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The Honey Quality of *Apis mellifera* with Extrafloral Nectar in Lombok West Nusa Tenggara Indonesia

Erwan*[1], Muhammad Harun[1], Muhammad Muhsinin[1]

[1] Faculty of Animal Science, University of Mataram, Jalan Majapahit No. 62, Mataram 83125, Indonesia
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Abstract: The purpose of the present study was to analyze Apis mellifera honey's quality given extrafloral nectar feed from *Arenga pinnata* sap and *Cocus nucifera* sap as stimulatory nutrition. The chemical compositions of honey, such as reducing sugar content, sucrose content, the acidity of honey, moisture content, and diastase enzyme activity, were measured. The comparison between A. mellifera honey's chemical compositions on Arenga pinnata sap and Cocus nucifera sap ware was analyzed using the student t-test (GraphPad Instant Statistical Program). The result from the analysis of reducing sugar content showed that the *A. mellifera* honey from *Cocus nucifera* sap (73.69± 0.21%) had a higher ($P<0.05$) than the *Arenga pinnata* sap (60.15±2.13%). The significant differences ($P<0.05$) in the acidity of *A. mellifera* honey from *Arenga pinnata* sap (43.00±7.48) compared with *Cocus nucifera* sap (22.00±2.14). The sucrose content, moisture content, and diastase enzyme activity were not significant differences between the *A. mellifera* honey from *Arenga pinnata* sap compared with the *A. mellifera* honey from *Cocus nucifera* sap. In conclusion, the chemical compositions of *A. mellifera* honey with extrafloral nectar (*Arenga pinnata* sap and *Cocus nucifera* sap) are good quality and indicate that the honey falls under the limits of international standards. The *A. mellifera* honey from *Cocus nucifera* sap has a higher sugar reduction content and lower acidity than the *A. mellifera* honey from *Arenga pinnata* sap.

Keywords: *Apis mellifera*; extrafloral nectar; honey quality; chemical compositions of honey

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Introduction

Honey is a natural product used by humans both as a food source and medical product from long periods to modern culture [1]. It applies to human diet health is due to its chemical composition. The honey composition is varied and linked to factors that directly affect its composition and quality, such as the bee species, floral source, and environmental and storage term [2]. Honey is a rich source of carbohydrates, making it widely used as a natural sweetener, as well as an important source of other minors constituents, which are more related to its biological properties such as polyphenols, carotenoids, minerals, proteins, free amino acid, enzymes and vitamins [3].

The world honey production and consumption are based on the product obtained from the species *A. mellifera*, whose producers are principally located in Europe and Asia. The *A. mellifera,* also called the Italian bee, is an important insect that **produces** high economic and ecological values for humans as a key pollinator of plants [4] and producer of bee products, including honey, royal jelly, pollen, propolis, and beeswax [5]. Each of these different bee products is becoming economically important and additionally, is known to have several potent bioactivities. Indeed, bee products have been used in traditional medicine throughout society. For instance, bee pollen is reported to boost energy and stamina [6], propolis to help maintain good health [7], royal jelly to support the immune system and increase energy [8], whilst honey, mainly used as a

Email: apiserwan@gmail.com *(*Corresponding Author)*

natural sweetener in every food culture, is also used traditionally used for the treatment of burns, sore throats and as an antiseptic [9]. More recent studies have found that several bee products have potential anticancer activity *in vitro* and *in vivo* [10].

According to [11], the average for honey production of *A. mellifera* is 25-40 kg per colony, *A. dorsata* is 50-80 kg per colony, *A. florea* is 0.2-0.9 kg per colony, *A. cerana* is 8-10 kg per colony, and stingless bee (*Trigona sp*) is 0.3-0.4 kg per colony per year. The honey production of *A. mellifera* can be increased by providing an additional feed of extrafloral nectar, which is the source of nectar outside the flower sector. One of the plants that can be an alternative to producing this nectar is palm (*Arenga pinnata*) and coconut (*Cocos nucifera*) plants. Both plants can release sweet liquid throughout the year called the extrafloral nectar or *nira*, obtained from the tap. The production sap or *nira* of *Arenga pinnata* per day per tree ranged from 8-12 liters [12] and 4-6 liters for *Cocos nucifera* sap [13]. These plants are widely available and grow well in the area of Lombok West Nusa Tenggara and spread throughout the region in Indonesia. Based on data from [14] the area of *Arenga pinnata* and *Cocos nucifera* plants in Lombok West Nusa Tenggara are ±724 Ha with ±112.665 trees and ±11.684 Ha with 2.763.547 trees, respectively.

With the source of alternative feed is expected to produce honey of *A. mellifera* can be improved. Increased A. mellifera honey production must be followed by honey quality standards established by national and international agencies. Therefore, the present study aimed to analyze the quality of *A. mellifera* honey given extrafloral nectar feed from *Arenga pinnata* sap and *Cocus nucifera* sap as a stimulatory nutrition in Lombok West Nusa Tenggara, Indonesia.

Materials and Method

Sample Collection

A total of 16 *A. mellifera* honey samples, 8 for each honey type (*Arenga pinnata* and *Cocus nucifera*) were used in the study. The honey samples were collected from North Lombok district, West Nusa Tenggara Province, Indonesia.

Reduced Sugar Content

The estimation of reducing sugars was carried out using the using Luff Schoorl method. About 2 g of honey was weighed and diluted to 5 mL with Pb acetate ½ base. Ten milliliters (10 mL) of solutions were transferred to a 500 mL Erlenmeyer flask containing 15 mL of water and 25 mL of Luff Schoorl's solution. The Erlenmeyer flask was heated to 2 min and 10 mL of KI 30%, and 25 mL H_2SO_4 25% was added, then titrated with a 0.1 N Na-thiosulfate solution. Make a blank solution using 25 mL of water and 25 mL of Luff solution. The content of reducing sugar was calculated by the difference between the blank titration and the titration of the sample with the formula:

$$\textbf{Reduced sugar level} = \frac{\textbf{mg sugar x dilution factor}}{\textbf{mg example}} \textbf{x 100\%}$$

Sucrose Content

Inversion, sucrose content was determined by adding 5 mL of dilute HCl 0.25 M, 50 mL of diluted honey solution, and water in a 100 mL volumetric flask. The solution was then warm in a water bath, cooled, and diluted to the mark. Finally, the sucrose content was obtained by calculating the sugar content difference after inversion and before inversion, then multiplied by 0.95.

$$Sucrose\,(\%) = (\%\ sugar\ after\ the\ inversion - \%\ sugar\ reduction)\ x\ 0.95$$

$$Sugar\ after\ the\ inversion = \frac{mg\ sugar\ x\ dilution\ factor}{mg\ example}\ x\ 100\%$$

Acidity of honey

Honey samples (5 g) were dissolved in 100 mL of volumetric flask. The filtrate (25 mL) was mixed with 50 mL of water, and 3-4 drops of phenolphthalein indicator were added. Titrate with 0.1 N NaOH solution for 10 s. The sample was titrated with 0.1 N NaOH solution at a rate of 5.0 mL/min. Titration was completed at pH = 8.5. The acid content in honey was calculated using the formula:

$$\text{Acidity}\ (ml\ N\ NaOH/kg) = \frac{a\ x\ b}{c}\ x\ 1000$$

a = The volume of NaOH in mL
b = Normality of 0.1 N NaOH in mL
c = Sample weights in g

Moisture Content

Honey samples (3 g) in the cup were put into the oven at 105 °C for 4 h and cooled into the desiccator for 20 min, then put again into the oven for one h. The following formula calculated water content:

$$\text{Moisture content} = \frac{X - Y}{Z}\ x\ 100\%$$

X = Weight sample before in oven (gr)
Y = Weight sample after in oven (gr)
Z = Weight sample of honey (gr)

Diastase Enzyme Activity

Diastase enzyme activity in the fresh honey was determined using the Phadebas method using the spectrophotometer. The first step of the Phadebas method was to prepare acetate buffer by dissolving 87 g

of sodium acetate trihydrate in 400 mL of water. The pH of the solution was adjusted to 5.3, with 10.5 of glacial acetic acid. The solution was diluted to 500 mL with distilled water and stored in a glass bottle. Ten gram (10 g) of honey was weighed, quantitatively transferred to a 50 mL volumetric flask, and made up to volume with 5 mL of acetate buffer. Five milliliters of the sample was transferred to the test tube and placed in a water bath at 40 ^{o}C. At the same time, under the same conditions, the blank (5 ml of acetate buffer) was heated in a water bath at 40 ^{o}C. After 15 min, 1 Phadebas tablet was added to the two solutions, stirred (approx. 10 s), and placed back into the water bath at 40 ^{o}C. After exactly 30 min, 1 mL of sodium hydroxide solution was added to interrupt the enzyme reaction.

The solutions were centrifuged in the next step (5 min; 1500 rpm), and the absorbance was measured at 620 nm against distilled water as the reference sample. The diastase number (DN) was calculated as 300 divided by t_x, required to reach the specified absorbance, 0.235. The Schade unit is defined as that amount of enzyme that will convert 0.01 g of starch to the prescribed endpoint in one hour at 40°C under the test conditions.

Honey Quality Standard

As a reference to know the quality of honey required standards established by national and international agencies. Honey quality standards were presented in Table 1.

Table 1. Honey quality standard based on SNI 3545:2013 and CODEX STAN 12-1981

Constituent Content	SNI 3545:2013[1]	CODEX STAN 12-1981[2]
Moisture (water)	Max. 22% (< 22%)	Max. 20% (< 20%)
Reducing sugars	Min. 65 g/100 g (> 65%)	Min. 60 g/100 g (> 60%)
Sucrose	Max. 5 g/100 g (< 5%)	Max. 5g/100 g (< 5%)
Free acidity	Max. 50 ml NaOH/kg	Max. 50 mval/kg
Diastase activity (DN)	Min. 3 Schade units	Min. 8 Schade units

[15]; [16]

Statistical Analysis

The data obtained in the study were analyzed statistically using student t-test (using GraphPad Instant Statistical Program). Differences between mean values were considered significant at values of P<0.05

Result and Discussion

The chemical compositions of *A. mellifera* honey, such as reducing sugar content, sucrose content, the acidity of honey, moisture content, and diastase enzyme activity, were presented in Table 2.

Table 2. The chemical compositions of A. mellifera honey from extrafloral nectar

Variable	Feed sources	
	Arenga pinnata sap (n=8)	*Cocus nucifera* sap (n=8)
Reducing Sugar (%)	60.15±2.13^{a}	73.69± 0.21^{b}
Sucrose (%)	4.40±2.04^{a}	4.21±0.83^{a}
Acidity (NaOH/kg)	43.00±7.48^{a}	22.00±2.14^{b}
Moisture (%)	19.34±0.29^{a}	20.94±0.51^{a}
Diastase Enzyme (Schade units)	17.12±0.83^{a}	16.48±0.53^{a}

n: Number of samples, the different superscript within the same row shows significant (P<0.05) difference

The reducing sugar content of *A. mellifera* honey from *Cocus nucifera* sap was significantly higher (P<0.05) than the *A. mellifera* honey from *Arenga pinnata* sap. The significant differences (P<0.05) in the acidity of *A. mellifera* honey from *Arenga pinnata* sap (43.00±7.48) compared with *Cocus nucifera* sap (22.00±2.14). The sucrose content, moisture content, and diastase enzyme activity were not significant differences between the *A. mellifera* honey from *Arenga pinnata* sap compared with the *A. mellifera* honey from *Cocus nucifera* sap.

The result of the analysis of reducing sugar content in *A. mellifera* honey from extrafloral nectar in accordance with CODEX STAN 12-1981 standard is not less than 60% (g/100 g), but lower than the SNI 3545:2013 standard for *A. mellifera* honey from *Arenga pinnata* sap. Bee honey's properties and compositions depend on its geographical floral origin, season, environmental factors, and treatment of beekeepers [17]. Bogdanov [18] found more than 22 sugars in honey; however, fructose and glucose are the major sugar content. Primary sugars that existed in honey are fructose and glucose, and in nectar honey, the fructose content should exceed that of glucose [19]. The sugar content of *Arenga pinnata* sap and *Cocus nucifera* sap is 10.5% and 10.9% respectively [20]. The high sugar content in bees feed can reduce honey sugar content [21]. Sugar reduction of honey results from the hydrolysis process by enzyme invertase of honeybees converts sucrose into glucose and fructose [22]. Bees convert the sugars in the nectar and add

microorganisms and reduce the water content to prevent fermentation. The high and low sugar reduction in honey is influenced by the perfect or not hydrolysis process at the time of honey formation. Pourakbari [23] reported reducing sugar content in *A. mellifera* honey from Persimmon sap is 46.00±2.71%, 63.89±0.25% from *Acacia mangium* and 61.17±0.17% from *Ananas comosus* [24] and 70.34±7.49 from multi-floral [25].

The *A. mellifera* honey showed a sucrose content of 4.40±2.04% (*Arenga pinnata* sap) and 4.21±0.83% (*Cocus nucifera* sap), which is within the Indonesia national standard (SNI 3545:2013) and international parameters (CODEX STAN 12-1981) recommended for this honey (<5%). The results show that the *A. mellifera* honey samples generally have higher sucrose content than Algerian honey (1.80 to 2.54%) [26]; [25] and comparatively similar to Malaysian honey (4.51%) [24]. The significant carbohydrates of honey are glucose, fructose, and sucrose. They are frequently attended by complex sugars [27]. The rifest disaccharide in the plant's world is sucrose. In the nectar honey, its content normally does not outpace 3% [28]. It is affected that mature nectar honey should not include more than 5% of sucrose. According to [29], although honey contains an active sucrose separation enzyme (sucrase, glucosidase), honey's sucrose content never reaches zero. The sucrose contents acquired in this study are within the range of values reported for Argentine and Turkish [30], Venezuelan in Vit [31], American [32], and Pakistani in Zafar [19] honey.

The values obtained for the acidity of *A. mellifera* honey from extrafloral nectar were all within the limits of national and international standards (Max. 50 ml NaOH/kg). Kowalski [33] reported the acidity values for honey having a range from 12.75±0.42 to 62.61±0.88 mval/kg. These results are similar to the results of other researchers [34]; [35]; [36]; [37]. The acidity is another parameter that plays an important role in honey quality and freshness. Although the acidic character is related to honey antimicrobial properties [1], high acidity levels can indicate sugar fermentation processes, thus affecting the organoleptic characteristics and quality of honey. The excessive acidity is the feature of fermented honey, and generally is the outcome of the several microorganisms development on their surface [38]. Honey acidity depends mainly on the type of material, maturity level, and season in which it was produced [39].

Moisture content is a necessary parameter of honey quality and necessary the amount of water provide in honey. In the present study, the percentage moisture content was between 19.34±0.29% (Arenga pinnata sap) and 20.94±0.51% (Cocus nucifera sap), which is under the limit of ≤22% set by the Indonesia national standard (SNI 3545:2013) for honey quality. Usually, the moisture contents for *A. mellifera* honey from extrafloral nectar in this study were relatively similar to those of other honey, such as Portuguese honey (15.9-17.2%) [40], Anatolian honey (17.0-19.4%) [41], Romanian honey (15.4-20.0%) [42] and Indian honey (17.2-21.6%) [43]. The moisture content provides in honey samples is important as it contributes to its capability to refuse fermentation and granulation through storage [44]. The moisture content was within the standard helps to encourage longer shelf life during storage [45]. Overall, the moisture content within the standard (SNI 3545:2013 and CODEX STAN 12-1981) in our honey samples shows their good storage capability and quality.

The diastase enzyme is the common name for the enzyme a-amylase. It is found in nectar and is also added by the honeybee during the collection and ripening of nectar. The diastase enzyme digests starch into simpler compounds. The diastase enzyme activity of *A. mellifera* honey obtained in this study was within the limits of minimal eight schade units specified by international norms and minimal three schade units for national norms. The results of the diastase enzyme activity of *A. mellifera* honey from extrafloral nectar samples showed that diastase enzyme activity between *Arenga pinnata* sap (17.12±0.83 Schade units) and *Cocus nucifera* sap (16.48±0.53 Schade units) were no significant differences (P>0.05). The floral origin of honey also influences its diastase content. For example, citrus and clover honey tend to contain less diastase enzyme [46]. Other factors may affect diastase values: the natural difference in pH among honey, nectar flow, and the bees' foraging patterns. Long storage at moderate temperatures and exposure to high temperatures will inactivate diastase in honey [47].

According to [48], honey's enzyme content may differ based on the age of the bees that vary in race, the nectar gathering time, the colony's physiological period, the quantity of nectar flow and its sugar content and pollen consumption. The diastase enzyme activity of *A. mellifera* honey with extrafloral nectar was higher than the previous report on Azerbaijan honey (9.69 Schade units) [39], Ethiopian honey (13.60 Schade units) [49], and comparatively similar to Venezuelan honey (16.13 Schade units) [31] and Algerian honey (15.10 Schade units) [50].

Conclusion

In this study, the values of quality parameters for *A. mellifera* honey from extrafloral nectar are good quality and coincide with those specified by the international honey regulations. The *A. mellifera* honey from *Cocus nucifera* sap has a higher sugar reduction

content, and lower acidity compared the *A. mellifera* honey from *Arenga pinnata* sap. The results also reported extrafloral nectar as an alternative feed for honeybee in the topical area.

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