Amino acids characterization of forest honeys from some area of South Sulawesi

A Noor1,*,# M Mahmud2,3,#, A Ahmad2,3 and R A Arfah2

1Radiation Chemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, 90245, Makassar, Indonesia
2Biochemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, 90245, Makassar, Indonesia
3Laboratory of Research Centre and Developing of Sciences, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, 90245, Makassar, Indonesia

*Email: alfiannoor744@gmail.com; #author with equal contribution

Abstract. Amino acid characterization of forest honey was carried out in eight samples from Terasa, Mallawa and Bontojai, South Sulawesi Province. Analytical method of amino acid concentration in honey is using Ultra Performance Liquid Chromatography (UPLC). 16 amino acids with an average amino acids concentration in mg/kg dominated by histidine (589.20 – 2776.81), glutamic acid (992.76 – 1527.40), and arginine (674.10 – 1038.76) followed by alanine (424.84 – 707.41), aspartic acid (256.51 – 595.42), phenylalanine (200.59 – 711.29) and serine (419.18). Other amino acids were tyrosine (215.33 – 333.54), glycine (240.26 – 402.00), leucine (259.63 – 315.27), proline (209.51 – 333.73), threonine (217.09 – 279.36), valine (198.08 – 290.88) and lysine (157.01 – 275.86). Amino acids in small amounts were methionine (86.46 – 129.89) and isoleucine (98.24 – 125.94). Forest honey from Terasa, Mallawa and Bontojai can be a source of essential amino acids.

1. Introduction
Honey is a natural food, mainly composed of sugars and other constituents such as enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, lipid, aromatic substances and also the presence of flavonoids and phenolic acids emphasizes the role of honey, along with fruits and vegetables, as a nutritional source of natural antioxidants responsible for protecting human health [1,2].

Honey contains amino acids in small proportions, nitrogen compounds and amino acids derived from the main content of flower nectar from insect pollination in plant and sap floem. The type and concentration of amino acids in honey is linked to the amino acid content of bee food sources, which in turn depends on the source of interest, geographical origin and season [3,4].

Proteins and amino acids in honeys are attributable both to animal and vegetal sources, the major of these being pollen. Amino acids account for 1% (w/w) [3], and proline is the major contributor with 50–85% of the total amino acids [5,6]. Paramás, et al., also explained that proline has the potential for honey maturity is associated with proline content in honey [7]. Proline in honey is also used as a
criterion to estimate the antioxidant quality and activity of honey as well as the characterization of the botanical origin [6]. In addition to proline, there are 26 amino acids in honeys, their relative proportions depending on the honey origin (nectar or honeydew) [3]. Some researchers report that other amino acids contained in honey are glutamic acid, alanine, phenylalanine, tyrosine, leucine and isoleucine generally. Honey also contains aspartic acid, glutamine, histidine, glycine, threonine, β-alanine, arginine, α-alanine, γ-aminobutyric acid, valine, methionine, cysteine, tryptophan, ornithine, lysine, serine, and asparagine [2].

Research on amino acid at various examples of honey that is based on regional origin or country in the world already exists, but the honey specifically from South Sulawesi has not been studied nor the characterization of amino acid in honey that are in the forest of South Sulawesi. The aim of this work was, to contribute to the scarce knowledge about the amino acid characterization of forest honeys from South Sulawesi Province.

2. Materials and Methods

2.1. Materials

The materials used in this study were honeys from forest of Terasa, Bontojai and Mallawa village, South Sulawesi Province, aquabidest, hydrochlorid acid (HCl) from Merck, natrium carbonat (Na₂CO₃) from Merck, mix standard of amino acid from Fluka Analytical, fluor borate reagent from Merck, fluor A reagent from Merck, L-α-aminobutyric acid (AABA) from Merck.

2.2. Instrumentations

The instruments used in this study were Acquity Ultra Performance Liquid Chromatography H Class (Waters), vortex (Barnstead), analytical balance (Ohaus), and laboratory equipment commonly used.

2.3. Methods

The research was conducted from Mei 2017 to June 2017. Honey sampling was conducted in Terasa, Sinjai Barat sub-district, Sinjai district; Sabila village, Mallawa sub-district, Maros district; and Bontojai village, Bontocani sub-district, Bone district of South Sulawesi province. Honey was obtained from honey collectors from the area. All samples were placed on clean and dry containers.

The honey samples used for measuring amino acids were Terasa honeys (TR-1, TR-2, TR-3), which were harvested in November 2016, December 2016 and January 2017, Mallawa honeys (MW-1, MW-2) were harvested in October 2016 and January 2017, and Bontojai honeys (BJ-1, BJ-2, and BJ-3) were harvested in November 2016, December 2016 and January 2017. Amino acids analysis was done at PT. Saraswanti Indo Genetech, Bogor.

Honey samples and extraction of amino acid compounds: The analysis was performed using a honey sample ± 0.1 gram and inserted in a capped reaction tube, then added 5 mL of HCl 6 N and homogenized using a vortex. Then hydrolyzed at 110 °C for 22 hours, then cooled to room temperature and transferred in 50 mL flask. Then added aquabides to the boundary marks, then filtered with a 0.45 µm filter. 500 µL filtrate was piped and added 440 µL AABA and 460 µL aquabides then plucked 10 µL, then add 70 µL AccQ Fluor Borate and homogenized. Added 20 µL Flour A reagents and homogenized, then stuck for 1 minute and incubated for 10 minutes at 55°C. Then injected on UPLC.

Direct injection of the derivatized samples, was made on a Waters H Class Ultra Performance Liquid Chromatograph, fitted with a Waters AccQ.Tag Ultra C18 column (1.7 µm, 100 x 2.1 mm), thermostatted at 49°C. Detection was at 260 nm with a PDA cλ Waters detector. Identification was by means of the retention times obtained from pure compounds. Quantification was achieved by using calibration curves obtained from amino acid and ammonium solutions of known concentrations containing the same amount of internal standard as added to samples [8].
3. Results and Discussions

UPLC ensured the qualification and evaluation of 16 amino acids plus ammonium ion in the analysed honeys and amino standard certified as can be seen in Figure 1-2. The summarized data of eight honey samples for the amino acid concentration are presented in Table 1. The amino acids are classified into essential amino acids and non-essential amino acids.

Table 1. Distribution of amino acid concentration of forest honeys from Terasa, Mallawa and Bontojai, South Sulawesi Province. Results are given as mg/kg.

| Amino acids       | TR-1 | TR-2 | TR-3 | Mean TR | MW-1 | Mean MW | BJ-1 | BJ-2 | BJ-3 | Mean BJ |
|-------------------|------|------|------|---------|------|---------|------|------|------|---------|
| Essential amino acids |      |      |      |         |      |         |      |      |      |         |
| Histidine         | 1428.87 | 650.56 | 612.54 | 897.32 | 700.37 | 478.02 | 589.20 | 6520.35 | 989.87 | 720.42 | 2776.81 |
| Threonine         | 371.42 | 227.96 | 238.7 | 279.36 | 268.15 | 239.36 | 253.76 | 243.23 | 217.7 | 180.34 | 217.08 |
| Leucine           | 416.69 | 259.07 | 270.05 | 315.27 | 293.44 | 221.86 | 256.30 | 341.79 | Not detected | 221.89 | 281.84 |
| Lysine            | 298.88 | 259.7 | 258.99 | 275.86 | 187.61 | 156.34 | 171.98 | 168.17 | Not detected | 109.45 | 193.42 | 157.01 |
| Arginine          | 1486.02 | 801.96 | 827.71 | 1038.76 | 683.11 | 665.08 | 674.10 | 247.32 | 1015.16 | 804.27 | 718.92 |
| Valine            | 337.47 | 247.75 | 287.43 | 290.88 | 226.03 | 170.12 | 198.08 | 228.39 | 149.84 | 224.92 | 201.05 |
| Tryptophan        | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected |
| Phenylalanine     | 1025.09 | 435.01 | 673.77 | 711.29 | 288.11 | 174.74 | 231.43 | 270.55 | Not detected | 130.63 | 200.59 |
| Non essential amino acids |      |      |      |         |      |         |      |      |      |         |
| Proline           | 433.1 | 300.66 | 267.43 | 333.73 | 257.44 | 161.58 | 200.51 | 302.68 | 245.06 | 253.98 | 267.24 |
| Tyrosine          | 397.7 | 269.38 | Not detected | 333.54 | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected |
| Aspartic acid     | 584.87 | 625.96 | Not detected | 595.42 | 399.37 | 399.37 | 399.37 | 256.51 | Not detected | 218.89 | 240.26 |
| Glycine           | 332.28 | 230.74 | 211.14 | 258.05 | 402.00 | 402.00 | 402.00 | 261.63 | Not detected | 764.85 | 546.69 | 600.86 |
| Alanine           | 1055.21 | 520.52 | 546.51 | 707.41 | 466.14 | 383.33 | 424.84 | 518.05 | Not detected | 1180.08 | 1029.26 |
| Glutamic acid     | 2111.97 | 1324.72 | 1145.52 | 1527.40 | 1006.06 | 928.83 | 902.76 | 442.77 | Not detected | Not detected | Not detected |
| Serine            | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | 419.18 |
| Methionine        | 151.27 | 100.22 | 138.19 | 129.89 | 103.09 | 69.82 | 85.46 | 107.08 | Not detected | 138.47 | 122.78 |

Amino acid content of forest honey which is multiflora honey from these three regions is also compared with the amino acid content of monoflora honey. Amino acids contained in honey monoflora have been reported by Kivrak, et al., in Turkish eucalyptus honey and Polish acacia honey reported by Janiszewska, et al. [9,10]. Comparison of the average concentration of amino acid content of forest honey from Terasa, Mallawa, Bontojai, and Turkish Eucalyptus honey and Polish acacia honey are described in Table 2.

The essential amino acid which given the highest yield was histidine, which was 6620.35 mg/kg found in BJ-1 from Bontojai, and MW-2 from Mallawa gives the smallest concentration of 478.02 mg/kg. Other essential amino acids detected in all samples in mg/kg were threonin (190.34 – 371.42), lysine (109.45 – 298.88) and valine (149.84 – 337.47). Isoleucine was detected in small quantities in four samples, TR-3, MW-1, BJ-1 and BJ-3 with concentrations of 98.24 mg/kg, 125.94 mg/kg, 123.12 mg/kg and 77.73 mg/kg whereas leusin and phenylalanine were not found in BJ-2.

The abundant non essential amino acids in all honey samples were glutamic acid ranging from 442.77 - 2111.97 mg/kg with an average concentration of 1206.94 mg/kg. TR-1 gave the largest concentration of 2111.97 mg/kg. Proline was also contained in all samples analyzed, with an average concentration of 277.74 mg/kg. This amount is greater than the limit of the proline content to determine honey purity which is at least 180 mg/kg [3,11]. Serine was only detected in BJ-1 with a concentration of 419.18 mg/kg.
Honey from Bontojai (BJ-1) provides a complete profile of 16 amino acids measured in this study. TR-1 from Terasa provides higher amino acid levels than honey from Mallawa and Bontojai. The difference in amino acid content in all honey samples was due to bee food sources, namely pollen which contains proteins and amino acids where the composition depends on the type of plant, environmental conditions including seasons and different locations.

The concentrations of each honey amino acid listed in Table 2 show that forest honey which is multi flora honey containing amino acid levels is generally greater than mono flora honey from Poland and Turkey, this is due to among others the biodversity of the source of nectar and pollen which is food from bees, as explained that the types and concentrations of amino acids in honey are related to the amino acid content of bee food sources, which in turn depends on the source of interest, geographical origin and season [4].

![UPLC chromatogram of the amino acids and the ammonium ion isolated from 15 amino acid standard certified](image)

**Figure 1.** UPLC chromatogram of the amino acids and the ammonium ion isolated from 15 amino acid standard certified
Table 2. Comparison of the average concentration of amino acid content of forest honey from Terasa, Mallawa, Bontojai and Eucalyptus honey from Turkey and acacia honey from Poland in units of mg/kg [9,10]

| Amino acids     | Terasa | Mallawa | Bontojai | Turkey | Poland |
|-----------------|--------|---------|----------|--------|--------|
| Essential amino acids |        |         |          |        |        |
| Histidine       | 897.32 | 589.20  | 2776.81  | 13.96  | 1.45   |
| Threonin        | 279.36 | 253.76  | 217.09   | 10.75  | 3.33   |
| Leucine         | 315.27 | 259.63  | 281.84   | 532.52 | 2.41   |
| Lysine          | 275.86 | 171.98  | 157.01   | 51.42  | 0.51   |
| Arginine        | 1038.76| 674.10  | 718.92   | 13.28  | 1.90   |
| Valine          | 290.88 | 198.08  | 201.05   | 151.24 | 5.67   |
| Isoleucine      | 98.24  | 125.94  | 100.43   | 490.68 | 2.83   |
| Phenylalanine   | 711.29 | 231.43  | 200.59   | 3748.54| 0.44   |
| Non essential amino acids |      |         |          |        |        |
| Proline         | 333.73 | 209.51  | 267.24   | 1305.35| 225.74 |
| Tyrosine        | 333.54 | Not detected | 215.33  | 612.24 | 51.01  |
| Aspartic acid   | 595.42 | 399.37  | 256.51   | 7.54   | 7.77   |
| Glycine         | 258.05 | 402.00  | 240.26   | 39.98  | 2.79   |
| Alanine         | 707.41 | 424.84  | 609.86   | 205.44 | 7.26   |
| Glutamic acid   | 1527.40| 992.76  | 1029.26  | 78.86  | 9.12   |
| Serine          | Not detected | Not detected | 419.18 | Not detected | 6.20 |
| Methionine      | 129.89 | 86.46   | 122.78   | 38.35  | 1.58   |

Amino acid in honey is one component of honey which provides antioxidant activity in addition to the enzymes glucose oxidase, enzyme catalase, ascorbic acid, flavanoids, phenolic acids, derivatives of carotenoids, organic acids, Maillard reaction products, and proteins [12]. Amino acid which has a high correlation with antioxidant activity, namely aspartic acid, glutamic acid, glycine, threonine and glutamine where aspartic acid and glutamic acid are negatively charged polar amino acids while glycine, threonine and glutamine are amino acids without charge so as to make an important contribution to electric conductivity of honey which is a parameter associated with antioxidant activity of honey [5]. In this study the average yields in mg/kg for aspartic acid were 256.51 - 595.42, glutamic acid were 992.76 - 1527.40, glycine were 240.26 - 402.00 and threonin were 217.09 - 279.36, while glutamine was not detected. Pérez, et al. found that amino acid levels in honedew honey from Spain which gave a high correlation to antioxidant activity were aspartic acid (132.2 mg/kg), glutamic acid (276.0 mg/kg), glycine (21.0 mg/kg), threonin (34.5 mg/kg) and glutamine (162.2 mg/kg) [5]. Based on the comparison above, further research related to the relationship of amino acid concentrations and antioxidant activity of forest honey from Terasa, Mallawa and Bontojai needs to be done.
Figure 2. UPLC chromatogram of the amino acids and the ammonium ion isolated from 16 amino acid of forest honey: (a) Mallawa; MW-1, (b) Bontojai; BJ-1 (c) Terasa; TR-1.
4. Conclusion

The amino acid characterizations of eight forest honeys from some areas of South Sulawesi Province demonstrated that although most of the amino acid concentrations were similar, some significant differences could be found. The concentrations of histidine was apparently higher than that in other amino acids. In conclusion, our results support the characterization of amino acids in forest honeys from South Sulawesi Province and can be a source of essential amino acids.

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