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DOI: [10.31965/infokes.Vol22.Iss2.1526](https://doi.org/10.31965/infokes.Vol22.Iss2.1526)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****Quality Evaluation and Flavonoid Content of Honey from Riau Forest, Indonesia****Eva Yuniritha<sup>1a</sup>, Nur Ahmad Habibi<sup>1b\*</sup>, Hasneli<sup>1c</sup>, Alsri Windra Doni<sup>1d</sup>**<sup>1</sup> Department of Nutrition, Health Ministry Polytechnic of Padang, Padang, West Sumatra, Indonesia<sup>a</sup> Email address: [yunirithaeva2010@gmail.com](mailto:yunirithaeva2010@gmail.com)<sup>b</sup> Email address: [nahindo2022@gmail.com](mailto:nahindo2022@gmail.com)<sup>c</sup> Email address: [hasneli.darwis@yahoo.com](mailto:hasneli.darwis@yahoo.com)<sup>d</sup> Email address: [alsriwindradoni79@gmail.com](mailto:alsriwindradoni79@gmail.com)

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**Abstract**

Honey is a naturally sweet liquid produced by bees from flower nectar, which varies in quality and flavonoid content due to the food source, environment and the bees that produce it. This study aims to evaluate the quality and flavonoid content of honey from the Riau Forest of Indonesia. Quality testing was conducted in accordance with SNI 8664:2018 with sensory, physical, chemical and microbiological parameters. Flavonoid testing was carried out qualitatively using HPLC. The test results showed that the sensory profile of honey was in accordance with the standard, sweet taste, distinctive aroma, brown colour and thick shape. Chemical parameters obtained values of moisture content of 21.92%, diastase enzyme 3.13 DN and HMF 4.14 mg/kg have met the requirements. However, the reducing sugar of 54.1% is still below the requirement. Heavy metal parameters (Pb, Cd, As and Hg) were not detected and microbiological ALT 3.0 x 10 and yeast mould <10 colonies/gr that have met. Furthermore, the results of flavonoid qualitative tests detected several types including Luteolin, Mangiferin, Smiglanin, maltol, isoflavones, quercetin, Cnidimol C, Norcimifungin, Apigenin, Methyl ophiopogonanone B, Pectolarigenin, Kusenol C, 3,5,6-trihydroxy-4',7-dimetoxyflavone and Lupinifoline. The conclusion of this study shows that honey from Riau forests fulfils most of the quality requirements and has a diverse flavonoid content.

**Keywords:** Honey, Flavonoid, Food Quality and Safety.

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## 1. INTRODUCTION

Honey is a naturally sweet liquid produced by bees (*Apis dorsata*.) from flower nectar (Edo et al., 2023). Honey generally contains a large amount of carbohydrates, mainly in the form of fructose and glucose more than 60%. In addition, honey also contains proteins (including enzymes), minerals, vitamins, organic acids, polyphenols (flavonoids and phenolic acids), volatile compounds, waxes, and other phytochemicals (Tafere, 2021). Phenolic compounds (polyphenols) are secondary metabolites produced from plants. Flavonoids and phenolic acids are phenolic compounds that are found in honey (Cheung et al., 2019).

Flavonoids present in honey come from flower nectar, propolis, and pollen (Tomás et al., 1993). Honey that contains a lot of flavonoids and phenolic compounds is of better quality because it presents a potential natural antioxidant in the treatment of various diseases and contributes to individual health (Ahmed et al., 2018). Flavonoids and phenolic acids are able to prevent oxidation through the release of hydrogen or electrons, thus stabilizing free radicals. (Hassanpour, 2023). Many studies have shown that regular consumption of honey with its flavonoids and phenolic content can reduce the risk of heart disease (Bt Hj Idrus et al., 2020). In addition, these compounds also have biological activities as anticancer, anti-inflammatory, antioxidant, and antiatherogenic properties (Erejuwa et al., 2012).

Indonesia is one of the countries that has a high production of forest honey (Food Review Indonesia, 2020). This is due to the vast forest potential. Riau Province, precisely in the Kampar area, is an area in Indonesia that is able to produce honey in large quantities. Generally, the honey produced is cattle honey by utilizing forest nectar, such as calliandra and rubber flowers (Yunianto, 2020).

Despite the extensive research on the general composition and health benefits of honey, there remains a significant gap in the specific evaluation of honey from Riau Forest, Indonesia, particularly concerning its flavonoid content and overall quality. Previous studies have broadly covered the phenolic compounds in honey and their antioxidant properties, but there is limited detailed analysis of the flavonoid content in honey from this specific region. Additionally, while the health benefits of flavonoids and phenolic acids in honey are well-documented, there is a lack of localized research that ties these benefits to the unique floral sources and environmental conditions of Riau Forest. This study aims to fill this gap by providing a thorough evaluation of the quality and flavonoid content of honey from Riau Forest, contributing valuable data that can enhance our understanding of its potential health benefits and support the promotion of forest honey from this region as a high-quality product.

## 2. RESEARCH METHOD

This research is quantitative in nature with descriptive data analysis that observes the honey that comes from the forests of Riau province. The honey used in the study was multiflora honey produced from *Apis dorsata* bees taken from the forests of Riau province in the period January-June 2024. Honey was extracted from the hive through a centrifugation process, then put into glass bottles and stored at +4°C in a closed room. Prior to analysis the honey was gently stirred, when crystallization occurred the honey was warmed at 35°C to 38°C.



**Figure 1.** Sampling location of Riau Forest honey

In the sample collection stage, sterile sample bottles, honey filters, labeling tools, and portable coolers are used. Evaluation of honey quality requires a refractometer, pH meter, UV-Vis spectrophotometer, sugar analysis kit, water bath, micropipette, analytical balance, and thermometer. Analysis of flavonoid content using HPLC. Supporting equipment such as filter paper, test tubes, drop pipettes, and glassware are also required.

**Organoleptic Test Analysis.** The test used limited trained panelists of 3 people who have experience in honey testing. Honey was tested organoleptically for taste, aroma, colour and texture and the resulting sensory profile was described (Cabrera & Santander, 2022).

**Diastase Enzyme Test Analysis.** The exposed honey and starch solutions were incubated and the time required to reach the endpoint was measured photometrically. The result is expressed in ml of 1% hydrolyzed starch equivalent to the enzyme in 1 g honey in 1 (one) hour. Pipette 10 ml of sample solution, then put it into a 50 ml test tube. Continue by pipetting 5 ml of starch solution through the inner wall of the tube then place it in a  $40^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$  water bath for 15 minutes. Shake and switch on the stopwatch. At every 5-minute interval, pipet 1 ml of the sample mixture and add it to 10.00 ml of iodine solution. Mix, then dilute to the volume as before and determine the absorbance value at a wavelength of 660 nm. Plot the absorbance value against time (minutes) on millimeter paper. A straight line is drawn through several points. From the graph, determine the time required to reach an absorbance value (A) = 0.235. The value of 300 divided by the time required to reach the absorbance value (A) indicates the diastase enzyme activity (DN)(BSN, 2018).

**HMF Analysis.** HMF measurements were carried out based on the SNI 8664: (2018) reference, starting with 5g of honey weighed in a small glass cup, then put into a 50 ml volumetric flask and added aquadest until the volume of the solution reached 25 ml. Then 0.5ml Cerrez I solution was added, shaken, and then 0.5ml Cerrez II solution was added, shaken, and diluted with distilled water up to the line mark. Then a drop of alcohol was added to remove the foam on the surface. Then filtered using filter paper and discarded 10ml of the first filter. A total of 5ml of the filter was pipetted and each was put into a test tube. Then 5 ml of water was pipetted and put into one tube (example) and 5 ml of 0.2% NaHSO<sub>3</sub> into the other tube (comparison). The solution was shaken well and the absorbance of the sample was determined against the reference at wavelengths of 284nm and 336 nm (BSN, 2018).

**Moisture Content Analysis.** Moisture content was determined and measured using a honey refractometer. The way to use a honey refractometer is to open the light plate and drop a few drops of honey until the honey covers the entire blue area. The result is the value on the water scale shown on the viewfinder. The water content value is expressed in percent (%). The test results were compared with the standard value of honey according to SNI 8664:2018 (BSN, 2018).

**Sugar Content Analysis.** A total of 1g of honey was weighed and dissolved in 20 ml of distilled water. A solution of 0.1ml was taken and added to 1.5ml of DNS reagent. Then the solution was shaken evenly and heated in a water bath at 100<sup>o</sup> C for 5 minutes. After that the solution was cooled in running water, 0.5ml was taken and then 15ml of sterile distilled water was added. The solution was shaken evenly and measured at a wavelength of 540 nm. The results obtained were entered into the equation obtained from the standard curve and then divided by the weight of the sample used. The percentage of glucose content was calculated by multiplying the results obtained by 100% (BSN, 2018).

**Ash Content Analysis.** The percentage of ash content was measured through an ashing process using a *muffle furnace* at 550<sup>o</sup> C for 6 hours.

**Heavy Metal Analysis.** Test for metal contamination in food in accordance with SNI 2896: 1992. The prepared sample solution was determined by absorbance with Uv-Vis Spectrophotometer at  $\lambda = 283.3$  nm. The absorbance obtained was substituted in the standard equation obtained from the standard curve with the standard series solution of Pb, Cd, Hg and As (BSN, 1998).

**Microbiological Analysis Total Plate Number.** Honey samples were diluted with 0.85% physiological NaCl solution starting from dilution 10<sup>-1</sup> - 10<sup>-6</sup>. Each of the dilution samples was taken as much as 1 ml and then put into a petri dish and made duplo. Plate Count Agar (PCA) media that has been made as much as 15-25 ml was taken and poured into a petri dish. The Petri dish containing the media was then rotated back and forth with the aim that the sample could be mixed homogeneously and left to solidify. After the media becomes solid, the Petri dish containing the media is then incubated for 24-48 hours at a temperature of 35-45<sup>o</sup> C with an inverted position. If there are colonies that grow, the colonies are observed and counted (Hasanah et al., 2023).

**Microbiological Analysis Mould Yeast Numbers.** Herbal medicine samples were diluted with 0.85% physiological NaCl solution starting from dilution 10<sup>-1</sup> - 10<sup>-4</sup>. Each of the dilutions taken as much as 1 ml was put into a Petri dish and made duplo. Potato Dextrose Agar (PDA) media that has been made is taken as much as 15-25 ml put into a petri dish. Petri dishes are rotated back and forth with the aim that the sample can be mixed homogeneously and then left to solidify. After the PDA media becomes solid, the Petri dish is then incubated in an inverted position at a temperature of 20-25<sup>o</sup> C for 5 days. If there is colony growth on the media after 5 days of incubation, the number of colonies is then observed and counted (Hasanah et al., 2023).

**Qualitative Analysis of Flavonoid Content.** Flavonoids were extracted with ethyl acetate (Karabagias et al., 2014). 10 g of honey was dissolved in 50 mL of water to a final concentration of 20% (b/v). The solution was then acidified by adding 0.2M HCl and saturated with 30% (w/v) sodium chloride. The solution obtained was then filtered through cotton to remove solid particles and extracted three times with ethyl acetate using a separatory funnel. The organic layer was then dried in a vacuum dryer at 40<sup>o</sup>C with a low pressure of 100 mbar until the entire solution evaporated. The residue was redissolved in 5 mL of methanol. Prior to HPLC analysis, all honey sample solutions were filtered through a 0.45  $\mu$ m pore size *polytetrafluoroethylene* (PTFE) filter.

Agilent 1200 series HPLC system was used for flavonoid analysis. The wavelength used for detection was from 256 nm to 350 nm. Flavonoid separation was performed using a

WondaCract ODS-2 reversed-phase column (250 mm × 4.6 mm, particle size 5 µm) at room temperature. The gradient elution flow rate was 1 mL/min with a solution of 2.0% (v/v) acetic acid (solvent A) and acetonitrile (solvent B) as mobile phases. The injection volume was 20 µL. Flavonoid analysis started with 10% solvent B and then increased to 30% from 0 to 20 min. Then, solvent B was increased to 40% from 20 to 30 min, and then 50% from 30 to 50 min. In addition, the column was eluted with 50% acetonitrile for 10 minutes before the next sample injection. To identify the peaks, 100 µg/mL stock solution of each standard was prepared by dissolving the flavonoid standards in methanol. One mL of the stock solution was used and injected into the HPLC for peak identification. By comparing the retention time, flavonoids can be identified (Cheung et al., 2019).

The data obtained will be analyzed using a descriptive statistical approach to calculate the mean, standard deviation, minimum, and maximum values of each parameter measured, including moisture content, pH, total sugar, enzyme activity, and flavonoid content. The data will be presented in tables to facilitate interpretation, and the results of the analyses will be interpreted to provide a deeper understanding of the quality and flavonoid content of honey from Riau forests. This research has also received ethical approval from the Tanjungkarang Health Polytechnic Research Ethics Commission with No.879/KEPK-TJK/XII/2023.

### 3. RESULTS AND DISCUSSION

Riau is a region in Indonesia that is able to provide large amounts of forest and livestock honey. The honey produced includes rubber honey, calliandra honey, and other multiflora honey. Generally, the honey is sold directly by farmers to honey processing industries for commercialization on a large scale.

Regulation and standardization of honey is regulated by the Food and Drug Administration and the National Standardization Agency. There are parameters that must be met by producers, including organoleptic, physical, chemical, and microbiological. This aims to protect consumers from product adulteration and food safety risks. Table 1 shows the quality of honey from the Riau forest.

The quality of Riau honey in terms of taste, aroma, colour, and texture meets the set standards. The honey has a sweet taste, typical honey aroma, brown colour, and thick texture. The sweetness of honey comes from the sugar content (sucrose, fructose, and glucose) (Melina et al., 2023). The content of reduced sugar reaches 54.16%, which includes 33.19% fructose. This content is below the SNI requirements. The low content of reducing sugar is influenced by moisture content, humidity, and harvesting period. Research shows that high water content stimulates yeast activity to grow. *Yeast* in the form of *osmophilic yeast* of the genus *Zygosaccharomyces*, which is resistant to high sugar concentrations, can live and thrive in honey. Yeast in honey will degrade sugars, especially dextrose and levulose into alcohol and CO<sub>2</sub>, thus affecting the dextrose (glucose) and levulose (fructose) content of honey. This is thought to be the cause of the lower glucose content in honey (Dygas et al., 2021). Sucrose content in honey can be influenced by the presence of the enzyme invertase, an enzyme that converts sucrose into glucose and fructose. The optimum temperature of the invertase enzyme ranges from 30-50°C (Manoochehri et al., 2020). The sucrose content of room-temperature honey is lower than that of cold-temperature honey because the invertase enzyme is more active at room temperature than at cold-temperature (Wulandari, 2017).

The research results obtained honey moisture content of 21.92%, this value has met the SNI requirements, which is a maximum of 22%. The moisture content of honey is influenced by the humidity of the environment. This is because honey has hygroscopic properties, which is easy to absorb water. The higher the humidity, the higher the moisture content of the honey. If the humidity is 51%, the moisture content of honey is 16.1%. If the humidity is 81%, the moisture content of honey is 33.4% (Wulandari, 2017). A low moisture content will keep the

honey from spoiling for a relatively long period of time (Chirife et al., 2006). High moisture content in honey will stimulate yeast activity to grow and develop in honey. Harvesting age also affects the water composition of honey. Honey harvested at an older age has less water content than honey harvested at a younger age. The longer the honey is in the honeycomb, the more complete the evaporation of water content in the honey (Evahelda et al., 2017).

**Table 1.** Honey Quality Results based on SNI 8664:2018 Standard

No.	Parameters	Unit	Results	Honey Standard
1	Organoleptic Flavour Aroma Colour Texture		Sweet Typical Honey Chocolate Thick liquid	Typical Honey Typical Honey Chocolate Thick Liquid
2	Diastase enzyme	DN	3.13	Minimum 3
3	Hydroxymethylfurfural (HMF)	mg/kg	4.41	Maximum 50
4	Water Content	%	21.92	Maximum 22
5	Fructose	%	33.19	
6	Saccharose	%	NI	Maximum 5
7	Reducing Sugar	%	54.61	Minimum 65
8	Chloramphenicol	mg/kg	NI	NI
9	Acidity	ml NaOH 1N/kg	17.40	Maximum 50
10	pH		4.36	<4.5
11	Undissolved solids	%	0.12	Maximum 0.5
12	Ash Content	%	0.48	Maximum 0.5
13	Heavy Metals Cd Hg As Pb	mg/kg mg/kg mg/kg mg/kg	NI NI NI NI	Maximum 0.2 Maximum 0.03 Maximum 1.0 Maximum 2.0
14	Microbiology ALT Moulds	Colony/g Colony/g	$3.0 \times 10^1$ < $10^1$	< $5 \times 10^3$ < $1 \times 10^1$

Enzyme diastase and Hydroxylmethylfurfural (HMF) are specific parameters commonly used to determine the purity of honey. The test results obtained honey diastase enzyme 3.13 DN and HMF 4.41 mg/kg, these values have met the established quality requirements. The minimum diastase enzyme value is 3. Honey with undetectable diastase enzyme is suspected to be fake honey. There are several factors that affect the diastase enzyme content, including post-harvest honey processing and honey storage temperature. A good harvest time is when the honey is old, if it is young, or when the honey has not been completely covered by wax, it will produce honey with high water content due to the lack of evaporation. Usually, to reduce the water content in honey, heating is done. The optimal storage temperature for honey is at room temperature (26°C), during post-harvest handling, storage, and transport, honey is avoided from direct sun exposure and heat exceeding 28°C and open-air (Ichsan et al., 2022).

Heavy metal content is a critical parameter regarding the safety of honey. The test results showed no detectable content of heavy metals Cadmium (Cd), Mercury (Hg), Arsenic (As), and Lead (Pb). These values fulfill the quality requirements set by the regulator. The content of heavy metals is strongly influenced by the environment from which the honey is produced, honey produced in areas polluted by industrial pollutants, whether from air, water, or soil will

be at risk of heavy metal contamination (Demaku et al., 2023). Riau forest honey is produced from forests that are free from pollutants, so the honey is not contaminated with heavy metals.

Furthermore, the results of microbiological testing obtained the number of total plate numbers (ALT)  $3.0 \times 10^1$  colonies/g and yeast mould  $<10^1$  colonies/g. These values fulfill the requirements of SNI. Microbial contamination in honey comes from primary and secondary sources. Primary sources of microbial contamination may come from pollen, bee digestive tract, dust, air, soil, and nectar. On the other hand, secondary sources come from personal, cross-contamination, equipment, and the environment. Secondary sources of contamination can be controlled through *good manufacturing practices* (GMP) (Snowdon & Cliver, 1996).

Flavonoids (from Latin *flavus* meaning yellow) are a group of naturally occurring heterocyclic compounds that contain oxygen. These compounds are found in plants in free form and bound to sugar components in several glycoside forms. Flavonoids are generally water-soluble and in nature are found in fruits, vegetables, tea, grapes, wine, honey, propolis, nectar and others. Currently, more than 5000 kinds of flavonoids have been identified in various plants (Panche et al., 2016; Ververidis et al., 2007).

**Table 2.** Flavonoid content in honey riau

Parameters	Identification
<i>Luteolin</i>	+
<i>Mangiferin</i>	+
<i>Smiglanin</i>	+
<i>Leucodelphinidin</i>	-
<i>Maltol</i>	+
<i>(-)-epi-afzelechin</i>	-
<i>5,6,7,3-tetrahydroxy-4-methoxyisoflavone</i>	+
<i>Sec-O-glucosylhamaudol</i>	-
<i>Robinetin</i>	-
<i>3-O-B-D-Galacopyanosyl quercetin</i>	+
<i>2,5-dimethyl-7-hydroxychromone</i>	+
<i>Khellol-b-D-glucoside</i>	+
<i>Cnidimol C</i>	+
<i>Norcimifungin</i>	+
<i>Isorhamnetin-3-O-b-rutinoside_1</i>	+
<i>Patuletin-7-O[6-(2-methylbutyryl)]-glucoside</i>	+
<i>Isoophiopogonone A</i>	+
<i>Apigenin-6-C-glucosylglucoside</i>	+
<i>Isoscoparin-3-O-glucopyranoside</i>	+
<i>Schaftoside</i>	+
<i>Cnidimol A</i>	+
<i>3,7-dihydroxy-6-methoxyflacanonol</i>	+
<i>Methyl ophiopogonanone B</i>	+
<i>Pectolarigenine</i>	+
<i>Kusenol C</i>	+
<i>3,5,6-trihydroxy-4',7-dimethoxyflavone</i>	+
<i>Lupinifoline</i>	+
<i>6-methoxy-2-(2-phenylethyl)chromone</i>	-
<i>Chromone</i>	-
<i>Cianidine 3,5-diglucoside_1</i>	-
<i>Adenine</i>	-

<i>Trigoneline</i>	-
<i>Gentiatibetine</i>	-
<i>Salviamiltamide</i>	-
<i>Guvacoline</i>	-
<i>19-epi-3-iso-ajmalicine</i>	-

Description: (-) not detected (+) Detected

The structural diversity of flavonoids is due to the modification of the basic skeleton structure. These modifications are conditioned by various reactions of hydrogenation, hydroxylation, O-methylation of hydroxyl groups, dimerization, or glycosylation of hydroxyl groups (O-glycosides). Depending on the degree of oxidation of the pyran ring, as well as the  $\alpha$ -position of the secondary aromatic ring. Flavonoids can be classified into several subgroups, including flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydro flavonols, and chalcones based on differences in their basic molecular structure (Harborne & Williams, 2000). Table 2 shows the flavonoids contained in Riau forest honey.

The amount of flavonoids in honey can reach 6 mg/kg, while the amount is much higher in pollen (0.5%) and propolis (10%)(Gašić et al., 2017). Based on the test results, the flavonoids detected include *Luteolin*, *Mangiferin*, *Smiglanin*, *maltol*, *isoflavones*, *quercetin*, *Cnidimol C*, *Norcimifungin*, *Apigenin*, *Methyl ophiopogonanone B*, *Pectolarigenin*, *Kusenol C*, *3,5,6-trihydroxy-4',7-dimetoxyflavone* and *Lupinifoline*. The flavonoid content in each honey varies greatly influenced by many factors, including the flower nectar, and the propolis present. (Tomás-Barberán et al., 2001). Riau Forest Honey generally takes its nectar from the sialang tree (Suhesti & Hadinoto, 2015).

Flavonoids have a number of health benefits, including anti-cancer, antioxidant, anti-inflammatory, and anti-viral activities. They also have neuroprotective and cardioprotective effects. These biological activities depend on the type of flavonoid, its mode of action, and its bioavailability. Research shows that in the form of isoflavones, quercetin and luteolin are able to exert a favourable influence on the health of the cardiovascular system through the inhibition of thrombokinase activity, the lowering of LDL cholesterol levels and the prevention of inflammatory activity caused by free radicals (Olas, 2020). The flavonoids in honey can prevent cancer through the mechanism of increasing *caspase 3* activity which has an impact on cancer cell apoptosis (Ahmed & Othman, 2013).

#### 4. CONCLUSION

Forest honey from Riau has good quality and safety, fulfilling the requirements set by SNI. The reducing sugar content needs to be improved with a good storage process. The flavonoid content in Riau forest honey is quite varied, including Luteolin, Mangiferin, Smiglanin, maltol, isoflavones, quercetin, Cnidimol C, Norcimifungin, Apigenin, Methyl ophiopogonanone B, Pectolarigenin, Kusenol C, 3,5,6-trihydroxy-4',7-dimetoxyflavone and Lupinifoline. The type of flavonoids produced is due to the source of nectar and propolis provided. The flavonoid content in honey has potential health benefits.

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