

# Evaluation of Physicochemical and Antioxidant Properties of Kelulut (*Heterotrigona itama*) Honey and Forest Honey Authentic to West Borneo

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**Abstract:** The study aimed to evaluate the physicochemical characteristics and antioxidant properties of Kelulut (*Heterotrigona itama*) honey and forest honey authentic to West Borneo. The organoleptic properties of honey testing with the multiple comparison test (MCTs) methods. The analysis of the chemical properties of the samples was based on the quality requirements of Indonesian honey standards (SNI 8664:2018), including testing for water content, pH, reducing sugar, ash, metal contamination, arsenic contamination (As), and chloramphenicol content. Antioxidant properties are measured by the DPPH method. The honey quality standards in the test samples still meet SNI 8664: 2018. The characteristics result of honey samples are known to contain total phenolics and flavonoids. The total phenolic content of kelulut honey and forest honey were 13.87 and 17.82 mg/kg, respectively. Flavonoid levels in kelulut honey and forest honey samples were 46.49 and 49.92 mg/kg, respectively. Antioxidant test results showed that the value of free radical scavenging for Forest and Kelulut honey was 85 ppm ± 2.45 and 115 ppm ± 1.27, respectively. Kelulut (*Heterotrigona itama*) honey and forest honey authentic to West Borneo have characteristics that meet Indonesian national standards (SNI 8664: 2018) with potential free radical scavenging abilities.

**Keywords:** Kelulut (*Heterotrigona itama*) honey; forest honey; physicochemical characteristics; antioxidant properties.

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## 1. Introduction

West Borneo Province is the world's lungs, with a forest area of 8.389.600 ha or reaching 57.14% of the province's total area. One of the potential forest products with high economic value is honey. West Kalimantan is the largest honey producer in Indonesia. In addition to honey harvested in nature, honey bee cultivation is also being developed in West Borneo. The high consumption of honey in West Borneo encourages people to meet quality standards and the health benefits of honey itself.

Many studies today are about functional foods and beverages. Research on functional foods tends to provide benefits for supportive therapy and prevention of diseases such as cancer and cardiovascular and neurodegenerative diseases [1–3]. One of the potential functional foods

because of its bioactive compound content is honey. Honey is known to have been used by many civilizations and even listed in many books of various religions [4,5]. The bioactive content of honey is very complex, with varied compositions. Honey is a result of the bees obtaining a variety of plants that provide different nectar and pollen. The main component of honey consists of monosaccharides (75%), disaccharides (10-15%), and small amounts of other sugars. Polyphenols, vitamins, enzymes, amino acids, minerals, organic acids, and essential oils are minor honey compounds and are considered necessary [6–9]. The composition of the bioactive components of honey is largely determined by its botanical origin. Therefore, honey's most important bioactive fraction consists of secondary metabolites present in the nectar, including phenolic acids and flavonoids [10,11]. These bioactive compounds are responsible for honey's antibacterial, antifungal, wound healing, and other properties. Publications on honey with antiproliferative, immunomodulatory, and antioxidant activities have also been reported [5,8–10,12–14].

*Trigona (Heterotrigona itama)* or kelulut honey is one of West Borneo's leading commodities from non-timber forest products. Many studies have shown that honey from stingless bees has higher antioxidant activity than honey from stingless bees, such as *Apis sp.* [15–17]. The antioxidant activity of honey can prevent hepatoprotective, cardioprotective, anti-tumor, and anticancer effects [18,19]. *Trigona* bee honey or kelulut was reported to have high antioxidant content because of its high total phenolic content [20,21]. In contrast to honey that is often found in the market, kelulut honey has a sourer taste with higher water content. The quality standard of kelulut honey in Indonesia refers to SNI 8664: 2018.

## 2. Materials and Methods

### 2.1. Materials.

The equipment used in this study is a digital analysis balance (Ohaus PA214, USA), glassware (pyrex), a vortex mixer (Thermolyne), UV-VIS Spectrophotometer (Shimadzu type 2450), a 1 cm-sized quartz cuvette, a magnetic stirrer (Stuart CB162), pH meter (HANNA), micropipette Socorex® (0,5 –10; 5–50; 50-200, 200–1000 $\mu$ L), an Eppendorf tube, a rotary evaporator (Heldolph type Hei-VAP), an oven (Mettler), a water bath (Mettler type WNB14). Ethanol 70% (Merck), a n-hexan (Merck), ethyl acetate (Merck), chloroform (Merck), methanol (Merck), TLC Silica gel 60 F 254 (Merck), aquadest, quercetin (Sigma aldrich), Gallic acid (Sigma aldrich), DPPH (Smartlab).

### 2.2. Preparation sample.

Two honey samples were selected from national Parks, Danau Sentarum (Kapas Hulu district) and Balai Karangan (Sanggau district), West Kalimantan, Indonesia. Honey is tested for taste, aroma, and texture. Analysis of chemical characteristics based on the quality requirements of Indonesian honey (SNI 8664: 2018) as water content, reducing sugars, sucrose, acidity, insoluble solids, ash, metal contaminants (Pb, Cd, Hg, As), microbial contamination; which was held at the Central Lab-Universitas Padjajaran.

### 2.3. Water content and ash assay.

The moisture content test on the powder was carried out using the gravimetry method. The honey sample is weighed as much as 1-2 grams and put into a weighing bottle with a

known weight. The sample was put into the oven at 105-110°C for 2 hours. The samples were placed in a desiccator for 10 minutes, then weighed and put back in the oven for 1 hour. Repeat heating in the oven and weighing until constant weight (difference in successive weighing  $\leq$  0.2 mg) [22–24].

#### 2.4. Assay of antioxidants.

2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to measure the free radical scavenging activity of Kelulut honey. Preparation of 100 ppm DPPH Solution was performed with 10 mg of DPPH in 100 mL of methanol. The test sample was 0.75 mL of honey and methanol solution with a concentration of 20-50 mg/mL, and then the absorbance was measured at a wavelength of 528 nm. 1 mL sample was added to 2 mL of 0.1 mM DPPH. The mixture was homogenized and incubated at room temperature for 30 minutes in a dark room. The absorbance of the solution was measured at the wavelength obtained in the previous stage using a UV-Vis spectrophotometer. The same treatment is for the blank solution (DPPH solution that does not contain the test material). The blank solution consisted of 2 mL of 0.1 mM DPPH and 1 mL of methanol p.a. [25–27].

### 3. Results and Discussion

Differences in feed sources affect the quality of honey, including honey color [28]. Stingless bees can forage around their nests as far as 2 kilometers [29]. This reference becomes essential for the area coverage for the vegetation survey. When choosing a food source, bees choose the most profitable source and replace that preference according to availability when the primary source is exhausted. Many factors, including season, environmental conditions, flowering season, and flower age, influence the availability of nectar as a source of bee feed. The number of flowers around the nest shows that honey bees have many food sources and depend on many types of plants to support their lives. The difference in feed sources affects the characteristics of honey, including honey color. Locations with different vegetation conditions are known to produce honey with different colors. This color difference is closely related to local vegetation types. However, this study did not identify the species or plant families that determine the color of the honey sampled. The melissopalynological analysis is needed to identify the plant species that are the source of honey and determine the color, one of which is the identification of pollen in honey [30].

Kelulut honey is a honey produced by Kelulut bees (*Trigon sp*) and forest honey by Asian giant honey bees (*Apis dorsata*). The test results (table 1) show that different types of honey also show different characteristics.

**Table 1.** Quality assay of samples honey compared SNI 8664:2018.

No.	Type of assay	Unit	SNI requirement 8664:2018	Kelulut honey	Forest honey
A.	Organoleptic Smell Taste Color Consistency Textures		Typical honey Typical honey	Typical honey Typical honey Dark Liquid Mild	Typical honey Typical honey Golden yellow Thick Mild
B.	Laboratory Water content pH Reducing sugar (calculated as glucose)	% b/b  % b/b	max 22  min 65	5.56±0.004 3.5 94.27	5.20±0.002 3.8 72.07

No.	Type of assay	Unit	SNI requirement 8664:2018	Kelulut honey	Forest honey
	Ash				
	Metal contamination	% b/b	max 0.5	0.20	0.24
	a. Timbale (Pb)				
	b. Cadmium (Cd)	mg/kg	max 2.0	< 0.0001	< 0.0001
	c. Mercury (Hg)	mg/kg	max 0.2	< 0.0001	< 0.0001
	Arsenic contamination (As)	mg/kg	max 0.03	< 0.0001	< 0.0001
	Chloramphenicol	mg/kg	max 1.0	< 0.0001	< 0.0001
			undetected	-	-

The difference is mainly in the water content, reducing sugar and acidity, exceeding the SNI 8664:2018 honey quality standards. That character is because kelulut honey has a distinctive sour taste compared to honey in general. In addition, Kelulut honey has a higher water content than other forest honey types [31]. The quality of honey is highly dependent on external and internal factors. External factors influencing it are the source of nectar, season, soil or geographical conditions, processing, and storage conditions. The type of bee is the internal factor that influences it. Based on Table 1, the honey quality standards in the test samples still meet SNI 8664:2018: sucrose content, solids not soluble in water, lead, copper, and arsenic. The same results are also shown in test results; water content, reduced sugar quality, and metal contamination analysis meet the standards set by SNI (8664:2018).

### 3.1. Organoleptic result.

This honey was collected in July 2022, which is the harvest period. The honey selected for this study was fresh from Paloh (Sambas district) and Danau Sentarum (Kapas Hulu district) with a yellow to golden brown color, liquid and thick consistency, and a typical honey aroma, as shown in Table 1.

### 3.2. Water and heavy metal content assay.

In addition, geographical conditions can affect the high water content of honey. It has been shown that tropical climates usually receive more rain than average. Dry air can cause inferior honey during the dry season. Water content increases the sugar content of nectar. Honey is harvested or collected. During the dry season, the ambient air is drier, so honey may contain less water than honey harvested during the wet season. Water content is affected by the fact that honey absorbs water from the surrounding air. Harvest time also affects the water composition of honey [31]. Honey harvested during ripening has a lower moisture content than honey harvested when young. The longer the honey stays in the hive, the more perfect its evaporation. The water content test sample obtained met the SNI 8664: 2018 standard. The water content of forest honey and Kelulut honey were  $5.20 \pm 0.002$  and  $5.56 \pm 0.004$ , respectively. Good-quality honey should have less than 20 g/ 100 g moisture content. Honey with a high water content has a more significant potential for fermentation, so the fermentation must be considered, and appropriate storage is needed [32]. Water content is the second-largest constituent of honey.

Honey contains several antioxidant enzymes, such as catalase and glucose oxidase [33,34]. A study on the phenolic acid profile of 12 types of honey collected from different regions of Greece showed those honey types are rich in phenolic acids, particularly protocatechuic acid and p-hydroxybenzoic acid [35]. Several studies have proposed chemical markers to determine the botanical origin of honey based on the presence and abundance of

one or more certain phenolic compounds [11]. Several studies have been published on honey's bioavailability and metabolism of phenolic and flavonoid compounds. The value of total phenolic and flavonoid levels in forest honey and kelulut honey can be seen in Table 2. Total phenolic plasma concentrations increased along with antioxidants and reduced plasma capacity after consuming 15 g honey/kg body weight for two types of honey in forty subjects. In addition, it was also reported that the absorption and metabolism of honey phenolic compounds require a place in the small intestine [36].

### 3.3. Antioxidant assay result.

The antioxidant ability of the sample was measured by looking at the free radical scavenging activity with DPPH in vitro. The IC<sub>50</sub> value of the sample is shown in Table 2.

**Table 2.** The IC<sub>50</sub> value sample, total phenol, and flavonoid content.

Sample	IC <sub>50</sub>	Total Fenolic (mg/Kg)	Flavonoid (mg/Kg)
Forest honey	85 ± 2.45	17.62	49.92
Kelulut honey	115 ± 1.27	13.87	46.49

Based on the table, antioxidant assay results show that forest honey has a more potent free radical scavenging effect than Kelulut honey. The result is caused by the DPPH radical scavenging activity of phenolic and flavonoid compounds. Many studies show flavonoid and phenolic compounds in forest honey and Kelulut honey are known as antioxidants. The antioxidant mechanism is known because flavonoid compounds have hydroxyl groups that can release protons from hydrogen ions. The hydrogen ion has only one proton and no electrons, so the radical electrons contained in the nitrogen atom in the DPPH compound bind to hydrogen ions and produce reduced DPPH [25]. Radicals in DPPH can be reduced when reacted with hydrogen donors in flavonoids and phenolic compounds [62]. In several studies, honey's phenolic and hydroxymethylfurfural (5-(hydroxymethyl)furfural) content correlated with the increased inhibitory effect on cancer cells [35,37]. In another study, the flavonoid content of honey was reported to induce cell death in some cancer cells [6,38,39]. Other components in honey, such as amino acids, glucan acid, proteins, and vitamins, have also been described as having an essential role in inhibiting the growth of lung cell tumors [38].

The method used in activity testing antioxidants in this study is the method of DPPH radical uptake. Antioxidant activity of honey: This is expressed as a percentage of inhibition against DPPH radicals. A UV-Vis spectrophotometer measured the percentage of inhibition derived from DPPH and sample absorbance differences. The honey antioxidant activity test yielded an absorbance value that consistently decreased with increasing sample concentration. The results of the honey antioxidant activity test showed that the higher the sample concentration, the lower the absorbance value of the sample at each increase in concentration. Deterrence of free radicals from DPPH occurs due to the presence of compounds that can provide hydrogen radicals to DPPH radicals so that they are reduced to DPPH-H. Furthermore, it causes the DPPH color to decay from purple to yellow. A decrease in the absorbance value of DPPH means that the sample has captured the DPPH radical. The presence of antioxidant activity from the sample resulted in a color change in the methanol DPPH solution, which was initially violet to pale yellow. Data from the measurement of absorbance values can be analyzed to determine the effect of sample concentration on the percentage of inhibition. The increase in the percentage of inhibition is proportional to the increase in sample concentration. Then, the linear regression and equation are used to determine the effective concentration (IC<sub>50</sub>).

Results of sample analysis with the DPPH method showed that samples of Kelulut honey and forest honey had antioxidant activity. These activities differ from one another because various factors influence them. Factors that affect this antioxidant activity also include the different compositions of honey, which depend on the flower source used to collect nectar by honey bees, seasonal and climatic factors, and processing [34,37,39].

This analysis also showed that the highest antioxidant activity came from forest honey samples, followed by Kelulut honey. Generally, biological antioxidants are easily absorbed and transported to cells and are not toxic in the proper intake [40–42]. Enzymes in honey also function as antioxidants by inducing the removal of oxygen radicals [19]. The consumption of honey has also been reported to be effective in increasing total plasma antioxidants [6,43]. The components of honey responsible for antioxidant activity are mainly flavonoids, phenolic acids, catalase, peroxidase, carotenoids, and non-steroidal constituents [10]. The amount of these components varies significantly according to the floral and geographical origin of the honey, processing, handling, and storage of honey.

#### 4. Conclusions

Kelulut (*Heterotrigona itama*) honey and forest honey authentic to West Borneo have characteristics that meet Indonesian National Standards (SNI 8664: 2018) with potential antioxidant properties.

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#### Conflicts of Interest

All authors declare that they contributed equally.

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