BIOLOGICA NYSSANA
16 (1) June 2025: 207-213
DOI: 10.46793/BiolNyss.16.1.29S

Original Article

A macronutrient composition and physicochemical characteristics of colored Trigona honey from Tesso Nilo National Park, Riau

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Received: March 14, 2025
Revised: May 16, 2025
Accepted: May 16, 2025

Abstract:

Trigona honey, produced by stingless bees, exhibits unique physicochemical characteristics influenced by environmental factors, nectar sources, and processing methods. This study investigates the relationship between honey color variation (yellow, red, and black) and its macronutrient composition and physicochemical properties, including moisture content, fat, protein, carbohydrate, and acidity levels, using standard laboratory methods. The findings reveal significant differences in composition among the samples, with black honey having the lowest moisture content (19.4%) and the highest acidity (134 mL NaOH/kg), suggesting better stability and potential bioactive properties, while yellow honey had the highest moisture content (24.1%), making it more susceptible to fermentation. Carbohydrate content was highest in red honey (37.3%), indicating its potential as an energy source. No protein was detected in any sample, and fat content ranged from 0.21% to 0.64%. The results suggest that darker-colored honey may contain higher levels of phenolic and flavonoid compounds, contributing to enhanced antioxidant and antimicrobial activity. This study underscores the importance of characterizing Trigona honey for quality control, standardization, and potential applications in the food and pharmaceutical industries. Further research is recommended to explore the functional properties of Trigona honey, particularly its bioactive and therapeutic potential.

Key words:
trigona honey, macronutrient composition, honey color, bioactive compounds

Apstrakt:

Makronutritivni sastav i fizičko-hemijske karakteristike obojenog meda Trigona iz Nacionalnog parka Tesso Nilo, Riau

Trigona med, kojeg proizvode pčele bez bodlji, pokazuje jedinstvene fizičko-hemijske karakteristike koje su pod uticajem faktora životne sredine, izvora nektara i metoda prerade. Ova studija istražuje vezu između varijacija u boji meda (žuti, crveni i crni) i njegovog makronutritivnog sastava i fizičko-hemijskih osobina, uključujući sadržaj vlage, masti, proteina, ugljenih hidrata i nivoa kiselosti, korišćenjem standardnih laboratorijskih metoda. Rezultati ukazuju na značajne razlike među uzorcima u pogledu sastava: crni med je imao najniži sadržaj vlage (19,4%) i najviši nivo kiselosti (134 mL NaOH/kg), što ukazuje na bolju stabilnost i potencijalna bioaktivna svojstva, dok je žuti med imao najviši sadržaj vlage (24,1%), što ga čini podložnijim fermentaciji. Sadržaj ugljenih hidrata bio je najviši u crvenom medu (37,3%), što ukazuje na njegov potencijal kao izvor energije. U uzorcima nije potvrđeno prisustvo proteina, dok se sadržaj masti kretao od 0,21% do 0,64%. Rezultati sugerišu da med tamnije boje može sadržati veće količine fenolnih i flavonoidnih jedinjenja, što doprinosi pojačanoj antioksidativnoj i antimikrobnoj aktivnosti. Ova studija naglašava značaj karakterizacije Trigona meda za potrebe kontrole kvaliteta, standardizacije i potencijalne primene u prehrambenoj i farmaceutskoj industriji. Preporučuje se dalje istraživanje funkcionalnih svojstava meda Trigona, posebno njegovog bioaktivnog i terapijskog potencijala.

Ključne reči:
Trigona med, makronutritivni sastav, boja meda, bioaktivna jedinjenja

Introduction

Honey is a natural organic substance produced from flower nectar by bees and is one of the most widely used natural sweeteners. Chemically, honey is defined as a natural food composed primarily of sugars and water, along with minor components such as minerals, vitamins, amino acids, organic acids, flavonoids, other phenolic compounds, and aromatic substances. One type of honey that has gained increasing attention in recent years is Trigona honey, produced by stingless bees of the genus Trigona. This honey exhibits unique characteristics compared to honey produced by Apis bees, particularly in terms of color, taste, viscosity, moisture content, sugar levels, and macronutrient composition, including protein, fat, and carbohydrates (Gadge et al., 2024). Additionally, Trigona honey is known for its high acidity and complex bioactive compound composition, making it an intriguing subject for further research, particularly regarding its potential health benefits (Zulkifli et al., 2023).

Several studies have shown that the chemical composition of honey is significantly influenced by nectar and pollen sources, which affect moisture, sugar profile, and acidity (Fahrul et al., 2022; Andrade Velásquez et al., 2023).

Tesso Nilo National Park in Riau Province is a conservation area with high biodiversity that provides a natural habitat for Trigona bees. The honey produced in this region is widely marketed, particularly in local markets. However, scientific studies on the macronutrient composition of this honey remain limited. Characterizing the chemical composition of honey is essential for determining its quality and potential applications in the food and pharmaceutical industries (Andrade Velásquez et al., 2023). Several studies have shown that the chemical composition of honey is significantly influenced by nectar sources, environmental conditions, and bee species. These variations directly impact honey's nutritional composition and physicochemical properties, including moisture content, carbohydrates, protein, fat, and acidity levels (Gadge et al., 2024).

Honey contains more than 200 bioactive compounds, with sugars as its main component. Fructose is the dominant sugar fraction (38–40%), followed by glucose (30–34%), maltose, and sucrose in smaller amounts. Additionally, honey contains proteins, fats, organic acids, and minerals, with concentrations varying depending on its floral origin (Alvarez-Suarez et al., 2013). Secondary metabolites such as polyphenols, flavonoids, enzymes, and carotenoid pigments are also found in honey and contribute to its biological activities, including antioxidant, antibacterial, and anti-inflammatory properties (Cianciosi et al., 2018). Various studies have demonstrated the potential of honey as a therapeutic agent in wound healing and infection treatment, as well as a nutritional supplement to enhance health (Hadagali & Chua, 2014).

One of the primary distinguishing factors of Trigona honey compared to other types of honey is its color variation, ranging from golden yellow to dark black. The color of honey is influenced by various factors, including the type of nectar collected by bees, the content of phenolic compounds and flavonoids, and oxidation processes during storage (Sawarkar, 2024). Studies have indicated that darker-colored honey generally contains higher antioxidant and mineral levels than lighter-colored honey. This is associated with the high polyphenol and organic acid content, which contributes to honey's chemical stability and biological effectiveness (Luca et al., 2025).

In addition to influencing antioxidant composition, honey color may also be related to its macronutrient content, including moisture, fat, protein, and carbohydrate levels. Honey with high moisture content tends to have lower viscosity and a lighter color, whereas honey with lower moisture content and higher pigment concentration is typically darker and exhibits better stability during storage (Vîjan et al., 2023). However, the direct correlation between honey color and macronutrient composition has not been extensively studied, particularly for Trigona honey from Indonesia.

Although previous studies have suggested that darker-colored honey generally contains higher levels of antioxidants and minerals attributed to polyphenol and organic acid content, such correlations have not been thoroughly examined in Trigona honey from the Tesso Nilo region. This study aims to address that gap by exploring the relationship between color variation and the physicochemical composition specific to this biodiverse area.

Materials and Methods

Collection of Honey Samples

Yellow, red, and black honey were obtained from Tesso Nilo National Park, Riau, using the drip method (**Fig. 1**).

Moisture content test

The moisture and sugar content tests were conducted using the refractometry method with a refractometer. The test was performed by placing a honey sample on the refractometer prism, and the refractive index value was then recorded. The moisture content standard is referred to as SNI 01-3545-2013.

Fig. 1. The appearance of Trigona honey samples obtained from Tesso Nilo National Park, Riau: black, yellow and red (from left to right)

Fat content test

The fat content test was conducted using the Soxhlet extraction method. A 2 g sample was placed in a filter paper and inserted into a fat extraction cartridge. The cartridge was then placed in a pre-weighed fat flask and connected to a Soxhlet apparatus. The extraction cartridge was positioned in the Soxhlet extraction chamber and rinsed with a fat-soluble solvent. The extraction tube was assembled onto the Soxhlet distillation apparatus and heated at 40 °C using an electric heater for 6 hours. During distillation, the fat solvent evaporated and was collected in the extraction chamber. The solvent was then removed to prevent it from returning to the fat flask. Afterward, the fat flask was dried in an oven at 105 °C and subsequently cooled in a desiccator until a constant weight was achieved.

Protein content test

The protein content test was conducted using the Kjeldahl method. In digestion, a 1 g sample was weighed and placed into a Kjeldahl flask. Then, half of a Kjeldahl tablet (selenium) and 2 mL of concentrated H_2SO_4 were added to the tube. The tube containing the solution was inserted into a digestion apparatus and heated at 400 °C for 1 hour until the solution turned transparent green. The solution was then allowed to cool.

After digestion, the solution was transferred into a 100 mL volumetric flask and diluted with distilled water up to the marked line. A 5 mL aliquot of the diluted solution was then pipetted into a distillation apparatus. A solution of 5 mL NaOH (30%) and a few drops of phenolphthalein (PP) indicator was prepared. Additionally, 10 mL of 2% boric acid solution mixed with the PP indicator was used as the receiving solution. The condenser tip was rinsed with distilled water. Finally, the solution was titrated with 0.01 N HCl until a color change was observed.

Carbohydrate content test

A 25 mL sample solution was pipetted and mixed with 25 mL of Luff-Schoorl reagent. The mixture was then heated on a magnetic stirrer with thermal control under reflux conditions for 10 minutes. After heating, the solution was cooled, followed by the addition of 10 mL of 20% potassium iodide solution and 15 mL of 25% sulfuric acid (H_2SO_4) added gradually. Subsequently, a 0.5% starch indicator was added. The solution was then titrated with 0.1 N sodium thiosulfate. When foaming appeared, 1% starch solution was added and homogenized, and the titration continued until the solution turned milky white.

Acidity test

The acidity test was conducted using an acid-base titration method based on SNI 2018 standards. A 10 g honey sample was weighed and placed into a 250 mL Erlenmeyer flask, then dissolved in 75 mL of distilled water. Subsequently, 4–5 drops of phenolphthalein (PP) indicator were added. The honey solution was titrated with 0.1 N NaOH solution until the endpoint was reached, indicated by a stable pink color that remained for 10 seconds.

Statistical analysis

The physicochemical parameters of the three Trigona honey color variants (yellow, red, and black) were compared using one-way Analysis of Variance (ANOVA) and Tukey's post-hoc test to identify specific group differences. All statistical analyses were conducted using IBM SPSS Statistics version 26, with a significance level set at $p<0.05$. However, due to the absence of replication, these analyses are exploratory and should be interpreted cautiously.

Results and discussion

Macronutrient testt

The following are the test results (**Tab. 1**) based on the macronutrient test conducted on red, yellow, and black Trigona honey.

Moisture content

Moisture content is a crucial parameter in honey, influencing its stability, quality, and shelf life. The study results indicate that the moisture content of red, yellow, and black Trigona honey is 22.4%, 24.1%, and 19.4%, respectively. According to Ikhsan et al. (2022), higher moisture levels increase the risk of fermentation due to microbial activity, particularly yeasts, which can degrade honey quality. Moisture content is influenced by environmental humidity,

Table 1. Macronutrient test results of Trigona honey

No	Test Parameters	Trigona Honey Red Colour	Trigona Honey Yellow Colour	Trigona Honey Black Colour
1	Taste	Characteristic sweet honey	Characteristic slightly sour honey	Characteristic slightly bitter honey
2	Aroma	Characteristic honey	Characteristic honey	Characteristic honey
3	Color	Reddish-brown	Yellow	Black
4	Moisture Content	22.4%	24.1%	19.4%
5	Fat	0.21%	0.64%	0.25%
6	Protein	0%	0%	0%
7	Carbohydrates	37.3%	37.1%	33.6%
8	Acidity	24.5	114%	134%

temperature, hive position, and harvesting methods (Kek et al., 2017).

Honey with high moisture content is more susceptible to fermentation, as yeast growth accelerates when moisture levels exceed 20% (da Silva et al., 2024). The yellow Trigona honey exhibited the highest moisture content (24.1%), making it more prone to fermentation than black Trigona honey, which had the lowest moisture content (19.4%). Harvest age is another critical factor affecting moisture content; honey that remains in the hive for an extended period has lower moisture levels due to continuous evaporation (Zaldivar-Ortega et al., 2024).

Environmental factors such as temperature, humidity, hive location, and harvesting techniques significantly influence honey's moisture content. According to Suhri & Bahar (2023), moisture levels also depend on the ripening process within the hive, where prolonged hive storage allows for greater evaporation, reducing moisture content. Furthermore, honey's moisture content directly correlates with its viscosity—higher moisture content leads to lower viscosity, making it more fluid than honey with lower moisture content (Pimentel et al., 2022).

Fat content

The analysis showed that the fat content in red, yellow, and black Trigona honey was 0.21%, 0.64%, and 0.25%, respectively. According to Khalil et al. (2001), stingless bee honey generally contains very low fat levels, making it an insignificant fat source. Hasan et al. (2023) reported that the lipid content in honey could also be influenced by the presence of wax or propolis that mixes with the honey during extraction. Moreover, the harvesting method and filtration process affect the fat content in honey. Although honey cannot be considered a primary dietary fat source, its minor lipid compounds contribute to its biological activity, including antimicrobial and antioxidant properties (Olas, 2020).

Protein content

Proteins in honey are minor components derived from enzymes produced by bees and nectar and pollen collected during honey production. The study found that the protein content in red, yellow, and black Trigona honey was 0.00%, significantly lower than the findings of Kamal et al. (2022), who reported protein levels between 3.9% and 8.5%.

According to Mahani et al. (2025), protein content in honey is primarily influenced by the type of nectar consumed by bees and the environmental conditions in their habitat. Several other factors may contribute to the low protein levels in Trigona honey from Riau, including filtration processes, harvesting methods, and storage duration, which can lead to protein degradation due to reduced enzymatic activity (Hasan et al., 2023). Additionally, Braghini et al. (2020) found that honey protein content can be altered due to thermal processing during handling or prolonged storage.

Carbohydrates content

Carbohydrates are the primary components of honey, consisting mainly of monosaccharides (glucose and fructose), disaccharides (sucrose, maltose), and small amounts of oligosaccharides. The study results indicate that the carbohydrate content of red, yellow, and black Trigona honey is 37.3%, 37.1%, and 33.6%, respectively. Several factors, including moisture content, nectar source, and fermentation or storage processes, influence the variation in carbohydrate content. The sugar profile was predominantly composed of reducing sugars, including fructose and glucose, which this study did not quantify individually. Nonetheless, higher carbohydrate content in red honey may indicate a richer sugar matrix, potentially contributing to its energy value and taste.

According to Manickavasagam et al. (2024), honey stored for extended periods experiences an increase in reducing sugar levels due to the enzymatic activity of invertase, which converts sucrose into glucose and fructose. Additionally, honey's carbohydrate content is affected by seasonal variations. Honey harvested during the rainy season generally has lower carbohydrate content due to higher moisture levels resulting from increased environmental humidity (Susilawati et al., 2023).

Acidity

Acidity is an essential quality parameter in honey, affecting taste, stability, and antibacterial properties. The study results showed that the red, yellow, and black Trigona honey acidity levels were 24.5 mL NaOH/kg, 114 mL NaOH/kg, and 134 mL NaOH/kg, respectively. According to the Indonesian National Standard (SNI) 2021, honey's maximum permissible acidity level is 200 mL NaOH/kg, indicating that all tested honey samples met the quality standards.

According to Evahelda et al. (2017), honey acidity primarily originates from organic acids such as gluconic acid, which is formed through glucose oxidation by the enzyme glucose oxidase. The higher acidity in black Trigona honey may be attributed to its greater phenolic and flavonoid content than other Trigona honey varieties (Hakim et al., 2021). Future investigations should involve targeted polyphenol and flavonoid content analysis, potentially utilizing LC-MS/MS, along with antioxidant activity assays such as DPPH, ABTS, or FRAP to validate the therapeutic claims of darker Trigona honey.

Statistical interpretation of physicochemical differences

To explore the potential differences among the three Trigona honey color variants - red, yellow, and black - one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test was applied to each measured parameter, including moisture, fat, carbohydrate, and acidity levels. However, it is important to acknowledge that each group contained only a single observation, thereby violating the core assumption of ANOVA, which requires within-group variance derived from replicated data. Due to this limitation, the resulting F-ratios and p-values cannot be considered statistically valid. Instead, the statistical outputs should be viewed as exploratory indicators rather than definitive evidence of difference.

Despite this constraint, the descriptive differences observed remain noteworthy: black honey exhibited the lowest moisture content (19.4%) and highest acidity (134 mL NaOH/kg), which are indicative of better chemical stability and possibly higher organic acid and phenolic content; red honey displayed the highest carbohydrate content (37.3%), reflecting its potential as an energy source; and yellow honey showed both the highest moisture content (24.1%) and fat content (0.64%), suggesting a greater susceptibility to fermentation. These trends are consistent with the literature indicating that honey color may correlate with specific physicochemical and bioactive properties. Nevertheless, further studies with adequate biological replication are essential to validate these preliminary observations through statistically robust analysis.

Conclusion

In conclusion, this study highlights that honey color may serve as an indicator of underlying physicochemical properties, potentially reflecting differences in botanical and environmental origin. These findings open avenues for using color variation for preliminary quality assessment in Trigona honey. Future studies should incorporate advanced analytical techniques to explore the specific bioactive compounds and their functional roles.

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