Free amino acids profile of honey produced by the Indonesian stingless bee: *Tetragonula laeviceps*

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Abstract. The objective of this study was to determine free amino acids profile of honey produced by the Indonesian stingless bee: *Tetragonula laeviceps*. Honey was obtained from meliponiculture result in the Ngrandu Katongan Village Nglipar Gunungkidul. The free amino acids was determined by LC-MS/MS method and the data was analyzed by descriptive analysis. The results showed that free amino acids content from honey *Tetragonula laeviceps* were arginine 591.83, histidine 561.93, lysine 882.03, phenylalanine 232.74, isoleucine 12.34, leucine 73.55, tyrosine 9.24, methionine 0.29, valine 20.39, proline 60.56, glutamic acid 119.82, aspartic acid 77.31, threonine 45.72, serine 168.65, alanine 62.46, and glycine 60.26 mg/kg while cysteine not detected. It can be concluded that the free amino acids content of *Tetragonula laeviceps* honey has 16 free amino acids with large quantity were lysine, arginine, histidine, phenylalanine, serine and glutamic acid.

1. Introduction

Tetragonula laeviceps also known as *Trigona* bees, are a stingless bee species found in Indonesia that nests in bamboo [1]. The main products of the stingless bee *T. laeviceps* are honey, bee pollen or bee bread, and propolis [1–3]. Honey is a sweet natural substance (natural food) produced by honeybees or stingless bees from the nectar of plant flowers (floral nectar), the extrafloral nectar of plants and honeydew [4–5]. Honey is mainly composed of sugars and other constituents, such as enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, and aromatic substances [4–6].

The chemical composition of honey from *T. laeviceps* have been studied by researchers [7–9], stingless bees species [10–12] and setting a quality standard for stingless bees honey from several countries [13]. The recent report that the profile of amino acids of honey from nine species stingless bees (*Meliponinae*) from Brazil are obtained 16 amino acids consists of acids aspartic, glutamic, asparagine, glutamine, serine, arginine, glycine, threonine, alanine, proline, tyrosine, valine, leucine, isoleucine phenylalanine, and tryptophan. The large content of amino acids were phenylalanine 5.20 to 1231 mg/kg and proline 12.1 to 762 mg/kg, but histidine was not identified [14].

The honey from Indonesian stingless bees has been commercialized by beekeepers but has not been studied with regard to its chemical composition. The recent studied about sugar profile of honey from Indonesian stingless bee *T. laeviceps* [7] but amino acids content not yet studied. Thus, the aim of this study was to determine the free amino acids content of honey produced by the Indonesian stingless bee *T. laeviceps*.

2. Material and methods

2.1. Material

Honey sample was used in the study has a bitter taste and obtained from Indonesian stingless bee *T. laeviceps* which meliponiculture in Ngrandu, Katongan Village, Nglipar Sub-district, Yogyakarta.

2.2. Methods

The profile of amino acids was determine by liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was reported by Kowalski *et al.* [15] with some modification. Briefly, sample preparation 2 g of honey was transferred into a 50 mL tube with a cap. Hydrogen chloride 6 N about 20 mL was added and was hydrolyzed using autoclave at temperature 110°C for 12 hours. Then, was neutralized by sodium hydroxide 6 N and was added up to 50 mL. The solution was filtered through 0.22 μm pore size nylon syringe filter prior and working solutions of amino acids prepared by 10 time dilution of stock solutions in acidified water was 100 lg/mL 10 time dilution of stock solutions in acidified water was 100 lg/mL. Then, 2 μL solution was injected to LC-MS/MS to analysis. Honey test was carriout in one test in duplo.

Preparation of standard solutions of amino acids and internal standard Mixed stock solution of amino acids and a stock solution of an internal standard were prepared in concentration 1000 lg/mL of acidified water (0.1% acetic acid). Working solutions of amino acids and the internal standard prepared by 10 time dilution of stock solutions in acidified water was 100 lg/mL. A calibration curve of amino acids was prepared in the range from 0.02 to 6 lg/mL, internal standard concentration was 0.5 lg/mL (prepared in acidified water).

The data amino acid profile was analyzed using descriptive analysis.

3. Results and discussion

Amino acids are responsible for 1% (w/w) of the constituents of honey and their content depends on the geographical origin (plant flowers as the source of nectar to produce honey and honeydew) [4–16]. The results showed that amino acids were detected in honey from the Indonesian stingless bee *T. laeviceps* were 16 amino acids and cysteine not detected was shown by Table 1.

Table 1. Profile of amino acids of honey from the Indonesian stingless bee *T. laeviceps*

Amino acids	Content (mg/kg)	% of amino acids
Essential amino acids		
Arginine	591.83	19.87
Histidine	561.93	18.86
Lysine	882.03	29.61
Phenylalanine	232.74	7.81
Isoleucine	12.34	0.41
Leucine	73.55	2.47
Methionine	0.29	0.01
Valine	20.39	0.68
Threonine	45.72	1.53
Non essential amino acids		
Tyrosine	9.24	0.31
Proline	60.56	2.03
Glutamic acid	119.82	4.02
Aspartic acid	77.31	2.60
Serine	168.65	5.66
Alanine	62.46	2.10
Glycine	60.26	2.02
Cysteine	Not detected	-
Total of amino acids	2,979.12	100

The total of amino acids in honey *T. laeviceps* is about 2,979.12 mg/kg and was higher to those previously reported [14–15] and was lower than reported by Sun et al. [19]. The amino acids in the large quantity (dominant) in honey *T. laeviceps* were lysine about 882.03 mg/kg (29.61%), arginine 591.83 mg/kg (19.87%), and histidine 561.93 mg/kg (18.86%) was higher to those previously reported [14–15–19]. The medium amount of amino acids were phenylalanine 232.74 mg/kg (7.81%), serine 168.65 mg/kg (5.66%), and glutamic acid 119.82 mg/kg (4.02%) was higher to those previously reported [15–19] and was lower than Biluca et al. [14]. The small amount of amino acids were aspartic acid 77.31 mg/kg (2.60%), leucine 73.55 mg/kg (2.47%), alanine 62.46 mg/kg (2.10%), proline 60.56 mg/kg (2.03), glycine 60.26 mg/kg (2.02%), threonine 45.72 mg/kg (1.53%), valine 20.39 mg/kg (0.68%), isoleucine 12.34 mg/kg (0.41%), tyrosine 9.24 mg/kg (0.31%), and methionine 0.29 mg/kg (0.01%) was similar to those previously reported [14–15–19] except proline was higher than our study is lower. In addition, in the study amino acid cysteine not detected for honey *T. laeviceps*.

In honey, proline responsible about 50 to 85% from a total of amino acids [4–17–18], 80% [16] but in our study proline included small quantity is about 2.03%. Proline has been used as the criterion to evaluate the maturation of honey, and in some cases to detect adulteration of honey which made from sugar. The minimum content of proline in honey is 180 mg/kg is accepted as the limit content for pure honey [4–16], while in our study proline content very low is about 60.56 mg/kg was not acceptable. Thus, the honey in our study might be not complete maturity and might be indicate adulteration, but needed advance study.

The different of amino acids each honey influenced by geographical origin for the beekeeping or meliponiculture (different plant flowers as the source of nectar), bee species involved in produce honey, and especially pollen as the major of protein source [4–15–16–19–20]. In addition, in stingless bee honey, the amino acids content can also be influenced by the presence of the microorganisms that work in symbiosis with the bees inside the hive [21].

4. Conclusion

Free amino acids content of *T. laeviceps* honey has 16 free amino acids were arginine, histidine, lysine, phenylalanine, isoleucine, leucine, tyrosine, methionine, valine, proline, glutamic acid, aspartic acid, threonine, serine, alanine, and glycine with large quantity were lysine, arginine, histidine, phenylalanine, serine and glutamic acid.

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