Food Research 8 (5) : 8 - 12 (October 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Physicochemical analysis of trigona honey produced by *Tetragonula biroi* in Soppeng Regency, Indonesia

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Article history:
Received: 28 May 2022
Received in revised form: 12 July 2022
Accepted: 4 May 2023
Available Online: 2 September 2024

Keywords:
Trigona honey,
Stingless bee honey,
Physicochemical

DOI:
https://doi.org/10.26656/fr.2017.8(5).281

Abstract

Indonesia is a country with a tropical climate that is rich in biodiversity. Various types of bees are discovered in the country, including the trigona bees, whose honey offers numerous health benefits. This study aimed to analyze the nutritional and phytochemical content of trigona honey produced by *Tetragonula biroi* in Soppeng Regency, South Sulawesi, Indonesia. The honey samples are extracted directly from the hives, filtered and examined through laboratory tests. The results of this study showed that trigona honey from Soppeng has a low pH (4.5) and a high vitamin C content compared to other vitamins. The calcium content is higher than magnesium and zinc, while its high polyphenols contain flavonoids and antioxidants. The water content of 26.67% is indicated in trigona honey, which is within the acceptable range of the Indonesian National Standard (SNI). The sugar content of trigone honey indicates 6.99% w/w glucose, 12.96% w/w fructose and a fructose/glucose ratio of 1.85.

1. Introduction

Indonesia is the largest archipelago in the world consisting of more than 17,000 islands. Its tropical climate has blessed the country with rich flora and fauna diversity. A large variety of bees can be found in Indonesia, including stingless bees in Soppeng, a regency in South Sulawesi Province, whose natural conditions are relatively free from air pollution.

Trigona honey is produced by stingless bees, known as kelulut bees, trigona bees, stingless bees, or Melliponia bees (Yaacob *et al*., 2018). Trigona bees consist of several species, including *Tetragonula biroi* which can be found in Soppeng Regency, South Sulawesi, Indonesia. Trigona honey has a sweet and sour taste that distinguishes it from other types of honey. It also has a thinner consistency and contains more bee bread. The nutritional content of trigona honey is comparable to apis honey. The total phenol of trigona honey is higher than apis honey (Kek *et al*., 2014). Research on three types of honey with similar feed source, namely rubber trees, found that apis honey and trigona honey contain saponins, phenols and flavonoids (Adalina *et al*., 2020).

Trigona honey offers various health benefits. It is a chemopreventive agent in rats induced by colorectal cancer (Yazan *et al*., 2016). Trigona honey is also effective in inhibiting the growth of harmful bacteria in

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

wounds, increasing cytokine expression and accelerating the wound healing process (Medeiros *et al*., 2016). It also reduces obesity indicators, such as body mass index and fat index and displays hepatoprotective potential (Mohd Rafie *et al*., 2018). Trigona honey from 6 different plant species is suitable for the treatment of diabetes mellitus since it reduces amylase and glycosidation (Ali, 2020). This study aimed to analyze the nutritional and phytochemical content of trigona honey produced by *Tetragonula biroi* in Soppeng Regency, South Sulawesi, Indonesia.

2. Materials and methods

2.1 Sample collection

The trigona honey samples in the study were obtained from Soppeng Regency, which is a highland region. The area is less polluted since it is dominated by rice fields, plantations and residential areas. The honey samples were obtained from multiflora plants such as coconut, gamal and cashew. The samples were extracted from the beehives and manually squeezed while ensuring sterility to minimize contact through the use of disposable gloves. The honey was subsequently filtered using clean cloths to remove dirt.

2.2 Laboratory tests

The honey was placed in a bottle and taken to the Animal Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University Makassar for content and phytochemical examination. The sample was also sent to the Integrated Research and Testing Laboratory of Gajah Mada University (LPPT UGM) in Yogyakarta for examination of glucose, fructose and fructooligosaccharides (FOS) levels.

2.2.1 Total polyphenol

The total phenolic component of the honey samples was tested using Folin-Ciocalteu reagent with gallic acid as standard. A mixture of 10 mL methanol, 0.5 mL of Folin Ciocalteu reagent dissolved in water, and 0.5 mL of 7.5% $NaHCO_3$ were used. The samples were subsequently incubated at 45℃ for 60 mins and centrifuged. The results were measured on a spectrophotometer with a wavelength of 725 nm (Syamsul *et al*., 2020).

2.2.2 Flavonoid

One gram of the sample was dissolved with 10 mL of distilled water for 24 hrs. Approximately 2 mL of the solution was added to 100 µL of 10% $AlCl_3$ and 100 µL of 1 M sodium acetate. The mixture was incubated for 30 mins and the absorbance was measured at a wavelength of 430 nm. Subsequently, standard curves were created using quercetin.

2.2.3 Vitamin C

Approximately 10 g of the sample were added to 100 mL of aquadest. The mixture was subsequently shaken and left for 30 mins. After filtering, 25 mL of filtrates were extracted and added with 2 mL of 1% starch and 20 mL of distilled water, followed by titration with 0.01 N iodine. The results listed on the tool were subsequently recorded.

2.2.4 Vitamin A (Beta carotene)

Approximately 1 g of the sample was added with 10 mL of acetone, extracted, and filtered using Whatman filter paper no.1. The content of beta carotene pigment was measured using a spectrophotometer with a wavelength of 460 nm.

2.2.5 Measurement of glucose, fructose and fructooligosaccharides

The measurement of glucose, fructose and fructooligosaccharides was carried out using high-performance liquid chromatography (HPLC) method (Lindqvist *et al*., 2018). The extracted sample was added with water until it reached 5 mL and then centrifuged for 5 mins. The mixture was filtered using millex 0.45 uM, diluted 40× and injected into 20 L HPLC. After a temporary wait, the results listed on the screen were subsequently recorded.

3. Results and discussion

Trigona honey produced by *Tetragonula biroi* in Soppeng Regency contains 605.65 mg/kg polyphenols (Table 1), whereas, in previous studies, Trigona honey from Bone Regency contained 133.52 ppm polyphenols (Syamsul *et al*., 2020), Trigona honey from Masamba contained 106 mg/100 g polyphenols (Syam *et al*., 2016). Flavonoids and phenolic acids are the most common types of polyphenols in honey. The types of polyphenols discovered in stingless bee honey from Brazil include quercetin, gallic acid, coumaric acid and vanillic acid (Jibril *et al*., 2019).

Many studies have presented the health benefits of polyphenols (Grosso, 2018). Polyphenols have been discovered to be effective in losing weight among patients with cardiovascular risks such as obesity (Guo *et al*., 2017). Polyphenols are also inversely related to hypertension (Godos *et al*., 2017), while other studies indicate the role of polyphenols as anti-inflammatory drugs, anti-diabetic, anti-cancer, anti-inflammatory, anti-aging, and hepatoprotective (Ganesan and Xu, 2017).

Trigona honey contains vitamins and minerals, in the

https://doi.org/10.26656/fr.2017.8(5).281

form of vitamin C (196.97 mg/kg) and beta carotene (7.93 mg/kg), as well as minerals such as calcium (301.12 mg/kg), magnesium (237.71 mg/kg) and zinc (7.42 mg/kg) (Table 1). It is deemed good for health since it helps supply the needed vitamins and minerals for the body.

Table 1. Nutritional and phytochemical content of Trigona honey from Soppeng-South Sulawesi, Indonesia

No	Parameter	Unit	Rate
1	Water	(%)	26.67
2	Ash	(%)	1.31
3	Crude Protein	(%)	0.06
4	Crude Fat	(%)	0.3
5	Carbohydrate	(%)	61.66
6	Total Sugar	(%)	49.55
7	Total Energy	kcal/kg	2679
8	Calcium (Ca)	mg/kg	301.12
9	Magnesium (Mg)	mg/kg	237.71
10	Zinc	mg/kg	7.42
11	Vitamin C	mg/kg	196.97
12	Beta Carotene	mg/kg	7.93
13	Polyphenol	mg/kg	605.65
14	Flavonoids	mg/kg	93.57
15	Antioxidant (IC_{50})	ppm	113.27
16	pH		4.5

The acidic nature of trigona honey, with a pH of 4.5 (Table 1), indicates that it is good enough to inhibit microorganisms. Its high acidity is not caused by the fermentation process, but due to the free acid content, acid minerals and amino acids (Adalina *et al*., 2020). Several studies have also proven the antibacterial properties of trigona honey, suggesting its effectiveness in inhibiting bacterial growth in wounds, increasing cytokine expression and accelerating the healing process (Medeiros *et al*., 2016). Trigona honey displays high antibacterial and antibiofilm effectiveness against the growth of *Pseudomonas aeruginosa* and *Streptococcus pyogenes* bacteria (Al-kafaween *et al*., 2020)

This study discovered the water content of 26.67% in trigona honey (Table 1), while a study on stingless bee honey in Bazil indicated that the water content of 23.9-28.9 g/100 g (De Sousa *et al*., 2016). The water content in trigona honey is also within the Indonesian National Standard (SNI) honey standard 8664-2018, with a maximum of 27.5% (Badan Standardidasi Nasional (BSN), 2018).

The main sugar content in honey is monosaccharides (glucose and fructose), hence they are able to be quickly transported into the blood and used as a source of energy. Trigona honey indicates glucose content of 70.17 ppm or 6.99% w/w with fructose and fructooligosaccharides of 130.04 ppm or 12.96%w/w (Table 2). A daily dose of 20 g of honey can meet 3% of daily energy requirements (Bogdanov, 2016). Regional conditions, climate and the type of bee food affect the composition of sugar. High humidity encourages the production of a large amount of nectar with low sugar content. On the other hand, dry air causes low nectar production but high sugar content (Adalina *et al*., 2020). This study discovered fructose/glucose ratio of 1.85 (Table 2), while a study in Brazil indicated F/G ratio of monofloral stingless bee honey of 1.1-1.5. This ratio directly affects the sweetness of honey since fructose is sweeter than glucose (De Sousa *et al*., 2016).

Table 2. Sugar content (glucose and fructose+FOS) Trigona honey from Soppeng-South Sulawesi, Indonesia

No	Parameter	Unit	Amount of Content
1	Glucose	ppm	70.17
2	Fructose + FOS	ppm	130.04
3	Glucose	% w/w	6.99
4	Fructose + FOS	% w/w	12.96
5	Fructose/Glucose ratio		1.85

Trigona honey is also discovered to be good for the intestines since it contains fructooligosaccharides (FOS), which are prebiotics supporting the growth of good bacteria in the intestines. Prebiotics are food ingredients or compounds contained in food that cannot be digested by the body, particularly by pancreatic amylase enzymes or brush border enzymes, such as dextrinase and glucoamylase. Prebiotics are beneficial for the body to support the growth of gut microbiota, especially in the intestines that have lost the role of lactobacilli and bifidobacteria (Mohan *et al*., 2017). The increasing growth of these two bacteria causes an increase in short-chain fatty acids (SCFA) such as acetate, butyrate and propionate. SCFA plays a role in maintaining the integrity of the epithelial barrier by regulating tight junction proteins (claudin-1, occludin and Zonula occludens-1), thereby preventing bacterial translocation and inflammatory reactions due to increased lipopolysaccharide (LPS) (Sivamaruthi *et al*., 2019).

4. Conclusion

Trigona honey produced by *Tetragonula biroi* in Soppeng has a low pH and a high vitamin C content compared to other vitamins. Its calcium content is higher than magnesium and zinc. The trigona honey in Soppeng also displays a high polyphenol content and water content of 26.67%, within the acceptable range of the Indonesian National Standard (SNI).

https://doi.org/10.26656/fr.2017.8(5).281

Conflict of interest

The authors declare that there is no conflict of interest in the conduct of this study.

Acknowledgements

The authors would like to express gratitude to the Animal Feed Chemistry Laboratory (Faculty of Animal Husbandry, Hasanuddin University, Makassar, Indonesia) and the Integrated Research and Testing Laboratory (Gajah Mada University (LPPT UGM), Yogjakarta, Indonesia) for the facilities provided for the study.

References

Adalina, Y., Kusmiati, E. and Pudjiani, M. (2020). Phytochemical test and physical chemical properties of rubber honey from three types of bees (*Apis mellifera, Apis dorsata* and *Trigona itama*). *IOP Conference Series: Materials Science and Engineering*, 935, 012007. https://doi.org/10.1088/1757-899X/935/1/012007

Ali, H., Abu Bakar, M.F., Majid, M., Muhammad, N. and Lim, S.Y. (2020). *In vitro* anti-diabetic activity of stingless bee honey from different botanical origins. *Food Research*, 4(5), 1421-1426. https://doi.org/10.26656/fr.2017.4(5).411

Al-kafaween, M.A., Hilmi, A.B.M., Jaffar, N., Al-Jamal, H.A.N., Zahri, M.K. and Jibril, F.I. (2020). Antibacterial and Antibiofilm activities of Malaysian Trigona honey against *Pseudomonas aeruginosa* ATCC 10145 and *Streptococcus pyogenes* ATCC 19615. *Jordan Journal of Biological Science*, 13(1), 69-76.

Badan Standardidasi Nasional (BSN) (2018). Madu (SNI 8664: 2018). Jakarta, Indonesia: BSN. [In Bahsa Indonesia].

Bogdanov, S. (2016). Honey as nutrient and functional food. Retrieved from Bee Hexagon website: https://www.bee-hexagon.net/app/download/11112227973/8HoneyNutrientFunctionalReview.pdf?t=1609254889

De Sousa, J.M.B., de Souza, E.L., Marques, G., de Toledo Benassi, M., Gullón, B., Pintado, M.M. and Magnani, M. (2016). Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region. *LWT-Food Science and Technology*, 65, 645-651. https://doi.org/10.1016/j.lwt.2015.08.058

Ganesan, K. and Xu, B. (2017). A critical review on polyphenols and health benefits of black soybeans. *Nutrients*, 9(5), 455-471. https://doi.org/10.3390/nu9050455

Godos, J., Sinatra, D., Blanco, I., Mulè, S., La Verde, M. and Marranzano, M. (2017). Association between dietary phenolic acids and hypertension in a Mediterranean cohort. *Nutrients*, 9(10), 1069-1081. https://doi.org/10.3390/nu9101069

Grosso, G. (2018). Effects of polyphenol-rich foods on human health. *Nutrients*, 10(8), 1089-1094. https://doi.org/10.3390/nu10081089

Guo, X., Tresserra-Rimbau, A., Estruch, R., Martínez-González, M.A., Medina-Remón, A., Fitó, M. and Lamuela-Raventós, R.M. (2017). Polyphenol levels are inversely correlated with body weight and obesity in an elderly population after 5 years of follow up (the randomised PREDIMED study). *Nutrients*, 9(5), 452-470. https://doi.org/10.3390/nu9050452

Jibril, F.I., Hilmi, A.B.M. and Manivannan, L. (2019). Isolation and characterization of polyphenols in natural honey for the treatment of human diseases. *Bulletin of the National Research Centre*, 43, 4. https://doi.org/10.1186/s42269-019-0044-7

Kek, S.P., Chin, N.L., Yusof, Y.A., Tan, S.W. and Chua, L.S. (2014). Total phenolic contents and colour intensity of Malaysian honeys from the *Apis* spp. and *Trigona* spp. bees. *Agriculture and Agricultural Science Procedia*, 2, 150-155. https://doi.org/10.1016/j.aaspro.2014.11.022

Lindqvist, D.N., Pedersen, H.Æ. and Rasmussen, L.H. (2018). A novel technique for determination of the fructose, glucose and sucrose distribution in nectar from orchids by HPLC-ELSD. *Journal of Chromatography B*, 1081-1082, 126-130. https://doi.org/10.1016/j.jchromb.2018.02.019

Medeiros, V.D.F.L.P., Azevedo, Í.M., Rêgo, A.C.M., Egito, E.S.T.D., Araújo-Filho, I. and Medeiros, A.C. (2016). Antibacterial properties and healing effects of *Melipona scutellaris* honey in MRSA-infected wounds of rats. *Acta Cirúrgica Brasileira*, 31, 327-332. https://doi.org/10.1590/S0102-865020160050000006

Mohan, A., Quek, S.Y., Gutierrez-Maddox, N., Gao, Y. and Shu, Q. (2017). Effect of honey in improving the gut microbial balance. *Food Quality and Safety*, 1 (2), 107-115. https://doi.org/10.1093/fqsafe/fyx015

Mohd Rafie, A.Z., Syahir, A., Wan Ahmad, W.A.N., Mustafa, M.Z. and Mariatulqabtiah, A.R. (2018). Supplementation of stingless bee honey from *Heterotrigona itama* improves antiobesity parameters in high-fat diet induced obese rat model. *Evidence-Based Complementary and Alternative Medicine*, 2018, 6371582. https://doi.org/10.1155/2018/6371582

Sivamaruthi, B.S., Kesika, P., Suganthy, N. and Chaiyasut, C. (2019). A review on role of microbiome in obesity and antiobesity properties of probiotic supplements. *BioMed Research International*, 2019, 3291367. https://doi.org/10.1155/2019/3291367

Syam, Y., Usman, A.N., Natzir, R., Rahardjo, S.P., Hatta, M., Sjattar, E.L. and Sa'na, M. (2016). Nutrition and pH of Trigona Honey from Masamba, South Sulawesi, Indonesia. *International Journal of Sciences: Basic and Applied Research*, 27(1), 32-36.

Syamsul, T. D., Natzir, R., As'ad, S., Hadju, V., Hatta, M., Pratiwi, S. and Tenriola, A. (2020). The Chemical Composition of Trigona Honey in Bone, South Sulawesi. *Indian Journal of Public Health Research and Development,* 11(9), 258-262.

Yaacob, M., Rajab, N.F., Shahar, S. and Sharif, R. (2018). Stingless bee honey and its potential value: a systematic review. *Food Research,* 2(2), 124-133. https://doi.org/10.26656/fr.2017.2(2).212

Yazan, S.L., Muhamad Zali, M.F.S., Mohd Ali, R., Zainal, N.A., Esa, N., Sapuan, S., Ong, Y.S., Tor, Y.S., Gopalsamy, B., Voon, F.L. and Syed Alwi, S.S. (2016). Chemopreventive properties and toxicity of Kelulut honey in Sprague Dawley rats induced with Azoxymethane. *BioMed Research International*, 2016, 4036926. https://doi.org/10.1155/2016/4036926

