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PROFILE OF AMINO ACIDS OF FOREST HONEYS FROM MALLAWA SOUTH SULAWESI

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ABSTRACT

Mallawa is one of the areas that has production forests as the second largest honey producer in Maros. The aim of this research determined the profile of amino acids in honey. Amino acids of two forest honeys were analyzed using ultra performance liquid chromatography (UPLC). The main amino acids detected honeys were glutamic acid (1060.66 mg/kg, 928.85 mg/kg), histidine (700.37 mg/kg, 478.02 mg/kg), and arginin (683.11 mg/kg, 665.08 mg/kg), but tyrosine and serine not detected. Some other amino acids, including threonine, leucine, valine, phenylalanine and proline, displayed similar values contents of 239.36 mg/kg, 268.15 mg/kg; 225.81 mg/kg, 293.44 mg/kg; 170.12 mg/kg, 226.03 mg/kg; 174.74 mg/kg, 288.11 mg/kg; and 161.58 mg/kg, 257.44 mg/kg, respectively.

Keywords: Honey, Forest Mallawa, ultra performance liquid chromatography (UPLC), glutamic acid

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1. INTRODUCTION

Honey is a sweet liquid derived from plant nectar that is processed by bees and stored in honeycomb cells. Honey has unique characteristic and is one of the best sources of food, which is influenced by different types of honey-containing compounds[1], such as amino acids, carbohydrates, proteins, certain types of vitamins and minerals, volatile compounds, and secondary metabolite compounds. There are at least 181 types of compounds in honey[2].

One of the biochemical compositions of honey is the amino acid. Amino acids, being an important constituent of food, serve as structural compounds in protein synthesis, increase food flavor, and are precursors to aroma compounds[3]. 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[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), and proline is the major contributor with 50–85% of the total amino acids. Besides proline, there are 26 amino acids in honeys, their relative proportions depending on the honey origin (nectar or honeydew)[5].

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[4]. Pirini et.al., 1992 ;. Bouseta et.al., 1996 has found that amino acids in honey come from bees and food sources[5]. Hermosin, I., et.al., 2003 and Keckeš, S., et.al., 2013 mention that the relative amino acid ratio (about 0.3-1% w / w) in honey is related to the source (nectar or honey dew). Paramás, et.al., 2006 and Ohe, et al., 2004 also explained that proline is a major contributor to total amino acids in honey and the potential for honey maturity is associated with proline content in honey[3][5].

Mallawa is one of the districts in Maros which has a forest area of 14,610.68 ha. The area lies at 119.30 'East longitude and 5.00' South latitude is one of the areas that has production forests as the second largest honey producer in Maros district of 4,058.64 ha[6]. Most of Mallawa residents undertake honey bee harvesting activities in large quantities of 1.601.25 kg/year[7].

Geographical location Mallawa topographical districts that are in the ground slope between 3-70% and the altitude above sea level between 15-458 M. stretch of rocks making up includes karst hills, volcanic mountains, hills intrusion and sediments. Rock constituent dominated by karst hills composed of limestone is one sign that the area was once a sea. Spermonde Islands are located around the Makassar Strait is thought to have a correlation with the geographical conditions Mallawa, thus indirectly may affect the presence of floral origin in Mallawa. 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 districts Mallawa Maros has not been studied nor the profile of amino acid in honey that are in the forest of Mallawa[8]

The aim of this research was, to determine profile of amino acid of Mallawa forest honeys.

2. MATERIAL AND METHOD

2.1 Materials and Tools

The materials used were Mallawa honey, aquabidest, hidrochlorid acid (HCl) from Merck, natrium karbonat (Na_2CO_3), standart of amino acid PIERCE H No. NC1 0180 Sigma Chemical-Company USA, fluor borate reagent, fluor A reagent, L-α-amino butyric acid (AABA). The tools used in this study were Acquity UPLC H Class (Waters), vortex, analytical balance (Ohaus), and laboratory equipment commonly used.

2.2 Time and Place Research

The research was conducted from Mei 2017 to June 2017. Amino acids analysis was done at PT. Saraswanti Indo Genetech, Bogor.

2.3 Sampling

Honey sampling was conducted in Sabila village, Mallawa sub-district, Maros district, South Sulawesi. Honey is obtained from honey collectors from the area. All samples are placed on clean and dry containers.

2.4 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 divortex. Added 20 µL Flour A and divortex reagents, then stuck for 1 minute and incubated for 10 minutes at 55º C. Then injected on UPLC.

2.5 Analytical methods

Direct injection of the derivatizated 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 49ºC. Detection was at 260 nm with a PDA eλ 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.

3. RESULT AND DISCUSSION

3.1 Identification of amino acids

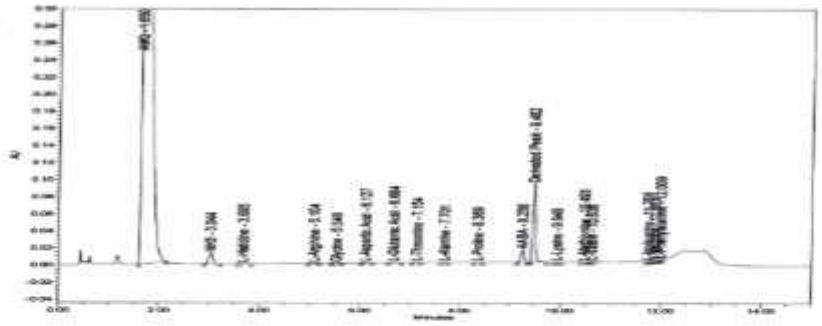
UPLC ensured the qualification and evaluation of 15 amino acids plus ammonium ion in the analysed honeys, as can be seen in Fig. 1. The summarized data of two honey samples for the amino acid content are presented in Table 1. The essential amino acids (Histidine, Threonin, Leucine, Lisin, Arginine, Valine, Phenylalanine) which were present in honey samples but isoleucine was detected in one of the honey samples. The abundant amino acids revealed for the studied honey samples were histidine and arginin. The non-essential amino acids (Proline, Aspartic acid, glycine, alanine, glutamic acid), were detected in Mallawa honey, but tyrosine and serine were not detected in sample honey. Amino acids contained in both samples of honey showed the highest glutamate acid content of 1060.66 mg/kg

Table 1. Comparison of different Amino acid of honeys from Mallawa and single botanical origin honeys from Spanish and Turkish according to the content (as mg/kg of honey)

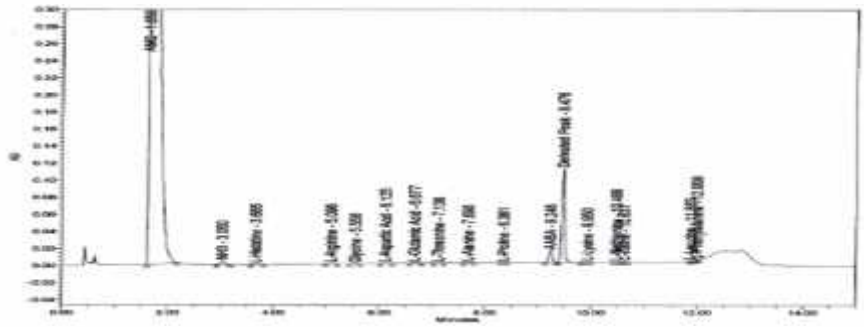
Amino acids	Unit	Mallawa 1	Mallawa 2	Spanish	Turkish
Essential amino acids					
Histidine (His)	mg/kg	700.37	478.02	35.7	69.02
Threonine (Thr)	mg/kg	268.15	239.36	6.5	45.75
Leucine (Leu)	mg/kg	293.44	225.81	27.9	2033.25
Lysine (Lys)	mg/kg	187.61	156.34	33.1	141.75
Arginin (Arg)	mg/kg	683.11	665.08	96.4	55.24
Valine (Val)	mg/kg	226.03	170.12	34.1	483.92
Isoleucine (Ile)	mg/kg	125.94	Not detected	22.6	1438
Phenylalanine (Phe)	mg/kg	288.11	174.74	886	5299.05
Non essential amino acids					
Proline (Pro)	mg/kg	257.44	161.58	879	1290.01
Tyrosine (Tyr)	mg/kg	Not detected	Not detected	404	1047.44
Aspartic acid (Asp)	mg/kg	399.37	399.37	33.9	51.55
Glycine (Gly)	mg/kg	402.00	402.00	6	126.75
Alanine (Ala)	mg/kg	466.14	383.53	37.6	322.85
Glutamic acid (Glu)	mg/kg	1060.66	924.85	153	85.13
Serine (Ser)	mg/kg	Not detected	Not detected	2.52	62.45

and 924.85 mg/kg. Some other amino acids, including threonine, leucine, valine, phenylalanine and proline, displayed similar mean values contents of 239.36 mg/kg – 268.15 mg/kg, 225.81 mg/kg – 293.44 mg/kg, 170.12 mg/kg – 226.03 mg/kg, 174.74 mg/kg – 288/11 mg/kg, and 161.58 mg/kg – 257.44 mg/kg, respectively. These amino acids had been found in honeys from some areas, such as certain Turkish and Spanish honeys. Hermosin et al. determined the main amino acids in Spanish honeys were proline, phenylalanine, tyrosine and lysine, followed by arginine, glutamic acid, histidine and valine[5] and Turkish honey were phenylalanine (4024.53 mg/kg), proline (1138.18 mg/kg), tyrosine (693.42 mg/kg), and isoleucine (749.81 mg/kg) was research by Kivrak et al.[3]. The comparison of different amino acid of honeys from Mallawa, Spanish and Turkish showed in Table 1.

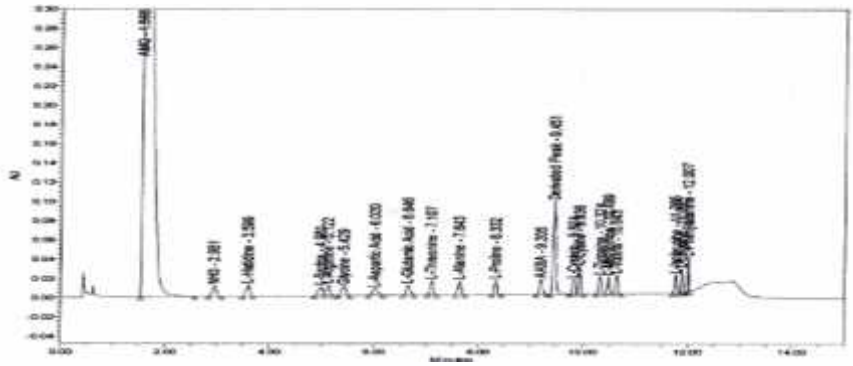
Table 1 showed that amino acids in



A. Mallawa 1



B. Mallawa 2



C. Amino acid standard

Figure 1. UPLC chromatogram of the amino acids and the ammonium ion isolated from two sample of Mallawa honey and 15 amino acid standard certified

Mallawa honey were histidine, threonin, lysine, arginine, aspartic acid, glycine, and glutamate acid, more abundant than honey from Spain and Turkey. Leucine, isoleucine, phenylalanine, tyrosine and proline showed a greater amount in honey from Spain and Turkey. Serine was detected in small quantities from both countries. Amino acids in honey play a central role as building blocks of proteins and as intermediates in the metabolism. They are precursors for the production of the key flavour compounds[9].

4. CONCLUSION

The amino acid profiles of two Mallawa honeys demonstrated that although most of the amino acid contents were similar, some significant differences could be found. The contents of glutamic acid in samples was apparently higher than that in other samples. In conclusion, our results support the profile of amino acids in Mallawa honeys.

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