**Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan**
**ISSN 2303-2227 eISSN 2615-594X**
**Accredited by National Journal Accreditation No. 105/E/KPT/2022**

**Vol. 13 No. 3 October 2025, Page: 159-165**
DOI: https://doi.org/10.29244/jipthp.10.2.159-165
Available online at https://journal.ipb.ac.id/index.php/ipthp/index

# Phytochemical and Antioxidant Characteristics of Pineapple-Fermented Trigona Honey from Indonesian Tropical Nectar Sources

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*(Received 24-07-2025; Revised 15-09-2025; Accepted 21-10-2025)*

## ABSTRACT

**Trigona honey is a stingless bee product rich in secondary metabolites and recognized for its antioxidant properties. Pineapple also contains bioactive phytochemicals. Therefore, their combination through fermentation has the potential to produce a functional product. This study aimed to evaluate the phytochemical profile and antioxidant activity of Trigona honey fermented with pineapple for seven days. The honey originated from nectar nutmeg (*Myristica fragrans*), mangosteen (*Garcinia mangostana*), and durian (*Durio zibethinus*). Analyses included pH, moisture, alcohol content, sugar profile, total phenolics, saponins, and antioxidant activity. Fermentation decreased pH due to microbial acid production and increased moisture. Glucose and sucrose declined from 6.13% and 4.83% on day 4 to 3.99% and 1.47% on day 7, respectively. Alcohol content rose to 6.21% w/w, indicating active *Saccharomyces cerevisiae* metabolism. Total phenolic content decreased from 1.508 to 0.541 mg TAE/g on day 4 and rose slightly to 0.707 mg TAE/g on day 7. Saponin content dropped from 573.16 to 147.66 mg KOH/g on day 4, increasing to 429.84 mg KOH/g on day 7. Antioxidant activity rose significantly from 3.17% to 24.36%. These results show that seven day fermentation enhances antioxidant activity while modifying honey's phytochemical properties, making it a potential functional product from tropical Indonesia.**

**Keywords: fermentation, metabolite secondary, pineapple, trigona honey**

## ABSTRAK

**Madu Trigona merupakan produk hasil ternak yang kaya metabolit sekunder dan memiliki aktivitas antioksidan. Nanas juga mengandung fitokimia bioaktif, sehingga kombinasi keduanya melalui fermentasi berpotensi menghasilkan produk fungsional. Penelitian ini bertujuan mengevaluasi profil fitokimia dan aktivitas antioksidan madu Trigona yang difermentasi dengan nanas selama tujuh hari. Madu ini berasal dari lebah yang mengumpulkan nektar dari pohon pala (*Myristica fragrans*), manggis (*Garcinia mangostana*), dan durian (*Durio zibethinus*). Analisis yang dilakukan meliputi pH, kadar air, kadar alkohol, profil gula, total fenolik, saponin, dan aktivitas antioksidan. Fermentasi menyebabkan penurunan pH akibat produksi asam oleh mikroba serta peningkatan kadar air. Kadar glukosa dan sukrosa menurun dari 6,13% dan 4,83% pada hari ke-4 menjadi 3,99% dan 1,47% pada hari ke-7. Kadar alkohol meningkat hingga 6,21% b/b, menandakan aktivitas metabolisme *Saccharomyces cerevisiae*. Total fenolik menurun dari 1,508 menjadi 0,541 mg TAE/g pada hari ke-4 dan sedikit meningkat menjadi 0,707 mg TAE/g pada hari ke-7. Kandungan saponin turun dari 573,16 menjadi 147,66 mg KOH/g pada hari ke-4, lalu meningkat menjadi 429,84 mg KOH/g pada hari ke-7. Aktivitas antioksidan meningkat signifikan dari 3,17% menjadi 24,36%. Hasil menunjukkan bahwa fermentasi selama tujuh hari dapat meningkatkan aktivitas antioksidan dan sifat fitokimia madu, sehingga berpotensi menjadi produk fungsional dari Indonesia tropis.**

**Kata kunci: fermentasi, metabolit sekunder, madu trigona, nanas**

## INTRODUCTION

Trigona honey is a type of honey produced by stingless bees of the genus Trigona (*Trigona sp*.). It is a natural liquid, typically sweet in taste, produced by both wild and cultivated Trigona bees from the nectar of plant flowers or other parts of plants (Badan Standarisasi Nasional 2018). Trigona honey generally has a dark color and a slightly sour taste. Its characteristics differ from those of honey produced by *Apis mellifera*, particularly in terms of color, taste, viscosity, moisture content, and sugar composition (Syamsul *et al.* 2021). The maximum moisture content of Trigona honey is higher than that of conventional honey, with a limit of 27.5%, compared to 22% for Apis mellifera honey (Badan Standarisasi Nasional 2018). The chemical and biological characteristics of honey are largely influenced by the bees foraging sources, particularly the type of nectar collected. As a tropical megabiodiversity hotspot, Indonesia offers a wide range of natural floral resources for Trigona bees. In this study, Trigona honey was sourced from bees foraging on nutmeg (*Myristica fragrans*), mangosteen (*Garcinia mangostana*), and durian (*Durio zibethinus*) three indigenous tropical species known for their high content of bioactive compounds. However, studies specifically examining the characteristics of Trigona honey derived from these floral sources remain limited. According to Rafa *et al.* (2024) Trigona honey contains higher levels of polyphenols than other types of honey, as indicated by its greater total phenolic content (TPC) and total flavonoid content (TFC) compared to *Apis mellifera* honey. Furthermore, Trigona honey contains vitamins that function as antibiotics, antitoxins, and antioxidants, and contribute to enhancing the immune system.

Trigona honey can be processed into fermented honey through a biological process involving microorganisms such as bacteria or yeast. According to Liu *et al.* (2023) fermentation induces alterations in the composition and content of various compounds in honey, including a significant reduction in sugar levels, the breakdown of large proteins into smaller peptides, an increase in polyphenol and vitamin content, and the formation of additional volatile organic compounds. The application of fermentation in honey processing has the potential to extend shelf life and enhance nutritional quality. This process involves chemical transformations of organic compounds under aerobic or anaerobic conditions through the enzymatic activity of microorganisms (Ulviena *et al.* 2023).

Trigona honey can be fermented with pineapple. This type of honey naturally contains lactic acid bacteria (LAB), such as *Lactiplantibacillus plantarum* and *Pediococcus acidilactici*, which act as probiotics (Fatma *et al.* 2022). In addition, honey also harbors yeasts such as *Candida magnoliae*, *Rhodotorula mucilaginosa*, *Zygosaccharomyces mellis*, and *Saccharomyces cerevisiae*. The addition of pineapple increases the moisture content of Trigona honey, thereby accelerating the fermentation process and creating an ideal substrate for microbial activity due to its high glucose and fructose content. Honey with a moisture content of ≥17% is susceptible to fermentation, producing alcohol and carbon dioxide. Although fermentation may reduce shelf life, controlled fermentation can improve the nutritional value, enrich bioactive compounds, and develop distinct flavor, aroma, and health benefits in honey-based products (Zahoor *et al.* 2021). Several studies have shown that fermented Trigona honey contains higher levels of polyphenols, antioxidants, and other bioactive compounds that contribute to cognitive health, mainly due to the phenolic content of the honey (Medina *et al.* 2024).

Phenolic compounds and saponins present in Trigona honey have potential as bioactive constituents beneficial to human health. Phenolics are compounds containing hydroxyl groups attached to aromatic rings and are responsible for the color and flavor of food products. These compounds are commonly found in vegetables, fruits, and legumes, and they contribute significantly to antioxidant activity, which increases in line with higher phenolic content (Agustin *et al.* 2024; Ardila, 2020; Zhang *et al.* 2022). Saponins are a class of triterpene and steroidal glycosides that are water-soluble, foamforming, and exhibit antioxidant, anti-inflammatory, and anticancer activities. Moreover, saponins are known to enhance probiotic growth and inhibit pathogenic bacteria (Ravelliani *et al.* 2021). Therefore, fermented Trigona honey beverages with pineapple addition have the potential to serve as functional drinks with antioxidant activity capable of enhancing the immune system. This study aims to analyze the phytochemical profile and antioxidant activity of fermented Trigona honey supplemented with pineapple, focusing on changes in phenolics, saponins, and antioxidant properties during a seven day fermentation.

## MATERIALS AND METHODS

Trigona honey derived from Indonesian nectar sources such as nutmeg, mangosteen, and durian, was obtained from the Madu Pak Lebah beekeeping farm located in Bogor, West Java, Indonesia, while ripe pineapples (*Ananas comosus*) with bright yellow flesh were purchased from a local traditional market in Ciampea, Bogor, West Java, Indonesia.

**Production of Fermented Trigona Honey with Pineapple.** The fermentation process of Trigona honey with pineapple addition began with peeling the pineapple, followed by cutting the fruit into small pieces (1 × 1 × 1 cm) to ensure homogeneity. The pineapple pieces were then placed into 400 mL plastic containers containing Trigona honey. The experimental formulations were prepared by mixing Trigona honey and pineapple in ratios of 25:75 (TrNa1), 50:50 (TrNa2), and 75:25 (TrNa3), respectively. All formulations were left to ferment at room temperature for 7 days (Nora *et al.* 2022).

**pH.** pH testing was conducted using a pH meter. The pH electrode was rinsed with distilled water and dried using tissue paper. The electrode tip was then immersed in 10 mL of the sample. The measurement was repeated three times, and the electrode was cleaned afterward (Primandasari *et al.* 2021).

**Moisture Determination.** Moisture content analysis of

the Trigona honey beverage with pineapple addition was conducted at the Instrumentation and Analysis Laboratory of Animal Product Technology, IPB University. The moisture content was determined using a refractometer. A sample was placed on the daylight plate of the refractometer, and the refractive index was read at a temperature of 20 °C (Badan Standarisasi Nasional 2018).

**Alcohol Determination.** Alcohol content analysis was conducted at the Integrated Laboratory of IPB University, Baranangsiang. The TrNa1 sample was selected due to its moisture content (70%–80%), which provides a thinner viscosity suitable for functional beverages that require a lighter, more fluid consistency than typical honey. The sample was measured using a GC/FID instrument. A 0.10 g sample was weighed into a 20 mL HS vial, followed by the addition of 5 mL of deionized water. The sample was then incubated, and 400 µL of HS was injected into the GC/FID (Costa *et al.* 2022).

**Sugar Profile Analysis.** Sugar profile analysis was conducted at the Integrated Laboratory of IPB University, Baranangsiang, using HPLC. The TrNa1 sample was selected due to its moisture content (70%–80%), which provides a thinner viscosity suitable for functional beverages that require a lighter, more fluid consistency than typical honey. Dissolved sugars were separated using a Waters Sugar-Pak I column (6.5 × 300 mm) at 80 °C, with a Ca-EDTA solution (50 $mgL^{-1}$) as the elution solvent. Sucrose, glucose, and fructose were used as standards to identify and quantify the dissolved sugars. A series of 10 concentrations ranging from 0.042–41.64 mg $mL^{-1}$ for sucrose, 0.038–38.06 mg $mL^{-1}$ for glucose, and 0.040–40.20 mg $mL^{-1}$ for fructose were used to create calibration curves for the quantification of each sugar in the sample (Li *et al.* 2021).

**Total Phenolic Content.** Total phenolic content analysis was performed at the Integrated Laboratory of IPB University, Baranangsiang. The TrNa1 sample was selected due to its moisture content (70%–80%), which provides a thinner viscosity suitable for functional beverages that require a lighter, more fluid consistency than typical honey. The analysis of total phenolic content (TPC) was conducted using the Folin-Ciocalteu method. A total of 50 µL of the sample was mixed with 0.5 mL of Folin-Ciocalteu reagent, followed by homogenization using a vortex mixer. After a 3-minute reaction time, 400 µL of sodium carbonate solution (75 $gL^{-1}$) was added, and the mixture was vortexed again until homogeneous. The resulting mixture was incubated for 30 minutes at room temperature, after which the absorbance was measured at a wavelength of 765 nm against a blank. All measurements were performed in triplicate. Tannic acid was used as the standard, and TPC values were expressed as mg tannic acid equivalents (TAE) per 100 g of sample (Priyanthi *et al.* 2021).

**Saponin Determination.** Saponin content was determined using a spectrophotometric method. The TrNa1 sample was selected due to its moisture content (70%–80%), which provides a thinner viscosity suitable for functional beverages that require a lighter, more fluid consistency than typical honey. A 0.25 mL sample was added to 1 mL of a reagent mixture (1:1 v/v acetic acid/sulfuric acid). The mixture was vortexed and reacted at 60°C in a water bath for 30 minutes, then cooled. The absorbance of the sample was measured at a wavelength of 527 nm using a spectrophotometer. Oleanolic acid was used as the standard (0–1,000 µg $mL^{-1}$). The total saponin content was expressed in grams per 100 grams as oleanolic acid equivalents (Lim *et al.* 2020).

**Antioxidant Activity.** Antioxidant activity was tested at the Integrated Laboratory of IPB University, Baranangsiang. The TrNa1 sample was selected due to its moisture content (70%–80%), which provides a thinner viscosity suitable for functional beverages that require a lighter, more fluid consistency than typical honey. The Trigona honey beverage with pineapple addition was evaluated for its ability to scavenge the 1,1- diphenyl-2-picrylhydrazyl hydrate (DPPH) radical. A 2 g sample was dissolved in 10 mL of distilled water, then homogenized and filtered. Next, 0.75 mL of the sample solution was mixed with 2.25 mL of 0.1 mmol L -1 DPPH solution in methanol. The solutions were homogenized and incubated at room temperature, protected from light, for 60 minutes. Absorbance was measured at a wavelength of 517 nm using a spectrophotometer (Majewska *et al.* 2020).

### Data Analysis

This study was designed using a completely randomized design with a factorial pattern, consisting of two factors: the percentage of pineapple addition to Trigona honey and the fermentation duration. The treatments included formulations of Trigona honey and pineapple at ratios of 25:75 (TrNa1), 50:50 (TrNa2), and 75:25 (TrNa3), with three replicates for each treatment. Data analysis was performed using Microsoft Excel and R Studio software. The statistical test used was analysis of variance (ANOVA). If a significant effect was observed, post hoc testing was carried out using the Tukey test.

## RESULTS AND DISCUSSION

### pH

Based on the pH analysis of fermented Trigona honey, a notable interaction was observed between fermentation duration and pineapple additions (Table 1). The data revealed a marked decline in pH values throughout the fermentation process, indicating active microbial metabolism, particularly by lactic acid bacteria and yeasts, which contribute to the production of organic acids. As reported by Nurhayati *et al.* (2020), the reduction in pH is a result of microbial metabolic pathways in which sucrose is metabolized into ethanol, while bacteria synthesize organic acids. The accumulation of these acids facilitates the dissociation of protons, thereby decreasing the pH. Variations in formulation may influence the composition and predominance of specific microbial communities, thus altering fermentation dynamics and the extent of acidification. This substantial pH reduction could potentially influence the sensory attributes of honey and enhance or modify the bioactive potential of the final fermented product (Putra *et al.* 2024).

Table 1. pH characteristics of pineapple-fermented Trigona honey

| Fermentation Duration | Variables | | |
|---|---|---|---|
| | TrNa1 | TrNa2 | TrNa3 |
| 1 | 3.49 ± 0.12a | 3.50 ± 0.00 ab | 3.33 ± 0.06 bcdef |
| 2 | 3.28 ± 0.06 abc | 3.20 ± 0.10 defg | 3.17 ± 0.15 efg |
| 3 | 3.28 ± 0.06 bcde | 3.17 ± 0.06 efg | 3.30 ± 0.20 bcdefg |
| 4 | 3.30 ± 0.06 efg | 3.33 ± 0.06 bcdef | 3.43 ± 0.12 abcd |
| 5 | 3.28 ± 0.00 fg | 3.30 ± 0.00 bcdefg | 3.43 ± 0.06 abcd |
| 6 | 3.20 ± 0.06efg | 3.23 ± 0.06 cdefg | 3.23 ± 012 cdefg |
| 7 | 3.16 ± 0.06 g | 3.17 ± 0.06 efg | 3.23 ± 0.06 cdefg |

Note: Values are mean ± SD of three replicates.Values with different superscript letter sin the same row are significantly different ($p \leq 0.05$). TrNa1 = Trigona honey with pineapple (25:75); TrNa2 = Trigona honey with pineapple (50:50); TrNa3 = Trigona honey with pineapple (75:25)

Table 2. Moisture determination of pineapple-fermented Trigona honey

| Fermentation Duration | Variables | | |
|---|---|---|---|
| | TrNa1 | TrNa2 | TrNa3 |
| 1 | 75.43 ± 0.25 c | 57.20 ± 1.20 d | 43.00 ± 2.65 e |
| 2 | 75.73 ± 0.21 c | 57.50 ± 1.30 d | 37.00 ± 1.00 f |
| 3 | 76.17 ± 0.35 bc | 57.83 ± 1.23 d | 37.83 ± 0.29 f |
| 4 | 74.87 ± 0.85 c | 57.07 ± 1.40 d | 36.67 ± 0.58 f |
| 5 | 78.17 ± 1.24 abc | 56.67 ± 0.85 d | 37.33 ± 0.58 f |
| 6 | 81.10 ± 2.42 a | 58.17 ± 1.35 d | 37.67 ± 0.58 f |
| 7 | 79.73 ± 2.30 ab | 59.97 ± 1.05 d | 37.67 ± 0.58 f |

Note: Values are mean ± SD of three replicates.Values with different superscript letter sin the same row are significantly different ($p \leq 0.05$). TrNa1 = Trigona honey with pineapple (25:75); TrNa2 = Trigona honey with pineapple (50:50); TrNa3 = Trigona honey with pineapple (75:25)

**Moisture Determination**

Moisture determination in fermented Trigona honey revealed a significant interaction between fermentation duration and pineapple supplementation. As illustrated in Table 2, the water content increased proportionally with the amount of pineapple added during fermentation. This rise is attributed to the inherently high moisture content of pineapple, which contributes directly to the overall water content in the fermented product. Utari *et al.* (2024) reported that microbial metabolism of carbohydrates during fermentation not only serves as an energy source but also leads to elevated water content as a byproduct of metabolic activity.

Formulations enriched with greater quantities of pineapple exhibited increased moisture determination, which may influence the viscosity and overall texture of the final product. Given that pineapple contains approximately 85.3% water, its incorporation into the fermentation matrix significantly contributes to the moisture level of the end product (Indriaty *et al.* 2016). Moreover, microbial processes such as polysaccharide hydrolysis or the formation of volatile metabolites can alter the water balance during fermentation. As noted by Riyana *et al.* (2022), honey with water content exceeding 22% becomes susceptible to spontaneous fermentation during storage due to the activity of *Zygosaccharomyces*. This yeast metabolizes sugars to produce ethanol and carbon dioxide, which are subsequently oxidized into water and acetic acid, thereby contributing to a tangier, more acidic flavor profile.

**Alcohol Determination**

As presented in Table 3, the ethanol content in Trigona honey fermented with pineapple increased markedly, reaching 6.21% w w $^{-1}$ by the seventh day of fermentation. This concentration remains well below the maximum allowable limit of 24% w/w stipulated by Indonesia's National Agency of Drug and Food Control (BPOM) under Regulation No. 14 of 2016 for alcoholic beverages (BPOM 2016), including fermented honey. The rise in ethanol is primarily the result of yeast-driven anaerobic fermentation, during which *Saccharomyces cerevisiae* metabolizes fermentable sugars via the glycolytic pathway, producing ethanol as the major end product (Meisela *et al.* 2016). The concurrent decline in sugar content indicates its active consumption as a carbon and energy source to sustain yeast proliferation and metabolic activity throughout the fermentation process.

**Sugar Profile**

As presented in Table 4, the concentrations of glucose and sucrose in Trigona honey with added pineapple declined progressively during fermentation. By the fourth day, glucose and sucrose levels decreased to 6.13% and 4.83%, respectively, and continued to drop to 3.99% and 1.47% by the seventh day. This downward trend reflects

Table 3. Alcohol determination of pineapple-fermented Trigona honey

| Sample | Alcohol (%) w/w | | |
|---|---|---|---|
| | Methanol | Ethanol | Iso Alcohol |
| Control | n.d | 0.07 | n.d |
| TrNa1_D4 | n.d | 14.33 | n.d |
| TrNa1_D7 | 0.04 | 62.06 | n.d |

Note: Control is Trigona honey without pineapple; TrNa_D4 = Trigona honey with pineapple in 4th day of fermentation; TrNa_D7 = Trigona honey with pineapple in 7th day of fermentation; and n.d is not detected

Table 4. Sugar profile of pineapple-fermented Trigona honey

| Sample | Sugar Profile % | | |
|---|---|---|---|
| | Glucose | Sucrose | Fructose |
| Control | 20.48 | 13.11 | <0.08 |
| TrNa1_D4 | 6.13 | 4.83 | <0.08 |
| TrNa1_D7 | 3.99 | 1.47 | <0.08 |

Note: Control is Trigona honey without pineapple; TrNa_D4 = Trigona honey with pineapple in 4th day of fermentation; TrNa_D7 = Trigona honey with pineapple in 7th day of fermentation; and n.d is not detected

the active involvement of fermentative microorganisms in metabolizing these sugars into secondary metabolites such as ethanol and organic acids. Conversely, fructose levels remained relatively unchanged throughout the fermentation period, consistently measured at below 0.08%. According to Puspaningrum *et al.* (2022), the reduction in sugar concentrations during fermentation is primarily driven by yeast activity, especially their role in converting sugars into alcohol. The decline in sucrose is further enhanced by its enzymatic hydrolysis into glucose through the action of invertase, a process that is accelerated under acidic conditions. Sugars serve as the principal carbon sources for microbial metabolism; hence, those available in the fermentation matrix are utilized as substrates and are biochemically transformed into ethanol, carbon dioxide ($CO_2$), and carbonic acid via fermentative metabolic pathways.

## Total Phenolic Content, Saponin, and Antioxidant Activity

**Total Phenolic Content.** The total phenolic content (TPC) of Trigona honey exhibited a notable fluctuation during the fermentation process. Initially measured at 1.508 $mgTAEg^{-1}$ in the unfermented honey, the TPC significantly decreased to 0.541 $mgTAEg^{-1}$ after four days of fermentation, followed by a slight increase to 0.707 $mgTAEg^{-1}$ after seven days. These variations are likely attributable to the enzymatic activity of fermentative microorganisms, which can degrade certain matrix components and thereby facilitate the release or transformation of phenolic compounds. According to Puspaningrum *et al.* (2022), microbial enzymes can hydrolyze specific materials within the matrix, promoting the generation of new phenolic derivatives. Additionally, the interaction of phenolics with macromolecules such as proteins or polysaccharides within the substrate may result in their temporary binding. Naturally, many phenolic compounds exist in glycosylated forms. During fermentation, polyphenols may also undergo polymerization, particularly in the early phases of the process (Marco *et al.* 2025), which contributes to fluctuations in TPC.

**Saponin.** Similarly, the saponin concentration showed dynamic changes. The initial content of 573.16 $mgKOHg^{-1}$ in pure Trigona honey dropped substantially to 147.66 $mgKOHg^{-1}$ after four days of fermentation, before rising again to 429.84 $mgKOHg^{-1}$ by the seventh day. The early reduction in saponin levels may result from the hydrolytic activity of glycosidase enzymes that cleave glycosidic bonds within saponin molecules. As reported by Sharma *et al.* (2023), food processing often induces such enzymatic degradation, leading to the formation of prosapogenins, free aglycones, and sugar residues, thus altering the chemical structure and reducing saponin content. The subsequent increase in saponins by day seven could be due to the release of previously bound saponins within the honey matrix or potentially the biosynthetic activity of the fermenting microorganisms. Najihudin *et al.* (2023) noted that the structure of saponins is susceptible to enzymatic transformation during processing and storage, particularly in the glycosidic linkages between sugar moieties and aglycone cores.

**Antioxidant Activity.** In terms of antioxidant activity, the fermentation process resulted in a marked enhancement. The antioxidant capacity, measured via DPPH assay, increased from 3.17% in unfermented honey to 12.17% after four days and reached 24.36% after seven days of fermentation. Despite this improvement, the observed values remain significantly lower than those reported by Fu *et al.* (2023), who recorded an antioxidant activity of 83.17% on the 16th day of fermentation. This comparison suggests that a longer fermentation period may further optimize antioxidant efficacy. The progressive increase in antioxidant potential is likely linked to the transformation of phenolic compounds and the accumulation of bioactive metabolites, including phenolic acids and peptides, synthesized by fermentative microbes (Munoz *et al.* 2023; Hassmy *et al.* 2017).

Table 5. Total phenolic content, saponin, and antioxidant activity of pineapple-fermented Trigona honey

| Sample | Total Phenolics (mgTAE/g) | Saponin (mgKOH/g) | Aktivitas Antioksidan (%) |
|---|---|---|---|
| Control | 1.508 | 573.16 | 3.17 |
| TrNa1_D4 | 0.541 | 147.66 | 12.17 |
| TrNa1_D7 | 0.707 | 429.84 | 24.36 |

Note: Control is Trigona honey without pineapple; TrNa_D4 = Trigona honey with pineapple in 4th day of fermentation; TrNa_D7 = Trigona honey with pineapple in 7th day of fermentation; and n.d is not detected

## CONCLUSION

The present study demonstrated that the fermentation of Trigona honey supplemented with pineapple induced substantial alterations in physicochemical and bioactive properties, including pH, moisture content, ethanol concentration, sugar composition, and levels of bioactive constituents. The metabolic activity of Saccharomyces cerevisiae facilitated the bioconversion of sugars into ethanol, while the antioxidant capacity increased, likely due to the liberation of phenolic compounds and the accumulation of secondary metabolites. The observed variations in phenolic and saponin content are indicative of ongoing degradation, biotransformation, and potentially de novo biosynthesis by fermentative microbial populations. Seven-day fermentation yielded the highest antioxidant activity and altered phytochemical properties, indicating its potential as a functional product from tropical Indonesia.

## ACKNOWLEDGEMENT

This research was funded by the Ministry of Research, Technology, and Higher Education through the National Collaboration Research (Ri-Na) scheme of IPB University.

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