# **Physicochemical Properties of Stingless Bee Honey**

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Stingless bee honey (SLBH) has a high moisture content, making it more prone to fermentation and leading to honey spoilage. Dehydration of SLBH after harvest is needed to reduce the moisture content. This review compiles the available data on the dehydration methods for SLBH and their effect on its physicochemical properties.

Keywords: stingless bee honey; dehydrated honey; chemical composition

### 1. Introduction

Stingless bee honey (SLBH) is an emerging functional food due to its many health benefits. SLBH is rich in flavonoid and phenolic content, which contributes to its high antioxidant activity  $^{[\underline{1}]}$ . The common phenolic compounds in SLBH are the same as *Apis mellifera*, such as salicylic acid, p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid and quercetin  $^{[\underline{2}]}$ . Despite its benefits, SLBH has generally higher moisture content than *Apis* spp.  $^{[\underline{3}]}$ . A previous study showed that the moisture content in SLBH is the highest with 33.24%, compared to *Apis* spp. which is between 21.96–27.41%  $^{[\underline{4}]}$ . SLBH of *Melipona* spp. has a moisture content above 24.8% compared to *Apis* spp. with 18.6%  $^{[\underline{5}]}$ . A comprehensive review reported that SLBH contains more moisture (21.52–31%) than Tualang and Gelam honey (17.53–26.51%)  $^{[\underline{6}]}$ . Furthermore, SLBH has a high water activity of 0.76 compared to a range between 0.60–0.67 in *Apis* spp.  $^{[\underline{4}]}$ .

The high moisture content in SLBH makes it more susceptible to alcoholic fermentation contributing to honey acidity  $^{[I]}$ . The rapid fermentation by microorganism growth in SLBH leads to honey spoilage  $^{[B]}$ . Apart from high water content, SLBH has higher free acidity, electrical conductivity and lower diastase activity compared to Apis spp.  $^{[3]}$ . Hence, it is difficult for SLBH to follow the honey standard. Several studies have proposed a different standard for SLBH given the difficulty for SLBH to follow the International Honey Commission (IHC) standard  $^{[9]}$ .

Owing to the high water content, it is a challenge to maintain the quality of SLBH. Therefore, dehydration of SLBH upon collection is suggested to lower the moisture content. Microbial stability can be achieved through dehydration, thus, prolonging the shelf life of honey  $^{[8]}$ . Additionally, lowering the moisture content will help the SLBH adhere to the standard. However, previous studies have shown that the dehydration process can reduce the phenolic content  $^{[10]}$ . In addition, thermal treatment can increase hydroxymethylfurfural (HMF) content in honey  $^{[11]}$ . The phenolic content is an essential source of antioxidants in honey  $^{[1]}$ . Meanwhile, HMF is a potential carcinogenic and genotoxic agent  $^{[12]}$ . Therefore, a suitable dehydration method is needed to obtain the maximum benefit from SLBH by reducing its moisture content without compromising its phenolic content and ensuring a safe level of HMF.

Globally, almost 500 species of SLBH are distributed in South America, Africa, Australia and Southeast Asia [13]. Despite the numerous species, the most commonly domesticated stingless bee honey by beekeepers worldwide are from *Melipona* and *Trigona* genera [14]. To the best of the knowledge, there are limited publications on the dehydration of SLBH that provide information on the changes in its physicochemical properties. Researchers provide an overview of the available information on the physicochemical properties of SLBH before and after the dehydration process. This will help to determine the most optimal setting and method of dehydration for SLBH without compromising its benefit. The physicochemical information retrieved includes moisture content, water activity, pH, free acidity, hydroxymethylfurfural (HMF), ash, electrical conductivity, diastase, sugar content, total soluble solids, total phenolic content and total flavonoid content.

# 2. Physicochemical Properties of Dehydrated Stingless Bee Honey

#### 2.1. Moisture Content

Moisture content is the amount or percentage of water present in the honey [15]. Water in honey is the key factor for honey quality as it determines the ability of honey to resist spoilage by microorganism fermentation [16]. Previous studies

presented in **Table 1** showed that the percentage of moisture content of raw SLBH was between 23.9 and 40%. However, another study showed that the moisture content of raw SLBH was between 13.26 and 45.8%  $^{[\underline{9}]}$ . The wide range in the percentage of moisture content was due to environmental factors such as seasonal weather and humidity  $^{[\underline{8}]}$ . Harvest and storage conditions also influenced the moisture content in SLBH  $^{[\underline{9}]}$ .

Table 1. Moisture content of raw and dehydrated stingless bee honey (SLBH).

Method of Dehydration		SLBH Species	T (°C)/ PL	Time	Moistu (%)	re Content	Water Reduction (%)	Author			
		Species			Raw	Dehydrated	Academon (70)				
		Tetragonisca angustula	52 °C	470 min	23.9	21.6 *	9.6				
		T. angustula	55 °C	170 min	23.9	22.7 *	5.0				
		T. angustula	57 °C	60 min	23.9	23.3 *	2.5				
		T. angustula	60 °C	22 min	23.9	23.3 *	2.5	[ <u>17]</u>			
		T. angustula	66 °C	8 min	23.9	23.3 *	2.5				
Therma	al treatment	T. angustula	66 °C	3 min	23.9	23.4	2.1				
		T. angustula	68 °C	1 min	23.9	23.4	2.1				
		T. angustula	71 °C	24 s	23.9	23.5	1.7				
		Melipona bicolor	90 °C	15-60 s	30.8	29.5 *	4.2	[ <u>18]</u>			
		M. bicolor	95 °C	15-60 s	30.8	29.5–29.6 *	3.9-4.2				
		-	45–90 °C	30–120 min	30.93	28.8	6.9	[19]			
Thermo	osonication	-	45–90 °C	30-120 min	31.06	25.9	16.6				
	Drying (5% moisture)	Heterotrigona itama	40–60 °C	-	31.9	5	84.3				
Vacuum	Drying (11% moisture)	H. itama	40–60 °C	-	31.9	11	65.5	[ <u>20]</u>			
	Evaporation (11% moisture)	H. itama	40–60 °C	-	31.9	11	65.5				
Freeze-dryin	ng (5% moisture)	H. itama	−54 °C	24 h	31.9	5	84.3				
		H. itama	20 PL	15-60 s	31.47	25.24-26.46	16-20				
Microw	ave heating	H. itama	60 PL	25-60 s	31.47	15.04–20.3 *	35–52				
		H. itama	100 PL	5–15 s	31.47	22.29–24.32 *	23–29	[ <u>21</u> ]			
Dehun	nidification	H. itama	35 °C	1–2 days	31.47	17.21–17.48 *	44-45				
		H. itama	40 °C	36 h	40	<8	>80				
Food dehydrator		H. itama	55 °C	18 h	40	<8	>80				
		H. itama	55 °C	36 h	40	0	100	[ <u>22</u> ]			
		H. itama	70 °C	18 h	40	<8	>80				
		H. itama	70 °C	36 h	40	0	100				
MARDI	dehydrator	H. itama	30 °C	8 h	29	19	35	[ <u>23</u> ]			

Method of Dehydration		SLBH Species	T (°C)/ PL	Time	Moistu (%) Raw	re Content Dehydrated	Water Reduction (%)	Author
Glass bottle storage		Geniotrigona thoracica	25 °C	1–10 days	26.21	25–26	0.8-4.62	
	Small surface area	G. thoracica	25 °C	1–10 days	26.21	24.32 *	7.21	[24]
Clay pot	Large surface area	G. thoracica	25 °C	1–10 days	26.21	23.35 *	10.9	
storage		H. itama	25 °C	1–21 days	25.82	18.13–25.13 *	2.7–29.8	[25]
		H. itama	35 °C	1–3 days	25.82	19.56–23.68 *	8.3–24.2	

Several studies summarized in **Table 1** showed that reduction in the water content of the SLBH after harvesting could be achieved either by increasing the temperature through various dehydration methods or via passive diffusion. The temperature used in the dehydration process was between 30 and 95 °C, while the temperature for the passive diffusion method was between 25 and 35 °C. As a result, the moisture content of SLBH was reduced between 29.6 and 5% after the dehydration process using these various methods. Moisture content below 17% could prevent the fermentation process by the microorganisms [26].

As conclusion, according to the data presented in **Table 1**, the dehydration process using thermal treatment will only cause less than a 10% reduction in water content. Meanwhile, another study showed that the thermosonication method of the dehydration process caused a 16.6% reduction in water content compared to 6.9% using the thermal method <sup>[19]</sup>. These findings suggest that thermosonication is the better method for the dehydration process for SLBH compared to thermal treatment. However, both methods could not reduce the moisture content below 17% (25.9% for thermosonication and 28.8% for the thermal treatment method).

A study showed that the moisture content of the SLBH was reduced from 31.9 to 11 and 5% after the dehydration process using vacuum and freeze-drying methods [20]. As presented in **Table 1**, the vacuum drying and freeze-drying at 5% moisture setting could achieve an 84.3% reduction in water content. Meanwhile, a 65.5% reduction in water content of the SLBH was observed after dehydration using vacuum drying and evaporation at 11% moisture setting. These findings suggest that both vacuum treatment and freeze-drying are the best methods in reducing the moisture content of SLBH. In addition, both methods could achieve a safe level of moisture content below 17%.

A study by Yegge et al. <sup>[21]</sup> showed that the dehydration process using microwave heating and dehumidification methods could reduce water content by up to 52% and 45%, respectively, as presented in **Table 1**. In the study, both methods could reduce almost half of the water content in raw SLBH. The microwave heating method used a power level of energy (PL) of 20, 60 and 100. However, only the microwave heating method at 60 PL for 60 s could reduce the moisture content below 17% (from 31.47 to 15.04%). Meanwhile, the dehumidification process was performed for 1 to 2 days. Therefore, microwave heating at 60 PL for 1 min was the best method for achieving the recommended moisture content level below 17%. In addition, this method was more practical because it takes less time to prepare the dehydrated SLBH.

From the data provided in **Table 1**, a previous study has also shown that the dehydration process of SLBH using a food dehydrator could reduce the water content of SLBH up to 80–100% [22]. The food dehydrator could achieve 80% water reduction at 40 °C for 36 h or at 55 °C and 70 °C for 18 h. Complete water reduction was achieved at 55 °C and 70 °C by prolonging the duration of the dehydration process to 36 h [22]. Another study showed that a dehydrator developed by the Malaysian Agricultural Research and Development Institute (MARDI) could reduce 35% of the water content [23]. However, the MARDI dehydrator set at 30 °C for 8 h was unable to reduce the moisture content below 17% [23]. Meanwhile, the conventional food dehydrator set between 40 and 70 °C for the duration of 18 to 36 h could achieve recommended moisture content level below 17% [22]. These findings suggest that a higher temperature would result in a higher reduction in moisture content.

Several studies summarized in **Table 1** showed that the dehydration process of the SLBH can be performed via passive diffusion by storage in a clay pot. A study by Ghazali et al. [24] showed that the reduction in the moisture content was significant in the clay pot compared to the glass container. In addition, the storage in a clay pot with a larger surface area resulted in a 10.9% reduction in water content compared to a smaller clay pot with only 7.21%. This finding suggests that

the larger the surface area of the container, the more effective the passive diffusion process will occur. On the other hand, the storage of SLBH at 35 °C for three days could reduce up to 24.2% of water [25]. Meanwhile, the storage of SLBH at room temperature (25 °C) for 21 days could reduce water content by up to 29.8% content [25]. These findings suggest a higher temperature would expedite the passive diffusion process. However, the dehydration process via passive diffusion requires a long duration to reduce the moisture content of the SLBH. Furthermore, the moisture content after storage in the clay pot was between 18.13 and 25.13%, which was still above the recommended moisture content level at 17%.

Various dehydration methods of SLBH can reduce moisture content depending on the temperature and duration of the dehydration process. Researchers concluded that the higher the temperature setting, the more reduction in water content. For that, researchers suggest the dehydration method of SLBH at a high temperature setting to achieve at least less than 17% moisture content to retard the fermentation process. The methods that yield low moisture contents are vacuum treatment, freeze-drying, food dehydrator and microwave heating at 60 PL. In conclusion, researchers observed that the food dehydrator is the best method because it could remove up to 80 to 100% water content, resulting in moisture content of less than 17%. However, it takes up to 18 to 36 h in duration. Therefore, microwave heating at 60 PL is the method of choice due to the short duration of 60 s with the moisture content of less than 17%. Although the vacuum treatment could reduce the moisture content to 5 and 11%, the duration of the dehydration process was not mentioned by the authors.

#### 2.2. Water Activity

Water activity is a measurement of free unbound water that can be utilized by microorganisms for growth  $^{[27]}$ . Water activity gives a better prediction of the likelihood of the fermentation process occurring compared to moisture content  $^{[16]}$ . Therefore, water activity is used as an indicator of food stability, which is important for the determination of honey spoilage due to microbial growth  $^{[4]}$ . Water activity (aw) is expressed in decimals and calculated from equilibrium relative humidity (ERH) divided by 100 (aw = ERH (%)/100)  $^{[28]}$ . ERH is the equilibrium of humidity of the food product with its environment.

Microorganisms will not grow below a particular water activity level, which is 0.90 for bacteria and 0.70 for molds. A water activity of less than 0.6 will halt all types of microbial growth <sup>[27]</sup>. Hence, it is crucial to maintain the water activity of SLBH below 0.6. Water activity is strongly correlated with moisture content <sup>[16]</sup>. Therefore, the dehydration process of SLBH is needed to reduce moisture content and water activity in SLBH. Subsequently, the dehydration process will help to prevent the likelihood of fermentation due to the inability of the microorganism to grow in the SLBH. A previous study reported that SLBH has the highest water activity of 0.76 compared to *Apis* spp. and commercialized honey with water activity ranges between 0.54–0.67 <sup>[4]</sup>. Several studies compiled in **Table 2** showed that the water activity of raw SLBH was between 0.79 and 0.807. After the dehydration process, the water activity was reduced between 0.28 and 0.785 as presented in **Table 2**.

Table 2. Water activity of raw and dehydrated stingless bee honey (SLBH).

Method of Dehydration		SLBH	т	Time	Water /	Activity	Water Activity	Author	
		Species	(°C)	(°C)		Dehydrated	Reduction (%)	Author	
Thermal treatment		-	45– 80	30–100 min	0.795	<0.767	<3.5		
		-	90	120 min	0.795	0.767	3.5	[ <u>19]</u>	
Thermo	Thermosonication		45– 80	30–100 min	0.807	<0.743	<7.9	-	
		-	90	120 min	0.807	0.743	7.9		
	Drying (5% moisture)	Heterotrigona itama	40- 60	-	0.79	0.28-0.29 *	63.3-64.6		
Vacuum	Drying (11% moisture)	H. itama	40- 60	-	0.79	0.45-0.48 *	39.2-43	[ <u>20</u> ]	
	Evaporation (11% moisture)	H. itama	40- 60	-	0.79	0.47-0.5 *	36.7–40.5		
Freeze-dryir	ng (5% moisture)	H. itama	-54	24 h	0.79	0.3 *	62		

Method of Dehydration		SLBH	т	Time	Water	Activity	Water Activity	Author
		Species	(°C)	Time	Raw	Dehydrated	Reduction (%)	Author
		H. itama	40	36 h	0.788	<0.6	>23.9	
Food	dehydrator	H. itama	55	18 h	0.788	<0.6	>23.9	[ <u>22</u> ]
		H. itama	70	12 h	0.788	<0.6	>23.9	
Glass bo	ottle storage	Geniotrigona thoracica	25	1–10 days	0.8	0.782-0.785	1.9–2.25	
	Small surface area	G. thoracica	25	1–10 days	0.8	0.679-0.774 *	3.3–15.1	[ <u>24</u> ]
	Large surface area	G. thoracica	25	1–10 days	0.8	0.632-0.737 *	7.9–21	
Clay pot storage		H. itama	25	1 day	0.79	0.79	-	
		H. itama	25	7–21 days	0.79	0.63-0.7 *	11.4–20.3	[25]
		H. itama	35	1–3 days	0.79	0.7-0.76 *	3.8-11.4	

According to the data summarized in **Table 2**, thermosonication causes a 7.9% reduction in water activity compared to 3.5% for the thermal method  $\frac{[19]}{}$ . This suggests that thermosonication is the better method in reducing water activity compared to thermal treatment. However, the water activity was 0.743 and 0.767 for thermosonication and thermal treatment, respectively, which was still above 0.6.

A study by Chen et al. [20] showed that dehydration processes using vacuum and freeze-drying methods at a 5% moisture setting were able to reduce the water activity level from 0.79 to less than 0.3, as presented in **Table 2**. Meanwhile, the water activity level of the vacuum drying and evaporation at an 11% moisture setting could reduce the water activity level to less than 0.5. These findings suggest that both vacuum treatment and freeze-drying methods could reduce the water activity level of SLBH to less than 0.6. It is observed that vacuum drying and freeze-drying at a 5% moisture setting was the best dehydration process for reducing the water activity level. However, the freeze-drying method at a 5% moisture setting needs 24 h to achieve a 0.3 water activity level. Meanwhile, the duration of the vacuum treatment was not mentioned by the author.

A study showed that dehydration of SLBH using a food dehydrator could reduce water activity levels from 0.788 to less than 0.6  $^{[22]}$ . In the study, the water activity of less than 0.6 was achieved with 40 °C for 36 h, 55 °C for 18 h, and 70 °C for 12 h, as summarized in **Table 2**. The study findings showed that the dehydration process using a food dehydrator at a higher temperature will take less time to reduce the water activity level to less than 0.6.

From the data provided in **Table 2**, the dehydration of SLBH via passive diffusion could reduce water activity levels from a range between 0.79–0.8 to 0.63–0.785 [24]. The study showed that SLBH stored in a clay pot could reduce water activity by up to 21% compared to storage in a glass container, which was only up to 2.25%. The surface area of the clay pot also plays an important role in the reduction in water activity. The study showed up to 21% reduction in the water activity level in a clay pot with a larger surface area compared to 15.1% for a clay pot with a smaller surface area [24]. Another study has also shown more reduction in water activity will be achieved in a clay pot at 35 °C compared to 25 °C [25]. In the study, the water activity level of the SLBH in a clay pot at 35 °C was 0.7 after three days. Meanwhile, the water activity level of the SLBH in a clay pot at 25 °C was 0.7 after seven days. Therefore, the duration of the dehydration process is shorter as the temperature setting in the clay pot storage increases. However, this passive diffusion method of the dehydration process was unable to reduce the water activity level below 0.6.

All the dehydration methods could reduce water activity levels. In addition, a higher temperature setting of the dehydration process progressively reduces the water activity level of the SLBH. The methods of dehydration that produced a water activity level below 0.6 were the vacuum method, freeze-drying, and food dehydrator. The food dehydrator at 70 °C was the best method of dehydration to achieve a water activity level below 0.6 within a shorter duration. Meanwhile, the vacuum method could reduce water activity levels below 0.5, but the duration was not mentioned by the authors.

#### 2.3. Hydroxymethylfurfural

Hydroxymethylfurfural (HMF) is a chemical compound from the furan group that indicates the freshness, overheating and ageing of honey  $^{[Z]}$ . HMF and diastase activity are indicators of overheating. However, HMF is a more reliable parameter for overheating compared to diastase activity  $^{[29]}$ . The HMF content can provide information regarding total heat exposure to honey  $^{[29]}$ . Apart from overheating, prolonged honey storage also promotes the formation of HMF by degradation of the honey sugar into HMF  $^{[30]}$ . A previous study showed that raw SLBH has a lower HMF content than *Apis mellifera* honey because raw SLBH has higher acidity and water activity that can slow down the Maillard reaction  $^{[31]}$ .

Codex Alimentarius Standards (2001) has set that the HMF level should not exceed 40 mg/kg for honey, except for that from the tropical region, which should not exceed 80 mg/kg. A high concentration of HMF is potentially carcinogenic and genotoxic [12]. A previous study showed that the heat from thermal treatment can increase the HMF content in honey [11]. Therefore, the dehydration process needs to be controlled to ensure strict adherence to the maximum permitted amount of HMF. Several studies summarized in **Table 3** showed that the HMF was not detected in raw SLBH, except for in a study by Syariffuddeen et al. [23] that reported a HMF level of 2.27 mg/kg. After the dehydration process, the HMF content either remained unchanged or increased between 2.39 and 238.18 mg/kg, as presented in **Table 3**.

Table 3. Hydroxymethylfurfural (HMF) content of raw and dehydrated stingless bee honey (SLBH).

	SLBH			HMF (m		
Method of Dehydration	Species	T (°C)	Time	Raw	Dehydrated	Author
	Tetragonisca angustula	52	470 min	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>	
	T. angustula	55	170 min	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>	
	T. angustula	57	60 min	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>	
	T. angustula	60	22 min	<loq< td=""><td><l0q< td=""><td>[<u>17]</u></td></l0q<></td></loq<>	<l0q< td=""><td>[<u>17]</u></td></l0q<>	[ <u>17]</u>
	T. angustula	66	8 min	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>	
	T. angustula	66	3 min	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>	
	T. angustula	68	1 min	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>	
Thermal treatment	T. angustula	71	24 s	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>	
	Melipona bicolor	90	15-60 s	<loq< td=""><td><loq< td=""><td>[18]</td></loq<></td></loq<>	<loq< td=""><td>[18]</td></loq<>	[18]
	M. bicolor	95	15-60 s	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>	
	-	75–95	20-60 s	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>	
	-	75	15 min	<loq< td=""><td><l0q< td=""><td>[<u>31</u>]</td></l0q<></td></loq<>	<l0q< td=""><td>[<u>31</u>]</td></l0q<>	[ <u>31</u> ]
	-	75	24 h	<loq< td=""><td>238.18</td><td></td></loq<>	238.18	
	-	45–67.5	30-75 min	0	0	
	-	67.5–90	100-120 min	0	↑ up to 42.40 *	
Thormogeniaction	-	45–67.5	30-75 min	0	0	[ <u>19</u> ]
Thermosonication	-	67.5–90	100–120 min	0	↑ up to 62.46 *	
	Heterotrigona itama	40	-	0	9.3 *	
Vacuum drying (5% moisture)	H. itama	50	-	0	10.71 *	
	H. itama	60	-	0	12.18 *	[ <u>20</u> ]
Freeze-drying (5% moisture)	H. itama	-54	-	0	9.29 *	

Method of Dehydration	SLBH	T (%C)	Time	HMF (m	Author	
	Species	T (°C)	Time	Raw	Dehydrated	Autiloi
	H. itama	40	18–36 h	0	0	
	H. itama	55	18 h	0	<5.81	
Food dehydrator	H. itama	55	36 h	0	5.81	[22]
	H. itama	70	18 h	0	<50	
	H. itama	70	36 h	0	83.19	
MARDI dehydrator	H. itama	30	8 h	2.27	2.39	[23]

According to the data provided in **Table 3**, the HMF content remained below the detection level after thermal treatment. However, as the duration of thermal treatment was prolonged at 75 °C for 24 h, the HMF content increased to 238.18 mg/kg, which exceeded the standard set by Codex [31]. Similarly, a study by Chong et al. [19] showed that the HMF level remained undetected at a low temperature and short duration in the thermal treatment and thermosonication method. However, the HMF content increased as the temperature and duration of dehydration increased to 67.5–90 °C for 100–120 min. HMF level was higher after the thermosonication process (62.46 mg/kg) compared to the thermal treatment (42.40 mg/kg). These findings suggest that the thermal treatment is the better dehydration method for SLBH compared to thermosonication. However, prolonged duration and higher temperature settings in both methods can increase HMF level above the permitted level of 40 mg/kg.

A previous study showed that HMF content increased after the dehydration process using vacuum drying and freezedrying methods from zero to up to 12.18 mg/kg, and 9.29 mg/kg, respectively [20]. The HMF content increased as the temperature of vacuum drying increased, as shown in **Table 3**. Both dehydration methods were able to increase the HMF content even at low temperatures, as low as 40 and -54 °C. However, the increase in HMF content was far below the permitted level of 40 mg/kg.

A study by Yap and colleagues <sup>[22]</sup> showed that HMF content remained undetected after the dehydration process using a food dehydrator at low temperature as presented in **Table 3**. However, as the temperature and duration increased to 55 and 70 °C for 36 h, the HMF content increased to 5.81 and 83.19 mg/kg, respectively. These findings suggest that the HMF content increases along with the increase in temperature and duration of the dehydration process. On the other hand, the dehydration process using the MARDI dehydrator at 30 °C for 8 h showed a slight increase in HMF from 2.27 to 2.39 mg/kg <sup>[23]</sup>. Therefore, the settings in which HMF content remained below 40 mg/kg were a food dehydrator at 40 and 50 °C for 18 to 36 h and the MARDI dehydrator. The HMF content exceeded the permissible limit using a food dehydrator above 70 °C.

In conclusion, the HMF content increased as the temperature and duration of the dehydration process increased. These were observed in thermal treatment, thermosonication, vacuum drying and dehydration using food dehydrator methods. The dehydration method that could maintain HMF content below the permitted level of 40 mg/kg were the thermal treatment for a short duration, thermosonication at 45–67.5 °C for 30–75 min duration, vacuum drying, freeze-drying, food dehydrator at 40 and 55 °C for 18–36 h, and MARDI dehydrator. The best dehydration method was the food dehydrator at 40 °C for 36 h duration setting, because the HMF content remained undetectable, although heated for many hours.

#### 2.4. The Optimal Setting for Each Method of Dehydration

**Table 4** summarized the optimal temperature and duration for each dehydration method used in previous studies. According to the data presented in **Table 4**, the most optimal dehydration processes for SLBH are thermal treatment at 90–95 °C for 15–60 s, thermal treatment at 45–90 °C for 30–120 min, thermosonication at 45–90 °C for 30–120 min, vacuum drying at 5% moisture content and 60 °C, freeze-drying at 5% moisture content and –54 °C, microwave heating at 60 PL for 60 s, dehumidification at 35 °C for two days, food dehydrator at 55 °C for 18 h, MARDI dehydrator at 30 °C for 8 h, dehydration by passive diffusion by storage in a clay pot with a large surface area at 25 °C for 10 days and storage in a clay pot at 35 °C for three days.

**Table 4.** The optimal setting for each dehydration method and impact on physicochemical properties of Stingless bee honey (SLBH).

Method of Dehydration	T (°C)/ PL	Time	MC (%)	WR (%)	WA	HMF (mg/kg)	рН	FA	Ash	EC	DA	TSS	TRC	TPC	TFC
	90− 95 °C	15– 60 s	>17	4.2	-	<loq< td=""><td>NC</td><td>NC</td><td>-</td><td>NC</td><td>NC</td><td>1</td><td>ļ</td><td>1</td><td>1</td></loq<>	NC	NC	-	NC	NC	1	ļ	1	1
Thermal treatment	45− 90 °C	30– 120 min	>17	6.9	>0.6	42.40	-	-	-	-	-			1	
Thermosonication	45− 90 °C	30– 120 min	>17	16.6	>0.6	62.46	-	-	-	-	-	-	-	1	-
Vacuum drying (5% moisture)	°C	-	<17	84.3	<0.6	12.18	1	1	-	-	-	-	1	1	1
Freeze-drying (5% moisture)	−54 °C	24 h	<17	84.3	<0.6	9.29	NC	NC	-	-	-	-	1	1	<b>†</b>
Microwave heating	60 PL	60 s	<17	52	-	-	NC	-	-	-	-	1	-	1	-
Dehumidification	35 ℃	2 days	>17	45	-	-	NC	-	-	-	-	1	-	1	-
Food dehydrator	55 ℃	18 h	<17	80	<0.6	<5.81	-	-	-	-	NC	-	-	1	-
MARDI dehydrator	°C	8 h	>17	35	-	2.39	-	-	-	-	-	-	1	1	-
Large Clay surface pot area	25 ℃	10 days	>17	10.9	>0.6	-	NC	NC	1	-	-	1	1	-	-
storage	35 ℃	3 days	>17	24.2	>0.6	-	1	NC	NC	1	-	1	-	-	-

NC: no changes of value in the parameter; †: increase; ‡: decrease; T: temperature; PL: power level; MC: moisture content.; WR: water reduction; WA: water activity; HMF: hydroxymethylfurfural; FA: free acidity; EC: electrical conductivity; DA: diastase activity; TSS: total soluble solids); TRC: total reducing sugar; TPC: total phenolic content; TFC: total flavonoid content.

A suitable dehydration method is a method that can reduce the moisture content to below 17% and water activity to less than 0.6. These conditions prevent the fermentation process and microorganism growth. At the same time, the HMF content must be below the permitted level of 40 mg/kg. The pH level should be increased or maintained. Hence, the SLBH will not be too acidic, and the sourness can be prevented. Meanwhile, the free acidity, ash content, electrical conductivity and diastase activity should be increased or maintained. As a result, honey's organic acids, enzymes, and minerals can be improved or preserved as well as in fresh SLBH. The water loss in SLBH through the dehydration process is reflected by an increase in the total soluble solids and total reducing sugar. On the other hand, a good dehydration method will increase the antioxidant activity in SLBH by increasing the total phenolic and flavonoid content.

**Table 4** shows that vacuum drying at 5% moisture content and 60 °C, freeze-drying at 5% moisture content and −54 °C for 24 h, and food dehydrator at 55 °C for 18 h could extract 80% and more water content in SLBH. As a result, these methods could decrease both moisture content below 17% and water activity to less than 0.6. The HMF value remains within the permissible range of below 40 mg/kg. Microwave heating at 60 PL for 60 s could reduce moisture below 17%. However, there was a lack of data on water activity and HMF content. On the other hand, the total phenolic content increased after dehydration by these methods.

### 3. Conclusions

Regardless of the dehydration method used, it was observed that the dehydration process at a high temperature resulted in a greater moisture content reduction. However, a very high temperature and prolonged honey exposure to extreme heat can increase the undesirable HMF content. Therefore, the dehydration process should be performed at an optimal temperature that can extract the maximum amount of water feasible while maintaining a low HMF level within the permitted amount. This review compiles data on the dehydration of SLBH by thermal treatment, thermosonication,

vacuum method, freeze-drying, microwave heating, dehumidification, dehydration using the MARDI dehydrator and dehydration via passive diffusion by a clay pot. This review found that the dehydration process using vacuum drying at 5% moisture content and 60 °C, freeze-drying at 5% moisture content and -54 °C for 24 h, and food dehydrator at 55 °C for 18 h could remove 80% and more water content in SLBH. As a result, these methods could decrease moisture content below 17% and water activity to less than 0.6. The HMF values were within the permissible range set by Codex Alimentarius Standards (2001) of below 40 mg/kg. The total phenolic content increased after dehydration by these methods. The physicochemical parameters of dehydrated SLBH are not comprehensive. Therefore, we suggest that future studies on dehydration of SLBH include moisture content, water activity, HMF, pH, free acidity, ash content, electrical conductivity, diastase activity, total soluble solids, total reducing sugar, total phenolic content and total flavonoid content as the parameters. Furthermore, we suggest more studies to evaluate phenolic compounds before and after the dehydration of SLBH. By this, we can compare and choose the best dehydration method to maximize the nutritional benefits of SLBH.

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