

Free Amino Acids Content of Pakistani Unifloral Honey Produced by *Apis mellifera*

SAMINA QAMER, MOHAMMED EHSAN, SHAHID NADEEM AND ABDUL RAUF SHAKOORI*

School of Biological Sciences, University of the Punjab, New Campus, Lahore (SQ, ME, ARS) and Nuclear Institute of Agriculture and Biology, Jhang Road, Faisalabad (SN)

Abstract.- Pakistani honey samples of different botanical and geographical origin were analyzed for free amino acids profile. A total of seventeen free amino acids were detected in sunflower honey sample from Honeybee Research Farms of University of the Punjab, Phulai honey sample from Islamabad and sidder/ber honey samples from Bannu, Karak and Chunian. Proline was found to be the most dominant amino acid followed by aspartic acid and glutamic acid. Methionine was exclusively present in three sidder honey samples collected from different localities of Pakistan. Lysine and tryptophan were exclusively found in sunflower honey sample. Arginine was the only essential amino acid found in "Phulai" honey sample. Free amino acids have been found to be good indicators of botanical origin of honey.

Key words: Pakistani honey, essential amino acids, sidder honey.

INTRODUCTION

Honey contains amino acids in small proportions (Crane, 1980). Nitrogenous compounds and amino acids are the common constituents of floral nectar of insect pollinated plants and originate from phloem sap (Baker and Baker, 1973). Variation in the type and concentration of amino acids in honey is related to the amino acids content in the bee's diet, which in turn depends upon floral source, and season of the year. During spring when the plants sprout and in autumn when leaves change colour the concentration of amino acids and nitrogenous compounds become very high in the phloem sap, resultantly it increases in nectar and finally it enters the honey (Maurizio, 1975). White (1979) detected 11 to 21 different free amino acids (FAA) in honey. Komamine (1960) noted that proline was the predominant amino acid in honey, which is 50-85 % of total amino acid content. It has been found that amino acids in honey originate both from the bees and the food source (Pirini *et al.*, 1992; Bouseta *et al.*, 1996).

In the past few years, amino acid analysis has become the most popular method for determination of honey nectar source. The total amino acid content ranges from 20mg/100g to 300mg/100g. Large

0030-9923/2007/0002-0099 \$ 8.00/0

Copyright 2007 Zoological Society of Pakistan.

fluctuations have been observed both in the number and the concentration of amino acids in honey of different botanical and geographic origins (Pawlowska and Armstrong, 1994; Pirini *et al.*, 1992). Cometto *et al.* (2003) found various FAA in different groups of honey representing different floral and three geographic regions of Argentina. Iglesias *et al.* (2004) reported phenylalanine as major essential amino acid followed by lysine, arginine, histidine, valine, isoleucine, leucine and tryptophane in floral honey samples of Spain. The aim of the present study was to assess the usefulness of the FAA composition of honey as a marker for *Apis mellifera* unifloral honey. This is the first attempt to characterize Pakistani honey of various floral origins with reference to FAA composition.

MATERIALS AND METHODS

Sources of honey samples

Honey produced by *Apis mellifera*, using nectar sources of *Acacia modesta* (phulai), *Helianthus annuus* (sunflower) and *Ziziphus jojoba* (sidder or ber) were included in this study. *Acacia modesta* commonly known as "Phulai" is a native forest tree in the foothills areas, and plains of Punjab and Sind in Pakistan. *Ziziphus jojoba* is another economically important plants in Pakistan. It is commonly known as 'sidder' or 'ber' and is

* Corresponding author: Phone: +92-42-9231248.
E-mail: arshak@brain.net.pk

indigenous plant species to Pakistan. It is a bush like tree found in Karak, Kohat and Bannu districts of N.W.F.P, Attock, Chakwal and Mianwali districts of Punjab, Karachi, Hyderabad and Nawabshah districts of Sind province. *Helianthus annuus* is known as "sunflower". It is an oil seed crop and grown throughout the country mainly for oil (Anonymous, 1985; Ahmad *et al.*, 1986).

For analysis *Ziziphus jojoba* (sidder) honey samples were collected from Karak and Bannu districts of N.W.F.P, and Chunian, 100km north of Lahore, Punjab. *Acacia modesta* (Phulai) honey samples were collected from Rawalpindi area, whereas crop of sunflower was cultivated on Honeybee Research Farms within University campus.

Free amino acids analysis

The qualitative and quantitative analysis of FAA in honey was done by High Performance Liquid Chromatography according to the procedure of Pawlowska and Armstrong (1994). The column (150 x 4.6 mm) used was Hypersil® ODS. Mobile phase, 1% acetonitrile; gradient, isocratic elution; flow rate, 1 ml/min; temperature, 40°C; detection wavelength 266 nm. 1 ml of standard amino acid (3 mg/ml) was added in 1.5 ml reaction mixture (9 ml acetonitrile + 1 ml 1% Na₂CO₃ + 20 mg FMOCCl reagent) and diluted with 7.5 ml blank (9 ml acetonitrile + 1 ml 1% Na₂CO₃). 20 µl of each of the amino acids were run separately. The retention time and height of the peak were noted.

Honey (10 g) was diluted with 9 ml of a water-acetonitrile mixture (1/2, v/v). Then 1 ml of 1% Na₂CO₃ was added and the sample was derivatized at room temperature with 20 mg of FMOCCl reagent. After 5 minutes the sample was acidified with 50% acetic acid solution to stabilize the FMOCCl group. This honey sample (20 µl) was analyzed for amino acid contents through HPLC. The chromatographic peaks were identified by comparing the retention times of the standards and the sample components.

Statistical analysis

The data was subjected to statistical analysis in which different physicochemical and biochemical

parameters of honey were compared using analysis of variance, student's 't' test and 'F' test (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

Table I shows the FAA profile of Pakistani honey samples with nectar source from sunflower, phulai and sidder/ber honeys, whereas Figure 1 shows the chromatograms of FMOC derivatized honey samples.

Honey samples of phulai floral source showed a total of five detectable FAA (34.05 mg/100g), whereas seven amino acids could be detected in sunflower (42.93mg/100g) and sidder honey from Bannu (45.57mg/100g). The honey samples of Ber floral origin from Karak, however, showed a total of 13 detectable amino acids (49.59 mg/100g), and the one from 'ber' floral origin from Chunian showed a total of 8 detectable FAA (41.57 mg/100g). In sidder honey samples of different habitats, methionine, aspartic acid, cysteine, proline and hydroxyproline seem to be universal component. Phulai and sunflower honey sample had proline and glutamic acid. There are some amino acids such as isoleucine, leucine, methionine, valine, glycine and serine are absent from Phulai and sunflower honey samples.

Essential amino acids

Methionine was the only essential FAA found exclusively in all sidder honey samples from Bannu (1.66 mg/100g), Karak (1.19 mg/100g) and Chunian (1.75 mg/100g), while it was absent in Phulai and sunflower honey samples. Arginine has been detected in Phulai (1.45mg/100g) honey sample and in two sidder (Karak, 0.23mg/100g and Chunian, 0.79mg/100g) honey samples. In sidder sample, leucine and lysine were absent. Sidder honey samples of Bannu and Chunian had no isoleucine, phenylalanine and tryptophane. Sidder honey sample from Karak had exclusively isoleucine (0.07mg/100g) and phenylalanine (0.21mg/100g), while other sidder samples did not have these amino acids. Bannu and Karak areas have wild sidder plantation, whereas Chunian contains cultivated sidder plantation. Moreover, the flower blooming period of wild sidder plants is followed by

cultivated sidder species. A different geographic region, climatic conditions and sidder species appear to be the main reasons for variation in the

Table I.- Amino acid profile of Pakistani honey samples (mg per 100g honey).

Amino acids	Floral source				
	Phulai honey	Sunflower honey	Bannu ber honey	Karak ber honey	Chunian ber honey
Arginine	1.45	-	-	0.23	0.79
Isoleucine	-	-	-	0.07	-
Leucine	-	-	-	-	-
Lysine	-	1.3	-	-	-
Methionine	-	-	1.66	1.19	1.75
Phenylalaine	-	-	-	0.21	-
Tryptophane	-	1.9	-	1.07	-
Valine	-	-	1.61	-	-
Alanine	-	0.91	-	0.47	0.11
Aspartic acid	8.7	-	8.3	4.5	7.5
Cystein	5.8	-	0.71	0.57	0.13
Glutamic acid	5.8	2.9	-	-	-
Glycine	-	-	-	0.96	-
Hydroxyproline	-	0.11	2.95	1.5	0.89
Proline	12.3	35.6	29.7	33.01	27.6
Tryosine	-	0.21	-	5.6	2.8
Serine	-	-	0.64	0.21	-
Total amino acids	34.05	42.93	45.57	49.59	41.57

composition of FAA in sidder honeys.

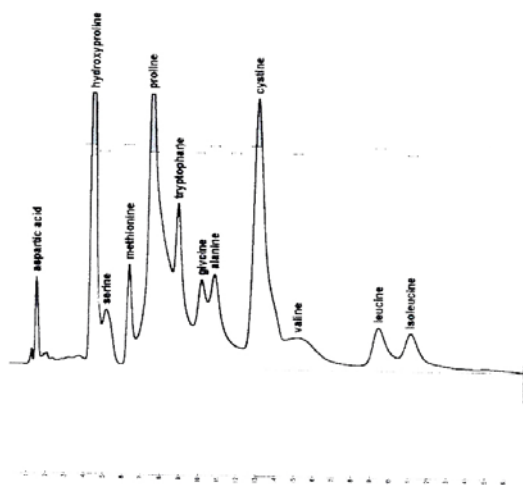
Sunflower honey had lysine (1.3mg/100g) and tryptophane (1.9mg/100g), and phulai honey had only one (arginine, 4.25mg/100g) essential amino acid. In the case of Pakistani unifloral honey the number of essential amino acids were found to be less than those reported by Komamine (1960), Meada *et al.* (1962), Michelotti and Margkeri (1969), Bauseta *et al.* (1996) and by Iglesia *et al.* (2004). The essential FAA profile of Pakistani honey is close to Biino (1971) who detected a total of 8 amino acids in *Acacia* honey in which 0.52mg/100g each of isoleucine and leucine were reported.

Non-essential amino acids

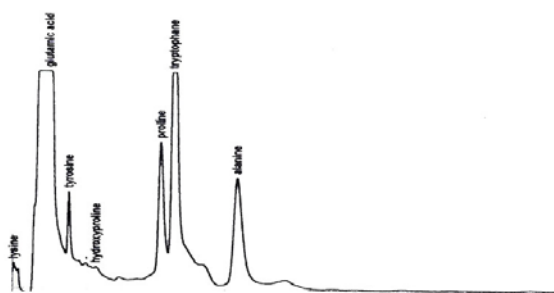
Proline was the dominant amino acid in Pakistani unifloral honey. It was 36% (12.3mg/100g) of the total amino acid content in Phulai, 83% (35.6 mg/100g) in sunflower, 65% (29.7mg/100g) in Bannu sidder, 66% (33.01mg/100g) in karak sidder and 66.5% (27.6 mg/100g) in Chunian sidder honey. Aspartic acid

was 26% (8.7mg/100g) in phulai honey sample, 18% (8.3 mg/100g) in Bannu sidder, 9% (4.5 mg/100g) in Karak sidder and 18% (7.5 mg/100g) in Chunian sidder honey sample. Glutamic acid was 5.8mg/100g (17%) and 2.9mg/100g (7%), respectively, in Phalui and Sunflower honey samples, while it was altogether absent in three sidder honey samples. Cystein was found in Phulai (5.8 mg/100g), Bannu sidder (0.71 mg/100g), Karak sidder (0.57 mg/100g) and in Chunian sidder honey (0.13 mg/100g). Hydroxyproline was detected as 2.95 mg/100g in Bannu sidder, 1.5 mg/100g in Karak sidder, 0.89mg/100g in Chunian sidder and 0.11 mg/100g in sunflower honey. The proline amount in Pakistani unifloral honey is close to that of Mizusawa and Matrumur (1968), who reported 20-28.71mg/100g proline. Meada *et al.* (1962) recorded proline much higher (134-249mg/100g) as compared with that of Pakistani unifloral honey. Bouseta *et al.* (1996) reported 167-722 ppm proline in eucalyptus and lavender honey. Iglesias *et al.* (2004) found higher concentration of proline (67.15mg/100g). Pakistani honey samples showed less

amount of glutamic acid and slightly higher concentration of aspartic acid as compared with that of Iglesias *et al.* (2004).



A



B

Fig. 1. Chromatograms of standard amino acids (A), and sunflower honey sample from Pakistan (B).

REFERENCES

- BAKER, H. G. AND BAKER, I., 1973. Amino acids in nectar and their evolutionary significance. *Nature*, **241**: 543-545.
- BIINO, L., 1971. Ricerca di alcuni aminoacidi in due variet  di miele. *Riv. ital. Essenze Proumi*, **53**: 80-84.
- BOUSETA, A., SCHEIRMAN, V. AND COLLIN, S., 1996. Flavor and free amino acid composition of lavender and eucalyptus honeys. *J. Fd. Sci.*, **61**: 687-694.
- CRANE, E., 1980. *A book of honey*. Oxford University Press, UK, 49 pp.
- CRANE, E., 1990. The traditional hive products: honey and beeswax. In: *Bees and beekeeping. Science, Practice and World Resources*: Heinemann Newnes, Oxford, UK. P 388-451.
- COMETTO, P. M., FAYE, P. f., DiPAOLA NARANJO, R. D., RUBIO, M. A. AND ALDAO, M-A., 2003. Comparison of free amino acids profile in honey from three Argentinian regions. *J. Agric. Fd. Chem.*, **51**: 5079-5087.
- KOMAMINE, A., 1960. Amino acids in honey. *Acta Chem.fenn.*, **33**: 185-187.
- IGLESIAS, M. T., De LORENZO, C., POLO, M. D. C., MARTIN-ALVAREZ, P. J. AND PUEYO, E., 2004. Usefulness of amino acid composition to discriminate between honeydew and floral honeys. Application to honeys from a small geographic area. *J. Agric. Fd. Chem.*, **52**: 84-89.
- MAEDA, S., MUKAI, A., KOSUGI, N. AND OKADA, Y., 1962. The flavour components of honey. *J. Fd. Sci.Tech.*, **9**: 270-277.
- MAURIZIO, A., 1975. How bees make honey? In: *Honey: A comprehensive survey* (ed. E. Crane), pp. 77-105, Heinemann, London.
- MICHELOTTI, P. AND MARGHERI, G., 1969. Studio sul contenuto in aminoacidi del miele. *Sci. Alimen.*, **15**: 170-180.
- MINH, H. VAN., MENDOZA, B. G. AND LAIGO, F. M., 1971. The chemical composition of honey produced by *Apis dorsata*. *J. Apic. Res.*, **10**: 91-97.
- MITRA, S. N AND MATHEW, T. V., 1968. Studies on the characteristics of honey. *Proc. Inst. Chem. India*, **40**: 26-30.
- MIZUSAWA, H. AND MATSUMURO, H., 1968. Studies on chemical composition of honey. IV. Composition of free amino acids. *Bull. Fac. Agric. Tamagawa Univ.*, **7-8**: 194-196.
- STEEL, R.G.D. AND TORRIE, J.H., 1981. Analysis of Variance factorial experiments. In: *Principles and procedures of statistics*. 2nd Ed., McGraw Hill International Book Company, Tokyo, pp. 336-375.
- PAWLOWSKA, M. AND ARMSTRONG, D. W., 1994. Evaluation of enantiomeric purity of selected amino acids in honey. *Chirality*, **6**: 270-276.
- PIRINI, A., LANFRANCO, S. C., FRANCIOSO, O. AND LERCKER, G., 1992. Capillary gas chromatographic determination of free amino acid in honey as a mean of discrimination between different botanical sources. *J. High Resol. Chromat.*, **15**: 165-170.
- WHITE, J. W. Jr., RIETHOF, M. L., SUBERS, M. H. AND

KUSHNIR, I., 1962. Composition of American honey.
Tech. Bull. U.S. Dept. Agric., **1261**: 124.

(Received 7 January 2006, revised 20 December 2006)