Jurnal Inovasi Pendidikan dan Sains
Vol. 5 No.3, Desember 2024: 77-81 ISSN 2721-9119 (Online)

DETERMINATION OF DIASTASE ENZYME ACTIVITY AND WATER CONTENT OF HONEY FOREST OF SULA ISLAND, NORTH MALUKU USING SPECTROPHOTOMETRY UV-Vis

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Accepted: October 11th 2024. Approved: December 1th 2024. Published: December 15th 2024

Abstract: The experiment that has been carried out has the aim of knowing the enzyme activity test and determining the water content of forest honey originating from Sula Islands Regency, North Maluku, based on the parameters set by the Indonesian National Standards Agency (SNI) 8864:2018. Which includes organoleptic parameters (odour and taste), water content and Diastase Number. These parameters can indicate the originality of a forest honey product. The research was conducted at the Forensic Laboratory Center of the Criminal Investigation Department of the Republic of Indonesia Police, using the laboratory experiment method, using the UV-Vis Spectrophotometry instrument, forest honey originating from the Sula Islands district, North Maluku Province was used as material in this experiment. Organoleptic tests including odour and taste were carried out using the sense of smell and taste, water content testing using an oven drying technique, and Diastese Number (DN) value determination using the UV-Vis Spectrophotometry instrument. The experiment results showed that some parameters met the requirements and did not meet the criteria. Tests on Sula Islands forest honey's taste and water content parameters did not meet the criteria, but the odour and Diastese Number (DN) parameters met the requirements.

Keywords: Forest honey, Diastase Enzyme, Water Content, Honey quality, UV-Vis spectrophotometry

INTRODUCTION

Indonesia is a country with abundant natural resources, and forests are one of the ecosystems that contribute natural resources for survival [1] , one of the areas that has a pristine forest is the Sula Islands Regency, which is one of the regencies in the administrative area of the North Maluku provincial government, and one of the regencies whose people utilise natural resources from the forest well. One form of natural resource that is currently enjoyed is the result of forests that are rich in benefits, namely forest honey.

Honey is a liquid produced by bees, which is taken by bees from flower nectar (floral nectar) or other parts of plants that are extracted (extract floral nectar) [2]. Honey generally has a sweet taste and is a very useful product [3]. This is because honey contains chemical compounds such as carbohydrates with a percentage of 80-85%, 15-17% water, 0.3% protein, and 0.2% consisting of ash, amino acids, and vitamins.

Honey has properties as a natural antioxidant needed by the body [4], which is what makes the demand for honey increase day by day because the majority of Indonesian people use honey as an additional ingredient in traditional herbal medicine to increase the efficacy of herbal medicine in healing disease. Honey is also beneficial in terms of tissue regeneration [5]. In addition, honey can also function as a non-pharmacological raw material which plays a role in protecting the body from cardiovascular disease [6].

The benefits of honey can be seen in the high demand during the Covid-19 pandemic in 2022. Given how important honey is for health, it is considered necessary to know the quality of the honey that will be consumed. The quality of honey can be determined based on the parameters recommended by the Indonesian National Standards Agency (SNI) 8864:2018, which states that the determination of the quality of a product is seen from parameters that include the content of hydroxymethylfurfural (HMF), reducing sugar, sucrose, diastase enzyme activity and H2O in honey [7].

After explaining the importance of paying attention to the quality of honey and the parameters used to determine the quality of a honey product produced by forest bees, this research carried out enzyme activity tests and determination of water content in forest honey from Sula. Islands Regency, North Maluku, based on SNI 886 parameters.

RESEARCH METHOD

The research was conducted using a type of laboratory experimental research with a type of research approach, namely quantitative analysis, which aims to determine the water content and diastase enzyme value in Sula Islands forest honey, North Maluku based on the parameters of the Indonesian National Standard (SNI) 8864: 2018. This study also uses materials and instruments including.

1. **Tools**

   The tools needed during testing include Erlenmeyer flasks, measuring cups, volumetric pipettes, measuring flasks, burettes, glass funnels, dropper pipettes, porcelain cups, test

tubes, beakers, analytical scales, ovens, pH meters, water baths, UV-Vis spectrophotometry.

2. **Materials**

The materials needed during testing include forest honey, 0.0007N iodine solution, 0.5M NaCl solution, starch solution, Carrez I solution, Carrez II solution, 0.20% sodium bisulfite solution, 0.05M NaOH solution, 0.05M HCl solution.

3. **Method**

a. **Organoleptic Test**

Organoleptic testing is carried out directly with the senses of smell (nose), taste (tongue), and sight (eyes). Testing is carried out by at least 3 trained panellists or 1 expert. Experts are those who have expertise related to honey analysis (honey traders, breeders, or researchers). If the results are found to meet the criteria for honey's smell, race, and colour, they are given a normal value.

b. **The water content of the honey test**

The sample was weighed as much as 2 grams and placed in a porcelain cup of known weight. The sample was placed in an oven at a temperature of 105-110°C for 2 hours. After that, the sample was cooled in a desiccator for 10 minutes, then weighed and put back in the oven for 1 hour. The sample was cooled in a desiccator for 10 minutes and then weighed again. Repeated heating in the oven and weighing until constant weight (difference in consecutive weighings ≤ 0.2 mg) then the water content of the sample was calculated.

c. **Diastase enyme activity test**

A total of 5 grams of honey was weighed, then 10-15 ml of distilled water and 2.5 ml of acetate buffer solution pH 5.3 (1.59 M) were added. In a cold state, the solution was stirred until the honey sample was completely dissolved. The solution was then added with 1.5 ml of 0.5 M NaCl solution and set to the calibration mark with distilled water. A total of 10 ml of this solution was then added with 5 ml of starch solution through the inner wall of the tube and then placed in a water bath (40±0.2°C) for 15 minutes. At every 5-minute interval, 1 ml of the mixture was added to 10 ml of 0.0007 N iodine solution. The absorbance value was measured at a wavelength of 660 nm. The time from mixing the starch with honey to adding the liquid to the iodine was recorded as the reaction time. The solution was taken continuously at certain intervals until an A< value of 0.235 was obtained. The absorbance value is then plotted against time (minutes) from the top of the millimetre paper. From the graph, the time required to reach the absorbance value (A) = 0.235 is determined. The value of 300 divided by the time required to reach the absorbance value (A) indicates the activity of the diastase enzyme (DN).

RESULTS AND DISCUSSION

The research that has been conducted is a laboratory experimental research with a type of analysis, namely quantitative analysis. This type of analysis is a stage of analysis with the aim of finding information in the form of numbers or amounts of an analyte contained in the sample being analyzed [8].

The results of the organoleptic test of honey (smell and taste) showed a distinctive smell in forest honey that smells sharp like sugar. Organoleptic tests were carried out using the sense of smell and taste, showing that the results for the honey still have a distinctive honey smell and meet the requirements, while for the taste it does not meet the requirements. The forest honey used in this study can be seen in Figure 1 and the results of organolipic tests on forest honey can be seen in Table 1.

Firuge 1. Frest honey originating from the Sula Islands district

Tabel.1 Forest honey test results based on SNI 886 parameters

Parameters	Unit	SNI 886:2018 Requirements	Test Determination	Result
Odour	-	Specific Honey	Specific Honey	Eligible
Taste	-	Specific Honey	Not specific	Not Eligible
Water Content	%b/b	Maks.22%	23.38%	Not Eligible
Diastase enzyme activity	DN	Min. 1	20	Eligible

Honey is a fluid that is semi-liquid and usually in Indonesia honey itself has a variety of smells and tastes. This is caused by disparities such as distance of area, season, type and way of life of bees, type of plant (nectar source), and how to harvest and handle after the harvest period. [3].

A water content test was conducted on forest honey samples. Water content is one of the most important parameters to note because the high or low water content in honey can inhibit microbial activity and the fermentation rate of a honey product [9].

The water content test of forest honey in this study obtained a value of 23.38%. This value indicates that the forest honey used in this study slightly exceeds the standard set by SNI, but the Sula Islands forest honey used in this study still has a fairly good water content. The water content of a product in the form of an extract is said to be quite good if it has a water content of no more than 30% [10]. Usually, the high water content of honey is influenced by various factors including the humid conditions of the production and storage areas and because honey is hygroscopic in nature, making it very easy for honey to absorb water at a higher percentage than that stipulated by SNI. [3].

The water content in honey determines the durability of honey. Low water content in honey causes rotting microbes to be unable to live in it, plus honey also contains antimicrobial substances. Honey with a high water content (water content of more than 25%) is easily fermented by yeast from the genus Zygosaccharomyces which is resistant to high sugar concentrations, so it can live in honey [11]. The water content in honey also affects the quality of the diastase enzyme activity of forest honey. [12].

The diastase enzyme is a biomolecular compound that has a chemical composition in the form of protein that comes from bees and is distributed when the honey harvest reaches old age [13]. The diastase enzyme is also a reference in determining the level of original quality of a forest honey product, and the activity of an enzyme in an original honey product from bees is determined using the Diastase Number abbreviated as DN [14]. DN is defined as the amount of enzyme that will convert 0.01 g of starch to a specified endpoint in 1 h at 40°C under the test conditions [15].

These results are supported by research conducted by [16] by comparing the quality of honey by estimating the activity of the diastase enzyme for honey samples in the Saudi market. Determination of the DN value was carried out by plotting the absorbance against time (minutes) on square paper. A straight line was drawn through at least the last three points on the graph to determine when the reaction mixture reached an absorbance of 0.235. Divide 300 by the time in minutes to obtain the diastase number (DN). This number expresses diastase activity as ml of 1 percent starch solution hydrolyzed by the enzyme in 1 g of honey in 1 hour at 40°C. This diastase number corresponds to the Gothe scale number

Diastase enzyme activity depends on ambient temperature, which if the production temperature or storage temperature increases, it will decrease the activity of the diastase enzyme. This statement is in line with the results of research conducted by [17]. On Nondestructive Determination of Diastase Activity of Honey Based on Visible and Near-Infrared Spectroscopy. The results showed that diastase activity decreased with increasing temperature and heating time, and the sensitivity of acacia and longan to temperature was higher than linen. The optimum temperature for production and processing is 60^0C.

The results of the DN value test on Sula Islands forest honey, North Maluku, obtained a diastase value of 20 DN. This value indicates that the diastase enzyme in the parameter meets the requirements set by SNI, where the lower limit of the DN value for honey is 1 DN [18].

Honey that has a DN value above 3 DAN has benefits as an immunomodulator. An immunomodulator is a chemical component that has pharmacological properties that modulate the function and structure of immune activity. [19].

Based on the Sula Islands forest honey, North Maluku has a DN value of 20, which also shows that this honey also has benefits as an immunomodulator that functions to increase the body's immunity to avoid attacks from things that can interfere with human health.

This statement is based on the results of previous research, namely Analysis of Honey Quality After Pasteurization Process and Fast Cooling, where the research obtained a Diastase Number of 7.98 and it was said that the honey used in the research had immunomodulatory properties [20].

CONCLUSION

Based on the results of the research that has been conducted, the researcher concluded that the water content and taste of the Sula Islands forest honey, North Maluku did not meet the requirements, while the odour and Diastase Number (DN) met the SNI requirements. The water content and taste of the

honey did not meet the requirements due to the influence of temperature and storage of Sula Islands forest honey, North Maluku, so further research is needed regarding this to obtain maximum results.

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