The Physicochemistry of Stingless Bees Honey
(*Heterotrigona itama*) from Different Meliponiculture Areas in East Kalimantan, Indonesia

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ABSTRACT

Almost all of the stingless honey bees in East Kalimantan are in the district/city. Various types of plants as sources of nectar in each region for stingless bees honey are interesting for research. This study’s purpose was the physicochemical analysis of *H. itama* honey from different meliponiculture areas in East Kalimantan. The data were analyzed using variance analysis at the 5% level and a further test of the smallest significant difference at the 5% level. The results showed that the physicochemistry of *H. itama* honey was the respondent’s response value to color 52-100%, aroma 74-92%, taste 56-88%, moisture content 30.80-33.67%, pH value 2.77-3.20, reducing sugar content 51.59-59.56%, sucrose content 1.82-3.82%, total dissolved solids content 67.23-69.7⁰Brix, ash content 0.17-0.35% and heavy metals were not detectable-0.01 mg / L).

Keywords: Different Meliponiculture, Physicochemistry, Stingless Bees Honey

1. INTRODUCTION

East Kalimantan has superior non-timber forest products, namely, rattan, cananga, sugar palm, jelutong, gaharu, wallet, and honey. Honey bees (*Apis dorsata*) in East Kalimantan are obtained from forests. Stingless bees in East Kalimantan are found in the Samarinda educational forest, namely *Trigona itama*, *T. incise*, *T. apicalis*, *T. melina*, *T. fusciabasis*, *T. fusciobalteata*, *T. laeviceps*, *T. drescheri*, and *T. terminate* [1]. Bees without stingers in East Kalimantan are now widely cultivated by breeders in Samarinda, Kutai Kartanegara, Balikpapan, Penajam Paser Utara, Paser Regency, Bontang, Sangata, West Kutai and Berau.

Various types of plants in East Kalimantan have the potential to produce multiple kinds of nectar. Different types of nectar from different plants have different nutrient content. Nectar is the main component used by bees to convert into honey, bees take the more various types of nectar, and it can cause the emergence of multiple types of honey available. The difference in place and source of nectar, which is different from *H. itama* honey cultivation, will give differences in the physicochemical of honey produced. For this reason, in this study 5 (five) locations of *H. itama* honey different meliponiculture areas were taken, namely Samarinda (Rimbawan Dalam) Tanah Merah sub-district (SMD), Kutai Kartanegara (Buana Jaya Village) Tenggarong Seberang (KKN), Balikpapan (Karya Merdeka Village) (BPN), Penajam Paser Utara (Penajam ) (PPU) and Paser District (Saing Prupuk Village) (KP). This study’s purpose was the physicochemical analysis of *H. itama* stingless honey from 5 (five) different meliponiculture areas in East Kalimantan.
2. MATERIAL AND METHODS

2.1. Materials

The materials used in this study were *H. itama* honey, clean water, distilled water, tissue, whatman filter paper no. 42, label paper, Luff solution, standard sea of mercury, Nitric Acid (HNO₃, Merck), Hydrogen Peroxide (H₂O₂, Merck), Sulfuric Acid (H₂SO₄, Merck), Potassium Iodide (KI, Merck) Sodium Thiocyanate (Na₂S₂O₃, Merck). The tools used include bottles, suction devices, gloves, filter cloth, filters, plastic trays, Erlenmeyer’s (Merck), measuring cups (Merck), measuring flasks (Merck), spatulas, funnels, test tubes, test tube racks, pipettes, drops, spray bottle, syringe (injection), refractometer (Atago), pH meter (Atago), scale (AND), refrigerator (Sharp), Wed Digester (Shaft Holder), Furnace (Thermolyne), Porcelain Cup, Lead Cathode Lamp (Pb), Cadmium (Cd), Mercury (Hg) and Arsenic (As), F-AAS (Atomic Absorption Spectrophotometer, Shimadzu), Water bath and spectrophotometer (Shimadzu).

2.2. Sampling

Location of honey sampling in Samarinda (Rimbawan Dalam) Tanah Merah Village, Kutai Kartanegara (Buana Village) Tenggarong Seberang District, Balikpapan (Sungai Merdeka Village) Karya Merdeka Village, Penajam Paser Utara (Penajam) Penajam Sub district and Paser (Aper Sejahtera Forest Farmers Group ) Saing Prupuk Village, Batu Engau District.

2.3. Physicochemical Analysis

Physicochemical analysis of honey, namely color, aroma, taste [2], reducing sugar content, sucrose content (3), and ash content [3,4] heavy metals [5,6], SNI: 01-2896; SNI:01-486: method of testing metal contamination in food; Arsenic Contamination Test Method in Food), pH [7], using a refractometer and water content and total dissolved solids using a refractometer.

3. RESULTS AND DISCUSSION

3.1. Color

The physical properties of *H. itama* honey are properties that can be observed using the five senses in the form of eyes for color, nose for aroma, and tongue for taste. The color of honey is one of the critical characteristics of honey. *H. itama* honey from 5 (five) cultivated areas in East Kalimantan meets the SNI requirements: 8664:Honey (honey bee without sting [2] is a distinctive aroma of honey (76%-92%) as shown in Figure 2. Respondents gave the impression of the smell of *H. itama* honey from 5 (five) cultivated areas in East Kalimantan in the form of a distinctive smell with a score of 74%-92%. This shows that the aroma of *H. itama* honey in the study sample is the same as honey in general. The fragrance’s sensory characteristic explains that monofloral honey has honey sensory by stingless bees [9,10]. In general, honey from the same flower source was observed to have similar sensory attributes and did not differ from the bees they produced. The physicochemical and sensory characteristics of honey vary according to plant origin, ripening time, weather, and storage conditions [9].

Generally, the light honey color is preferred by consumers over the dark honey color [8]. The dark color of honey has a strong and sharp flavor. The light color of honey has a more palatable taste. The dark and light colors indicate the source of the nectar and the honey’s quality and tell a chemical change in the honey, such as the darkhoney’s darker color. The honey used in this study is honey that has just been harvested and has not been heated, so it is suspected that the honey has not changed color. Physical properties in the form of color can be seen in Figure 1.
3.3. Taste

The taste of *H. itama* honey from 5 (five) from different meliponiculture areas in East Kalimantan fulfills the SNI requirements: 8644 [2] with a distinctive taste of honey as in figure 3, while *H. itama* honey from Penajam Paser Utara is not unique honey. Respondents gave an impression of the taste of *H. itama* honey from 5 (five) cultivated areas in East Kalimantan in the form of a typical honey taste with a score of 60% - 88%, while *H. itama* honey from Penajam Paser Utara was not ordinary for honey, with a score of 56%. This shows that the taste of *H. itama* honey in the study sample is the same as honey in general, except for the North Paser Sharpener. The difference in the typical taste of honey in *H. itama* honey from Penajam Paser Utara is thought to be a difference in the nectar source. Stingless bee honey has a characteristic taste [11,10]. Taste is closely related to the nectar from the collected flower sources [12,13].

3.4. Water Content

Chemical properties of the honey water content of honey from 5 (five) regions of origin shown in Figure 4 have a moisture content of 30.80% - 33.67%. All samples’ water content from 5 (five) areas of origin is higher than the SNI requirements: 8664 [2], a maximum of 27.5% for honey without the sting. The water content of honey also depends on the humidity in the area around the honeycomb. Honey has hygroscopic properties that can absorb water around it. According to [14] water content of honey varies greatly depending on the moisture content of the nectar source and climate. If the nectar’s water content is high, the resulting honey’s water content tends to be high. Indonesian honey’s high water content is caused by the very high humidity levels in the tropics (around 60% to 80%) [15].

The higher the honey’s water contents, the lower the honey quality because it is closely related to honey’s shelf life and microorganisms’ growth. The lower the honey content, the better the honey quality. This is closely related to preventing microorganism growth and honey’s long shelf life and fermentation. Honey with low water content can be stored longer because it prevents the growth of honey-destroying microorganisms.

3.5. pH

The pH of honey is influenced by the content of organic and inorganic acids. The dominant organic acid in honey is gluconic acid, which results from glucose breakdown by enzymes. Other organic acids found in honey are acetic, butyric, formic, gluconate, lactic, malic, maleic, oxalic, pyrogulamate, citrate, succinic, glycolic, α-ketoglutarate, pyruvate, 3-phosphoglycerate, β-gliserofaosfate, and glucosaphosphate [17]. The acidity level of honey can help honey’s resistance and stability to microorganisms [18]. Chemical properties in the form of pH can be seen in Figure 5.
3.6. Reducing Sugar

Reducing sugars are carbohydrate sugars such as fructose, glucose, maltose, and dextrin’s. According to [19], the dominant type of sugar in honey is glucose and fructose, reaching 85-90% of the honey’s carbohydrates. Reducing sugars are a group of sugars that can reduce electron-accepting compounds (glucose, mannose, fructose, lactose, maltose, the main reducing sugar content in honey is fructose and glucose. Reducing sugar (glucose) is one of the required quality standards for honey. In SNI 8664: 2018, at least 55% for honey without sting [2].

Reducing honey by bees itself is a complex process, so there is a high probability that there will be differences in the levels and composition of reducing sugars between the various types of honey. Fructose and glucose are the main reducing sugars in honey, reaching 85% -90% of the carbohydrates found in honey and only a small portion of oligosaccharides and polysaccharides [19]. In general, the fructose content is higher than the glucose content in honey. This fructose and glucose ratio is an essential factor for the crystallization process in honey. Honey, which has a higher glucose level than fructose, will crystallize more easily. Therefore, the fructose/glucose and glucose/fructose ratio is a parameter used to help estimate the tendency of honey to crystallize [20]. The reducing sugar content of honey from the 5 (five) regions of origin is between 51.58%-63.68%. Chemical properties in the form of reducing sugar can be seen in Figure 6.

3.7. Sucrose

Based on Figure 8, the sucrose content of H. itama honey is 1.82% -4.57% from 5 (five) regions of origin that meet the requirements of the Indonesian National Standard [2], a maximum of 5%. The sucrose levels in honey are small, and fructose and glucose levels are large [21]. According to [22], variations in sucrose level are thought to indicate the effect of different geographic origins so that they have different honey compositions. Chemical properties in the form of sucrose content can be seen in Figure 7.

3.8. Total Dissolved Solids

Total dissolved solids is a combined measure of the content of organic and inorganic substances contained in honey, including ionized molecules, microgranules (cloud solution), or suspended form. According to [24], the variability of honey TPT levels can be caused by the harvesting process, beekeeping techniques, and materials collected by bees while...
foraging in flora. Chemical properties in the form of total dissolved solids can be seen in Figure 8.

**Figure 8** Chemical properties of *H. itama* honey total dissolved solids

### 3.9. Ash Content

The ash content of *H. itama* honey from 5 (five) regions of origin tested in this study ranged from 0.17% to 0.35%. As in Figure 9 *H. itama* honey from 5 (five) areas of origin in this study each differs from one another. The study results [25-27] explain that the ash content of Malaysian stingless bees is in the number 0.22-0.41%. According to [28], the foraging activity for honey in different regions would affect the ash content. *H. itama* honey is influenced by plant nectar. It correlates with mineral content such as calcium, sodium, calcium, iron, and other essential minerals for the human diet. The minerals in the soil are given up by the plants and then end up in the nectar. Ash content can determine the quality of honey so that it can affect the taste, shape, texture, and stability of the honey.

**Figure 9** Chemical properties of *H. itama* honey ash content

### 3.10. Heavy Metal

In general, the test results for heavy metal contamination of Pb, Cd, Hg and As *H. itama* honey from 5 (five) areas of origin of cultivation products in East Kalimantan (table 1) meet the quality requirements of SNI: 8664 [2], because they show lower levels of metal contamination of the required SNI: 8664 [2]. Lead metal contamination (Pb) and Arsenic (As) *H. itama* honey from 5 (five) cultivated areas in East Kalimantan are under metal detection so that they meet the SNI requirements: 8664 [2] honeys bees without the sting. According to lead (Pb), a type of metal with a bluish-gray color has a relatively high density and is very soft. It quickly melts in a concentrated HNO3 solution with a little HCl H2PO4 solution. Arsenic (As) is an inorganic metal that is gray, low solubility in water. Arsenic at low concentrations is obtained in soil, water, food, and air [30].

The results of the Cadmium (Cd) metal contamination test for *H. itama* honey from Samarinda, Kutai Kartanegara, Balikpapan, Penajam Paser Utara fulfilled the SNI requirements: 8664 [2] because they were under metal detection as well as *H. itama* honey from Paser (0.01 mg/kg) fulfill the SNI requirements: 8664 (0.2) [2]. According to [31], cadmium is absorbed by planting from the soil through the roots and distributed in plant parts. The level of Cd that plants absorb is influenced by several factors, including soil pH, calcium (Ca) mineral content, and when fertilizing the soil in phosphate. In general, the Cd content found in grains, vegetables, fruits, and those not contaminated with Cd was very low. Cadmium is always released in the smelting of tin, iron, copper, and gold. Cd’s evaporation power in the metal industry can increase metal pollution in the air, soil, and plants.

The results of the metal contamination test (Hg) of *H. itama* honey from Samarinda, Kutai Kartanegara and Paser were below metal detection, Balikpapan and Penajam Paser Utara (0.003 mg/kg) met the SNI requirements: 8664 (0.03 mg/kg) [2]. Mercury is the only metal in liquid form at average temperatures. Mercury is called quicksilver because it is silvery-white and shiny. Metallic mercury is a pure metal that is liquid at room temperature 25°C and evaporates quickly. Inorganic mercury is commonly used for disinfectants, and organic mercury is alkyl mercury [32].
Table 1. Heavy metals found in H. itama honey

| Origin of honey       | Heavy metal (mg/L) | SNI:8664:2018 (mg/kg) |
|-----------------------|--------------------|-----------------------|
|                       | Pb     | Cd     | Hg     | As     |                     |
| Samarinda (SMD)       | nd     | nd     | nd     | nd     | Pb = 1,0            |
| Kutai Kartanegara (KKN) | nd     | nd     | nd     | nd     | Cd = 0,2            |
| Balikpapan (BPN)      | nd     | nd     | 0,003  | nd     | Hg =0,03            |
| Penajam Paser Utara (PPU) | nd     | nd     | 0,003  | nd     | As = 1,0            |
| Paser (KP)            | nd     | 0,01   | nd     | nd     |                     |

Information: not detected (nd), detection limit: Pb= 0,01 ppm, Cd= 0,001 ppm, Hg=0,0005 ppm, As=0,001 ppm.

4. CONCLUSION

The physicochemical properties of aroma, sucrose content, ash content, and heavy metal content of H. itama honey from different meliponiculture areas in East Kalimantan (Samarinda, Kutai Kartanegara, Balikpapan, Penajam Paser Utara, and Paser Regency) meet SNI: 8664: 2018: Honey. The physicochemical properties of taste and reducing sugar for H. itama honey from the cultivation of several areas in East Kalimantan (Samarinda, Balikpapan, and Paser Regency) meet SNI: 8664: 2018: Honey. Except for the taste for Penajam Paser Utara and reducing sugar for Kutai Kartanegara. The physicochemical properties of color, H. itama honey from different meliponiculture areas in East Kalimantan (Samarinda, Kutai Kartanegara, Balikpapan, and Paser Regency) is typical for honey color, except for H. itama honey from Penajam Paser Utara, pH 2.77-3.20 and Total Solids Dissolved 67.23-69.77°Brix. The moisture content of H. itama honey from different meliponiculture areas in East Kalimantan does not meet SNI: 8664: 2018: Honey. Most of the pollen found in bee and honey hives have similarities/similarities from 4 (four) regions (Samarinda, Kutai Kartanegara, Balikpapan, Penajam Paser Utara) except for Paser Regency. There are differences in plant species that produce pollen in bee hives and H. itama honey from different meliponiculture areas in East Kalimantan (Kutai Katanegara, Balikpapan, Penajam Paser Utara and Paser District).

ACKNOWLEDGMENTS

My gratitude goes to the head of the Forest Products Chemical Laboratory and Biotechnology Faculty of Forestry, the Head of the Forest Products Technology Laboratory, Faculty of Agriculture, the Head of the Biology Laboratory of the Faculty of the Department of Mathematics and Natural Sciences, Mulawarman University, and the Head of the Research and Standardization Industry center for providing the use of facilities in carrying out this research. My gratitude goes to Mr. Muhammad Hijrafie, Head of UPTD KPHP Kendilo East Kalimantan Forest service, Mr. Syarif Syam “Madu Ratu Trigona”. Mr. Lalu Fauzul Idhi “Woodygona”, Mr. Suwondo “Kelulut Animal Farm” and Rendry Arista Avimaro “Kelulut Park Borneo Etam” as a stingless honey meliponiculture (H. itama and others) who have helped facilitate the sampling location in my research. Thank you to all friends who helped in carrying out this research.

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