Jurnal Kimia Valensi, Vol 8(2), November 2022, 251-262
Available online at Website: http://journal.uinjkt.ac.id/index.php/valensi

Characteristics and Antibacterial Activity of Apis and Trigona Honey Types against *Escherichia coli* and *Staphylococcus Aureus* on Various Heating

La Ode Sumarlin[1*], Nurul Amilia[1], Anna Muawanah[1], Nadya Uswatun Hasanah[1], Hajar Hajar[2]

[1]Department of Chemistry, Faculty of Science and Technology, UIN Syarif Hidayatullah Jakarta, Jl. Ir. H. Juanda No. 95 Ciputat, South Tangerang, 15412, Indonesia
[2]Department of Animal Husbandry, Faculty of Agricultural Technology, University of Southeast Sulawesi, Captain Piere Tendean Street No. 109. A Kendari, 93121, Indonesia

**Corresponding author*: sumarlin@uinjkt.ac.id

Received: July 2022; Revision: August 2022; Accepted: September 2022; Available online: November 2022

Abstract

Heating in honey processing can inhibit fermentation, crystallization, and the growth of microorganisms, such as bacteria. However, the effect of the honey heating process on the properties of honey and its antibacterial activity has not been further studied. Therefore, in this study, the properties of honey of both Apis and Trigona species from Bogor, Kalimantan, Sulawesi, Sumatra, and Lombok, were tested. The properties of honey, including water content, acidity, reducing sugar, 5-hydroxymethylfurfural (HMF), and diastase enzyme activity, were tested at heating temperatures 50, 70, and 90 °C. The antibacterial activity was determined using the disc method against *Escherichia coli* and *Staphylococcus aureus*. The results showed that the average water content and acidity values decreased after heating. However, the values met the SNI quality requirements with a water content value of < 22% and the acidity value not exceeding 50 mL NaOH 0.1 N/kg in the Apis and Trigona types of honey. The reduced sugar content fluctuated after heating all samples, and the average HMF level of honey increased after heating. However, the activity of the diastase enzyme decreased, although the value was still within the SNI standard value. The selected honey samples of the Apis and Trigona types were active in inhibiting the growth of *Staphylococcus aureus* but were not active against *Escherichia coli*.

Keywords: Antibacterial, Apis, *Escherichia coli*, *Staphylococcus aureus*, Trigona.

DOI: *10.15408/jkv.v8i2.27241*

1. INTRODUCTION

Honey is a natural substance with various bioactivity compounds, including honey produced in Indonesia. For example, Trigona sp honey, Kaliandra, and Kelengkeng have the potential as supplements for lung cancer patients (Sumarlin *et al.*, 2019), and the presence of aurantiamide acetate (antiviral or anti-inflammatory and therapeutic agent for influenza) (Sumarlin *et al.*, 2021). In addition, the ability as an antibacterial has also been widely found. However, this antibacterial property depends on its type, geographical location, as well as the type and origin of the plant that is the source of honey nectar (Rio *et al.*, 2012). The antibacterial properties and mechanisms in honey are caused because honey contains flavonoids, honey osmolarity, acidity, and the presence of inhibin compounds, namely hydrogen peroxide (Nadhilla, 2014).

The physicochemical properties of honey are listed in the honey quality requirements of SNI-01-3545-2013. Water content, acidity, reducing sugar content, and 5-hydroxymethylfurfural (HMF) content are very important to know to determine its quality through the physicochemical characteristics of honey (Sjamsiah *et al.*, 2018). Water content is one factor that determines honey's durability or shelf life, where the maximum moisture content determined by SNI is 22%. In addition, another parameter that determines the quality of honey based on SNI is the determination of the activity of the diastase enzyme. Bees produce this

P-ISSN: 2460-6065, E-ISSN: 2548-3013

diastase enzyme during the honey maturation process, where generally, the value of the activity of this enzyme is used as an indicator of the freshness or purity of honey.

Previous studies have shown that the ability of honey as an antibacterial is due to the high concentration of sugars and low water activity to prevent honey from fermenting. Meanwhile, honey easily crystallizes due to the high concentration of monosaccharides in honey, especially glucose. During crystallization, part of the water is released from the solid phase, resulting in increased humidity that can multiply yeast cells naturally and make the honey ferment, thus affecting the quality of honey (Kowalski, 2013; Samborska & Czelejewska, 2014).

Indonesia has various types of honey because of the diverse and different floral sources in each region, so the antibacterial activity of honey will also vary. One of the obstacles honey farmers faced in Indonesia is because the air humidity is quite high, around 60-90%, so in general, honey in Indonesia has a high-water content. Honey has hygroscopic properties where it will absorb water if left exposed to moist air (Enggar, 2018). High water content honey causes its consistency to become thinner, which can also increase the risk of bacterial fermentation, which causes a decrease in the quality of honey.

Heating treatment in honey can be one of the efforts as a honey preservation technique expected to produce better antibacterial activity. The heating process in honey can dissolve sugar to slow down granulation, reduce honey's viscosity, and inhibit honey's crystallization process. This process also eliminates microorganisms that cause honey decay, reduces the moisture content to prevent fermentation, and destroys sugar-tolerant osmophilic yeast to extend the shelf life of honey (Blidi *et al.*, 2017; Zarei *et al.*, 2019).

Although studies on the antibacterial activity of honey have been widely carried out, the effect of heating honey to maintain its quality on antibacterial activity and the characteristics surrounding it are still limited in the report. Therefore, the information presented in this study is very useful, especially about the optimum temperature possible in heating honey in the process and its presentation while maintaining and maintaining the quality of its bioactivity.

2. MATERIALS AND METHODS

Materials And Instruments

The honey samples used came from several regions in Indonesia, namely AP LK (Apis Klengkeng); AP LMB (Apis Lombok north); AP MG (Apis mango) MAC (Madu Aceh, Buloh Seuma); MG KLS (Apis mangrove south Kalimantan), TR BB (Trigona Southeast Sulawesi); TR BIR (Trigona tetroginola biroi South Sulawesi); TR BGR (Trigona Bogor); TR LMB (Trigona North Lombok); TR SLS (Trigona Genotrigona Insica South Sulawesi). Meanwhile, the antibacterial activity of honey against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 25923 using the disc method and measured clear zones (mm). The chemicals used in this study were NaOH (Merck), phenolphthalein indicator (Merck), Mueller Hinton Agar (MHA) (Merck), acetate buffer, NaCl (Merck), amylum (Himedia), iodine (Merck), KI (Sigma-Aldrich), H_2SO_4 (Merck), $Na_2S_2O_3$ (Merck), $K_4Fe(CN)_6.3H_2O$ (Merck), $Zn(CH_3COO)_2.2H_2O$ (Merck), $NaHSO_3$ (Merck), $C_6H_8O_7$ (Merck) and Na_2CO_3 (Sigma-Aldrich). Instruments used Oven (Memmert), desiccator, water bath (Memmert), and Uv-Vis Spectrophotometer (Genesys 10S).

Honey Preparations

30 g of ten honey samples were put into a vial bottle and tightly closed. Then it is heated in the oven at 50, 70, and 80 °C for 10 days, respectively. Vials of samples of any temperature are quickly taken and cooled on ice to stop the reaction during heating and stored at 24 °C until the time of analysis (Turkmen *et al.*, 2006). Meanwhile, samples before heating are finished the same as for heated samples, and only they are not put in the oven. After that, all the samples prepared above are ready for various characteristic tests. The characteristics measured include water content, acidity value, reducing sugar content, 5-hydroxymethylfurfural (HMF) content, and diastase enzyme activity. In addition, it was continued with antibacterial activity tests against Staphylococcus aureus and Escherichia coli.

Water Content Test

2 g of each honey sample was put in a petri dish already known to weigh it. The sample was heated at 105-110 °C for 2 hours. Cool the sample in a desiccator for 10 minutes,

weigh it, and heat it for 1 hour. The sample was cooled for 10 minutes and then re-weighed (Wulandari, 2017). Repeat heating and weighing until a constant weight (consecutive weighing difference ≤ 0.2 mg). Then the moisture content of the sample is calculated using equation 1.

$$MC\ (\%) = \frac{(Mo-Mi)}{Mo} \times 100 \quad (1)$$

Where, MC is moisture content (% w/w), Mo is initial mass of sample, Mi is final mass of sample.

Acidity Value Test

10 g of honey sample was put into an Erlenmeyer glass and dissolved in 75 mL of distilled water. Then, 4-5 drops of phenolphthalein indicator (PP) were added and titrated with a 0.1 N NaOH solution until it reached the equivalence point (Prabowo *et al.*, 2019). Then the acidity value on the sample is calculated using equation 2.

$$AV = \frac{(a \times b)}{m} \times 100 \quad (2)$$

Where, AV is acidity value of honey (mL NaOH /kg honey), V is volume of NaOH 0.1 N (mL), N is normality of NaOH 0.1 N and m is mass of honey (g).

Reducing Sugar Content Test

The sample was weighed 2 g, put in a measuring flask of 250 mL, and added distilled water to the limit mark and shaken. The solution is pipetted 10 mL and put into Erlenmeyer, then added 15 mL of distilled water and 25 mL of Luff Schoorl solution are. Erlenmeyer was connected with a condenser and heated for 10 minutes, then removed and cooled in an ice-filled bath (it should not be shaken). After cooling, add 10 mL of KI 20% and 25 ml of H_2SO_4 25% (carefully formed CO_2 gas). The solution was titrated with 0.1 N of sodium thiosulfate with a starch solution indicator of 0.5% (V_1) (Wulandari, 2017). The blanks were determined with a sample containing 25 mL water and 25 mL Luff Schoorl solution (V_2). The reducing sugar content was calculated using equation 3-4.

$$\text{Volume of } Na_2S_2O_3 = (V_1\text{-}V_2) \times \frac{N}{0.1} \quad (3)$$

$$RSC\ (\%) = \frac{At \times fp}{m} \times 100\% \quad (4)$$

Where, V_1 is volume of blank solution (mL), V_2 is volume of sample solution (mL), N is the normality of $Na_2S_2O_3$ solution, RSC is reducing sugar content (%), At is number of $Na_2S_2O_3$ volume in SNI-01-3545-2013 table and m is mass of sample and fp is dilution factor.

5-hydroxymethylfurfural HMF Content Test

Each honey sample is weighed 5 g, then put into a 50 mL measuring flask and rinsed with water until the solution volume is 25 mL. Add 0.50 mL of Carrez I solution, shake and add 0.50 ml of Carrez II solution, shake again, and dilute with water until the line mark. Add a drop of alcohol to remove the foam on the surface. Strain using filter paper, and discard the first 10 mL of the filtrate. Pipette 5 mL filtrate and each put into an 18 x 150 mL test tube. Pipette 5 mL of distilled water into a test tube for a sample solution. 5 mL of $NaHSO_3$ 0.20% was put into another tube for reference. Shake it with a Vortex mixer. Furthermore, measure the absorbance of the sample to the reference in cells of 1 cm at 284 and 336 nm. The HMF concentration was calculated using equation 5.

$$HMF\ (mg/kg\ honey) = \frac{(A284-A336) \times 14.97 \times 5}{\text{mass of sample (g)}} \quad (5)$$

Diastase Enzyme Activity Test

The honey solution from the sample preparation was pipetted 10 mL and put into a 50 mL Erlenmeyer. A 5 mL starch solution was pipetted into the tube and placed in a water bath at 40±0.2 °C for 15 min. Mix the solution and turn on the stopwatch. Every 1-minute time interval, pipette the 1 mL of the sample mixture and add it to the 10 mL iodine solution of 0.0007 N, mix and dilute to the volume as before and set the absorbent value at 660 nm. The mixing time between amylum and honey was recorded until the addition of iodine as the reaction time. The solution collection in a certain time interval is continued until an A value of A < 0.235 (SNI 3545-2013) is obtained. The diastase enzyme activity was determined by calculated the disatase number (DN) using equation 6.

$$DN = 300/t \quad (6)$$

Where, DN is diastase number, t = time used to reach the absorbance value (A < 0.235).

Antibacterial Activity Test

A 24-hour bacterial swab (*Staphylococcus aureus* and *Escherichia coli*) was prepared. Put ose in a diluent (sterile TBS) to homogenize the suspension. Next, measured %T with a UV-Vis Spectrophotometer at 580 nm to 25%T (108 CFU/mL). Bacterial suspensions were taken as much as 0.2 mL each into the Petri, add 20-25 mL of homogenized TSA (Tryptic Soy Agar) media, let it solidify, and placed the disc containing the sample. K- (negative control) and K+ (positive control) for *E. coli* using distilled water and chloramphenicol, respectively. In contrast, *S. aureus* used distilled water and tetracycline over the surface of the solidified medium. It incubated for 1 hour until the test solution absorbs, incubating for 30-35 for 18-24 hours aerobically. The presence of a clear zone around the disc indicated antibacterial activity. Observe the clear zone/inhibitory zone. The samples tested for antibacterial activity were only four, AP LK (Apis Kelengkeng), AP LMB (Apis Lombok Utara), TR LMB (trigona Lombok), and TR BGR (trigona Bogor) (Nurhayati *et al.*, 2020).

3. RESULTS AND DISCUSSION

Moisture Content

Water content is the amount of water that contains in the substance. The water content in honey determines the quality and durability of honey (Ariandi & Khaerati, 2017; Zarei *et al.*, 2019). All honey samples have a decreasing water content result along with the high heating temperature (**Figure 1**), so it can be said that honey's moisture content and heating temperature are inversely proportional. Prabowo *et al.* (2019) state that when the honey is heated, the moisture content will decrease, and there will be a decrease in surface tension characterized by foam formation.

Most Apis honey, before heating, has a water content that matches SNI. While Trigona honey has not met SNI standards because its value is > 22%, this is because Trigona honey has a higher moisture content than Apis honey due to the natural conditions of Trigona bees which are moister (Gela *et al.*, 2021). The water content value decreases when heating 50 °C in Apis and Trigona types of honey appropriate to the SNI quality requirements of < 22%.

The type of Apis honey that run into the largest decrease in water content when heated, namely at 70 °C, is South Kalimantan Mangrove honey (MG KLS) by 14%, and the lowest decrease, namely at 80 °C in Kelengkeng Apis honey (AP LK) by 0.24%. The Trigona type of honey that underwent the largest decrease in water content was the Bogor Trigona (TR BGR) at 50 °C with a decrease in water content of 12.8% and the lowest decrease, namely Sulawesi Trigona honey (TR SLS) at 80 °C by 1.1%.

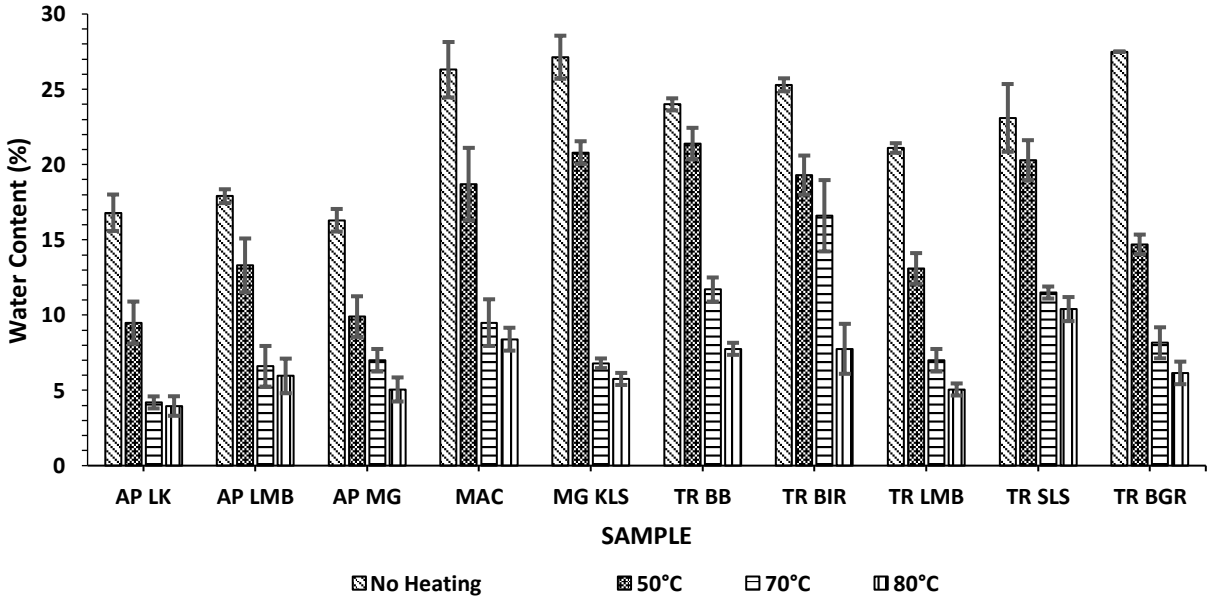

Figure 1. Water content in different types of honey

Acidity Value

Acidity affects the quality of honey because honey contains several organic acids, including formic acid, acetic acid, citric acid, lactic acid, butyric acid, oxalic acid, and succinic acid (Khasanah *et al.*, 2017). The acidity value of the different types of honey tested varies (**Figure 2**). Due to the different types and origins of honey, the content contained in it will also be different such as minerals, vitamins, and enzymes, as well as the fermentation process (Prabowo *et al.*, 2019). Apis and Trigona-type honey have different characteristics. Trigona honey has a honey flavor that tends to be sour and sharper. Trigona honey has a high acidity influenced by its content of free acids, minerals, and amino acids (Dan *et al.*, 2018). In addition, variations in organic acid content in plants that are the source of bee feed will also affect the acidity of honey (Sousa *et al.*, 2016).

All samples without heating except AP LK have an acidity value exceeding the SNI quality requirement limit, which is a maximum of 50 mL NaOH 0.1 N/Kg, with an acidity value of 27.6 mL NaOH 0.1 N/Kg (**Figure 2**). Honey with low acidity indicates a thick honey texture causing putrefactive microbes that make honey acidity not easy to grow. Samples with acidity exceeding SNI honey texture tends to be liquid and smell sour. It happens because the liquid honey texture is easier to ferment and will affect the acidity level (Savitri *et al.*, 2017).

The measurement results (**Figure 2**) show a decrease in the acidity value of various types of honey by increasing the temperature. At 50 °C heating, the acidity value in honey Apis samples AP LK, AP LMB, and MG KLS decreased and was appropriate to SNI. For Trigona types, TR BB samples decreased and met SNI quality requirements. Heating at 70 °C, all samples of Apis honey except MAC were appropriate to SNI. In contrast, all samples of Trigona honey decreased. However, only SLBB was appropriate to SNI, and at the heating of 80 °C, the acidity of all Apis honey was appropriate to SNI quality requirements.

At room temperature, the acidity of honey will increase compared to low temperatures because the humidity of honey is relatively high, so it is easier to absorb water (Prabowo *et al.*, 2019). Acidity can also be affected by the high moisture content of honey. Almost the entire sample of Trigona honey has a very high acidity value. It is because the water content of Trigona honey is higher than the water content of Apis honey (Maria *et al.*, 2021). When the moisture content is high, honey will ferment easily. The fermentation process of honey into organic acids is an indicator of increasing the acidity of honey (Wulandari, 2017). One of its fermentation processes is by yeast (*Zygosaccharomyces*) which will degrade sugar to alcohol. If alcohol reacts with oxygen, it will form free acids such as acetic acid and oxalic acid, thus affecting acidity, taste, and aroma levels (Budiwijono, 2008; Savitri *et al.*, 2017). According to Wusnah *et al.* (2018), his reaction was as follows:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

$$C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O$$

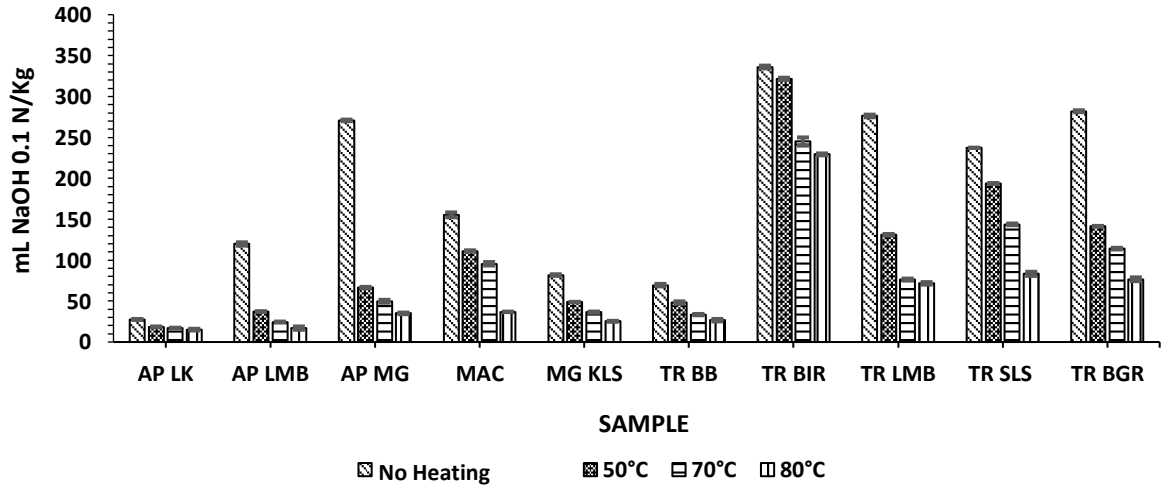

Figure 2. Acidity value in different types of honey

Reducing Sugar Content

Honey has the main components of carbohydrates from the monosaccharide group, namely glucose and fructose, called reducing sugars (Ariandi & Khaerati, 2017).

The average value of reducing sugar when heated at a temperature of 50 °C has increased (**Figure 3**). The increased level of reduced sugar in honey is due to heating that converts sucrose into reduced sugar (fructose and glucose). Not only has it increased, but at a temperature of 70 °C, the value of reducing sugar has decreased. It happens due to the formation of HMF during the heating process, and there will be further degradation into levulinic acid and formic acid (Dewi *et al.*, 2013) (**Figure 4**).

Apis-type honey that underwent the largest increase in reducing sugar content when heated was south Kalimantan mangrove honey (MG KLS) which increased by 21.24% at 80 °C and ran into the largest decrease, namely at 70 °C by 25.47%. Trigona honey that sustained the largest increase in reducing sugar content was Sulawesi Trigona honey (TR SLS) at 50 °C with a value of 13.84%. The largest decrease was south Sulawesi Trigona honey (TR BIR) at 70 °C by 16.31%.

5-Hydroxymethylfurfural (HMF) Levels

5-Hydroxymethylfurfural (HMF) is a fraction of sucrose and fructose in honey. This HMF is an indicator of freshness, heating process, and length of honey storage. The measurement results showed that the HMF value increased after a heating treatment (**Figure 5**).

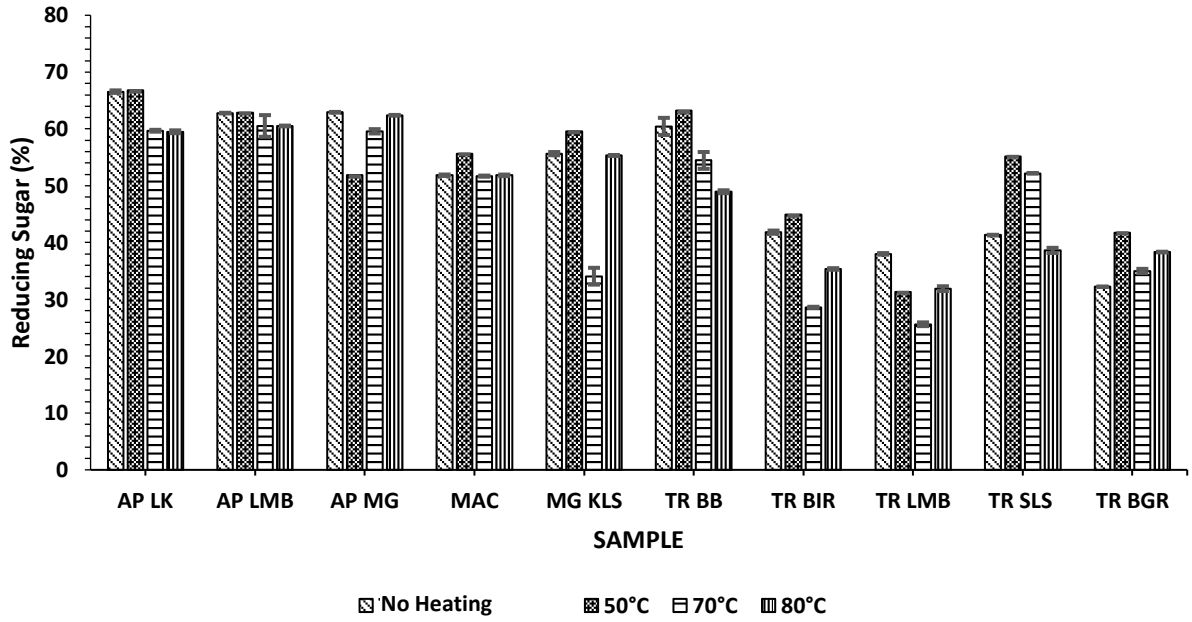

Figure 3. Graph of reducing sugar content of different types of honey

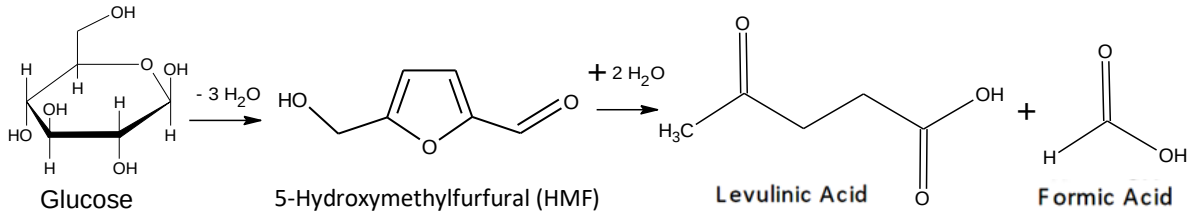

Figure 4. The reaction of glucose dehydration to 5-hyrdoxymethylfurfural (HMF) (Kupiainen *et al.*, 2011)

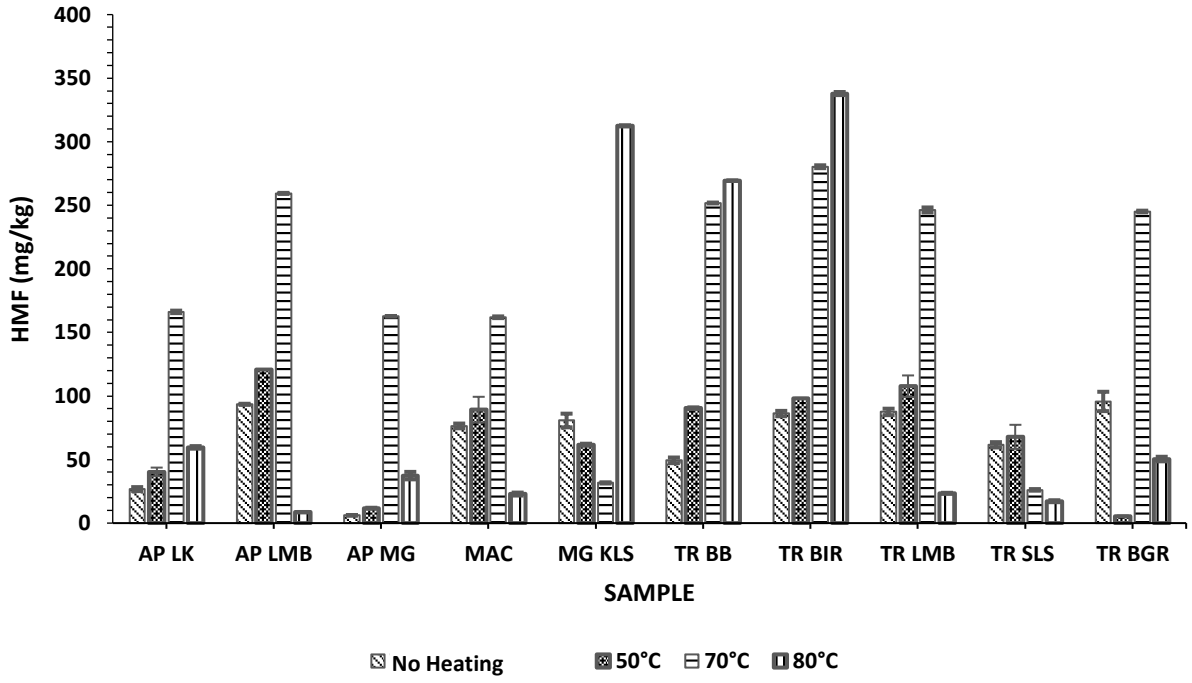

Figure 5. 5-Hydroxymethylfurfural (HMF) levels of various types of honey

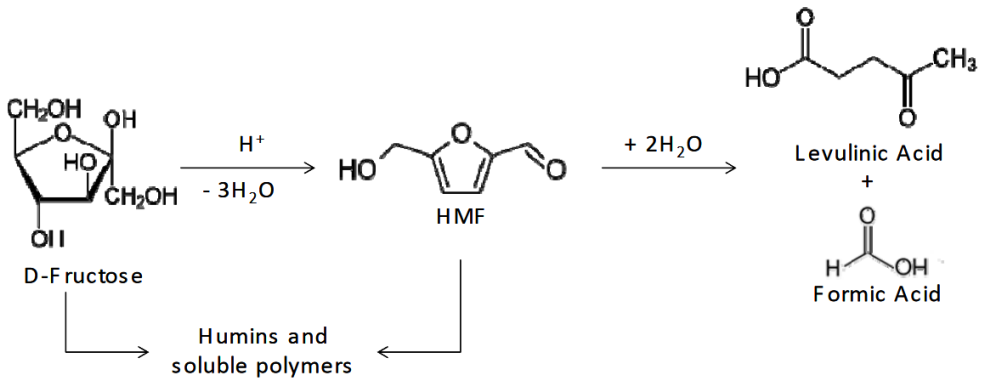

Figure 6. Reaction schemes for the dehydration of fructose into HMF and parallel reactions (Gomes *et al.*, 2015)

On average, after heating at 50 °C, the HMF content of honey increases. Apis-type honey undergoes the largest increase in HMF levels after being heated. While South Kalimantan Mangrove honey (MG KLS) of 280.84 mg/kg, Lombok Apis (AP LMB) of 250.77 mg/kg at 80 °C. Trigona-type honey that undergoes the largest increase in HMF levels was Bogor Trigona honey (TR BGR) at 70 °C with a value of 239.53 mg/kg. On the other hand, the largest decrease was Lombok Trigona honey (TR LMB) at 80 °C of 222.85 mg/kg.

HMF values in Apis and Trigona types have increased at an average temperature of 70 °C. It is in line with the reducing sugar indicator, which shows that the reduced sugar level decreases at that temperature due to the formation of HMF. Evahelda *et al.* (2015) stated that an increase in HMF values indicates a decrease in reducing sugar levels in honey due to dehydration.

Honey damage is caused by overheating or adding inverted sugar (a mixture of glucose and fructose hydrolyzed sucrose); both of these treatments will increase HMF levels in honey (Koesprimadisari *et al.*, 2016; Sumarlin *et al.*, 2018). The HMF value is 0 mg/kg, or it can be said that there is no, so there is a possibility of the further degradation process of HMF into levulinic acid and formic acid (**Figure 6**) (Kesić *et al.*, 2017; Sumarlin *et al.*, 2018).

Diastase Enzyme Activity

The activity of the enzyme diastase is closely related to its structure and can be modified by denaturation caused by heating. Diastases are a group of starch-digesting enzymes that include α-amylase and β-amylase. These enzymes convert complex carbohydrates or polysaccharides into monosaccharides like glucose and fructose (Sakac & Sak-bosnar, 2012). According to the quality requirements of honey SNI-01-3545-2013, the activity of the diastase enzyme is at least 3 DN (Diastase Number), where generally, the value of the activity of this diastase enzyme is used as an indicator of honey freshness. Bees produce diastase enzymes during the honey maturation process. The value of the enzyme diastase activity that is not appropriate to the SNI requirements can be influenced by the packaging process or the possibility of honey processing during harvesting (Akuba *et al.*, 2020).

The value of the diastase enzyme activity of 10 honey samples tested had fluctuations or irregularities (**Figure 7**). It is thought to be because when honey is heated, enzyme molecules will expend energy to overcome mutual repulsion forces between electrons so that the enzyme molecules will be close together and make transitions and then undergo denaturation (Nurmala, 2020).

Apis-type honey that ran into the largest increase in diastase enzyme activity when heated was mangrove Apis honey (AP MG), which increased at 50 °C with a diastase number of 98.06 DN and decreased at 70 °C with a diastase number of 59.92 DN. While, Trigona honey that underwent the largest increase in diastase enzyme activity was Bogor Trigona honey (TR BGR) at 70 °C with a value of 34.41 DN, and the largest decrease was Southeast Sulawesi Trigona honey (TR BB) at 50 °C with a diastase number of 51.15 DN.

Heating honey at a certain constant temperature over a while can cause the activation of the diastase enzyme to restore the value of diastase that has dropped (Tester, 2002). It is also shown in Hasan's research (2013) that the heating treatment of honey does not affect diastase and invertase rates, except for the effect of time storage. Research by Harjo *et al.* (2015) also explained that heating honey at temperatures above 40 °C causes the activity of the diastase enzyme to decrease and can even cause the enzyme to be inactive due to damage to the protein structure of the enzyme. Therefore, the indicator to determine if honey was damaged by heating is the increase in HMF value due to honey sugar degradation.

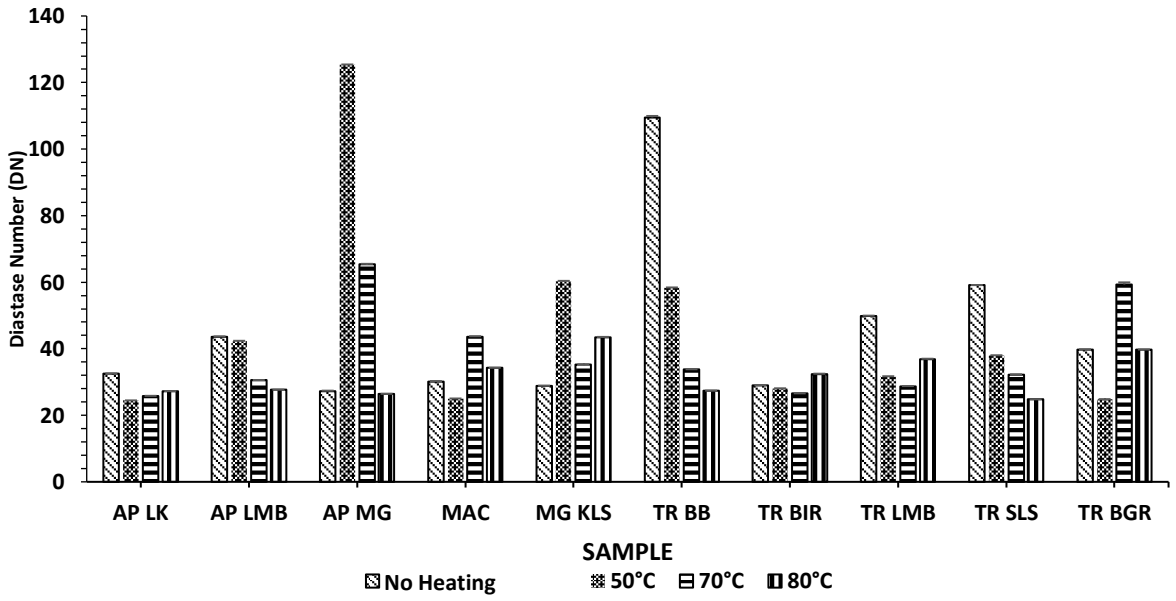

Figure 7. The activity of the enzyme diastase on various honey samples

Antibacterial Activity of Honey

The samples measured antibacterial are selected samples representing the Apis type (AP LK and AP LMB) and the Trigona type (TR BGR and TR LMB). This selection is based on analyzing the dominant characteristics of the water content, acidity value, HMF, and diastase enzyme activity.

Tables 1 and **2** show that AP LK, AP LMB, TR BGR, and TR LMB honey can inhibit the growth of gram-positive bacteria (*Staphylococcus aureus*) with strong inhibition activity. The diameter zone inhibition obtained is 10-20 mm. However, they did not inhibit the growth of gram-negative (*Escherichia coli*). Apis-type honey AP LK and AP LMB inhibit bacterial growth the most during heating at 70 °C with clear zone diameters of 11.4 and 18.1 mm, while Trigona TR LMB and TR BGR at a heating temperature of 50 °C with clear zone diameters of 11.1 and 10.1mm. Heating at 80 °C of all samples did not indicate the presence of a clear zone. It is possible because overheating damages the active antibacterial substances in honey, resulting in the absence of a clear zone. Gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria were used because of the different properties on their cell walls. The cell wall of gram-positive bacteria is composed of peptidoglycans. In contrast, gram-negative bacteria cell walls are made of protein, lipids, and only a small amount of peptidoglycan (Nurhayati *et al.,* 2020).

The honey antibacterial mechanism is directly influenced by hydrogen peroxide (H_2O_2), high osmolarity, osmotic pressure, low pH, non-peroxide factor (methylglyoxal (MGO), and phenol content (Putri *et al.,* 2017). Phenolic compounds inhibit microorganisms by disrupting cell membranes and inhibiting the synthesis of bacterial structural components (Prestianti *et al.,* 2018). In addition, the content of flavonoid compounds can also inhibit bacterial growth by damaging the bacterial cell wall, which will cause lysis in cells (Lutpiatina, 2015).

Inactive honey samples inhibit *Escherichia coli* bacteria (gram-negative). It can be seen from selected samples representing Apis (AP) and Trigona (TR) honey, which show clear zones or cannot inhibit bacterial growth. It was conducted by Rio *et al.* (2012), which tested honey from Sikabu and Lubuk. It showed the inability of antibacterial effects against *Escherichia coli* but affected *Staphylococcus aureus*. It is due to the nature of the two bacteria tested as well as the nature of the honey. In addition, the compound content in honey affects the activity of honey as an antibacterial agent.

Table 1. Diameter of the inhibitory zone against *Staphylococcus aureus*

T (°C)	Inhibitory zone diameter (mm) *Staphylococcus aureus*			
	AP LK	**AP LMB**	**TR LMB**	**Tr BGR**
0	10.7	16.1	10.5	9.3
50	10.8	16.3	11.1	10.1
70	11.4	18.1	-	-
80	-	-	-	-
K(+)	21.3	20.8	22.3	20.2

Table 2. Diameter of the inhibition zone against *Escherichia coli*

T (°C)	Inhibitory zone diameter (mm) *Escherichia coli*			
	AP LK	**AP LMB**	**TR LMB**	**Tr BGR**
0	-	-	-	-
50	-	-	-	-
70	-	-	-	-
80	-	-	-	-
K(+)	30.1	29.7	30.6	29.4

Aseron *et al.* (2019) research about the antibacterial effect on *Staphylococcus aureus*, using samples of *Trigona biroi* and *Apis mellifera* honey species, showed antibacterial effects on *Staphylococcus aureus* and prescribed to cure patients in the Philippines. However, it is not yet known which of the two is more effective in preventing the growth of *Staphylococcus aureus*. In addition, Aseron *et al.* (2019) also compared the antibacterial properties of *Trigona biroi* honey and *Apis mellifera* honey against *Staphylococcus aureus* through a gelatinous diffusion test and showed that *Trigona biroi* honey inhibits the growth of *Staphylococcus aureus* more than Apis mellifera honey, but the difference is not significant. Ewnetu *et al.* (2013 compared the anatar of stingless bee honey (*Trigona sp* type) and white honey (*Apis mellifera* type), also showing inhibition of *Staphylococcus aureus*. Thus, making honey a new source of

chemotherapeutic agents to treat drug-resistant bacteria in the future (Ewnetu *et al.*, 2013).

Eliza (2010) mentioned that another effect of the antibacterial activity of honey on *Staphylococcus aureus* is due to differences in the structure of gram-positive and gram-negative bacteria cell walls. In this case, the cell wall of gram-positive bacteria (*Staphylococcus aureus*) has a simpler structure than gram-negative bacteria (*Escherichia coli*). Gram-negative bacteria consist of three layers: lipoproteins, peptidoglycan, and lipopolysaccharides.

4. CONCLUSIONS

Honey samples before and after heating in Apis and Trigona types had significant differences in moisture content, acidity, and HMF (5-hydroxymethylfurfural). Reducing sugar and diastase enzyme activity were no significant differences. Selected honey samples Apis (AP LK and AP LMB) and Trigona (TR LMB and TR BGR) under conditions without heating at 50 °C were all able to inhibit the growth of *Staphylococcus aureus* bacteria. However, at 70 °C, only Apis (AP) can inhibit *Staphylococcus aureus*, while at 80 °C, all such samples do not show inhibition. It was inactive against *Escherichia coli* bacteria in selected samples before and after heating.

ACKNOWLEDGEMENTS

Thank you to Dr. Sri Yadial Chalid, M.Si, and Tarso Rudiana, M.Si, who have corrected some parts of this research. In addition, the authors also expressed their gratitude to the Research and Publication Center of UIN Syarif Hidayatullah Jakarta for financing this research in the fiscal year 2022 with contract number: UN.01/KPA/223/2022.

REFERENCES

Ariandi & Khaerati. (2017). Uji aktivitas enzim diastase, Hidroksimetilfurfural (HMF), kadar air pada madu hutan Battang. *Prosiding Seminar Hasil Penelitian (SNP2M)*, pp. 1-4.

Akuba, J., & Pakaya, M. S. (2020). Uji aktivitas enzim diastase madu hutan mentah Gorontalo sebagai imunomodulator. *Pharmaceutical Journal of Islamic Pharmacy*, *4*(2), 30–34. http://dx.doi.org/10.21111/pharmasipha.v4i2.4852.

Aseron, L. R., Atega, K. M., Beloso, T. B., Malacad, C. A., & Millena, C. M. (2019). Comparative analysis of the antibacterial properties of honey from *Trigona biroi* (Stingless bee) and *Apis mellifera* (Western honey bee) against *Staphylococcus aureus* strains. [Research output, De La Salle Medical and Health Sciences Institute]. GreenPrints. https://greenprints.dlshsi.edu.ph/grade_12/62/

Blidi, S., Gotsiou, P., Loupassaki, S., Grigorakis, S., & Calokerinos, A. C. (2017). Effect of Thermal Treatment on the Quality of Honey Samples from Crete. *Advences in Food Science and Engineering*, *1*(3), 1–9. https://doi.org/10.22606/afse.2017.11001

Badan Standarisasi Nasional Indonesia (BSN). (2013). SNI-01-3545-2013: Madu. Jakarta: Badan Standarisasi Nasional Indonesia.

Budiwijono, T. (2008). Evaluasi kadar gula pereduksi, derajat keasaman dan identifikasi enzim pada madu yang dipanaskan dengan oven udara kering sistem konveksi. Universitas Muhammadiyah Malang. http://publikasi.umm.ac.id.

Dan, P. N. S. M., Omar, S., & Ismail, W. I. W. (2018). Physicochemical Analysis of Several Natural Malaysian Honeys and Adulterated Honey. *IOP Conference Series: Materials Science and Engineering*, 440 (1): 5–10.

Dewi, A. D. R. & Susanto, W. H. (2013). Pembuatan lempok pisang (kajian jenis pisang dan konsentrasi madu). *Jurnal Pangan Dan Agroindustri*, *1*(1), 101–114.

Ewnetu, Y., Lemma, W., & Birhane, N. (2013). Antibacterial effects of Apis mellifera and stingless bees honeys on susceptible and resistant strains of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* in Gondar, Northwest Ethiopia. *BMC Complementary and Alternative Medicine*, 13,269. http://www.biomedcentral.com/1472-6882/13/269

Elliza, N. (2010). Pengaruh pemberian madu terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. [Skripsi], UIN Syarif Hidayatullah Jakarta.

Enggar, M. D. (2018). Karakteristik Madu Hutan Berdasarkan Jenis Pakan Lebah di Propinsi Riau. Inspirasi Kuok, Balai Penelitian Teknologi Serat Tanaman Hutan. 1(1), p22-27.

Evahelda, Pratama, F. Malahayati, N., & Santoso, B. (2015). Uji aktivitas enzim diastase , kadar gula pereduksi dan kadar air pada madu bangka dan madu kemasan yang dipasarkan di kota Palembang. P*rosiding Seminar Nasional Lahan Suboptima*, pp. 1-6, 2015.

Gela, A., Hora, Z. A., Kebebe, D., & Gebresilassie, A. (2021). Physico-chemical characteristics of honey produced by stingless bees (*Meliponula beccarii*) from West Showa zone of Oromia Region, Ethiopia. *Heliyon*, *7*(1), e05875. https://doi.org/10.1016/j.heliyon.2020.e05875 .

Gomes, F. N. D. C., Pereira, L. R., Ribeiro, N. F. P. &. Souza, M. M. V. M. (2015). Production of 5-Hydroxymethylfurfural (Hmf) Via Fructose Dehydration: Effect of Solvent And Salting-Out. *Brazilian Journal of Chemical Engineering*, *32*(1), 119 – 126. dx.doi.org/10.1590/0104-6632.20150321s00002914.

Hasan, S. H. (2013). Effect of Storage and Processing Temperatures on Honey Quality. Journal of Babylon University. 21(6).

Kesić, A., Zaimović, I., Ibrišimović-Mehmedinović, N., & Šestan, A. (2017). The Influence of Thermal Treatment on the Concentration of HMF in Honey. *International Journal of Environmental Chemistry*, 1(1), 14–18. https://doi.org/10.11648/j.ijec.20170101.13.

Khasanah, R. Parman, S. & Suedy, S. W. A. (2017). Kualitas madu lokal dari lima wilayah di Kabupaten Wonosobo. *Jurnal Biologi, 6*(1), 29-37.

Kupiainen, L., Ahola, J., & Tanskanen, J. (2011). Kinetics of glucose decomposition in formic acid. *Chemical Engineering Research and Design*, *89*(12), 2706–2713. https://doi.org/10.1016/j.cherd.2011.06.005

Koesprimadisari, A. R., Arrisujaya, D. & Syafdaningsih, R. (2016). Uji kandungan Hidroksimetilfurfural (HMF) sebagai parameter kualitas madu. *Jurnal Sains Natural Universitas Nusa Bangsa*, *2*(2), 44-51.

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.

Lutpiatina, L. (2015). Efektivitas Ekstrak Propolis Lebah Kelut (*Trigona* spp) dalam Menghambat Pertumbuhan *Salmonella typhi*, *Staphylococcus aureus* dan *Candida albicans*. *Jurnal Skala Kesehatan*, *6*(1), 1–8. https://doi.org/10.31964/jsk.v6i1.32

Maria, Aziz, H., & Nuh, M. (2021). Analisi Waktu Pemasakan dalam Proses Pembuatan Permen Madu Trigona biroi dan Apis dorsata. *Jurnal Penelitian Kehutanan Bonita*, *5*(1), 1–15. http://dx.doi.org/10.55285/bonita.v3i1.771

Nurmala, D., P. (2020). Pengaruh pemanasan menggunakan evaporator vakum terhadap fisikokimia madu karet, madu rambutan dan madu kayu putih. [Tesis]. Universitas Brawijaya.

Nadhilla, N., F. (2014). The activity of antibacterial agent of honey against *Staphylococcus aureus. J Majority*, *3*(7), 94–101.

Nurhayati, L., S. Yahdiyani, N. & Hidayatulloh, A. (2020). Perbandingan pengujian aktivitas antibakteri starter yogurt dengan metode difusi sumuran dan metode difusi cakram. *Jurnal Teknologi Hasil Peternakan*, *1*(2), 41-46. https://doi.org/10.24198/jthp.v1i2.27537.

Putri, A., P. & Asparini, R., R. (2017). Peran madu dalam menghambat pertumbuhan bakteri pada luka bakar. *Journal of Saintika Medika*, *13*(2), 63-68. https://doi.org/10.22219/sm.v13i2.5413.

Prabowo, S. Yuliani, Prayitno, Agus, Y., Lestari, K., & Kusevara, A. (2019). Penentuan karakteristik fisiko-kimia beberapa jenis madu menggunakan metode konvensional dan metode kimia. *Journal of Tropical AgriFood*, *2*(1), 66-73. http://dx.doi.org/10.35941/jtaf.1.2.2019.2685.66-73.

Prestianti, I., Baharuddin, M., & Sappewali, S. (2018). Uji Aktivitas Antibakteri Ekstrak Sarang Lebah Hutan (Apis dorsata) terhadap Pertumbuhan Staphylococcus aureus, Escherichia coli dan Pseudomonas aeruginosa. *ALCHEMY Jurnal Penelitian*

Kimia, *14*(2), 313. https://doi.org/10.20961/alchemy.14.2.13028. 314-322

Rio, Y. B. P., Aziz, D., & Asterina. (2012). Perbandingan efek antibakteri madu asli Sikabu dengan madu Lubuk Minturun terhadap *Escherichia coli* dan *Staphylococcus aureus* secara in vitro. *Jurnal Kesehatan Andalas*, *1*(2), 59–62. https://doi.org/10.25077/jka.v1i2.15.

Sakac, N., & Sak-bosnar, M. (2012). A rapid method for the determination of honey diastase activity. *Talanta*, *93*, 135–138. https://doi.org/10.1016/j.talanta.2012.01.063

Samborska, K. & Czelejewska, M. (2012). The Influence of thermal treatment and spray drying on the physicochemical properties of polish honeys. *Journal of Food Processing and Preservation*, 38(1), 413–419. https://doi.org/10.1111/j.1745-4549.2012.00789.x

Savitri, N. P. T., Hastuti, E. D., & Suedy, S. W. A. (2017). Kualitas madu lokal dari beberapa wilayah di Kabupaten Temanggung. *Buletin Anatomi dan Fisiologi*, *2*(1), 58-66. https://doi.org/10.14710/baf.2.1.2017.58-66.

Sjamsiah, S., Sikanna, R., Rifkah.A, A., & Saleh, A. (2018). Penentuan Sifat Fisikokimia Madu Hutan (Apis dorsata) Sulawesi Selatan. *Al-Kimia*, *6*(2), 191-199. https://doi.org/10.24252/al-kimia.v6i2.6668.

Sousa, J. M., Souza, E. L., Marques, G., Benassi, M. D., Gullón, B., Pintado, M. M., & Magnani, M. (2016). Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region. *Lwt - Food Science and Technology*, *65*, 645-651. https://doi.org/10.1016/j.lwt.2015.08.058.

Sumarlin, L. O., Ernita, N., Afandi, F. R., & Fathoni, A. (2021). Identification of Active Chemical Compounds of Honey from Some Regions in Indonesia. *Science and Technology Indonesia*, *6*(2), 74–84. https://doi.org/10.26554/sti.2021.6.2.74-84.

Sumarlin, L. O., Hendrawati, Bahalwan, I., Farhan, R. A., & Adawiyah. (2019). Anticancer Activity of Honey Extract from Indonesia By A549 Cell Inhibition In Vitro. *Ecology, Environment and Conservation, 25(3), 1196-1202*.

Sumarlin, L. O., Tjachja, A., Octavia, R., & Ernita, N. (2018). Aktivitas Antioksidan Ekstrak Metanol Madu Cair dan Madu Bubuk Lokal Indonesia. *Al-Kimia*, *6*(1), 10-23. https://doi.org/10.24252/al-kimia.v6i1.4333.

Tester, R. F. (2002). Starch: The Polysaccharide Frections In P.J. Frazier, P. Richmond and A.M. Donald. Starch,Structure and Functionally. *Royal Society of Chemistry*, *1*(6), 163–171.

Turkmen, N. Sari, F. Poyrazoglu, E. S., & 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.

Wulandari, D. D. (2017). Analisa Kualitas Madu (Keasaman, Kadar Air, dan Kadar Gula Pereduksi) Berdasarkan Perbedaan Suhu Penyimpanan. *Jurnal Kimia Riset*, *2*(1), 16–22. https://doi.org/10.20473/jkr.v2i1.3768.

Zarei, M., Fazlara, A., & Tulabifard, N. (2019). Effect of thermal treatment on physicochemical and antioxidant properties of honey. *Heliyon*, *5*(6), e01894. https://doi.org/10.1016/j.heliyon.2019.e01894.