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Quality Assessment on Honey Produced from Six Months Old *Acacia crassicarpa*

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Abstract. This study aimed to analyze the quality of *Apis mellifera*-produced honey generated from 6 mo old *Acacia crassicarpa* nectar at 28 d harvest period. The tests referred to the procedures required by the Indonesia National Standard (SNI) 8664-2018. The quality variables tested were of odor and taste and diastase enzyme activity as well as hydroxymethylfurfural (HMF), water, reducing sugar (glucose), sucrose, acid, water insoluble matter, and ash contents. The data were analyzed by using the student t test, where one sample group was prepared to compare each honey quality variable with the quality standard value. The results showed a distinctive smell and taste of honey, enzyme activity > 1.52 DN, HMF 0 mg kg^{-1}, water content 24.2 % w w^{-1}, reducing sugar content 66.56 % w w^{-1}, sucrose content 1.50 % w w^{-1}, acidity 113.05 NaOH kg^{-1}, water insoluble matter content 0.018 % w w^{-1}, and ash content 0.26 % w w^{-1}. It is concluded that the quality variables for honey samples that met the quality requirements were odor and taste, HMF, reducing sugar, sucrose, water insoluble matter, and ash. Meanwhile, those that do not meet the quality requirements are the activity of the attached enzyme, water content and acidity.

Keywords: Extrafloral nectar, functional food, honey bee farming, monofloral honey, pollinator

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Quality Assessment on Honey Produced from Six Months Old Acacia crassicarpa

by Artikel 2

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1 Introduction

Popular for its functions in producing honey and assisting pollination, *Apis mellifera* Linnaeus, 1758 is the most managed species of honey bee worldwide [1, 2]. Many apiarists in *Kabupaten Siak*, a regency in Riau Province, Indonesia, keep their beehives near and around *Acacia crassicarpa* A. cunn. ex. Bent, which happens to be the focal tree in its industrial plantation forest. Allowing bees to gather nectar not only from flowers but also from leaf nodes (extrafloral), the tree has become perpetual feed source for them regardless the season. The trees are laid out based on several groups of age, and some beekeepers – believing that in their fastest vertical growth stage, the trees should produce ample nectar – specifically set their hives up in a section where 6 mo old *A. crassicarpa* are growing.

Being the most essential product economically and quantitatively [3], honey is closely distinguished for its quality by keepers, consumers, and industries. Valued as a good nutrient source, it also has medicinal purposes as prophylaxis and antioxidant [4–6]. Among factors that control honey characteristics are geographical condition, feed source, climate, and bee variety [7, 8] as well as harvesting period [9]. Until this paper was written, no studies on the quality of *A. mellifera*-produced honey generated from 6 mo old *A. crassicarpa* nectar had been found.

The beekeepers in the observed locale collect their honey in disparate periods depending on weather, human resource, and other managerial considerations, but the most common one span between 21 d and 28 d.

2 Material and methods

Aiming to analyze the quality of honey produced by *A. mellifera* living around 6 mo old *A. crassicarpa* with harvest period of 28 d, the sample was obtained from apiaries in *Sungai Mandau* District of Siak Regency, Riau, Indonesia (0º48'17.102" N 101º40'1.11" E) and studied in a laboratory belongs to the Technical Implementation Unit on Product Quality Testing and Certification of the Department of Industry, Trade, Cooperatives and Small and Medium Enterprises of Riau Province, Indonesia in accordance with Indonesia National Standard (SNI). Chemicals utilized were of technical grade, purchased from *Toko Melisa Laborta* (a store in Pekanbaru, Indonesia). Nine variables were tested, covering organoleptic properties of aroma and taste, diastase activity, 5-hydroxymethylfurfural (HMF) content, water content, reducing sugar (glucose) content, sucrose content, acidity, water insoluble matter content, and ash content. Data gained were then run through student t-test by comparing each value with standardized one listed in SNI 8664-2018.

2.1 Organoleptic properties

As per required, the services of three experienced panelists and one honey expert were used to assess the aroma and taste of the sample [10]. The result was then stated as "distinctive of honey" and "not distinctive of honey".

2.2 Diastase activity

Spectrophotometry (MAPADA 6300 UV Vis Spectrophotometer – Germany) was performed to measure diastase activity. A volume of 10 mL to 15 mL water and 2.5 mL acetate buffer were added to 5 g honey in a 20 mL glass beaker, stirred thoroughly once cooled, and rested before transferred to a 25 mL volumetric flask containing 1.5 mL NaCl solution. Next, 10 mL sample solution in a 50 mL test tube was water-bathed for 15 min before 5 mL starch solution was added and homogenized. 1 mL of above emulsion was

dropped in 10 mL iodine solution every 5 min and made up to volume with water – a stopwatch was employed to record reaction time from mixing starch with honey to adding it to iodine solution. The absorbance was measured at 660 nm, taken constantly until a value of A < 0.35 was obtained. The absorbance rates were then plotted against time on a millimetre sheet as per Table 1, and lines were drawn to link one point to another in order to indicate the time required to reach absorbance rate (A) = 0.235 [11].

Table 1. Correlation between mixing time and absorbance.

Absorbance	Time (min)
0.7	> 25
0.65	20 to 25
0.60	15 to 18
0.55	11 to 13
0.50	9 to 10
0.45	7 to 10

The value of 300 was then divided by aforementioned rate to determine diastase activity (DN) as per Equation (1).

$$DN = \frac{300}{t} \tag{1}$$

Where,
DN = diastase activity
t = time required to reach absorbance rate (A)

2.3 HMF content

HMF was tested by employing a UV—VIS spectrophotometer (Shimadzu UV 1620, Japan). As much as 5 g honey was stirred in aquadest to make 25 mL mixture, then 0.5 mL Carrez I solution (15 g $K_4Fe(CN)_6 \cdot 3H_2O$ dissolved in water to the volume of 100 mL) was added. Once shaken thoroughly, 0.5 mL Carrez II solution (30 g $Zn(CH_3COO)_2$ $2H_2O30$ dissolved in water to the volume of 100 mL) was included. A drop of alcohol was used to get rid of foam formed on the surface before passed through a filter (Whatman WHA 1442042, USA) – the first 10 mL was discarded – and put in a number of 18 mL × 150 mL tubes, 5 mL each. 5 mL water was added to the sample tube and 5 mL 0.20 % natrium bisulfite to the reference tube, and then shaken well. Both were compared in a 1 cm cell at 284 nm and 336 nm. If the absorbance rates were of A > 0.6, the sample was diluted with water and the reference was diluted with NaHSO 0.1 %; the absorbance rates were then multiplied by the prior dilution factor. HMF content was established according to Equation 2 [12].

$$\text{HMF}\left(\frac{mg}{100}\ g\ honey\right) = \frac{A_{285} - A_{336} \times 14.97\ \times 5}{sample\ weight\ (g)} \tag{2}$$

$$\text{Factor} = \frac{126}{16\ 830} \times \frac{1\ 000}{10} \times \frac{100}{5} =\ 14.97$$

2.4 Water content

A Pal-Coffee (Atago BX/TDS, Japan) refractometer was utilized to measure the water content [13]. After calibrating it with aquadest, As much as 2 drops to 3 drops of honey

were placed on the glass prism and the device was faced to the light to make the scale readable. The water content was recorded in % w w^{-1}.

2.5 Reducing sugar content

To find out the reducing sugar content, Luff Schoorl method was engaged [14,15]. A total of 2 g honey in a 250 mL pyrex flask was diluted with aquadest to the volume. 10 mL of it was then mixed with 15 mL aquadest and 25 mL Luff Schoorl solution in an Erlenmeyer flask, connected to a condenser and heated on an electric heater to boil within 3 min. After heated for 10 min, the flask was rapid-cooled and kept still. 10 mL KI 20 % and 25 mL H_2SO_4 25 % were added, then the solution was titrated with natrium thiosulphate 0.1 N using starch 0.5 % as indicator (V_1). Blank (V_2) was made by mixing 25 mL water and 25 mL Luff Schoorl solution. The difference between V_1 from V_2 was then expressed in mL thio of 0.1000 N, then the amount of glucose was established in mg. The glucose content was calculated corresponding to Equation (3).

$$\%\,\text{inverted sugar} = \frac{W_i \times fp}{W} + 100\,\% \quad (3)$$

Where,
w_i = glucose (mg)
fp = dilution factor
W = dilution weight (mg)
% = total sugar = 0.59 × % inverted sugar (as sucrose)
% = sucrose = 0.59 × % sugar (after inversion – before inversion)

2.6 Sucrose content

Luff Schoorl method was also selected to reveal sucrose content [16, 17]. Amount of 2 g honey in a 250 mL flask was diluted with aquadest to the volume. 50 mL of it was then mixed in a 100mL flask with 25 mL HCl 25 %, fixed with a thermometer and hydrolyzed on a heater. Once reaching a constant temperature of 68 °C to 70 °C, the solution was kept for 10 min, gotten rid of the thermometer then cooled down. NaOH 30 % was then added to neutralize it – indicated by pink color on PP – before followed by aquadest to the volume and a good shake. 10 mL of it was then mixed with 15 mL aquadest and 25 mL Luff Schoorl solution in an Erlenmeyer flask, connected to a condenser and heated on an electric heater to boil within 3 min. After heated for 10 min, the flask was rapid-cooled and kept still. 10 mL KI 20 % and 25 mL H_2SO_4 25 % were added, then the solution was titrated with natrium thiosulphate 0.1 N using starch 0.5 % as indicator (V_1). Blank (V_2) was made by mixing 25 mL water and 25 mL Luff Schoorl solution. The difference between V_1 from V_2 was then expressed in mL thio, then the amount of sucrose was established in mg. The sucrose content was determined based on Equation (4).

$$\%\,\text{inverted sugar} = \frac{V_2 \times fp}{W} \quad (4)$$

Where,
v_2 = glucose produced (mg)
fp = dilution factor
w = dilution weight (mg)
% = total sugar = 0.59 × % inverted sugar (as sucrose)
% = sucrose = 0.59 × % sugar (after inversion – before inversion)

2.7 Acidity

Acidity was determined through neutralization method [18]. Amount of 10 g honey was mixed with 75 mL CO_2-free water in a 250 mL beaker and stirred with a magnetic stirrer (Ika type HS-7, Germany). The basic pH of the mixture was recorded before titrated with 0.05 M NaOH at 5 mL min^{-1} – titration was stopped once the pH rate was of 8.5. For the sample, 10 mL 0.05 M NaOH was rapid-titrated with 0.05 M HCl to reach pH rate of 8.3. The blank was made by titrating 75 mL CO_2-free water with NaOH to reach pH rate of 8.5. The results were then run through Equation (5).

$$Total\ Acidity = Free\ acid + lactone \tag{5}$$

Where,
Free acid = (mL 0.05M NaOH in the burette – mL blank) x N NaOH × 1 000 g^{-1} sample
Lactone = (10.0 mL 0.05 HCl in the burette) × N HCl x 1000 g^{-1}contoh

2.8 Water insoluble matter content

Filtration was the chosen method to study insoluble matter content [19, 20]. Amount of 20 g honey was diluted with 200 mL hot water in a 400 mL beaker, then strained using dried and measured filtering paper (Whatman WHA 1442042, USA) prior to use. The filter was then dried in an oven (Memmert UN 55, Germany) at 105 °C for 2 h and cooled before measured. The figures were processed through Equation (6).

$$Insoluble\ matter = \frac{W_1 - W_2}{W} \times 100\ \% \tag{6}$$

Where,
w = sample weight
$w1$ = filtering paper containing insoluble matter
$w2$ = filtering paper prior to use

2.9 Ash content

Gravimetry was presented to check the ash content [19, 20]. Amount of 2 g honey was put in a porcelain dish – measured prior to use – and steamed on a water bath to dry. The dried honey was then carbonized on a burner and thoroughly incinerated in a furnace (Payun Tech, Indonesia) at maximum temperature of 550 °C. Once cooled in an exciter, it was measured. The results were worked on Equation (7)

$$Ash\ content = \frac{W_1 - W_2}{W} \times 100\ \% \tag{7}$$

Where,
w = dish + sample weight after incinerated
w_1 = empty dish weight
w_2 = sample weight before incinerated

3 Result and discussion

Good quality honey should meet a set of requirements, which may differ in one country to another. The national standard in Indonesia, SNI 8664-2018, entails both organoleptic and laboratory tests for honey, which have become the variables of this study.

3.1 Organoleptic test

A technique to evaluate a food product, organoleptic test assesses appearance, aroma, flavor, and texture through sensory [10] which complements the physiochemical property analysis to confirm its feasibility. Specifically in honey, its smell and taste are subject to the sort of feed attained by bees. While having a specific organoleptic characteristic, honey may taste sweet, spicy, slightly spicy, even resin-like [21, 22]. The panelists and honey expert that examined the honey sample had confirmed it to be typical of honey.

3.2 Laboratory test

The test results on diastase activity as well as contents of HMF, water, glucose, sucrose, acid, insoluble matter, and ash are listed next to each of their standardized value in Table 2.

Table 2. The laboratory test results on *A. mellifera*-produced honey quality generated from 6 mo old *A. crassicarpa* nectar at 28 d harvest period.

Characteristic	Unit	Standard	Result	Statement on comparison
1. Diastase activity	DN	Min 3*)	1.52	Significantly different
2. Hydroxymetylfurfural (HMF)	mg kg^{-1}	Max 40	0	Significantly different
3. Water	% w w^{-1}	Max 22	24.2	Significantly different
4. Reducing sugar (Glucose)	% w w^{-1}	Min 65	66.56	Significantly different
5. Sucrose	% w w^{-1}	Max 0.5	1.50	Significantly different
6. Acidity	NaOH kg^{-1}	Max 50	113.05	Significantly different
7. Water insoluble matter	% w w^{-1}	Max 0.5	0.018	Significantly different
8. Ash	% w w^{-1}	Max 0.5	0.26	Insignificantly different

*) This requirement applies on newly-harvested honey.

3.2.1 Diastase activity

Diastase enzyme is formed in the hive during honey ripening process. Related to its function as antioxidant, diastase activity in honey helps to reduce oxidative damages in the body [23]. The sample contains significantly lower ($P < 0.01$) amount of diastase enzyme than the standard. It is presumably attributable to the bee age, nectar gathering time, sugar amount and composition in nectar or pollen consumed by the bee, and honey temperature [24, 25]. Further research on nectar composition in *A. crassicarpa*, the key source of bee feed, will be carried out in near future.

3.2.2 HMF content

Illustrating the freshness, HMF is an imperial aspect in honey quality evaluation. High HMF content may indicate tampering with syrup, since it is accessible through heating sugar [25, 26]. The HMF rate of 0 in the sample has stated it to be fresh, which is significantly lower ($P < 0.01$) than the standard of 40 mg kg^{-1}. Since HMF rate rises when honey is heated [27], the low HMF content is possibly due to the absence of heating process often performed to reduce water content.

3.2.3 Water content

The amount of water in honey affects its crystallization, viscosity, dilution, taste, and color [28]. Very low content of water in honey may lead to caramelizing, and very high one may initiate fermentation that accelerates spoilage when stored [29, 30]. Neither of those conditions is favored. The water content of 24.2 % w w^{-1} in the sample is below the required rate ($P < 0.05$). Yet, it is actually not far different from acacia, sunflower, and grass honeys made in Serbia containing 10.2 % to 24.1 % [31]. Harvest time, ripeness, temperature, and water content in plant are affective towards the water content in honey [5, 6]. In this case, the nectar produced by 6 mo old *A. crassicarpa* is deduced to be high. To lower the water rate, the harvest period should be set to be longer or harvesting in wet season should be avoided. Another attempt on it is by employing dehumidifier.

3.2.4 Reducing sugar content

The sugar composition varies in each kind of honey depending on nectar-producing plant, climate, location of the apiary, storing treatment, and handling [32, 33]. Out of assorted sugars found in honey, one is reducing sugar. Stated as glucose, the sample value of 66.62 % w w^{-1} meets the standard one of at least 65 % w w^{-1} despite being significantly different ($P < 0.05$). Glucose is not easily watered down compared to fructose [34], and its high content crystallizes honey more quickly. Since the glucose content is high compared to sucrose, much of the sucrose is modified into several other kinds of sugar – including glucose – during ripening process.

3.2.5 Sucrose content

Another parameter in testing the purity and maturity of honey, a high sucrose content may indicate tampering either by adding sugarcane or beetroot sugar in honey or by continuously feeding bees with artificial feeds like syrup [35, 28]. The sample's sucrose rate of merely 1.5 % w w^{-1} is significantly different ($P < 0.05$) from the standard, but conforms the maximum rate of 5 % w w^{-1}. The harvest period of 28 d, sufficient for the honey to ripen and turn sucrose into glucose and fructose, is apparently the cause of it.

3.2.6 Acidity

The sample acidity rate of 113.05 NaOH kg^{-1} is significantly higher ($P < 0.01$) and is unable to meet the maximum required rate of 50 NaOH kg^{-1}. The high acid content in *A.crassicarpa* is construed to be the reason, since the plant's habitat is peat soil known to be highly acidic. This finding is in sync with a research [5] on how honey acidity levels in Oman differed in regards of feed varieties. Honey acidity is also proven to depend on acid contents in flowers [36].

3.2.7 Water insoluble matter content

With water insoluble matter content rate as low as 0.016 % w w^{-1}, the sample is considered clean and easily meets the standardized rate of not higher than 0.5 % w w^{-1}. The honey was harvested by employing extraction method, so most of the solid matters were discarded prior to the experiment. This result is similar to one of the study on honey produced in Hatay District, Turkey, consisting (0.01 to 0.07) g 100 g^{-1} [26]. This aspect is notable to detect pollutants in honey, such as wax, pollen, and hive remains [3] due to incautious harvesting or storing process [37].

3.2.8 Ash content

Containing 0.26 % w w^{-1} ash, which is insignificantly different from the standard, the sample's feasibility is therefore confirmed. This value is even lower than the one of pine monoflora produced in Greece from diverse areas spanning between 0.31 % w w^{-1} and 0.92 % w w^{-1} [38]. Ash content represents the mineral level in honey. Since ash content also indicates the intensity of environmental and soil pollution of the site where honey is gained [38], it is evident that the nectar source plants grow in a relatively pollutant-free area.

4 Conclusion and suggestion

A. mellifera-produced honey in Siak Regency, Indonesia generated from 6 mo old *A. crassicarpa* nectar harvested in a 28 d period is concluded to agree with the Indonesia National Standard 8664-2018 in odor and taste as well as HMF, reducing sugar, sucrose, water insoluble matter, and ash contents.

As of activity of the attached enzyme, water content and acidity, further improvement is called for in order to meet the standardized rates. A research focused on enzyme activity will be conducted accordingly. Prolonging the harvest period and using honey dryer (dehumidifier) are recommended to reduce the water content.

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