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Evaluation of stingless bee honey quality (*Tetragonula laeviceps*) based on their physicochemical from different origins

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Abstract. *Agussalim, Sabir A, Sahlan M, Agus A. 2023. Evaluation of stingless bee honey quality (*Tetragonula laeviceps*) based on their physicochemical from different origins. Biodiversitas 24: 2134-2142.* Honey is a natural food mainly composed of sugars and other components such as enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, and aromatic substances. This study aimed to determine the quality of stingless bee honey (*Tetragonula laeviceps*) based on their physicochemical from different origins of Lombok, Magelang, and Purworejo (Indonesia). The honey was physicochemically analyzed on its moisture, fat, protein (by proximate analysis), energy (bomb calory meter), flavonoid, phenolic, and DPPH antioxidant (spectrophotometer Uv-Vis), organic and amino acids (high-performance liquid chromatography). Honey from Lombok was lower in moisture content ($p<0.01$) and higher in protein, energy, citric acid, propionic acid, phenolic, flavonoid, DPPH antioxidant, amino acids (aspartic acid, glutamic acid, asparagine, glycine, and threonine) than honey from Magelang and Purworejo ($p<0.01$). Also, the citric acid of honey from Lombok and Magelang was similar. It was higher than citric acid honey from Purworejo ($p<0.01$). The lactic acid of honey from Lombok and Purworejo was similar. Both were higher than the lactic acid of honey from Magelang ($p<0.05$), but not on fat, butyric acid, and other amino acids. Thus, *T. laeviceps* honey from Lombok has a better quality based on physicochemical than honey Magelang and Purworejo. Honey from *T. laeviceps* is rich in phenolic and flavonoids to support the higher antioxidant.

Keywords: Amino acids, antioxidant activity, honey, nectar, organic acids

INTRODUCTION

Stingless bee have been studied around the world are 500 species, and more than 100 has not been studied (Michener 2013), but in Indonesia is a minimum of 46 species (Kahono et al. 2018). However, the recently reported two stingless bee species found in Baluran National Park (East Java) are *Lepidotrigona terminata* and *T. laeviceps* (Rachmawati et al. 2022). One species is *Tetragonula laeviceps*, which has been meliponiculture by the beekeepers in the garden and forest to produce honey, pot-pollen, and propolis. In Indonesia, it is found chiefly nesting in bamboo. Stingless bee *T. laeviceps* has a good adaptation to the new environmental condition and can be meliponiculture in various types of hives such as bamboo, box, and *kendil* (local name) which the beekeepers practice (Agus et al. 2019, 2021; Agussalim et al. 2019a, 2019b, 2020, 2021, 2022; Erwan et al. 2020, 2021; Sabir et al. 2021; Supeno et al. 2021, 2022; Agussalim and Agus 2022). Honeybees or stingless bee workers produce honey from the nectar as the raw material obtained from plant flowers (floral nectar), extrafloral nectar, and honeydew (Codex Alimentarius 2001). Honey is contained sugars as the main component, followed by protein (amino acids and enzymes), ash (minerals), vitamins, organic acids, phenolic and volatile compounds (Da Silva et al. 2016). Honey from *T. laeviceps* has varying flavors, such as sweet, sour, bitter, and a mixture of the three flavors (Suntiparapop et al. 2012; Chanchao 2013; Agus et al. 2019; Agussalim et al. 2019a, 2021, 2022).

The honey's physicochemical, color, aroma, and flavor are affected by the nectar types (floral and extrafloral nectar), geographical origins, climate, bee species, and postharvest (processing, manipulation, and storage period) (Chanchao 2013; Escuredo et al. 2014; Juan-Borrás et al. 2014; Da Silva et al. 2016). The honey physicochemical of stingless bees from various countries have been studied (Biluca et al. 2016, 2019, 2020; Chuttong et al. 2016; Nordin et al. 2018; Ranneh et al. 2018; Mokaya et al. 2022), but the organic acids in stingless bees' honey are minimal. Furthermore, Da Silva et al. (2016) reported that organic acids present in honey consist of butyric, acetic, propionic, pyruvic, aspartic acid, succinic, fumaric, citric, formic, gluconic, galacturonic, glutamic, butyric, glutaric, 2-hydroxybutyric, α-hydroxyglutaric acid, glyoxylic, isocitric, malic, lactic, 2-oxopentanoic, malonic, α-ketoglutaric, methylmalonic, tartaric, quinic, shikimic, and oxalic. The organic acids of honey from *T. laeviceps* have not been studied, but *Trigona carbonaria* honey origin from Australia consists of D-gluconic acid, malic acid, and citric acid (Oddo et al. 2008).

The physicochemical of *T. laeviceps* honey have been studied such as sugars profile (Agussalim et al. 2019a; Agus et al. 2021), amino acids (Agussalim et al. 2021), minerals (Sabir et al. 2021), phenolic, flavonoid, and antioxidant activity (Agus et al. 2019; Agussalim et al. 2022), and moisture, ash, and protein contents (Agussalim et al. 2019b; 2021). Furthermore, several Indonesian regions have been used to meliponiculture of stingless bee *T. laeviceps*. Therefore, honey's physicochemical have been studied in such as Sleman Yogyakarta, Klaten Central Java, and Gunungkidul Yogyakarta (Agus et al. 2019, 2021; Agussalim et al. 2019a, 2019b, 2021, 2022; Sabir et al. 2021). However, the physicochemical of *T. laeviceps* honey from Sukadana (North Lombok), Kebonrejo (Magelang), and Sumberagung (Purworejo) have not been studied. The different locations for meliponiculture of stingless bees impact various types of plants as the nectar source to produce honey. Therefore, it influences honey quality, especially it's chemical composition (Nordin et al. 2018; Agus et al. 2021; Agussalim et al. 2021, 2022; Agussalim and Agus 2022). These three locations are included as the center of the meliponiculture (beekeeping in stingless bees) of *T. laeviceps.* Still, the beekeepers lack information on the chemical composition of honey to increase the selling value and attractiveness of their products. In addition, the organic acid from the *T. laeviceps* honey in our study is the first report in the world. Therefore, this study aimed to determine the quality of stingless bee honey (*T. laeviceps*) based on their physicochemical from three different origins.

MATERIALS AND METHODS

Geographical origins

Honey was collected from three different regions in Indonesia as the location for meliponiculture were Lombok (Sukadana Village, Bayan Sub-district, North Lombok District, West Nusa Tenggara Province), Magelang (Kebonrejo Village, Candimulyo Sub-district, Magelang District, Central Java Province), and Purworejo (Sumberagung Village, Grabag Sub-district, Purworejo District, Central Java Province). We selected the three regions because of their location as the center of meliponiculture of stingless bee, especially *T. laeviceps*. Still, the information on their honey physicochemical has not been studied. The dominant plant types were also identified as the nectar source to produce honey. Briefly, the flowers from the plants were taken, and the petals were removed to identify their nectar content; the liquid in the base of the flowers characterized it.

Procedures

Honey collection

Honey of *T. laeviceps* was collected from three samples from each location (Lombok, Magelang, and Purworejo) directly in the meliponiculture farm. Briefly, honey was harvested by cutting the propolis, which constructed a honey pot in the hive and was put in a plastic container. Afterward, the honey pot was squeezed to separate propolis and honey, and then the honey was filtered. Finally, the clean honey was put in a plastic bottle and stored at room temperature for a week until the samples were analyzed for their physicochemical.

Proximate and energy analyses

The honey's moisture, fat, and protein were analyzed by proximate analysis according to AOAC (AOAC 2005) and energy was analyzed using a bomb calorimeter method (Jensen et al. 2019). All samples were analyzed in three replicates and each in duplo.

Organic acids analysis

Honey organic acids were analyzed using high-performance liquid chromatography (HPLC) reported by Suárez-Luque et al. (2002) with some modifications. A sum of honey 0.5 g was dissolved in 5 mL of distilled water and mixed with a sonicator ultrasonic homogenizer for 15 minutes. Afterward, the mixture was added with distilled water up to 5 mL and was mixed. Ten milliliters of solution were filtered using millex filter 0.45 µm and was taken 20 µL and then injected into chromatography by auto syringe injector. The standard solution was prepared using malic acid, citric acid, propionic acid, acetic acid, butyric acid, and lactic acid with each concentration of 10,000 ppm. The standard solution of malic acid with the concentration of 0.125; 0.25; 0.5; 1; 2.5; 5; 10 ppm ($y = 1.12098\ e + 006\ x - 50825.2$ and $r^2 = 0.999434$), citric acid ($y = 11053.3\ x + 71357.1$ and $r^2 = 0.999615$), propionic acid ($y = 36954.4\ x + 102750$ and $r^2 = 0.999660$), acetic acid ($y = 4593.78\ x + 7840.62$ and $r^2 = 0.999550$), butyric acid ($y = 1.12098\ e + 006\ x - 50825.2$ and $r^2 = 0.999434$), and lactic acid ($y = 67600\ x + 39250$ and $r^2 = 0.999436$) with the concentration of 12.5; 25; 50; 100; 250; 500; 1000 ppm. All standards and honey samples were injected into HPLC in duplicate.

Total phenolic content (TPC)

The TPC of honey was analyzed by spectrophotometer UV-Vis method (Agus et al. 2019). Briefly, 2 g of honey was mixed with 0.5 mL reagent of Folin-Ciocalteu, followed by 7.5 mL of aquabidest, and then vortexed for 5 minutes. Afterward, the solution was stored for 10 minutes at room temperature, and then 1.5 mL of sodium carbonate (20%) was added and followed by the aquabidest up to the volume of 10 mL. Furthermore, we heat the solution in the water bath at 40°C for 20 minutes. Furthermore, the solution was immediately cooled and stored for 30 minutes to react before reading the absorbance at 760 nm. The standard curve of TPC was made by gallic acid solution at the concentration of 0.000; 6250; 12,500; 25,000; 50,000 ppm, where $y = 0.00511268x + 0.000000$ and $r^2 = 0.99941$. All analyses were done in triplicate, each in duplo.

Total flavonoid content (TFC)

The TFC of honey was analyzed by spectrophotometer UV-Vis method (Agus et al. 2019). Briefly, 0.2 g of honey was mixed with 4 mL of distilled water, followed by 0.3 mL of sodium nitrite (5%, w/v). After five minutes, 0.6 mL of aluminum chloride (10% w/v) was added, and two

milliliters of sodium hydroxide (1 M) was followed by the distilled water up to the volume of ten milliliters. Afterward, the solution was vortexed and read at the absorbance of 510 nm. The standard curve of TFC was made by quercetin solution at the concentration of 0.000; 1563; 3125; 6250; 12,500; 25,000; 50,000 ppm, where y = 0.00466915x + 0.000000 and r^2 = 0.99945. All analyses were done in triplicate, each in duplo.

DPPH antioxidant activity

The method spectrophotometer UV-Vis was used to assay the DPPH antioxidant activity of honey (Agus et al. 2019). Briefly, 100 µL of the honey solution was mixed with 900 µL of freshly DPPH methanol solution (0.1 mM) (as the control). Afterward, the solution was incubated for 30 minutes at room temperature (dark condition), and the absorbance was read at 517 nm. Scavenging activity (%) was calculated by the equation of DPPH (%) = [(Ac – As)/Ac] x 100, where Ac was the control absorbance and As was the sample absorbance. All analyses were done in triplicate, each in duplo.

Amino acids

The amino acid content of honey was determined by the high-performance liquid chromatography (HPLC) method. Briefly, 60 mg of honey sample was mixed with 4 mL of HCl 6 N and heated for 24 hours at 110°C. Afterward, the solution was neutralized by NaOH 6 N (pH was 7), aquabidest was added until the volume of 10 mL, and Whatman of 0.22 µM filtered the solution. Next, the sample solution was taken 50 uL and was mixed with 300 µL of ortho-phthalaldehyde solution, then vortexed for 5 minutes. Finally, ten microliters of the solution were taken to be injected into the HPLC injector. The standard curve concentration of 0, 50, 100, and 250 ppm of amino acids. The chromatogram of each honey sample is shown in Figure 1. The standard curve for each amino acid consists of aspartic acid (y = 5553x – 3050.7 and r^2 = 0.9996), glutamic acid (y = 5766.2x – 830.24 and r^2 = 0.9998), asparagine (y = 5220x – 3432.8 and r^2 = 0.9996), serine (y = 7636.8x – 7812 and r^2 = 0.9994), histidine (y = 2662.5x – 2676.3 and r^2 = 0.9983), glycine (y = 10332x – 44343 and r^2 = 0.9971), threonine (y = 6418.5x – 20893 and r^2 = 0.9987), arginine (y = 3725.3x – 11410 and r^2 = 0.9988), alanine (y = 7628.1x – 21419 and r^2 = 0.9991), tyrosine (y = 2299.7x – 438.75 and r^2 = 1), methionine (y = 4802x - 1044.7 and r^2 = 0.9999), valine (y = 7422.6x – 713.01 and r^2 = 1), phenylalanine (y = 3465.5x + 2079.5 and r^2 = 1), leucine (y = 5892.2x + 2562.4 and r^2 = 1), lysine (y = 1323.5x – 5192.4 and r^2 = 0.999). All samples were analyzed in three replicates and each in duplo.

Data analysis

The data of honey physicochemical were analyzed by analysis of variance using SPSS (Windows version of SPSS, release 23) and followed by honestly significant difference test.

RESULTS AND DISCUSSION

Moisture, fat content, and energy

The different regions for meliponiculture of stingless bee *T. laeviceps* had a high effect on the moisture ($p<0.01$) and energy ($p<0.05$) but not on the fat content. Honey of stingless bee *T. laeviceps* from Lombok was lower in moisture and was higher energy than honey from Magelang and Purworejo. Honey from stingless bee *T. laeviceps* from Lombok contains an energy of 3018.96 cal/g, higher than honey from Purworejo 2631.72 cal/g. However, it did not differ from honey from Magelang was 2752.17 cal/g (Table 1).

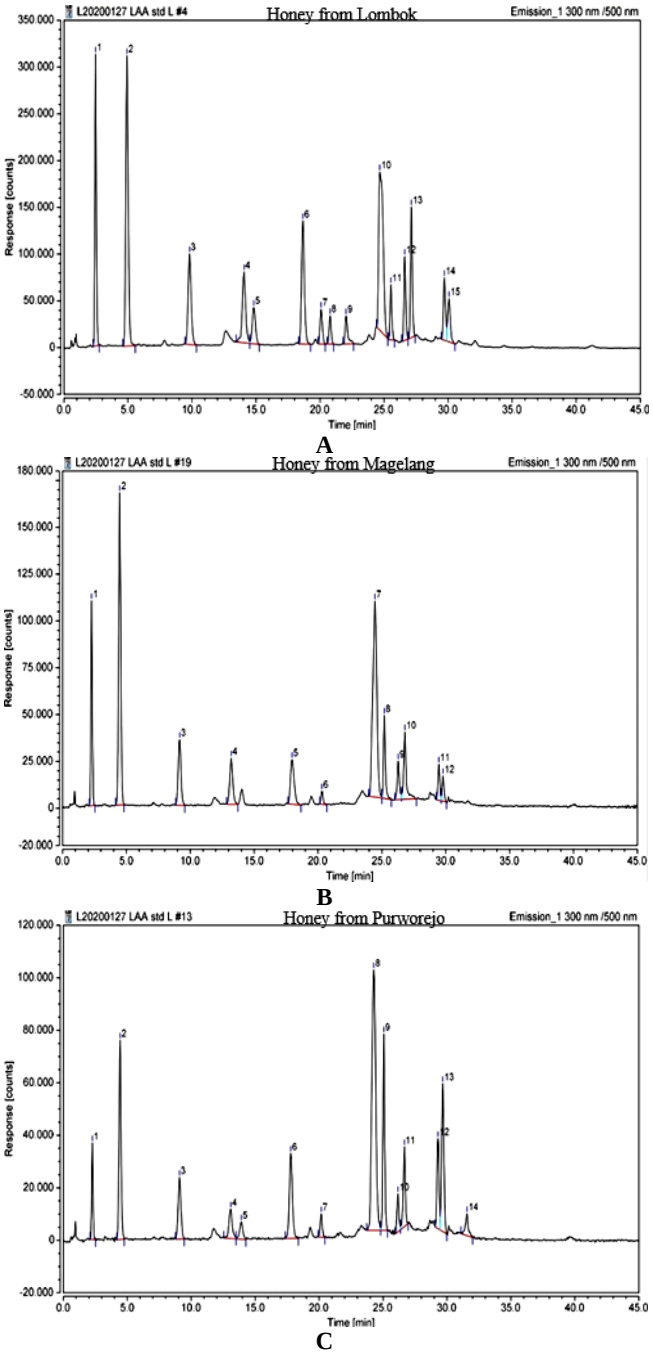

Figure 1. The chromatogram of amino acids of *Tetragonula laeviceps* honey from: A. Lombok; B. Magelang; and C. Purworejo by HPLC

Table 1. The proximate analysis, energy, total flavonoid content, total phenolic content, and antioxidant activity of honey from stingless bee *Tetragonula laeviceps* originate from different regions

Parameters	Regions for meliponiculture			SEM	*p*-value
	Lombok	Magelang	Purworejo		
Moisture, g/100 g	24.4[b]	29.4[a]	29.7[a]	0.78	0.000
Fat, g/100 g	0.33	0.34	0.33	0.04	0.994
Protein g/100 g	1.04[a]	0.63[b]	0.50[c]	0.08	0.000
Energy, cal/g	3018.96[a]	2752.17[ab]	2631.72[b]	62.24	0.020
Total flavonoid content (mg QE/g)	1550[a]	1065[b]	636[c]	0.133	0.000
Total phenolic content (% GAE w/w)	1.58[a]	0.76[b]	0.62[b]	0.152	0.000
Antioxidant activity of DPPH (%)	91.64[a]	87.97[a]	70.38[b]	3.565	0.004

Note: [a,b,c]Different superscripts within rows indicate differences at $p<0.05$; SEM: standard error of the mean

Table 2. The predominant plant types as the nectar source for the stingless bee *Tetragonula laeviceps* feed from Lombok, Magelang, and Purworejo of Indonesia

Region for meliponiculture	Plant species
Lombok	Mango (*Mangifera indica*)
	Cashew (*Anacardium occidentale*)
	Sunflowers (*Helianthus annuus*)
Magelang	Rubber (*Hevea brasiliensis*)
	Banana (*Musa paradisiaca*)
Purworejo	Mango (*Mangifera indica*)
	Rambutan (*Nephelium lappaceum*)
	Coconut (*Cocos nucifera*)

Moisture is the second constituent present in honey after carbohydrates or sugar (Da Silva et al. 2016). The different predominant plant types influenced the difference in moisture and energy from each honey from different origins as the nectar source to produce honey. For example, the plants as the nectar source from Lombok consist of mango, cashew, and sunflowers; Magelang consists of rubber and banana, while Purworejo consists of mango, rambutan, and coconut (Table 2). The different nectar sources impact the nectar content, especially moisture influencing honey moisture. Furthermore, environmental conditions like temperature and humidity also influenced the honey moisture. The high temperature and low humidity impacted the high sugar and low moisture content. On the contrary, the low temperature and high humidity impacted the low sugar and high moisture content (Agussalim et al. 2019a; Agus et al. 2021). Da Silva et al. (2016) explained that the chemical composition of honey is affected by the bee species, geographical origin, plant types (bee forage), temperature and humidity environment, level of honey maturity, postharvest processing (heating, manipulation), and storage time.

The honey moisture from Lombok was acceptable by the Indonesian National Standard (SNI 2018). The maximum moisture content for stingless bees is 27.5% (w/w), while the honey moisture from Magelang and Purworejo was unacceptable. The honey moisture from stingless bee species generally is higher because the foragers, besides collecting nectar, also collect the ripe fruit material with higher moisture content. Furthermore, stingless bee species do not have a behavior mechanism to evaporate water in honey in the nest (Suntiparapop et al. 2012). Therefore, the honey moisture in our study (Table 1) did differ from those previously studied (Oddo et al. 2008; Suntiparapop et al. 2012; Biluca et al. 2016; Chuttong et al. 2016; Ranneh et al. 2018; Agussalim et al. 2019b). The honey energy from our study differed from what was reported by Ranneh et al. (2018) for Kelulut honey ranging from 275.3 to 277.3 kcal/100 g of honey. The energy of honey is related to carbohydrate and sugar content (Da Silva et al. 2016; Ranneh et al. 2018). The honey moisture and energy differences were affected by the bee species involved in honey production, plant types as the nectar source, and environmental conditions, especially temperature and humidity. The high temperature with the low humidity impacted the high sugar content and low moisture of nectar that the plant flowers can produce. Furthermore, the low temperature and high humidity influenced the low sugar and high moisture content of nectar produced by the plant flowers (Da Silva et al. 2016).

TPC, TFC, and antioxidant activity

The different regions for meliponiculture stingless bee *T. laeviceps* had a high effect on the TPC, TFC, and antioxidant activity ($p<0.01$). The honey TPC from Lombok was 1.58% GAE (w/w), higher to the TPC of honey from Magelang and Purworejo. However, the honey TPC from Magelang was 0.76% GAE (w/w), similar to the honey TPC from Purworejo was 0.62% GAE (w/w). The honey TFC from Lombok was 1550 mg QE/g which differed from the honey TFC from Magelang and Purworejo. However, the honey TFC from Magelang was 1065 mg QE/g did differ from honey from Purworejo was 636 mg QE/g. The DPPH antioxidant of honey from Lombok was 91.64% at a 0.1 mM concentration, similar to the DPPH antioxidant of honey from Magelang at 87.97%. However, the DPPH antioxidants from Magelang and Lombok were higher than honey from Purworejo was 70.38% (Table 1).

Flavonoids and phenolics are bioactive honey compounds that may be used as a functional food to improve human health. The flavonoid compounds are divided into phenolic acid and flavonoid (flavanols, flavones, anthocyanins, flavanones, isoflavones, and chalcones) (Da Silva et al. 2016). Furthermore, the flavonoid compounds present in honey consist of vanillic acid, caffeic acid, quercetin, syringic acid, ferulic acid, kaempferol, pinobanksin, myricetin, p-coumaric acid,

gallic acid, pinocembrin, ellagic acid, galangin, chlorogenic acid, 3- and 4-hydroxybenzoic acid, rosmarinic acid, and benzoic acid (Da Silva et al. 2016; Scepankova et al. 2017).

The honey TPC difference from each geographical origin is affected by plant types, which produce nectar as the raw material to produce honey by the workers' bees (Table 2). Moreover, flower plants can produce nectar containing secondary metabolites affecting the *T. laeviceps* honey TPC. Therefore, the different plant types were impacted by the nectar chemical compositions of each plant flower, influencing the honey TPC (Da Silva et al. 2016; Agus et al. 2019). Furthermore, *T. laeviceps* honey was stored in honey pots made from propolis. Therefore, the honey TPC was also indirectly affected by the propolis phenolic content from the honey pots (Agus et al. 2019).

Aparna and Rajalakshmi (1999) explained that honey colors comprise chlorophyll, anthocyanin, xanthophyll, carotene, and tannin. Furthermore, Kuś et al. (2014) and Scepankova et al. (2017) explained that honey with a darker color generally has a higher TPC, TFC, and antioxidant activity than bright ones. This condition was found in honey from Lombok darker than honey from Magelang and Purworejo. Therefore, honey from Lombok was higher in TPC, TFC, and antioxidant activity than from Magelang and Purworejo. In addition, Brudzynski and Miotto (2011) also reported the relationship between phenolic content and honey color, indicating that honey's phenolics, sugars, and proteins may contain the high molecular weight of melanoidin and multi-component polymers, which exhibit antioxidant properties. In addition, Aparna and Rajalakshmi (1999) explained that the dark color of honey contains more mineral salts and vice versa. In contrast, the honey's sulfur and chlorine content can promote pigmentation degree.

The Pearson correlation shows that the TPC has a positive correlation with the honey DPPH antioxidant, whereas TPC/DPPH with r: 0.831 ($p<0.05$) (Table 3). This study result is similar to that of Agus et al. (2019) for stingless bee *T. laeviceps* in others origins (Lombok, Klaten, and Gunungkidul). On the other hand, the TPC/DPPH positively correlates with r: 0.79. Additionally, this result was also similarly reported by Gül and Pehlivan (2018) and Rodica et al. (2021), who stated that the TPC of monofloral honey has a positive correlation with antioxidant activity (DPPH).

The honey TPC (Table 1) in this study differed from those previously reported on several species of stingless bees. For example, ten species from Santa Catarina (Brazil) ranging from 10.3 to 98.0 mg GAE 100/g (Biluca et al. 2016), 57 to 214 mg GAE/100 g from six species from Kenya (*Liotrigona* sp., *Meliponula bocandei*, *Meliponula ferruginea*, *Meliponula lendliana*, *Meliponula togoensis*, and *Plebeina armata*) (Mokaya et al. 2022), 175 to 227 mg GAE/kg for *Melipona fasciculata* and 78 to 136 mg GAE/kg for *Melipona subnitida* (Sant'ana et al. 2020), and 97.88 to 101.5 mg CE/kg for Kelulut honey in Malaysia (Ranneh et al. 2018). The honey TPC was affected by the different stingless bee species, plant types as the nectar source to produce honey, and geographical origins (Nordin et al. 2018; Agus et al. 2019).

Table 3. The Pearson correlation among TPC, TFC, and DPPH antioxidant

	TPC	TFC	DPPH
TPC	1		
TFC	0.907**	1	
DPPH	0.831**	0.661*	1

Note: *Significant at $p<0.05$ (2-tailed) and **Significant at $p<0.01$ (2-tailed); TPC: total phenolic content; TFC: total flavonoid content; DPPH: antioxidant activity

The honey TFC of each geographical origin was caused by different plant types, which produce nectar as the raw material for honey by the workers' bee (Table 2), which was impacted by the different chemical composition of nectar from each plant flower (Da Silva et al. 2016; Agus et al. 2019). Furthermore, the flavonoids are rich in plants and trees' seeds, bark, leaves, and flowers. Thus, the flavonoids can be transferred from plants, especially from nectar to honey (Da Silva et al. 2016). Therefore, the honey TFC has been used as a floral marker to identify plant types from different geographical origins (Da Silva et al. 2016). Additionally, the honey from *T. laeviceps* was stored in the honey pots, which were made from propolis as the raw material, so indirectly, the honey TFC was affected by the propolis flavonoid content from the honey pots (Da Silva et al. 2016; Nordin et al. 2018; Agus et al. 2019).

The Pearson correlation showed that the TFC has a positive correlation with the DPPH antioxidant of honey, whereas TFC/DPPH with r: 0.661 ($p<0.05$). This study result was similar to that of Agus et al. (2019); the honey TFC from *T. laeviceps* was positively correlated with DPPH with r: 0.96 with the TFC ranging from 0.21 to 0.96 mg QE/g. Furthermore, the TFC in our study also differed from previously studied for several species of stingless bees, such as for *Melipona fasciculata* ranging from 78.3 to 135.7 mg QE/kg and 61 to 105 mg QE/kg for *Melipona subnitida* (Sant'ana et al. 2020). In addition, 97.88 to 101.5 mg CE/kg for Kelulut honey (Ranneh et al. 2018), 28.7 to 73.0 mg QE/100 g for honey from stingless bee *Liotrigona* sp. (Mokaya et al. 2022), and 45.42 to 470.70 mg GAE/100 g for monofloral honey in Turkey (Gül and Pehlivan 2018). The different honey TFC was affected by the different plant types as the nectar source, stingless bee species, and geographical origins (Da Silva et al. 2016; Nordin et al. 2018; Agus et al. 2019).

The higher honey DPPH antioxidant from Lombok and Magelang was supported by the higher TPC and TFC than honey from Purworejo (Table 1). In addition, phenolic and flavonoids are known as sources of stronger antioxidants (Da Silva et al. 2016; Gül and Pehlivan 2018; Rodica et al. 2021). Furthermore, the Pearson correlation revealed that the honey DPPH antioxidant from *T. laeviceps* had a positive correlation with the honey TFC and TPC, whereas DPPH/TFC with r: 0.661 ($p<0.05$) and DPPH/TPC with r: 0.831 ($p<0.01$) (Table 3). The honey DPPH antioxidant was also influenced by the honey TFC and TPC (Da Silva et al. 2016; Rodica et al. 2021). Scepankova et al. (2017) explained that darker color honey has a higher TPC, TFC,

and antioxidant activity than bright honey. Furthermore, Ferreira et al. (2009) reported that darker honey has a higher antioxidant concentration of flavonoid, phenolic, ascorbic acid, and β-carotene than bright honey. This condition was found in honey from Lombok and Magelang, darker than that from Purworejo.

The honey DPPH antioxidant (Table 1) in our study differs from previously studied for several species of stingless bees, such as the antioxidant DPPH of Kelulut honey from Malaysia was 90% at 40 mg/mL concentration (Ranneh et al. 2018). Furthermore, 47.3 to 91.2% at 0.1 mM concentration for *T. laeviceps* honey from Indonesia (Sleman, Nglipar, and Klaten) (Agus et al. 2019). In addition, the antioxidant activity was higher than honey from Afrotropical stingless bees ranging from 30.0 to 76.2% (Mokaya et al. 2022), and for monofloral honey with the DPPH antioxidant ranging from 12.01 to 65.52 mg/mL (Gül and Pehlivan 2018). The different antioxidant activity was affected by the stingless bee species involved in honey production and plant types as the nectar sources, which impacted the chemical composition of nectar, especially secondary metabolites.

Honey organic acids

The different regions for meliponiculture of stingless bee *T. laeviceps* had a high effect on the citric acid, propionic acid, acetic acid ($p<0.01$), and lactic acid ($p<0.05$), but not on the malic acid and butyric acid ($p>0.05$) of honey. Stingless bee honey of *T. laeviceps* from Lombok was higher in citric acid and propionic acid than from Magelang and Purworejo. In addition, the honey lactic acid from Lombok and Purworejo was similarly higher than from Magelang. Furthermore, the honey acetic acid from Lombok and Magelang was lower than that from Purworejo (Table 4).

The difference in organic acids from honey in each region was caused by different plant types, which produce nectar as the raw material for honey by the workers' bees (Table 2). For example, the plants as the nectar source from Lombok consist of mango, cashew, and sunflowers; Magelang consists of rubber and banana, while Purworejo consists of mango, rambutan, and coconut. Da Silva et al. (2016) and Nordin et al. (2018) explained that the chemical composition of honey is affected by the different species of bees involved in honey production and geographical origin. Furthermore, plant types, especially nectar sources, temperature, humidity environment, honey maturity level, postharvest processing (heating, manipulation), and storage time also affected honey chemical composition.

Organic acids in honey contribute to color, flavor, aroma, acidity, pH, and electrical conductivity. Organic acids are derived from the sugars by enzymes secreted by the bees when converting nectar into honey or obtained directly from nectar.

Organics acids present in honey are aspartic acid, butyric acid, citric acid, acetic acid, formic acid, fumaric acid, galacturonic acid, formic acid, gluconic acid, glutamic acid, glutaric acid, butyric acid, glyoxylic acid, 2-hydroxybutyric acid, α-hydroxyglutaric acid, isocitric acid, α-ketoglutaric acid, lactic acid, malic acid, malonic acid, methylmalonic acid, 2-oxopentanoic acid, propionic acid, pyruvic acid, quinic acid, shikimic acid, succinic acid, tartaric acid, and oxalic acid (Da Silva et al. 2016).

Table 4 shows organic acids from the stingless bee *T. laeviceps* studied are citric acid, malic acid, propionic acid, acetic acid, butyric acid, and lactic acid. Moreover, other organic acids may also be present in stingless bee *T. laeviceps* honey, but these facts need more advanced study.

Organic acids in honey from the stingless bee *T. laeviceps* (Table 4) differ from those reported by Oddo et al. (2008), for honey from the stingless bee *Trigona carbonaria* consists of D-gluconic, malic, and citric acids. The predominant organic acid in honey is gluconic acid, which originates from glucose oxidase secreted by the bees during honey ripening (Karabagias et al. 2014; Da Silva et al. 2016). A recent study by Vit et al. (2023) reported organic acids in honey in three stingless bee genera (*Geotrigona, Melipona, Scaptotrigona*) from Ecuador, consist of acetic acid, citric acid, formic acid, fumaric acid, lactic acid, malic acid, pyruvic acid, quinic acid, and succinic acid). *Geotrigona* honey was particularly high in acetic acid at 19.60 g/kg and lactic acid at 24.30 g/kg. In comparison, honey from *Melipona* and *Scaptotrigona* was contrasted namely 1.3 g/kg acetic acid and 1.6 g/kg lactic acid. Furthermore, Moreira et al. (2023) also reported that the organic acids in honey produced by *Cephalotrigona capitata* and *Melipona scutellaris* from Brazil consist of oxalic acid, citric acid, tartaric acid, malic acid, succinic acid, acetic acid, formic acid, and lactic acid. The concentration of both organic acids, gluconic and citric acids, has been used as the reliable criterion to differentiate honey from floral nectar and honeydew (Da Silva et al. 2016).

Table 4. The organic acids of honey from stingless bee *Tetragonula laeviceps* originate from different regions

Parameters	Regions for meliponiculture			SEM	p-value
	Lombok	Magelang	Purworejo		
Citric acid (µg/g)	1596.92^{a}	1331.79^{a}	421.43^{b}	186.37	0.001
Malic acid (µg/g)	0.62	0.60	0.73	0.04	0.517
Propionic acid (µg/g)	152.78^{a}	54.73^{b}	0.15^{c}	23.28	0.001
Acetic acid (µg/g)	3688.92^{b}	3771.24^{b}	5499.80^{a}	335.57	0.012
Butyric acid (µg/g)	274.11	173.24	253.28	43.37	0.668
Lactic acid (µg/g)	770.34^{a}	573.65^{b}	777.86^{a}	40.45	0.032

Note: ^{a,b,c}Different superscripts within rows indicate differences at $p<0.05$; SEM: standard error of the mean

Protein content and amino acids

The honey protein content from a stingless bee of *T. laeviceps* from Lombok was 1.04 g/100 g was higher, followed by the protein content of honey from Magelang was 0.63 g/100 g, and the lowest was 0.50 g/100 g for honey from Purworejo (Table 1). The amino acids in *T. laeviceps* honey from Lombok, Magelang, and Purworejo were found in 15 types consisting of aspartic acid, glutamic acid, asparagine, histidine, glycine, threonine, alanine, tyrosine, arginine, methionine, phenylalanine, valine, isoleucine, lysine, and leucine. However, histidine and arginine have not been detected in honey from Lombok, threonine, and leucine not detected in honey from Magelang, and glycine, threonine, alanine, tyrosine, and leucine have also not been detected in honey from Purworejo. Generally, the amino acid content of honey from Lombok was higher than that from Magelang and Purworejo (Table 5).

Honey gets its protein and amino acids from the nectar and honeydew (Escuredo et al. 2013; Da Silva et al. 2016); however, pollen is the primary source (Da Silva et al. 2016). Generally, the amino acids found in honey consist of proline, aspartic acid, glutamic acid, phenylalanine, arginine, histidine, tyrosine, glutamine, threonine, glycine, α-alanine, β-alanine, valine, ɣ-aminobutyric acid, cysteine, isoleucine, methionine, lysine, tryptophan, asparagine, ornithine, leucine, alanine, and serine (Truzzi et al. 2014; Da Silva et al. 2016).

Moreover, the protein content is affected by different plant types, which produce nectar as the raw material for honey by the workers' bees (Table 2). However, the bee's main protein source is plant flowers' pollen (Da Silva et al. 2016). Furthermore, the honey protein content from *T. laeviceps* is also affected by the bee bread, which is attached when their honey is harvested (Agussalim et al. 2021). Therefore, the protein content of honey from *T. laeviceps* in our study (Table 1) differed from previously studied (Ranneh et al. 2018; Agussalim et al. 2021; Villacrés-Granda et al. 2021). The protein content of honey was affected by the different stingless bee involved in honey production, plant types, and environmental condition, especially temperature and humidity (Agussalim et al. 2021). The amino acid content of each honey is influenced by different plant types, which produce nectar as the raw material for honey by the workers' bees (Table 2).

However, the primary protein source in honey is pollen obtained from plant flowers (Da Silva et al. 2016; Agussalim et al. 2021). In addition, the amino acid content in honey from Lombok was supported by a higher protein content than honey from Magelang and Purworejo (Table 1). Amino acids in honey positively correlate to honey's protein content (Agussalim et al. 2021). Therefore, it indicates that the honey amino acids are influenced by geographical origins, plant types (Biluca et al. 2019; Villacrés-Granda et al. 2021), and stingless bee (Villacrés-Granda et al. 2021).

This study revealed the honey amino acids that differ from those reported by Biluca et al. (2019), that stingless bee honey from Brazilian was found to have 16 amino acids arginine, glutamine, aspartic, serine, asparagine, glycine, glutamic, proline, threonine, alanine, valine, tyrosine, phenylalanine, isoleucine, tryptophan, and leucine. Furthermore, Agussalim et al. (2021) reported the amino acids detected in honey from stingless bee *T. laeviceps* from Indonesia consist of proline, glutamic acid, arginine, phenylalanine, histidine, isoleucine, lysine, leucine, tyrosine, methionine, valine, aspartic acid, cysteine, alanine, threonine, serine, and glycine.

Our study's predominant amino acids (Table 5) consist of glutamic acid, methionine, and lysine. This finding differed from what was reported by Agussalim et al. (2021), that the predominant honey amino acids from stingless bee *T. laeviceps* from originate from Sleman Indonesia, consisting of histidine, glutamic acid, arginine, and lysine. Furthermore, aspartic acid, glutamic acid, histidine, and lysine, predominantly in honey, originate from Lombok. While arginine, lysine, phenylalanine, and histidine are predominantly in honey from Gunungkidul.

Table 5. Amino acids of honey from stingless bee *Tetragonula laeviceps* from different regions for meliponiculture

Amino acids (mg/kg)	Regions for meliponiculture			SEM	p-value
	Lombok	Magelang	Purworejo		
Aspartic acid	6.822[a]	2.854[b]	1.403[b]	0.885	0.004
Glutamic acid	10.265[a]	5.218[ab]	2.727[b]	1.287	0.017
Asparagine	5.213[a]	2.575[b]	2.099[b]	0.558	0.015
Histidine	ND	2.723	2.568	0.597	0.093
Glycine	6.232[a]	1.533[b]	ND	1.046	0.008
Threonine	4.656[a]	ND	ND	0.781	0.000
Arginine	ND	3.850[ab]	5.606[a]	1.002	0.032
Alanine	6.638[a]	1.060[b]	ND	1.100	0.002
Tyrosine	2.208[a]	0.297[b]	ND	0.374	0.003
Methionine	12.018	7.078	7.763	1.740	0.517
Valine	1.138	1.536	1.693	0.344	0.837
Phenylalanine	1.718	1.505	0.200	0.402	0.277
Isoleucine	1.734[a]	0.736[ab]	0.616[b]	0.217	0.036
Leucine	3.472[a]	ND	ND	0.631	0.004
Lysine	10.797	6.477	10.464	1.009	0.147

Note: [a,b,c]Different superscripts within rows indicate differences at $p<0.05$. ND: not detected; SEM: standard error of the mean

However, it was reported that the honey from Brazilian stingless bees showed that histidine amino acid was less than the detection limit (Biluca et al. 2019). Furthermore, phenylalanine was the predominant amino acid in honey from Brazilian stingless bees (Biluca et al. 2019) and proline amino acid (Biluca et al. 2019; Villacrés-Granda et al. 2021). A recent study by Vit et al. (2023) found that the amino acids present in honey from three stingless bee genera (*Geotrigona, Melipona, Scaptotrigona*) from Ecuador. Those amino acids were quantified by the ^{1}H NMR method, and it found nine amino acids consisting of alanine, aspartic acid, glutamine, leucine, phenylalanine, proline, pyroglutamic acid, tyrosine, and valine. Furthermore, the predominant amino acids present in honey were proline and phenylalanine. The proline amino acid has been used to evaluate the maturity and adulteration of honey (Da Silva et al. 2016). In *Apis mellifera*, the proline amino acid of honey was a minimum of 180 mg/kg for pure honey (Manzanares et al. 2014; Da Silva et al. 2016); however, standardized honey has not been regulated for stingless bees. The honey amino acids were affected by the different bee species involved in honey production, plant types (nectar and pollen sources), and the protein content of honey. Thus, it can be concluded that honey from different geographical origins has different qualities based on its physicochemical. However, honey from stingless bee *T. laeviceps* from Lombok has better characteristic than that of Magelang and Purworejo. Honey from stingless bee *T. laeviceps* is rich in flavonoid and phenolic contents to support the higher honey DPPH antioxidant.

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