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Analysis of reducing sugar, diastase enzyme and acidity substances of stingless bees (*Trigona sp.*)honey based on time of Harvest in Layuh Village,Batu Benawa Subdistrict, Hulu Sungai Tengah Regency

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Key words: Stingless bees' honey, Harvesting age, Diastase enzyme activity.

Abstract

The superiority of *Trigona sp* bees is its high propolis production (3 kg / colony per year) compared to *Apis* bees which produce only 20-30 g of propolis per colony per year. This study aimed to determine the quality of stingless bees (*Trigona itama*) honey based on 1 month, 3 months and 5 months of harvesting. The method used is to observe when harvesting at the location where sampling of honey bee box has been prepared. The results showed that the product of stingless bees (*Trigona itama*) honey of KUBA farmers located in Layuh Village, Batu Benawa Sub district, Hulu Sungai Tengah Regency has fulfilled the Indonesian National Standard (Standar Nasional Indonesia – SNI 01-3545-2013) through laboratory test with 3 test parameters namely, diastase enzyme, reducing sugar and acidity. The results of laboratory analysis of stingless bees (*Trigona itama*) honey with 1 month, 3 months and 5 months of harvesting showed that the quality of honey was affected by the harvesting age, which can be seen from the diastase enzyme content, reducing sugar and the acidity of the honey which vary in every harvesting ages. The highest diastase enzyme activity (6.93 DN) was found at 5 months harvesting, the highest reducing sugar content (112.75% b/b) at 5 months harvesting, and the highest acid content (41.3) at 5 months harvesting.

Introduction

Honeybees are a very valuable resource in the world because of the honey they produce and their role in pollination of various types of plants. In Indonesia, beekeeping has a big enough opportunity to be developed in the community. In addition to the increasing demand for honey production in line with the increasing of the population, Indonesia also has considerable natural resource potential in the development of beekeeping business. The potential advantages of biological resources include the existence of various types of plant sources of bees feed and the condition of tropical agroclimate that strongly supports the sustainability of bee life (Engelsdorp and Meixner, 2009).

From studies conducted throughout the world, 150 species of stingless bees have been found including *Trigona scaptotrigona*, *Trigona laeviceps*, *Trigona apicalis*, *Trigona itama* and others.

In this research, the stingless bee type used by the Joint Venture Farmer Group (Kelompok Usaha Bersama - KUBA) in Batu Benawa Sub district is *Trigona itama* type, this type of bees has no stinger, but its body shape is similar to bees with stinger, but smaller.

The itama bees have sizes which range from 3 to 8 mm and are very agile to move, these bees are very different from the Apis bees type (*Apis dorsata* and *Apis cerana*).

Factors that can cause the quality of honey to decrease, among others are because the honey harvested before its old, wrong way of harvesting with increased temperature when stored (Kusuma, 2009). KUBA Farmers (Joint Venture Group) have been marketed their products out of Kalimantan city. The content and feasibility of the honey produced by these farmers need to be identified to be consumed by consumers. Therefore, this study was conducted to determine the content and quality of stingless bees honey consumed by the community.

Material and methods

The study was conducted at the Chemistry-Biochemistry Laboratory of the UNLAM's Medical Faculty.

The tools used in this study include honey sucking machine with battery power; Lidi made of bamboo; Bottles as honey containers; Electric heater; 500 ml Erlenmeyer flask; 10 ml, 25 ml and 50ml volumetric pipettes; 100 ml and 250 ml volumetric flask; Water bath, Standing cooler; Thermometer; 50 ml burette; Stopwatch, Camera. Materials used are stingless bees honey (*Trigona sp*), 20% KI (potassium iodide solution); 25% H₂SO₄ (sulfuric acid solution); 0.1 N Na₂S₂O₃ (sodium thiosulphate solution); 25% HCl (hydrochloric acid solution); 4N NaOH (sodium hydroxide solution); Phenolphthaline indicator solution; half acetate lead acid solution or zinc asetat solution; 10% (NH₄)₂HPO₄ (ammonium hydrogen phosphate solution) or potassium ferrocyanide solution; 0.5% starch indicator.

Observation was done during the harvest process, Sampling was done at 1 month, 3 months and 5 months harvesting age, honey quality test was conducted on 3 parameters (diastase enzyme, reducing sugar, and acidity).

The 3 parameters test done on stingless bees honey were based on SNI 01-3545-2013.

Diastase enzyme

Adding iod in starch solution will produce blue color. Diastase enzyme will change the starch to sugar. With the activity of diastase enzyme, blue color in starch solution will be lost. The higher the enzyme activity, the faster the blue color of the starch is lost.

The activity of diastase enzyme was tested according to AOAC Official Method 958.09-1999: 5 grams of honey were weighed in 20 ml cup, 10 ml - 15 ml water and 2.5 ml of daparacetate solution were added. In a cold state, the solution was stirred until the honey sample dissolved entirely.

The sample solution was moved in a 25 ml volumetric flask containing 1.5 ml of NaCl solution, keeps up to the water mark (the solution must be obtained before the NaCl solution).

Reducing sugar

Sugar was tested according to SNI 01-2892-1992, Sugar test method: Reducing sugar was tested using Luff Schoorl method and Lane and Eynon method. Saccharose/amount of sugar as saccharose was also tested using Luff Schoorl method and Lane and Eynon method. The determination of mono/disaccharide was done with high energy liquid chromatography method (HPLC).

Acidity

The acidity was tested according to CAC/Vol.III-Ed 1. Codex Standards for Sugars (including honey):10.0 grams of honey were weighed carefully, the honey was put into 250 ml Erlenmeyer then was dissolved with 75 ml of distilled water and 4 to 5 drops of PP indicator were added, sample was titrated with 0.1 N

NaOH to a fixed end point for 10 seconds, the volume of 0.1 N NaOH used for titration were recorded. Alternatively, pH meters can be used, and sample can be titrated up to pH 8.3 and the acidity in honey can be calculated.

Results and discussion

Diastase enzyme

The test results (Table 1) showed that all honey with different time of harvest meet the standard determined by the SNI with values of more than 3 DN. Standard determined by SNI is at least 3 DN. From these results, the value of diastase enzyme activity can be seen sequentially ranging from the highest to lowest: honey harvested in 5 months has diastase enzyme activity (6.93 DN), honey harvested in 1 month (4.64) and honey harvested in 3 months (4.11). The activity of diastase enzyme become criterion to determine the quality of honey, that is as a marker that the honey has undergone a heating process or due to long storage.

Table 1. The analysis result of diastase enzyme activity.

Replication	Treatment			SNI standard(DN)
	A	B	C	
1	4.64	3.97	7.48	Min 3
2	4.64	4.39	6.66	Min 3
3	4.64	3.96	6.66	Min 3
Total	13.92	12.32	20.8	
Average	4.64	4.11	6.93	

Explanation :

- A : harvested in 1 month
- B : harvested in 3 months
- C : harvested in 5 months

According to Hendrawan (2005) diastase enzyme is very sensitive to temperature rise. The higher the temperature, the lower the activity of the diastase enzyme. Heating a substance at a certain constant temperature over a period of time can restore a dropped diastase value.

This means that the diastase value can increase again with the increasing of heating time, unless heated at 100°C then diastase enzyme activity will not return at all. The lowest enzyme activity is found in honey with harvest time of 3 months. Environmental effects such as weather and sunlight also affect the activity of diastase enzymes. The higher the temperature, the

diastase enzyme activity will be lower. Sweet and bitter honey were mostly produced by *Apis dorsata* forest bee species in the forest of Battang which now cannot be cultivated properly. The results showed that diastase enzyme activity 5, 06 DN and hydroxymethylfurfural were not detected in honey sweet sample of Battang forest and have fulfilled honey quality requirement based on SNI 01-3545-2013. The result of moisture content analysis 22, 96%

and reducing sugar level 64, 91% almost reach the standard quality requirement of honey. The bitter honey sample of Battang forest, only hydroxymethylfurfural (not detected) analysis has fulfilled the quality requirement of honey based on SNI 01-3545-2013, but diastase enzyme activity <1 DN, moisture content 31,11% , and the reducing sugar content of 54,75% don't qualified based on SNI 01-3545-2013 standard (Ariandi & Khaerati, 2017).

Table 2. The analysis result of reducing sugar.

Replication	Treatment			SNI standard(% b/b)
	A	B	C	
1	79.75	99	110	Min 65
2	77	104.5	115.5	Min 65
3	74.25	104.5	112.75	Min 65
Total	231	308	338.25	
Average	77	102.67	112.75	

Explanation :

- A : harvested in 1 month
- B : harvested in 3 months
- C : harvested in 5 months

Reduced sugar contents

The reducing sugar is a group of sugars (carbohydrates) that can reduce electron receptor compounds, for example, glucose and fructose. The type of reducers found in honey are not only glucose and fructose, but also maltose and dextrin. Meanwhile, the process of honey production by bees itself is a complex process, so it is likely that there is a difference in the content and composition of reducing sugar in between various types of honey circulating in the community (Ratnayani, 2008).

Table 2 shows that the reducing sugar owned by each honey is at a value above the SNI standard that is at least 65, so this proves that the three types of honey are included in good honey types. In addition, this value is also above the reference of at least 60%. When compared to the three types of honey derived from the *Trigona* bees with different time of harvest, honey that was harvested in 5 months has a higher content

of reducing sugar than the other two types of honey. The high content of reducing sugar in *Trigona* honey that harvested in 5 months is because the honey was harvested in a completely mature condition.

The quality standard of honey is based on total reducing sugar content (glucose and fructose) that is at least 60%. Meanwhile, the type of reducing sugar found in honey is not only glucose and fructose, but also there is maltose and dextrin. Meanwhile, the production process in honey by bees itself is a complex process, so there is a possibility of different levels of content and the composition of sugar reduced among various types of honey circulating in the community. The composition of reducing sugar per honey is likely to affect the efficacy of honey, especially in the treatment process (Purbaya, 2002).

Acidity content

One the factor someone like honey is because of the sweetness possessed by the honey, as well as when

choosing the type of honey consumed. When going to consume a type of honey people will compare the taste of the honey, if honey has a sour taste, then people tend to leave it and choose honey that has a sweet taste. The National Standardization Body (BSN, 2013) has standardized the acidity of a honey to be distributed or sold.

There are seven types of free phenolic substances identified in the kelp honey. The presence of various

types of phenolic acids in hibernate honey may indicate that these honey have various properties. P-coumaric acid, for example, has the highest antioxidant activity followed by protokaechuic acid and therefore vanilla acid. P-koumarik acid is believed to reduce the risk of stomach cancer by reducing the formation of nitrosamine (carcinogens). (Suri, *et al.* 2012).

Table 3. The analysis result of acidity content.

Replication	Treatment			SNI Standard
	A	B	C	
1	40.0	41.0	41.0	Max 50
2	35.0	39.0	40.0	Max 50
3	37.0	39.0	43.0	Max 50
Total	112.0	119.0	124.0	
Average	37.3	39.7	41.3	

Explanation :

- A : harvested in 1 month
- B : harvested in 3 months
- C : harvested in 5 months

Honey that was harvested in 5 months has the highest acidity value of 41.3 and the lowest value is honey harvested in 1 month with 37.3 (Table 3). Honey that has a high moisture content will ferment easily so the acidity in honey is also increasing.

Conclusion

The results showed that the quality of stingless bee honey that was harvested in 1 month, 3 months and 5 months owned by farmer group venture (KUBA) in Layuh Village Batu Benawa Sub district Hulu Sungai Tengah Regency is in accordance with the quality requirement set by SNI (01- 3545-2013).

The best quality of honey was found at the honey that was harvested in 5 months, which can be seen from the results of its diastase enzyme, reducing sugar and acidity test.

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