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Quality test comparison for *Wallacetrigona incisa* and *Tetragonula biroi* honey in Mappedeceng District, North Luwu Regency, South Sulawesi Province

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Abstract

Cultivation of *Wallacetrigona incisa* and *Tetragonula biroi* in Mappedeceng District, Luwu Utara Regency, is growing rapidly, where the area is the center of cultivation in North Luwu Regency. Both of these stingless bees are endemic animals in North Luwu Regency. The purpose of this study was to analyze the quality of honey from *Wallacetrigona incisa* and *Tetragonula biroi*. This research uses honey quality testing based on SNI 8664: 2018. The results of the research on the quality test of *Wallacetrigona incisa* and *Tetragonula biroi* honey in Mappedeceng District which meet the requirements of SNI 8664: 2018 are water content, hydroxymethylfurfural (HMF), and acidity (pH). The test results for honey that do not meet SNI 8664: 2018 are reducing sugar and sucrose levels. The honey color produced by Wallaceatrigona incisa is darker dark brown and Tetragonula biroi has a clear light brown color. The honey texture produced by Wallaceatrigona incisa is thicker and the Tetragonula biroi is thin. The taste of honey produced by Wallaceatrigona incisa is more acidic than Tetragonula biroi honey.

Keywords: Honey Quality Test, *Wallacetrigona incisa, Tetragonula biroi*.

INTRODUCTION

Consumption of Trigona honey has been very popular with the public since the Covid-19 pandemic to keep the body's immune system healthy and immune to exposure to the Covid-19 virus. The quality of honey is a very important consideration for honey consumers, because it is very important to take into account that honey that is suitable for consumption is real honey, clean of other impurities. There are several quality variables for honey that are recognized by the Ministry of Health of the Republic of Indonesia and must pass the purity test and specifications for honey products, namely diastase enzyme activity, hydroxymethylfurfural (HMF), content water, reducing sugars, sucrose, acidity, insoluble solids, ash content., metal contamination (lead, cadmium and mercury) Arsenic and chloramphenicol contamination [1].

The production potential of the bees *Wallacetrigona incisa* and *Tetragonula biroi* is much higher, namely honey, because it is one of the natural ingredients that is believed to have many benefits empirically. [2]. From laboratory analysis it is known that honey contains 15% water, 1% ash, 8% sucrose, 41% fructose and 35% glucose. Honey is also antibiotics and various digestive enzymes. [3].

Morphology The species of bees in the Mappedeceng district, North Luwu Regency are *Wallacetrigona incisa* and *Tetragonula biroi* [4]. These two species are endemic to the North Luwu Regency because only these species can reproduce and produce an abundant production of honey.

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Picture 1: A.*Wallacetrigona incisa* Species; B. *Wallacetrigona incisa* Wing*;* C. *Wallacetrigona incisa* Limbs*;* D. *Wallacetrigona incisa* Feet

Picture 2: A. *Tetragonula biroi* Species*;* B. *Tetragonula biroi* Wing *;* C. *Tetragonula biroi* Limbs*;* D. *Tetragonula biroi* Feet

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Picture 3: A. Bee brood *Wallacetrigona incisa ;* B. Bee Brood *Tetragonula biroi*

In Indonesia, the quality of honey has been determined based on the Indonesian National Standard (SNI). Where the standard is a honey quality criterion that has been established by the National Standardization Agency (BSN). Honey in Indonesia is very diverse. The diversity of honey is influenced by differences in regional origin, seasons, types of bees, types of nectar source plants, the way of life of bees (cultivated or wild), collection methods and postharvest handling methods. Given this diversity, it is necessary to test the quality of honey quality standards as regulated in SNI 8664-2018. Therefore, it is necessary to have an SNI quality test to scientifically assure the content of the honey from Wallacetrigona incisa and Tetragonula biroi originating from Mappedeceng district, North Luwu Regency.

METHODS

Before the honey samples were analyzed at the Makassar Health Laboratory Center, honey from the bee species *Wallacetrigona incisa* and *Tetragonula biroi* was harvested at the Mappedeceng district culture site, North Luwu Regency. 1 colony of *Wallacetrigona incisa* bees and 1 colony of *Tetragonula biroi* bees were collected. The *Wallacetrigona incisa* colony maintained the colony for 8 months, while the bee colony *Tetragonula biroi* for 6 months. The harvesting technique is traditionally done using a knife to comb the honeycomb, then using a plastic baking dish to collect the honey, squeezing it (by hand using a handkun), and then filtering into a 200 ml sample bottle. The yield obtained, 1 colony 3 replicates as in **Picture 4** below:

Picture 4: Honey Samples A, B, C *Wallacetrigona incisa ;* Honey Samples D,E,F *Tetragonula biroi*

The data obtained from the analysis of the Makassar Health Laboratory Center is then tabulated and then viewed and compared with the Indonesian National Standard (SNI 8664: 2018) stingless honey. Then he descriptively concluded

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Table 1: Quality requirements for stingless honey according to SNI 8664: 2018

No	Type of test	Unit	Requirements
1	Diastase enzyme activity	DN	at least 1
2	Hydroxymethylfurfural (HMF)	mg/kg	Maximum 40
3	Water content	% bb	Maximum 27,5
4	Reducing sugar (counts as Glucose)	% bb	at least 55
5	Sucrose	% bb	Maximum 5
6	Acidity	Ml NaOH/kg	Maximum 200
7	Insoluble solid in water	% bb	Maximum 0,7
8	Ash	% bb	Maximum 0,5
9	Metal contamination 9.1 Lead (pb) 9.2 Cadmium (Cd) 9.3. Merkury (Hg)	 Mg/kg Mg/kg Mg/kg	 Maximum 1,0 Maximum 0,2 Maximum 0,03
10	Arsenic Contamination (As)	Mg/kg	Maximum 1,0
11	Chloramphenicol	Mg/kg	Not detected

Source: SNI 8664:2018

1. Determination of Water Content

The samples were weighed to 1-2 grams and placed in weighing bottles that knew the weight. The sample was placed in an oven at 105-110oC for 2 hours. After that, the sample was cooled in an excavator for 10 minutes, then weighed and put back in the oven for 1 hour. The sample was cooled in an exicator for 10 minutes and then reweighed. It was repeated heating in the oven and weighing it until the weight was constant (the difference in consecutive weights ≤ 0.2 mg) then the water content of the sample was calculated with equation (1) as follows:

$$\text{Water content} = \frac{\text{material weight (early – end)}}{\text{the weight of the starting material}} \text{ x } 100\% \quad \textbf{(1)}$$

2. Determination of Reducing Sugar Levels

Carefully weigh 2 g of honey in a weighted 250 ml volumetric flask, dilute with distilled water and add 5 ml of the half-alkaline pb acetate solution. To test the addition of half-alkaline pb acetate, 10% sodium phosphate solution was dropped, if a white precipitate appeared it meant the addition was sufficient. Then add 15 ml of 10% sodium phosphate solution to precipitate excess pb acetate. If a precipitate has appeared, it means that the addition of sodium phosphate is sufficient. After complete settling, then dilute the solution with distilled water to mark a line, then leave for 30 minutes and then filter the solution. Take with a pipette 5 ml of filter (solution) into 500 ml Erlenmeyer, add 15 ml of distilled water, boiling stone and 25 ml of luff solution. Connect the Erlenmeyer to the cooler upright, bring to a boil, and simmer continuously for 10 minutes. Remove and cool for 45 minutes. Then add 15 ml of 30% KI solution and 25 ml H2SO4 25% after the cold solution (the addition is done carefully), then measure it with 0.1 N sodium thio sulfate solution (starch solution indicator). Then work on blank determination using 25 ml of water and 25 ml of luff solution. Then calculate the reducing sugar using equation (2) as follows:

$$\textbf{Reducing Sugar} = \frac{W1 \; x \; fp}{W} \; \boldsymbol{x}\; \mathbf{100}\ \% \quad \textbf{(2)}$$

3. Determination of Sucrose Levels

Weighing the volumetric flask. Pipette 25 ml of filtrate in the previous reducing sugar determination (e, f) into a 100 ml volumetric flask having a known weight. Add 5 ml of 25% HCl. Put the flask in a water bath (680C - 750C) for 10 minutes. Then insert the thermometer into the flask to check the temperature. After that, remove the flask, cool for 45 minutes, neutralize the solution by adding 30% NaOH (Indicator PP), then add water to mark the line and shake. Pipet 5 ml of the solution and configure as in the determination of reducing sugar (g) with the luff solution and configure the blank. From this you can see the sugar content after the investment. Then calculate the sucrose content using equation (3) as follows:

Sucrose = (%sugar after inversion – %reducing sugars) x 0,95 **(3)**

4. Hydroxymethylfurfural Determination (HMF)

Weigh the measuring flask, then carefully weigh 5 g of honey into a 50 ml measuring flask of known weight and rinse with water until the volume of the solution is 25 ml. Then add 0.50 ml of Carrez I solution and 0.50 ml of Carrez II solution, shake and dilute with water to mark the line. Adding a drop of alcohol to remove the foam from the surface then filtering with filter paper, removing 10 ml of the first filter. Take with a 5 ml filter dropper pipet and put each one in a test tube. Take with a dropper 5 ml of water and put in one tube and 5 ml of 0.20% sodium bisulfite in the other tube (comparator), then shake until completely mixed and place the absorbent sample against the comparator in a 1 cm cell at wavelength. 284nm and 336nm. The same with the comparison solution, diluting the comparison solution in the same way, using a 0.1% NaHSO3 solution. The absorbance

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value obtained is multiplied by the diluent factor before calculation. Then calculate the HMF in honey using equation (4) as follows:

$$\textbf{HMFmg/Honey 100g} = \frac{(A284 - A336)\ x\ 14{,}97\ x\ 5}{sample\ weight\ (g)} \quad \textbf{(4)}$$

5. Determination of Acidity (pH)

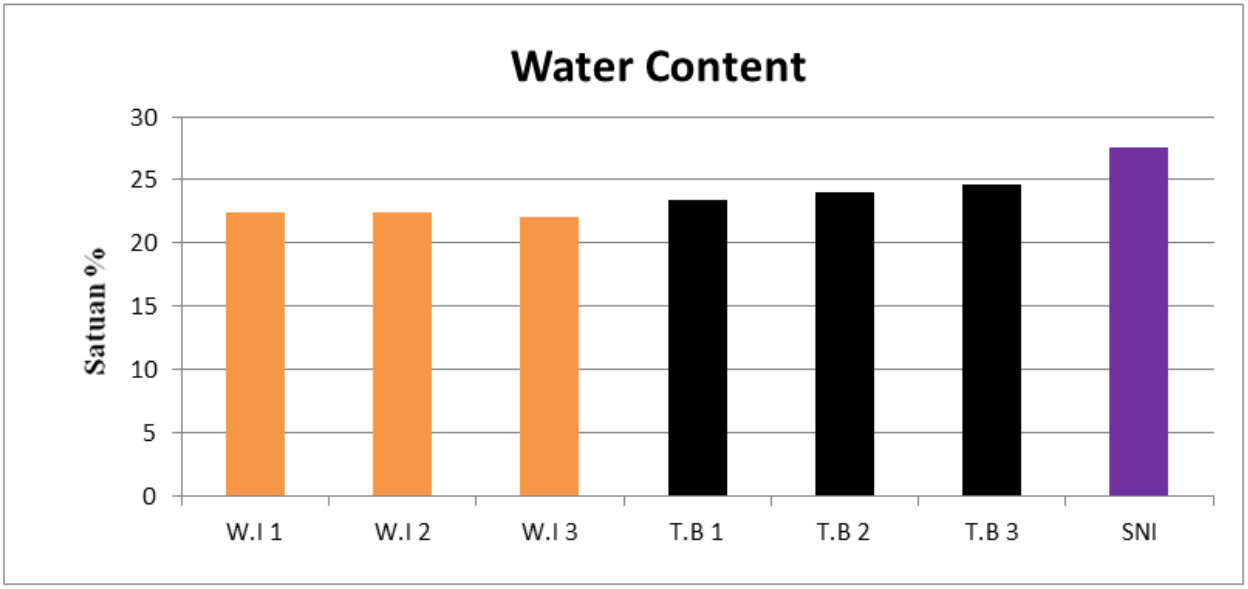
Erlenmeyer glass of 250 ml of weight. Then carefully weigh the 10 g sample into a known Erlenmeyer beaker. Then add 75 ml of distilled water and 5 drops of PP indicator. After that, stir with a magnetic stirrer while titrating with 0.1N NaOH to a fixed end point for 10 minutes. Then record the volume of 0.1 N NaOH used for the titration. Calculate the acidity using equation (5) as follows:

$$\textbf{Acidity}(ml\ NaOH\ 1\ N/kg) = \frac{a\ x\ b}{c} \quad \textbf{(5)}$$

RESULT AND DISCUSSION

Water content

Bees obtain water in a special way, namely from water nectar, which bees need to dissolve organic compounds and salts in the hive before being used for cell metabolism. During the dry season, the temperature in the nest is high, water is needed to control the temperature and humidity of the nest [5]. The water content in honey is due to several factors, namely the humidity, the type of nectar the bees feed, the production process and the storage of the honey. Nectar contains approximately 70% water [6]. High water content can be caused by high humidity levels in the tropics (around 60 - 80%). Unlike the subtropical areas where the humidity level is very low (below 50)**.**



Picture 5. Honey Water Content Graph *Wallacetrigona incisa* and *Tetragonula biroi*

The water content obtained through the quality test of the honey of *Wallacetrigona incisa* and *Tetragonula biroi* in the Mappedeceng district can be seen in **Picture 5**. The water content of the honey of *Wallacetrigona incisa* 1 was 22.41 %; *Wallacetrigona incisa* 2 was 22.41% and *Wallacetrigona incisa* 3 was 22.03%, while the water content of *Tetragonula biroi* 1 was 23.39%, *Tetragonula biroi* 2 was 24.05% and *Tetragonula biroi* 3 was 24.57 %. It can be seen in **Table 1** where the value given by this treatment meets the requirements of SNI 8664: 2018, with a maximum water content of 27.5%. The water content in honey is also an important aspect in determining the quality of honey to keep it fresh and avoid damage caused by fermentation, because the higher the water content of the honey, the easier it is to ferment the honey. . In contrast, the lower the water content of honey, the more suitable for consumption is the honey [7]. To obtain honey with low water content, it is necessary to pay attention to the harvest time, because the hygroscopic nature of honey is very influential with the humidity at the location of the farm. Honey sampling from *Wallacetrigona incisa* and *Tetragonula biroi* in Mappedeceng district in April, which is the dry season this month, so the time is very suitable for harvesting honey.

Reducing Sugar Levels

The presence of reducing sugars is very important, especially it facilitates the digestion of honey by the human digestive tract and provides high energy with the calories produced per 100 g, an average of 294 - 328 calories [8]. The main component of honey are carbohydrates from the group of monosaccharides consisting of glucose and fructose. When testing the quality of honey according to SNI, the two monosaccharides are called reducing sugars. Reducing sugar content is a group of sugars (carbohydrates) that can reduce electron-accepting compounds, eg glucose and fructose [9].

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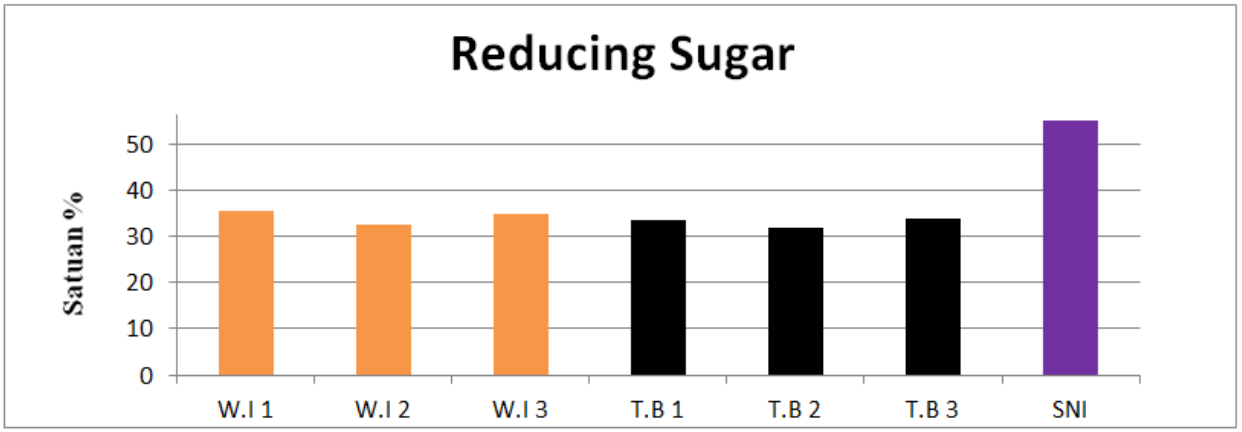


Picture 6. Graph of Honey Reducing Sugar *Wallacetrigona incisa* and *Tetragonula biroi*

The reducing sugar content obtained through the quality test of the honey of *Wallacetrigona incisa* and *Tetragonula biroi* in the Mappedeceng district can be seen in **Picture 6**. The reducing sugar content of the honey of *Wallacetrigona incisa* 1 was 35 , 41%; *Wallacetrigona incisa* 2 was 32.53% and *Wallacetrigona incisa* 3 was 34.91% while the reducing sugar content of *Tetragonula biroi* 1 was 33.70%, *Tetragonula biroi* 2 was 31.85% and *Tetragonula biroi* 3 was 33.75 %. It can be seen in **Table 1** where the value given by this treatment does not meet the requirements of SNI 8664: 2018 with a reducing sugar content of at least 55%.

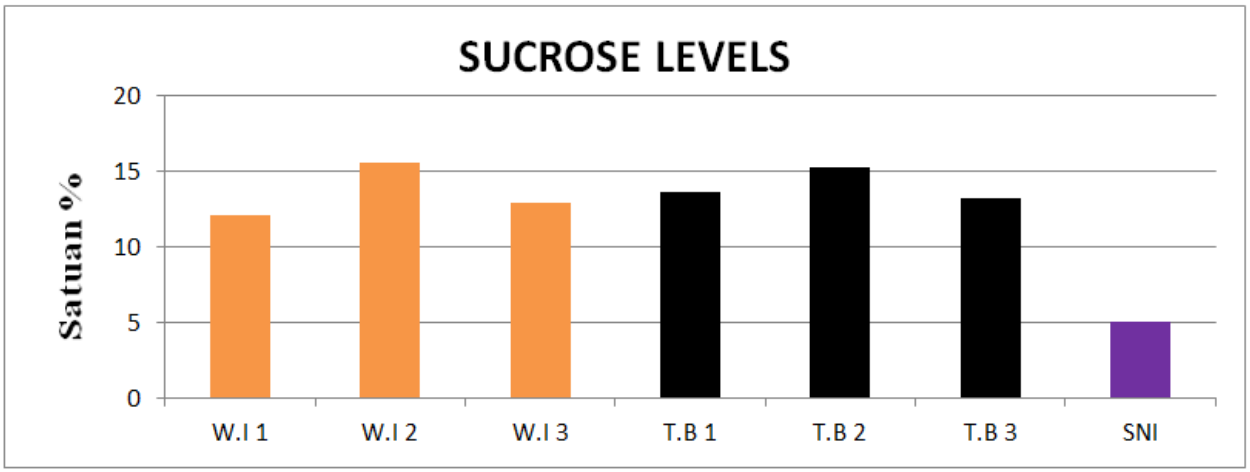
Results for reducing sugars from *Wallacetrigona incisa* and *Tetragonula biroi* in Mappedeceng district did not meet the requirements of SNI 8664: 2018, presumably due to storage time and ambient temperature in the dry season. Cold temperature honey has a better reducing sugar content than room temperature honey. There are several factors that affect the sugar-lowering levels of honey, including moisture content, moisture, and harvest time. There are studies that show that the high water content in honey can stimulate the activity of yeast to grow and develop in honey, triggering the fermentation process [5].

Furthermore, the cause of the low reducing sugar content in honey from *Wallacetrigona incisa* and *Tetragonula biroi* in Mappedeceng district is suspected to be due to the nectar feed source. At the grow site, most of the greenery is Melastoma flowers, embarrassed princesses, cat whiskers, and nails.

Sucrose levels

Sucrose is a sugar that cannot be reduced, but is present in mineral acids. Sucrose is a hydroxyl that is a combination of molecules with water molecules. In some countries, a maximum of 5% sucrose is available for use in food [10].

High-quality honey should also contain sucrose sugar that is not too high. Sucrose levels in honey occur when honey is harvested young or cooked once harvested. This causes the invertase enzyme in honey to die. In fact, the enzyme invertase works to convert long-chain sugar (sucrose) into monosaccharides [11].



Picture 7. Graph of Honey Sucrose Levels *Wallacetrigona incisa* and *Tetragonula biroi*

The test for sucrose content is done because most adulteration of honey, such as the addition of simple sugars, can increase the sucrose content of honey by more than 8%. The sucrose content obtained through the quality test of the honey of *Wallacetrigona incisa and Tetragonula biroi* in the Mappedeceng district can be seen in **Picture 7**. The sucrose content of the honey of *Wallacetrigona incisa* 1 was 12.04 %; *Wallacetrigona incisa* 2 was 15.58% and *Wallacetrigona incisa* 3 was 12.85%, while the sucrose content of Tetragonula biroi 1 was 13.63%, *Tetragonula biroi* 2 was 15.26% and *Tetragonula biroi* 3 was 13.17 %. It

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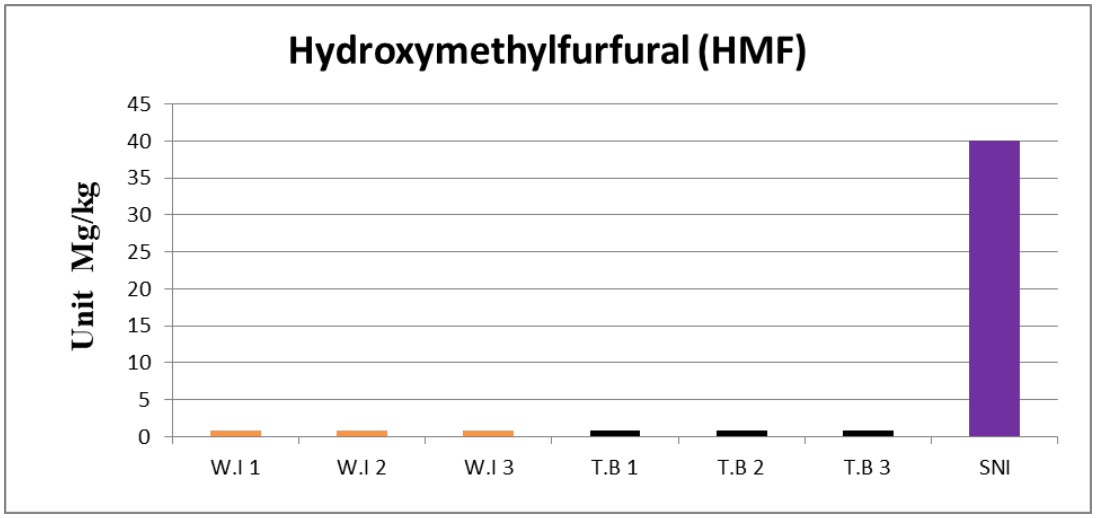
can be seen in **Table 1** where the value given by this treatment does not meet the requirements of SNI 8664: 2018 with a maximum sucrose content of 5%.
The results of the sucrose content of *Wallacetrigona incisa* and *Tetragonula biroi* in the Mappedeceng District did not meet the requirements of SNI 8664: 2018, allegedly due to the fermentation process in honey that yeast of the genus Zygosaccharomyces can perform.

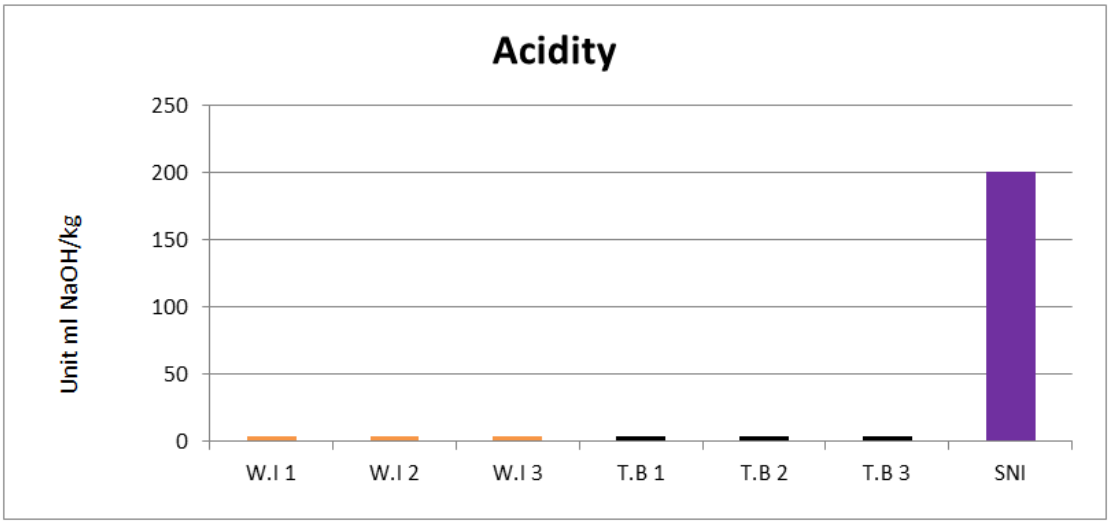
Hydroxymethylfurfural Content (HMF)
Hydroxymethylfurfural is a product of the breakdown of monosaccharides (glucose and fructose) by enzymes in honey. HMF is also known as a neurotoxin, which when abundant in honey makes honey bad [12]. HMF tests are carried out considering that the addition of sugar can increase the HMF value, in addition to that excessive heating will also increase the HMF value of honey. This heating is generally done to reduce the water content [5].



Picture 8. Graph of Honey Hydroxymethylfurfural (HMF) Content *Wallacetrigona incisa* and *Tetragonula biroi*.

The levels of hydroxymethylfurfural (HMF) obtained through the quality test of *Wallacetrigona incisa* and *Tetragonula biroi* honey in the Mappedeceng district can be seen in **Picture 8**. The levels of hydroxymethylfurfural (HMF) obtained from *Wallacetrigona incia* honey 1 is 0.88 Mg / kg; *Wallacetrigona incisa* 2 is 0.77 Mg / kg and *Wallacetrigona incisa* 3 is 0.81 Mg / kg, while the hydroxymethylfurfural (HMF) content of *Tetragonula biroi* 1 honey is 0.89 Mg / kg; *Tetragonula biroi* 2 is 0.81 Mg / kg and *Tetragonula biroi* 3 is 0.82 Mg / kg. It can be seen in **Table 1**. where the value given to this treatment meets the requirements of SNI 8664: 2018 with a maximum HMF level of 200Mg / Kg. This agrees with the opinion [13] that states that fresh or no damage contains a small or low amount of HMF.

Acidity (pH)
Honey can be classified in the acidic food group, because its pH is quite low, namely 3.4-6.1. Mineral-rich honey will have a high pH value. The low pH value of honey is due to part of the organic acid content of honey. The main acids that have been defined in honey include acetate, butyrate, formate, gluconate, lactate, maleic, oxalate, pyroglutamate, citrate, succinate, glycolic, α-ketoglutarate, pyruvate, 2/3-phosphoglucerate, α / β-glycerophosphate . and glucose-6-phosphate. Gluconic acid is the main acid in honey, produced by dextrose through an enzyme found in honey (glucose oxidase) [5].



Picture 9. Honey graph of acidity (pH) *Wallacetrigona incisa* and *Tetragonula biroi*

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The acidity (pH) obtained through the quality test of the honey from *Wallacetrigona incisa* and *Tetragonula biroi* in the Mappedeceng district can be seen in **Picture 9.** The acidity (pH) of the honey from *Wallacetrigona incisa* 1 was 3.5 ml NaOH / kg; *Wallacetrigona incisa* 2 is 3.5 ml of NaOH / kg and *Wallacetrigona incisa* 3 is 3.5 ml of NaOH / kg while the acidity (pH) of *Tetragonula biroi* 1 honey is 3.3 ml of NaOH / kg; Tetragonula biroi 2 is 3.3 ml NaOH / kg and *Tetragonula biroi* 3 is 3.3 ml NaOH / kg. It can be seen in **Table 1**. where the value given by this treatment meets the requirements of SNI 8664: 2018 with a maximum acidity (pH) of Trigona Sp Honey 200Mg / Kg.

CONCLUSION

The honey qualities of *Wallacetrigona incisa* and *Tetragonula biroi* in Mappedeceng district that comply with SNI 8664: 2018 are water content, hydroxymethylfurfural (HMF) and acidity (pH). The Trigona Sp honey test results that did not meet the requirements of SNI 8664: 2018 were reducing levels of sugar and sucrose. The honey color produced by *Wallacetrigona incisa* is darker dark brown and *Tetragonula biroi* has a light light brown color. The texture of the honey produced by *Wallacetrigona incisa* is thicker and *Tetragonula biroi* is finer. The taste of the honey produced by *Wallacetrigona incisa* is more acidic than the honey from *Tetragonula biroi.*

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