0.054	-0.718 0.021	0.046 0.037	1	
Antioxidant Activity	0.337 0.000	-0.558 0.066*	-0.291 0.716*	0.298 0.035	0.598 0.333*	1

*Correlations are not significant at the 0.05 level (2-tailed).

Based on the trend line in Figure 2, moisture loss in SBH increases ($P < 0.05$) with the increasing length of UV-C treatment duration, as expected because honey moisture loss accumulates with time. This finding was similar to Yap *et al.* (2019), who showed that as the moisture loss from honey increases, the yield of dehydrated honey decreases. These results proved that the UV-C could dry the SBH below its acceptable limit when the light energy emitted by the UV-C lamp was converted into radiation energy (where temperature increases are negligible). Furthermore, the aeration in the controlled chamber may have also assisted the drying process by removing the water from the surface of the honey. However, further study is needed to evaluate the drying rate of UV-C to different weights or thicknesses of SBH.

5-HMF is a typical metric for determining whether honey has been overheated or has aged (Chong *et al.*, 2017). Ros-Polski *et al.* (2016) did an extensive study regarding 5-HMF and explained that 5-HMF is a six-carbon compound with an aldehyde and alcohol functional group on the furan ring. It can be formed as an intermediate at high temperatures during the non-enzymatic browning Maillard reaction or caramelization of sugars (Ramos-Villarreal *et al.*, 2012). When hexoses are dehydrated in acidic circumstances, 5-HMF is formed, where keto-hexoses like fructose have the greatest dehydration selectivity. Because of its relatively fragile ring structure, fructose, unlike glucose and sucrose, is prone to 5-HMF conversion (Yang *et al.*, 2019). Honey from tropical regions should have a 5-HMF content of less than 80 mg/kg, while general honey must have less than 40 mg/kg (Chuttong *et al.*, 2016; Shamsudin *et al.*, 2019). Unfortunately, the results of this study have shown that the initial SBH has a significant ($P < 0.05$) value of 5-HMF, far beyond the standard value. The initial 5-HMF value was shown to be 2187.8 mg/kg (Figure 3).

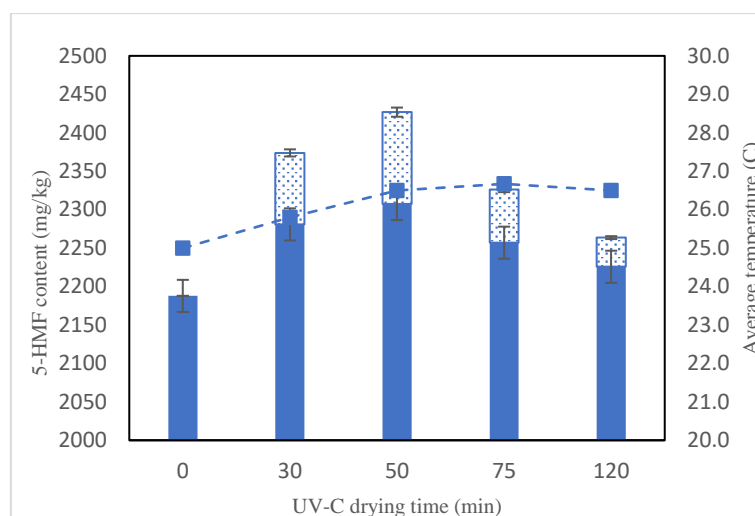


Figure 3. Effects of UV-C drying time and temperature on 5-HMF development of SBH

The 5-HMF initial finding in this study agrees with the findings of Chong *et al.* (2017) and Khalil *et al.* (2010), where the authors stated that the 5-HMF content of honey was highly correlated with longer storage time. Khalil *et al.* (2010) further noted that 5-HMF concentrations in Malaysian honey held at 25–30°C for over a year might reach extremely high levels, ranging from 118.47–1139.95 mg/kg, which showed that the extremely high value of honey found in this study might be due to long storage. However, adulteration with inverted sugars can also be indicated by high amounts of 5-HMF (more than 100 mg/kg) (Makawi *et al.*, 2009). Furthermore, various researches have reported that high ambient temperature during storage, thermal treatment, and low pH are the probable causes of furan synthesis in honey (Ros-Polski *et al.*, 2016). SBH from tropical places that have been subjected to high temperatures for an extended time can be attributed to high 5-HMF content (Chuttong *et al.*, 2016). Even though the authors found significant differences in 5-HMF content in all samples, the kinetics of 5-HMF formation were unaffected by the initial 5-HMF concentration in honey (Subramanian *et al.*, 2007). Thus, the effects of UV-C treatment on SBH in this study can still be evaluated. However, it is recommended that a sugar analysis is done to further confirm the root cause of high 5-HMF value of honey sample in this study.

The highest 5-HMF was recorded at 50 min (119.5 mg/kg), while the lowest 5-HMF value was shown at 120 min post-UV-C drying treatment (37.9 mg/kg). The results proved that the time and 5-HMF formation were a weak correlation ($R^2 = 0.11$; $P < 0.05$). However, the temperature and 5-HMF effects were strongly correlated ($R^2 = 0.89$; $P > 0.05$ Table 1). The insignificance of the correlation can be reasoned by other contributing factors besides temperature, such as the ratio of fructose to glucose content, which necessitates further study on the effect of UV-C drying treatment on the natural or unnatural sugar content (economically-driven adulteration). Yang *et al.* (2019) further stated that 5-HMF is a fructose break-down product that forms slowly during storage but rapidly when high temperature is involved. At the same time, the correlations between 5-HMF formation in UV-C dried SBH and moisture content (moderately correlated; $P < 0.05$). While brown pigment and antioxidant

activity were weakly correlated, these characteristics were not the contributing factors in the formation of 5-HMF in this study ($P < 0.05$).

SBH comes in various colours, including light yellow to black depending on its origin (Razali *et al.*, 2019). Chong *et al.* (2017) also emphasized that the colour of SBH plays a significant role since it influences customer perception and acceptance, with the darker colour of SBH being favoured in countries such as Germany, Austria, and Switzerland (Bogdanov *et al.*, 2004). In this study, the initial colour for SBH was dark amber, which is highly preferred and can indicate higher antioxidant content and pungent taste compared to lighter colour honey (Chong *et al.*, 2017). Based on Figure 4, the higher the UV-C drying duration, the lower the brown pigment of treated SBH ($R^2 = -0.953$, $P < 0.05$). At 120 minutes, the brown pigment formation was the lowest (0.0622 AU) due to the UV-C treatment (8.12% difference from the control sample).

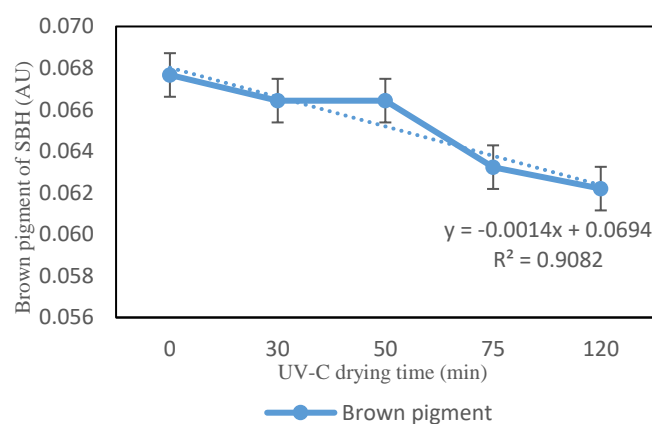


Figure 4. Effects of UV-C drying time on brown pigment of SBH

Further analysis has shown that the moisture content was significantly correlated to the brown pigment ($R^2 = 0.62$; $P < 0.05$) and was not linearly correlated to the antioxidant activity of SBH ($R^2 = 0.59$; $P > 0.05$) (Table 1). In comparison to this study, pasteurization of SBH at 90 °C resulted in a large difference in colour changes while HPP showed lower impact (Razali *et al.*, 2019). Both treatments showed similar results to this study, where the brown pigment of SBH was reduced after treatment. The decrement in brown pigment formation may be due to the presence of anthocyanin, which is sensitive to heat and pressure (Razali *et al.*, 2019). However, the findings in this study were in contrast with the previous research that resulted in darker SBH after heat treatment (Yap *et al.*, 2019; Kędzińska-Matysek *et al.*, 2016). The darker colour of SBH was due to sugar caramelisation, decomposition of volatile compounds, and brown melanoidins production (Yap *et al.*, 2019). In addition, the decrement of brown pigment may also be due to protein degradation in SBH, as UV-C treatment may have a negative effect on protein degradation (Koutchma, 2009). Further study should be conducted to determine the correlation between protein and brown pigment of SBH.

SBH is well known for its antioxidant properties that offer many health benefits, including strengthening the immune system and minimising the chances of heart disease and cancer (Liza A-Rahaman *et al.*, 2013). The presence of phenolic components such as flavonoids, peroxides, enzymes, phenolic acids, and Maillard reaction products is primarily responsible for this (Zulkhairi Amin *et al.*, 2018). According to Liza A-Rahaman *et al.* (2013), the botanical origin of SBH is a crucial element influencing its antioxidant activity. Based on Figure 5, the antioxidant activity of SBH fluctuated as UV-C drying times were increased ($P > 0.05$). The trend showed that a short UV-C treatment time, such as 30 min, may enhance the antioxidant activity of SBH, while longer UV-C treatment time resulted in lower antioxidant activity than untreated SBH ($R^2 = -0.56$). Further analysis also showed that the antioxidant activity of UV-C dried SBH was not significantly correlated to the moisture content of SBH and brown pigment ($P > 0.05$). At the same time, it was weakly correlated to the temperature during the UV-C drying treatment ($P < 0.05$). The result of this study agrees with a study by Csapó *et al.* (2019), where it was reported that UV-C light did not significantly influence the total antioxidant capacity or polyphenol oxidase or peroxidase activity compared to the equivalent heat treatment.

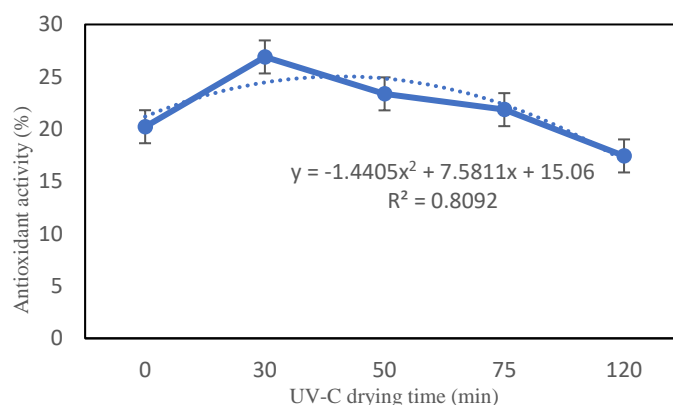


Figure 5. Effects of UV-C drying time on antioxidant activity of SBH

4. Conclusions

This study found that SBH moisture loss increases ($P < 0.05$) with the increasing length of the UV-C treatment period. However, the study also found an increased ($P > 0.05$) 5-HMF content while significant changes ($P < 0.05$) were found in brown pigment formation and antioxidant activity of treated SBH compared to untreated SBH. The highest moisture loss recorded during UV-C treatment was at the duration of 120 min (3.5%), where the least amount of 5-HMF formed (1.73%; $P < 0.05$) was shown, and minimal decrement was found in the brown pigment (8.12%; $P < 0.05$) and antioxidant activity (13.8%; $P > 0.05$) of SBH when compared to the control samples. However, it was demonstrated that the drying rate was the highest at 30 min ($P < 0.05$), and the amount of 5-HMF increment was among the highest (4.25%). In conclusion, UV-C technology offers the promise of lowering the moisture content to a below-acceptable level (20%), subsequently lowering the microbiological risks

while minimising the quality loss in terms of qualitative and nutritional content. However, its drying parameters must be carefully reviewed, given the wide range of results in this study that will need to be re-evaluated. UV-C lamps with various wavelengths and various sample heights should be considered in future studies. Other impacts of UV-C on various SBH quality characteristics, including diastase activity, total phenolic content, and viscosity of UV-C dried SBH, should be thoroughly examined.

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