Comparison of nutritional values of kelulut (*Heterotrigona itama*) honey derived from forest and sweet star fruit vegetations

LIES INDRAYANTI – NUWA NUWA – GRACE SISKA –
ERITA FIRDARA – AFENTINA AFENTINA – YELSA YELSA

Summary

The nutrients content of honey is affected by the plants used as the source of nectar and pollen. The nutritional values of kelulut (*Heterotrigona itama*) honey collected from the meliponicultures with forest vegetation (heterogenous) and sweet star fruit (homogenous) source were investigated. Biodiversity of pollen taxa in honey was estimated using the Shanon-Wiener method. The bee food analysis method was used to calculate the moisture content, total sugars, carbohydrates, protein, pH, ash, vitamin C and vitamin E of the samples. The presence of 17 species and 112 individuals at the level of seedling, sapling, pole and sawlog were identified. The bee food source was relatively sufficient with gerunggang (*Cratoxylon arborescens*) as the dominant species. The honey from heterogenous bee food sources had higher pH, total sugars, amino acids, vitamin E, potassium, calcium, magnesium, phosphorus and proteins, but lower ash, moisture and vitamin C content than the honey from homogenous food source. Generally, the nutritional value of honey from heterogenous bee food source was better. Regulation of particular plants as bee food sources can be done to obtain a targeted nutritional value.

Keywords

kelulut bee; *Heterotrigona itama*; pollen; nectar; meliponiculture; nutritional value; sweet star fruit

Kelulut beekeeping is a prospective program to support food independence at the community level. It is designated and implemented based on the concept of food estate, namely the integration of agriculture, plantations and livestock in an area. More than 600 species and 56 genera of kelulut bee, also known as stingless bee, are found in tropical and subtropical regions [1]. In Indonesia, approximately 40 species are identified and known as Klanceng. Stingless beekeeping is also common in USA, aimed to enhance production, consumption and economic growth [2]. Also known as meliponiculture, it is a quite promising sector in which kelulut honey is sold at a price of 40 USD or 600 Indonesian Rupiah (IDR) per litre in the Brazilian market [1]. Meanwhile, in Central Kalimantan, Indonesia, the price ranges from 300 IDR to 400 IDR per litre. Kelulut bees nest in the forest, specifically in living trees, dead trees and underground. The nesting preferences of kelulut, namely by 52 % on living trees, 20 % on dead trees and 28 % in the ground were identified [3]. The local communities adjacent to the forest usually target the nests for the purpose of harvesting, marketing and breeding. Nevertheless, the high rate of forest encroachment has an adverse impact on the existence of bee nests. Thus, kelulut farming becomes an urgent measure to preserve its existence.

Natural honey contains more than 300 bioactive substances [4]. The bioactive compounds of the stingless honey bee are superior to those of the *Apis* spp. due to the rich (heterogenous) vegetation as a bee food source, particularly in the tropi-

Lies Indrayanti, Department of Forestry, Faculty of Agriculture, Palangka Raya University, Yos Sudarso Street, 73112 Palangka Raya, Indonesia; Laboratory of Forest Product, Department of Forestry, Faculty of Agriculture, Palangka Raya University, Yos Sudarso Street, 73112 Palangka Raya, Indonesia.

Nuwa Nuwa, Grace Siska, Erita Firdara, Yelsa Yelsa, Department of Forestry, Faculty of Agriculture, Palangka Raya University, Yos Sudarso Street, 73112 Palangka Raya, Indonesia.

Afentina Afentina, Department of Forestry, Faculty of Agriculture, Palangka Raya University, Yos Sudarso Street, 73112 Palangka Raya, Indonesia; Center for the Study of Climate Change and Low Carbon Development LPPM, Palangka Raya University, Yos Sudarso Street, 73112 Palangka Raya, Indonesia.

Correspondence author:

Lies Indrayanti, e-mail: indayantilies@for.upr.ac.id

cal and subtropical regions [5]. The nutritional value of honey is based on carbohydrates, proteins, vitamins and minerals. The quality of honey is affected by the choice of bee food sources [6, 7].

Honey is known to promote health. It contains "natural antibiotics", boosts immune system, cures various diseases (e. g. ulcer and gastroesophageal reflux disease), contains antioxidants for anti-cancer effects, modulates cholesterol levels in human serum, has antiviral and antibacterial activities, prevents premature aging and contains flavonoids associated with reduced risk of cardiovascular diseases [8]. Honey is used for treatment of diseases related to oxidative stress, microbial infections or inflammation [9]. The antimicrobial activity of stingless bee honey overcomes bacterial infection and improves healing [5].

In Central Kalimantan, Indonesia, kelulut beekeeping or meliponiculture has been practiced in villages located on the outskirts of forest areas with relatively few inhabitants and low density residential zones. In Tuwung Village, kelulut bees (*Heterotrigona itama*) feed on forest plants and flowering plants intentionally cultivated as bee food sources. Meliponiculture is also practiced in villages relatively far from the forest and close to settlements. In Habaring Hurung Village, it is situated in the plantation of sweet star fruit (*Averrhoa carambola* L.). It is estimated that star fruit becomes the main and solely food source for kelulut bees since the plantation area is 750 m^2, while the maximum foraging range of bees is within a radius of 500 m^2.

Research on the nutritional value of honey in relation to the bee food source is still rare. Previous studies did not specifically compare the nutritional value of kelulut honey derived from homogenous and heterogenous bee food sources. Moreover, previous studies did not determine the possible differences in the nutritional value of kelulut honey, which can be dependent on the source of nectar and pollen as the bee food. The present study attempted to compare the nutritional value of kelulut honey derived from sweet star fruit (homogenous food source) and forest vegetation (heterogenous food source).

MATERIALS AND METHODS

Kelulut honey samples from two locations of meliponiculture were collected in March 2022. The first location was Habaring Hurung village (2°03'43.0"S 113°42'58.7"E) and the second was Tuwung village (2°02'43.1"S 113°57'54.5"E), Central Kalimantan, Indonesia.

The research was carried out in two locations of meliponiculture. The first was meliponiculture with forest vegetation as the bee food source with 38 stingless bee hives. The second was meliponiculture with sweet star fruit as the bee food source, in an area of 750 m^2 with approximately 476 trees and 33 stingless bee hives. The diversity of food source was identified in Tuwung Village where the bee food source was forest vegetation (heterogenous). The Shannon Wiener diversity index [10] was calculated using Eq. 1:

$$D = \frac{N}{A} \tag{1}$$

where D is area number density, N is number of individual trees, A is observed area (expressed in square kilometres).

Observations were carried out at each growth stage of vegetation (i. e. seedling, sapling, pole and sawlog). Despite the fact that seedlings and saplings had not produced nectar yet, they were included in the observation process due to their potential as nectar producer in the future. Purposive sampling was done by establishing two observation plots (20 m × 20 m) at the North, South, West and East with the apiary as the starting point. A total of eight plots were established with the distance between plots of 300 m and the distance from the apiary to the last plot of 600 m.

The parameters to characterize the nutritional value of kelulut honey were ash, pH, total sugar, moisture, lysine, carbohydrate, vitamin C, vitamin E and protein content according to the Indonesian National Standard SNI 3545-2013 [11]. The ash content of honey was tested using the Thermolyne muffle furnace (Chem-Mix Pratama, Yogyakarta City, Indonesia). The pH values were determined using a pH-meter. Total sugars were determined by the Nelson-Somogy method. Moisture content was determined using the graphimetric method. Lysine content was determined by the micro-Kjeldahl method. Vitamin C was determined using the iodine titration method. Vitamin E was determined by tocopherols analysis. The laboratory analyses were carried out in the Commanditaire Vennootschap (CV) Chem-Mix Pratama (Banguntapan, Yogyakarta, Indonesia).

RESULTS AND DISCUSSION

In Tuwung Village, the bee food sources for kelulut bees varied, ranging from forest plants to flowering plants intentionally planted at around 200 m^2 area. A total of 38 stingless bee hives were maintained at a distance of 3 m between each other. Results of identification of forest vegetation at the level of seedling, sapling, pole and sawlog in the meliponiculture in this location is presented in Tab. 1–4. Here, 17 species and 112 individuals were identified. The species of forest vegetation that produce nectar and pollen [12] in the meliponiculture area in Tuwung Village are presented in Tab. 5.

The nutritional values of kelulut honey derived from the meliponicultures of both heterogenous and homogenous food source were analysed. Subsequently, the results were compared to the reports of previous studies [1, 13–16] and the Indonesian National Standard (SNI) 3545-2013 [11]. The data and comparison are presented in Tab. 6.

The identification of biodiversity in the meliponiculture with forest vegetation as a heterogenous bee food source is documented in Tab. 1. The dominating karamunting is a potent bee food source since it blooms throughout the year with an attractive colour of flower. At sapling level, dominating was gerunggang with 13 individual trees (Tab. 2). At pole level, dominating were gerunggang with 12 individual trees and galam

Tab. 1. Forest tree seedlings in the meliponiculture in Tuwung Village.

Local name	Latin name	Number of individual trees	Area number density [km^{-2}]
Karamunting	*Rhodomyrtus tomentosa*	23	718 750
Mahang	*Macoranga pruinose* Miq.	9	218 250
Anakan Gerunggang	*Cratoxylum arborescens*	7	218 750
Jambu Burung	*Syzigium* sp.	5	156 250
Total		44	

Observed area was 0.000032 km^2.

Tab. 2. Forest tree saplings in the meliponiculture in Tuwung Village.

Local name	Latin name	Number of individual trees	Area number density [km^{-2}]
Gerunggang	*Cratoxylum arborescens*	13	65 000
Galam	*Melaleuca leucadendron*	7	35 000
Tumih	*Combretocarpus rotundatus* Miq.	7	35 000
Akasia Daun Lebar	*Acacia mangium* Willd.	3	15 000
Balangeran	*Shorea balangeran* Korth.	2	10 000
Total		32	

Observed area was 0.0002 km^2.

Tab. 3. Forest tree poles in the meliponiculture in Tuwung Village.

Local name	Latin name	Number of individual trees	Area number density [km^{-2}]
Gerunggang	*Cratoxylum arborescens*	12	15 000
Galam	*Melaleuca leucadendron*	7	8 750
Balangeran	*Shorea balangeran* Korth.	4	5 000
Akasia Daun Lebar	*Acacia mangium* Willd.	3	3 750
Tumih	*Combretocarpus rotundatus* Miq.	2	2 500
Total		28	

Observed area was 0.0008 km^2.

Tab. 4. Forest trees at sawlog level in the meliponiculture in Tuwung Village.

Local name	Latin name	Number of individual trees	Area number density [km^{-2}]
Akasia Daun Lebar	*Acacia mangium* Willd.	4	1 250
Gerunggang	*Cratoxylum arborescens*	2	625
Balangeran	*Shorea balangeran* Korth.	2	625
Total		8	

Observed area was 0.0032 km^2.

Tab. 5. Forest vegetation as the bee food source in the meliponiculture in Tuwung Village.

Species		Bee food source
Local name	Latin name	
Mahang	*Macoranga pruinose* Miq.	Nectar
Balangeran	*Shorea balangeran* Korth.	
Akasia Daun Lebar	*Acacia mangium* Willd.	
Karamunting	*Rhodomyrtus tomentosa*	Pollen
Gerunggang	*Cratoxylum arborescens*	
Jambu Burung	*Syzigium* sp.	
Galam	*Melaleuca leucadendron*	
Tumih	*Combretocarpus rotundatus* Miq.	

with 7 individual trees (Tab. 3). Both species are prospective as bee food sources as they bloom throughout the year. At sawlog level, eight individuals belonging to three species were identified (Tab. 4). The number of species found was relatively low probably due to the fact that the location of meliponiculture is a relatively open area. Of the three species, the dominant was gerunggang. This species is quite special for the meliponiculture since it blooms throughout the year, producing nectar and pollen. In addition, it also produces sap used by kelulut bees to make nests. Another sap-producing plant is tumih, which was found at both pole and sapling levels.

Fig. 1 demonstrates that most of tree individuals were identified at seedling level, followed by sapling, pole and sawlog. The location of meliponiculture is an open land hence only a few sawlogs were found in such area. The curve in Fig. 1 indicates that the location has the potential for succession and is prospective for meliponiculture due to the availability of bee food sources. The shape of the curve indicates that the condition of the forest is normal or balanced, in which the regeneration process is appropriate [17]. Moreover, the horizontal structure of forest vegetation forms an inverted J curve suggesting that a secondary succession process will improve over time [18].

The dominant vegetation at the seedling/understorey level was karamunting, at sapling and pole level was gerunggang and at sawlog level was akasia. Totally, eight species of plant were identified as the nectar and pollen sources for kelulut bee. Among the nectar-producing plants were mahang, balangeran and akasia, while the pollen-producing plants were karamunting, gerunggang, jambu burung, galam and tumih [12]. Sufficient bee food source will support the life of bee colonies to produce honey. The amount of the bee food source at the studied site had different values. Based on the analysis, the food source at sapling, pole and sawlog levels had high relationship with honey production. This finding is in line with previously reported positive relationship between the number of species and the abundance of flowers, varied flower colours and types of plants that attract the presence of insects [12].

The nutritional values of kelulut honey derived from the forest (heterogenous) and sweet star fruit (homogenous) vegetations were compared. The characteristics of honey are known to vary depending on the nectar or bee food source [5, 19–22].

Ash

The present study indicated that the ash content of honey derived from heterogenous bee food sources was lower than that of homogenous food source, namely 0.2 % and 0.5 %, respectively. In addition, both of them do not meet the SNI 3545-2013 [11]. The ash content of honey derived from both types of bee food source were higher than that previously reported [23], of 0.2 %. The ash content of honey derived from homogenous food source was also higher [13]. The mineral content affects the colour of honey [14, 24].

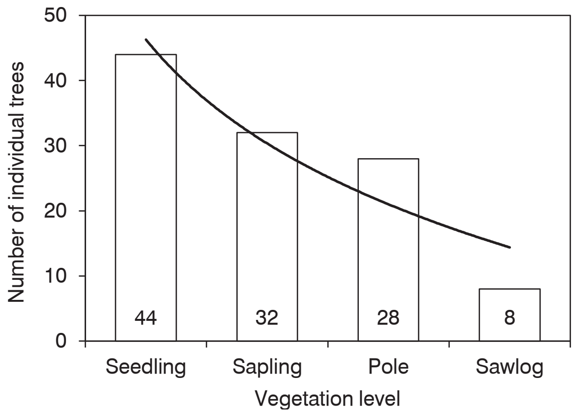

Fig. 1. Number of individual trees at vegetation level.

pH

Generally, the pH values of honey range from 3.4 to 6.1 with an average of pH 3.9. Tab. 6 shows that the pH value of kelulut honey derived from heterogenous bee food sources was higher than that of homogenous bee food source, specifically pH 3.63 and of pH 3.97, respectively. This was similar to the previously reported value of pH 3.92 [13] but lower than the value of pH 3.72 reported for kelulut honey in Thailand [25]. All of the pH values in the present and previous studies were classified as acceptable, as the SNI 3545-2013 [11] does not require any pH value of honey. The pH value is important for microbial stability of honey [5]. The pH of honey is influenced by the content of several types of acids, including amino acids and organic acids and is a significant parameter determining the quality of honey [26]. It affects the taste and aroma, as well as signifies the fermentation process.

Total sugar

The honey contains simple sugars, i. e. glucose and fructose, which have a higher relative sweetness than saccharose [14]. Moreover, the proportions of glucose and fructose in honey indicate the nature of honey [27]. The results on total sugars in Tab. 6 show the different sugar content in the honey derived from heterogenous and homogenous bee food source. Content of total sugars is influenced by the level of plant maturity, type of bee food and source of nectar. It is assumed that the high total sugars in the honey derived from heterogenous bee food sources is caused by varied bee food source and assorted sources of nectar. A study in the meliponiculture in Gembong Village showed that the total sugars in the honey derived from heterogenous bee food sources was higher than that from a homogenous bee food source. However, both met the requirement of the SNI 3545-2013 [11], of 65 % [28]. The high total sugars in honey are able to prevent the growth of microorganisms such as yeasts, thus preventing fermentation [29]. The high total sugars cause also a thicker texture of honey [29]. The different content of sugar is also characterized by the change in taste.

Moisture content

The moisture content of honey derived from the homogenous bee food source was higher than that from heterogenous bee food sources. Both of them exceeded the threshold set in the SNI 3545-2013 [11], of 22 %. In addition, the moisture content of the honey derived from this study was higher than those reported by RIDONI et al. [23] and MARDHIATI et al. [13], of 19.2 %. Stingless bee honey commonly has a higher moisture content than other *Apis mellifera* honeys [5]. It is highly affected by environmental factors, i. e. by weather and humidity of stingless bee hives, nectar conditions, harvesting and storage processes. Since honey is hygroscopic, absorbing moisture from a high humidity environment, the time of harvesting, humidity, air temperature and bee food sources will affect the level of moisture content [14, 15]. The higher the moisture content in honey, the more susceptible it is to fermentation by yeasts

Tab. 6. Parameters of kelulut honey.

Parameter	Experimental results		Literature data		SNI 3545-2013 [11]
	Forest vegetation	Sweet star fruit plantation	Value	Ref.	
Ash [%]	0.2 ± 0.1	0.5 ± 0.2	0.2	[23]	max. 0.5
pH	3.6 ± 0.0	4.0 ± 0.0	4.0	[13]	–
Total sugar [%]	85.4 ± 0.2	65.3 ± 0.2	74.5	[28]	min. 65
Moisture content [%]	28.8 ± 0.0	29.5 ± 0.0	25.0	[23]	max. 22
Lysine [%]	0.1 ± 0.0	0.0 ± 0.0	–	–	–
Vitamin C [$g\cdot kg^{-1}$]	43.7 ± 1.2	78.7 ± 1.2	0.3	[32]	–
Vitamin E [$g\cdot kg^{-1}$]	520.9 ± 0.7	272.5 ± 0.2	–	–	–
Potassium [$g\cdot kg^{-1}$]	227.8 ± 0.6	168.8 ± 1.4	–	–	–
Calcium [$g\cdot kg^{-1}$]	74.4 ± 0.1	33.3 ± 0.2	5.6	[16]	–
Magnesium [$g\cdot kg^{-1}$]	42.9 ± 0.1	23.7 ± 0.2	5.7	[16]	–
Phosphorus [$g\cdot kg^{-1}$]	151.9 ± 0.5	9.0. ± 0.8	31.7	[16]	–
Proteins [$g\cdot kg^{-1}$]	0.7 ± 0.0	0.5 ± 0.0	0.4	[16]	–

Experimental results are expressed as mean ± standard deviation, averaged from three samples or three replications for each parameter.

[1, 2]. Fermentation causes changes in the composition of honey, changing the taste to become more sour [30]. Meanwhile, honey with excessive water content is not suitable for long-term storage. Therefore, reducing the water content in honey is important to maintain its quality [14].

Amino acids

Tab. 6 shows data on the content of lysine in honey demonstrating higher contents in honey from heterogenous bee food sources than from the homogenous bee food sources. Meanwhile, the SNI 3545-2013 [11] does not require amino acids in honey. Amino acids in honey may be active at treatment of diseases and may regard the brain function. Amino acids in kelulut honey may potentially overcome mild cough problems frequently caused by irritation in the throat, reduce asthma symptoms by relieving respiratory cavities have positive effects on brain and nervous system [31]. The dominant acids in kelulut honey were proline and gluconic acid. Gluconic acid is formed by the enzyme glucose oxidase. Amino acids are able to lower serum cholesterol levels, participate in the formation of hormones and collagen as well as to help the body absorb calcium [31].

Vitamin C

Tab. 6 shows that the vitamin C content in kelulut honey derived from the homogenous bee food source was higher than that from heterogenous bee food sources. These data mean a very rich content compared to the content of vitamin C in randu honey, which was 16 $g \cdot kg^{-1}$ [32]. Meanwhile, the SNI 3545-2013 [11] does not require vitamin C in honey. The content of vitamin C in the samples of the present study might have been reduced by storage. Vitamin C is a well-established antioxidant helping to protect cells against oxidative damage [33].

Vitamin E

Kelulut honey derived from the homogenous bee food source contained less vitamin E than honey from heterogenous bee food sources (Tab. 6). Meanwhile, the SNI 3545-2013 [11] does not require vitamin E in honey. The high content of vitamin E in the honey from heterogenous bee food sources was allegedly due to the variety of nectar sources, which were numerous plants. Vitamin E is a an antioxidant, protecting body cells from damage and acting as a scavenger of free radicals and oxygen molecules that are important in preventing membrane peroxidation of unsaturated fatty acids [9].

Proteins

The proteins content in honey was relatively low but the essential and non-essential amino acids were quite diverse. Tab. 6 shows that the protein content of kelulut honey derived from heterogenous bee feed sources was higher than that from the homogenous bee food source. The values determined in the present study were higher than the protein content of kelulut honey reported previously [13, 16, 34]. Meanwhile, the SNI 3545-2013 [11] does not set a standard value for proteins content.

CONCLUSIONS

A comparison of the nutritional composition of kelelut honey derived from different bee food sources was performed. The findings indicated that the differences in bee food sources may potentially affect the nutritional composition of kelulut honey. Honey from the meliponiculture with forest vegetation (heterogenous) had higher level of pH, total sugars, amino acids, vitamin E, potassium, calcium, magnesium, phosphorus and proteins than the honey derived from the meliponiculture with sweet star fruit (homogenous). Meanwhile, ash, moisture content and vitamin C of kelulut honey derived from heterogenous bee food sources were lower than from homogenous bee food sources. In general, the nutritional value of kelulut honey from the meliponiculture with heterogenous food sources was higher than that from homogenous bee food source. Honey produced from the sweet star fruit plantation was characterized by high vitamin C. Kelulut bee keeping or meliponiculture can be carried out by regulating vegetation or plants as certain bee food sources to obtain kelulut honey with a targeted nutritional value.

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