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PHYSICOCHEMICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF MULTIFLORA HONEY FROM KERINCI, JAMBI

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ABSTRACT

Honey is a sweet, syrup-like substance that bees (Apis sp.) produce from the nectar of flowering plants. The aim of this study was to examine the physicochemical properties and antioxidant activities of multifloral honey (Apis dorsata forest honey) and (Apis cerana cultivated honey) from Kerinci, Jambi. The analysis consist of the intensity of colour, pH, viscosity, water content, ash content, sugar content, HMF content, acidity and antioxidant activity. The results showed that forest honey had light amber colour while cultivated honey had extra light amber colour. The pH, viscosity, water content, sugar content, HMF content, and acidity in forest honey were still in compliance with SNI standard, but the ash content was higher than the SNI standard. Cultivated honey had pH, sugar content, and HMF content in the range of SNI standar. However, the viscosity, water content, ash content, and acidity in cultivated honey were not in compliance with SNI standar. Forest honey and cultivated honey had antioxidant capacities about 16,74 mgVCE/g and 16,60 mgVCE/g, respectively. Meanwhile the antioxidant activity were 63,80% and 63,28%, respectively. Forest honey had more physicochemical aspects that were still in compliance with SNI standards compared to cultivated honey. The antioxidant activities of Kerinci honey, both forest honey and cultivated honey, were higher than the results of previous honey studies in other location.

Key words: Antioxidant activities; cultivated honey; forest honey; multifloral honey; kerinci honey.

INTRODUCTION

Indonesia is a country that has a high diversity of plants and can be a source of nectar for honey bees to produce various types of honey. According to SNI 3545:2013, honey is a sweet-tasting liquid made by honey bees from plant flower extracts (floral nectar) or other parts of plant (extra floral). Honey contains various nutrients that are good for the body, such as carbohydrates, proteins, amino acids, vitamins and minerals.

The vitamins contained in honey include Vit B1, B2, B3, B6, C, A, E, flavonoids. Honey also contains various minerals that are good for body health such as Na, Ca, K, Mg, Cl, Fe, Zn and others (Inayah et al. 2012). Honey contains antioxidants consisting of enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants in honey are catalase, glucose oxidase, and peroxidase enzymes (Pontis et al. 2014). Non-enzymatic antioxidants include carotenoids, amino acids, proteins, organic acids, products of the Maillard reaction, and more than 150 polyphenolic compounds including flavonoids, flavonols, phenolic acids, catechins, and cinnamic acid derivatives (Ferreira et al. 2009).

The characteristics of honey can be distinguished based on the source of nectar, geographical location and processing process. Types of honey based on the source of nectar can be divided into two types, monoflora honey and multiflora honey. Monoflora honey is honey obtained from bees with feed derived from one type of nectar source (bee feed). This honey is usually named based on the source of the nectar, such as longan honey, rambutan honey, randu honey, kapok honey, and so on. Monoflora honey has a specific fragrance, color and taste according to the source of the nectar. Multiflora honey comes from several types of nectar sources (bee feed). Multiflora honey comes from forests which is produced directly by wild bees such as Apis dorsata (Suranto 2007).

There are four species of honey bees including *Apis florea*, *Apis dorsata*, *Apis mellifera,* and *Apis cerana*. *Apis dorsata* is a very productive species. A colony of *Apis dorsata* bees can produce 20-40 kilograms of fresh honey per year. *Apis dorsata* is a wild bee that cannot be cultivated, thus it can only be harvested by hunting in forest areas (Adalina 2018). *Apis dorsata* has the largest body size. In addition, when compared to other honey bee species, this species is extremely aggressive. Forest bees' nests or habitats can take the form of combs hung from trees, rocks, caves, and other natural structures. The type of bee that has been widely cultivated is the local bee, *Apis cerana*. This beehive is shaped like a box (stup). In a single cultivation box, this bee can produce up to 2-5 kg of honey per year. *Apis cerana* is a honey bee species with a relatively short flight range of only about 300 meters. Morphologically, *Apis cerana* has the smallest body size then other species that form nests in closed place.

Kerinci Regency is a part of the Kerinci Seblat National Park (TNKS) where forests and agricultural land cover nearly half of the total area (Sari 2020). The Kerinci Regency area is also one of the Industrial Plantation Forest (HTI) areas in the form of cultivation and is overgrown with various types of plants so that multiflora honey is often found. The aim of this study was to compare the antioxidant activity of multiflora honey (*Apis dorsata* forest honey) and (*Apis cerana* cultivated honey) from Kerinci Jambi Regency. Most of people are unable to distinguish between high-quality and low-quality honey based on

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physical and chemical characteristics. Consumers should be informed about the quality of multiflora honey (forest honey and cultivated honey) produced in Kerinci Regency, Jambi as a result of this study. In addition, the research can be a way of promoting Kerinci honey's local products to community.

MATERIALS AND METHODS

Materials

The main material used in this study was honey which obtained from two different types of local honey, included forest honey (*Apis dorsata*) and cultivated honey (*Apis cerana*). Sample of forest honey (Apis *dorsata*) was bought from the UPTD office of the Production Forest Management Unit of Kerinci Regency, while cultivated honey (*Apis cerana*) was bought from Tunas Harapan Farmers Group, Kebun Baru Village, Gunung Raya Sub District, Kerinci Regency, Jambi. Both of them were harvested in November.

The majority nectar sources of forest honey came from several types of plants, such as cinnamon bark, coffee, cloves, and cocoa. While the source of cultivated honey nectar came from calliandra trees, passion fruit, sweet potatoes, dragon fruit, etc. During transportation, honey was packed in clear bottles that were tightly closed and wrapped with dark paper and bubble wrap to protect from the sun light.

Honey was kept at room temperature before analyzed. (Chayati and Miladiyah 2014). The equipments used in this research were pH meter, refractometer, furnace, oven, porcelain cup, beaker, desiccator, clear glass jar, VT-04 F viscometer, beaker, bulb, stopwatch, analytical balance, honey color guide, and HP 8453 UV/Vis spectrophotometer.

Furthermore, the sample materials used were Manis Salang Kerinci forest honey and *A. cerana* cultivation honey, while the analytical materials used included buffer solution, aquades, Carrez I solution, Carrez II solution, sodium bisulfite 0,20%, filter paper, 1% phenophthalein PP indicator, distilled water, 0.1 N NaOH.

Methods

Color Analysis

About 100 mL of honey was placed in a clear glass jar with with good lighting and then compared with the standard color. The color scale was divided into seven levels, such as Water White (0-8 mm, Pfund grader), Extra White (8-17 mm, Pfund grader), White (17-34 mm, Pfund grader), Extra Light Amber (34-50 mm, Pfund grade), Light Amber (50-85 mm, Pfund grader), Amber (85-114 mm, Pfund grade) and Dark Amber (114-140 mm, Pfund grade) (Winarno 1982).

pH Value

The pH measurement was carried out using a Waterproof pH tester. The use of this tool was to clean the pH tip first using distilled water and wipe clean and calibrate using a buffer solution. The 10 mL honey sample to be tested was placed in a container or cup. The pH tip was inserted into the sample and waited for the pH value to be read on the screen (Ockerman 1983).

Viscosity

Viscosity analysis was carried out based on SNI 01-2891-1992. The Rotational Viscoster VT-04 F was used to measure the viscosity. In the Rotational Viscoster, a 150 mL sample of honey was placed in a special container. The rotor was dipped into the honey samples and the rotor rotated inside the sample. Then, in the first 20 seconds, read the viscosity value.

Moisture Analysis

Moisture analysis was carried out based on SNI 01-3545-2013. A digital refractometer was used to determine the amount of water in the sample. After cleaning the prism, pressing start and calibrating it by pressing the zero button, dropping enough honey to fill the prism, pressing start, and viewing the results on the prism screen.

Ash Content Anaysis

Ash content was analyzed using AOAC 2015 method**.** The porcelain cup was heated at temperature of 105 °C for 24 hours, then cooled in a desiccator and weighed. About 2 g of honey was put into the porcelain cup and weighed. Then the samples were ashed at temperature 550 °C for 6 hours, cooled in a desiccator and weighed. The percentage of ash content was calculated by percentage of ash weight ratio before and after furnace process.

Sugar Content Analysis

The measurement of sugar content was carried out using a digital refractometer. After cleaning the prism, pressing start and calibrating it by pressing the zero button, dropping enough honey to fill the prism, pressing start, and the results will show on the prism screen.

Analysis of HMF Level

HMF level analysis was carried out based on SNI 01-8664-2018. About 5 g sample of honey was weighed and placed in a 50 mL volumetric flask. It was dissolved in water until the volume of the solution reached 25 mL. The Carrez I and Carrez II solutions were added in increments of 0.50 mL, shaken, and diluted with water to the desired concentration. A drop of alcohol was added to the solution's surface to remove the foam. The first 10 mL of the filter was discarded after filtering the solution. The filter results were pipetted into an 18 ml x 150 ml test tube in amounts up to 5 mL. For the sample solution, 5 mL of water was pipetted into a tube, and for the comparison solution, 5 mL of 0.20% sodium bisulfate was added, then homogenized until completely mixed (Vortex mixer). The absorbance of the sample was measured at wavelengths of 284 nm and 336.

Acidity Analysis

The honey sample was weighed 10 g and put into a 250 mL erlenmeyer flask. The sample was dissolved with 75 mL of distilled water and 4-5 drops of PP indicator were added. The sample was titrated with 0.1 N NaOH solution to a fixed endpoint for 10 seconds. Record the volume of 0.1 N NaOH required during the titration (SNI 01-3545-2013).

Antioxidant activity

About 7 mL of honey sample was mixed with 5 mL of methanol. Then put into a 10 mL volumetric flask and adjusted with methanol solvent. The honey extract was placed in a dark glass bottle and tightly closed and stored in a freezer (-25 °C) until analysis In a vial tube, 0.15 mL honey methanol extract was reacted with 0.1 mM DPPH (1,1-diphenyl-2-picyrl hydrazil) solution (in methanol solvent) to make 0.9 mL. After 30 minutes of incubation in a water bath at 37°C, the absorbance was measured using a UV-VIS 2453 spectrophotometer with a 515 nm wavelength. The free radical scavenging activity of DPPH is expressed in percent scavenging activity (% SA). (Tangkanakul *et al.* 2009).

Antioxidant Capacity

The %SA value is converted based on the standard curve. The standard curve was obtained by measuring the absorbance of the reaction between vitamin C (ascorbic acid) at concentrations 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg per 100 ml aquades with DPPH. A total of 0.15 mL of standard vitamin C solution was reacted with 0.9 mL of 0.1 Mm DPPH (in methanol solvent). The mixture was incubated in a water bath at 37°C for 30 minutes and analyzed using a UV-VIS 2453 spectrophotometer with a wavelength of 515 nm. The antioxidant capacity is expressed as mg equivalent of vitamin C 100 g^{-1} honey (Tangkanakul *et al*. 2009).

Data Analysis

The data obtained in this study were processed using descriptive analysis. The data from the analysis was the average value of three repetitions for each type of honey (Evahelda et al. 2017). The data obtained were presented in tabular form, compared

with the honey quality standard of SNI 01-3545-2013, and analyzed descriptively.

RESULTS AND DISCUSSIONS

Physicochemical Properties of Honey

Quality of honey are determined by their physicochemical properties In this study, the physicochemical properties of forest honey and cultivated honey were analyzed to determine the quality of these two honeys. Table 1 showed the results of physicochemical properties and antioxidant activity of honey compared to the honey standard SNI 01-3545-2013.

Honey Color

Table 1 showed that forest honey was light amber and cultivated honey was extra light amber, based on the honey color analysis. The color difference between the two kinds of honey was due to different nectar sources. Due to the high content of phenolic compounds in honey, the source of forest honey nectar is darker in color. This results were in line with the reserach of Kumazawa et al. (2012), who found that the amount of phenol compounds in honey was proportional to its color. Honey's color is determined by the presence of pigments such as carotenoids and flavonoids. Due to Apis dorsata bees' relatively longer range of feed, forest honey contains a high amount of phenolic compounds. Punchihewa et al. (1985) stated that the Apis dorsata bee flies for food at a distance of 400 meters from the nest, whereas the Apis cerana bee flies for food at a distance of 250 meters from the nest.

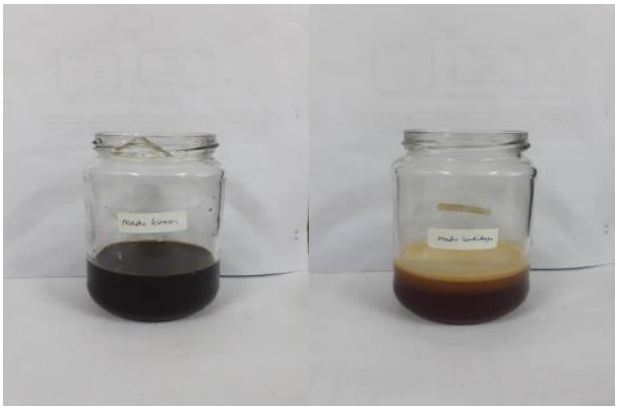
This result was consistent with the results of antioxidant activity and antioxidant capacity where forest honey had antioxidant activity and antioxidant capacity value slightly higher than cultivated honey. Moreover, the color of honey also influenced by sugar level and HMF level. High sugar level and HMF level in honey will enhance honey color (Chayati 2008). Forest honey had sugar level and HMF level higher than cultivated honey, it caused forest honey had a darker color than cultivated honey.

Table 1. Results of the physicochemical properties testing and antioxidant activity of honey

Test Type	Forest Honey	Cultivated Honey	Standard (SNI)*
Color	Light Amber	Extra Light Amber	-
pH	4,20±0,06	3,46±0,06	3,4-6,1
Viscosity (Poise)	13,6±0,58	5,33±0,57	Minimal 10
Water content (% b/b)	19,8±0,11	24,3±0,49	Max 22
Ash content (% b/b)	0,61±0,00	0,65±0,00	Max 0,5
Sugar content (% b/b)	80,13±0,1	75,6±0,11	Minimal 65
HMF content (mg/kg)	25,73±0,32	7,29±0,02	Max 50
Acidity (mLNaOH/kg)	42,3±0,56	69,9±0,53	Max 50
Antioxidant Capacity (mgVCE/g)	16,74±0,10	16,60±2,79	-
Inhibition Activity DPPH (%)	63,80±0,40	63,28±10,51	-

***** Honey SNI 01-3545-2013

According to Winarno (1982), the color of honey can be categorized into seven levels, from the lightest water white to the darkest dark amber. The figure 1 showed the different color image of forest honey and cultivated honey.



(a) (b)

Figure 1. The color of forest honey (a) and cultivated honey (b)

pH value

The results showed that the pH of forest honey was 4.20, while the pH of cultivated honey was lower at 3.46. The pH of forest honey and cultivated honey from Kerinci Regency has met the SNI which ranges from 3,4 to 6,1. The low pH of honey can prevent microbial growth and prolong the shel life of honey (Buba et al. 2013). Cultivated honey nectar was more acidic than forest honey.

The pH value can be influenced by organic acid content, honey processing process, stability and shelf life of honey (Kivrak et al. 2017). The content of acidic compounds in the nectar source affects the pH of honey. Muchtadi and Sugiyono (1992) support this statement that stated the presence of organic acidic compounds causes honey to have a low pH, such as malic acid, tartaric acid, citric acid, lactic acid and oxalic acid.

The differences in pH values can also be influenced by the bee's feed source (Kivrak et al. 2017). Soil conditions, geographical location, and climate condition where plants grow will affect the mineral and acid content of honey which will affect the pH of honey (Buba et al. 2013). Plants that have more acidic nectar will produce honey with a lower pH. The majority of nectar sources in the forests of Kerinci Regency are planted with several types of plants, such as cinnamon bark, coffee, cloves, and cocoa. Meanwhile, the source of cultivated honey nectar comes from calliandra trees, passion fruit, sweet potatoes, dragon fruit, and other plants.

Viscosity

The viscosity value of forest honey obtained in this study was 13,6 Poise which met the SNI standard, and cultivated honey had a viscosity value of 5,66 Poise which did not meet the SNI standard. The source of nectar, post-harvest handling, and environmental conditions can all affect the viscosity of honey (humidity). The low viscosity of cultivated honey can also be attributed to the lack of a post-harvest handling process, such as reduction in water content, which results in high water content and low viscosity values due to the watery texture of cultivated honey. The other factor that causes the low viscosity value in cultivated honey was the water content. Viscosity of honey can be influenced by water content. Honey with high water content will have low viscosity (Apriani et al. 2013)

Water Content

Honey with good quality must contain low water content, or a maximum of 22% based on SNI (2013). The results showed that forest honey had water content of 19.8% w/w which had met the SNI standard, which was less than 22% and cultivated honey had water content of 24.3% w/w, which was above the SNI standard of 22%. The difference in water content in the two honeys was influenced by different sources

of nectar. Other factors such as humidity, harvest and post-harvest handling of honey can also affect water content (Sarwono 2007). The high water content in cultivated honey was due to cultivated honey still in the form of raw honey which has not undergone a process of decreasing the water content, thus it will produce a more dilute honey, while forest honey has been treated to reduce water content after harvested. Furthermore, high water content in cultivated honey might be also caused by post-harvest handling such as packaging honey bottles that were not tightly closed, honey would absorbs water from the air and caused high water content. Honey is hygroscopic so it easily absorbs water in moister air and can increase the water content of honey (Sarwono 2007). This is in accordance with the study of Chasanah (2001), which states that the higher of humidity, the higher the water content and the lower the viscosity. The water content of honeys in Indonesia are generally higher than the SNI standard, therefore it is necessary to carry out a treatment to reduce the water content of honey (Apriantini et al. 2022).

Water content can affect the natural fermentation process in honey. High water content in honey can accelerate the rate of fermentation, thereby accelerating the deterioration of components in honey. However, low water content can extend the shelf life of honey because it can reduce the growth of yeasts that cause fermentation such as the genus Zygosaccharomyces. The fermentation process occurs because yeast will degrade sugar into alcohol, then alcohol will react with oxygen to form acetic acid which can reduce the quality of honey (Evahelda et al. 2015; Yap et al. 2019; Ariandi & Khaerati 2017).

Ash content

The results showed that the ash content of forest honey was 0.61% w/w and cultivated honey was 0.65% w/w. Both types of honey had ash content above the standard of SNI. Ash content can reflect the amount of mineral contained in honey (Abdulkhaliq and Swaileh 2017). Minerals contained in honey include K, Na, C, P, Mg. The mineral contained in the nectar source of cultivated honey was higher than the mineral contained in the nectar source of forest honey.

Ash content in honey can indicate the origin of honey, so geographical location, environmental conditions, soil conditions where plants as a source of nectar grow will affect the mineral content of honey (Bouhala et. al 2020).

Sugar Content

Sugar content in honey was measured to determine the quality of honey. High sugar content in honey can increase the shelf life of honey. Anand et al. (2019) stated that the average honey is composed of 81% sugar and 17% water and 2% small components such as enzymes, phenolic components, volatile components and others. The high sugar content and low water content in honey can inhibit the activity of yeast that is naturally found in honey (Yunus et al. 2020). Machado et al. (2020) mentions the main types of sugar in honey are glucose and fructose, while complex sugars contained in honey are included in small amounts.

The results of this study showed that the sugar content of forest honey was 80.13% w/w, while the sugar content of cultivated honey was 75.6% w/w. According to Mulu et al. (2004), the different types of plants which are source of food for bees will affect the characteristics of honey, such as flavor, aroma, color, quality, and sugar content in honey. Based on interviews with cultivated honey farmers, bees only get nectar sources from the environment without additional feed such as sugar solution. As a result, cultivated honey had lower sugar content. Rainfall and environmental humidity also have an impact on sugar content.

Sugar content in honey affects honey characteristics, such as viscosity, honey color and honey pH. The high sugar content

of honey in forest honey will reduce water content, increase viscosity and increase the color of forest honey. The results of this study were in accordance with Chayati's (2008) research that the water content of honey is negatively correlated with sugar content, viscosity and color of honey.

HMF (Hidroksimetilfurfural) Level

HMF (hydroxymethylfurfural) is an organic compound formed by the dehydration of reducing sugars. The results showed that forest honey had an HMF content of 25.73 mg/kg, and cultivated honey had an HMF level of 7.29 mg/kg. These results indicate that the levels of HMF in the two honey samples meet the SNI standard, which is below 50 mg/kg-1. The HMF value is an indication of prolonged heating during storage and preparation or potential adulteration. This compound is usually not found in fresh honey, but the amount of HMF content in honey will increase during the storage process (Zappala et al. 2005). Pure honey usually has an HMF level of 1 mg/kg at the beginning of harvesting and continues to increase when the temperature reaches 20˚C (Anjana et al. 2014).

HMF levels are influenced by several factors, such as temperature, heating time, storage conditions, pH and plant/flower source of honey. Forest honey has undergone postharvest treatment, such as heating process in order to reduce water content, cause the HMF in forest honey was higher than cultivated honey. This result was in accordance with the study of Minarti et al. (2016) that excessive heating can cause a decrease in the activity of the diastase enzyme and an increase in HMF levels. The higher the HMF value in honey, it indicates that the honey has undergone a high heating process. High HMF values can also be caused by heating both in the processing, storage, and shipping processes so that honey will produce a caramel-like color and aroma (Derndorfer 2015). This is consistent with the results obtained in this study that darker colored forest honey had higher levels of HMF when compared to light-colored cultivated honey.

Acidity Level

The results showed that forest honey had an acidity level of 42.3 mL NaOH/kg which had met the SNI standard and cultivated honey had a high acidity value of 69.9 mL NaOH/kg which above the SNI standard. The high acid level in cultivaed honey was due to the pH in cultivated honey higher than forest honey. The source of nectar, the location of cultivation, humidity, post-harvest management, and honey storage conditions can influence the acidity of the honey. Cultivated honey had high acidity also because the honey contained more water content than forest honey. Fermentation can be triggered by high water content and increase the acidity level of honey. Furthermore, high acidity in cultivated honey may also be caused by an unhygienic post-harvest processing process, this condition can lead contamination in honey. Karnia et al. (2019) stated that the acidity of honey is very important to keep the honey hygienic and safe for consumption.

Antioxidant Activity

The results of this analysis, it was found that forest honey had an antioxidant capacity of 16.74 mgVCE/g, and cultivated honey was 16.60 mgVCE/g. The amount and type of antioxidants in honey can be affected by the floral nectar. This results were supported by Suranto's (2007) statement, which stated that the amount and antioxidant content in honey were highly dependent on the nectar source. Forest honey and cultivated honey had similar antioxidant content, but different in the amount of compounds which responsible for antioxidant activity. Furthermore, it's possible that other compounds play a role in antioxidants but were not investigated further in this study. According to Khalil et al. (2010), antioxidant activity is influenced by the content of phytochemical compounds that have antioxidant properties. These

compounds include phenolic acids, flavonoids, enzymes (glucose oxidase and catalase), ascorbic acid, carotenoids, organic acids, amino acids and proteins.

The inhibition percentage of forest honey against free radicals was 63.80%, while cultivated honey was 63.28%. The percentage of DPPH inhibition in forest honey was higher than cultivated honey. In comparison to cultivated honey, the higher percentage of DPPH inhibition indicated the higher antioxidant content of forest honey. The antioxidant results in this study were higher than those obtained in Chayati and Miladiyah's (2014) study, which found antioxidant activity in honey in Java and Sumatra to be in the range of 21.06 to 32.14 %. The abundance of polyphenolic compounds in a sample indicates that they had a high antioxidant activity (Hossain et al. 2011).

CONCLUSION

Kerinci Forest Honey had pH value, viscosity, water content, sugar content, HMF level, and acidity level that meet the standards of SNI 01-3545-2013. However, in cultivated honey only pH, sugar content, and HMF levels have met the standard of SNI 01-3545-2013. Forest honey had a light amber color, while cultivated honey was lighter. Forest honey had the same high antioxidant content and free radical inhibition percentage as cultivated honey.

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