E-ISSN: 2278 – 179X

Journal of Environmental Science, Computer Science and Engineering & Technology

Available online at www.jecet.org

Engineering & Technology

Research Article

Amino acid characteristics of multifloral honey of indigenous bee *apis dorsata f.* and *apis cerana indica* from Udupi, Dakshina Kannada and Uttara Kannada districts of Karnataka

Balasubramanyam M.V.[1], Ramesha Iyyanahalli [1] and Jayaram, K.M. [2]

[1]Department of Zoology, Maharani's Science College for Women, Palace Road, Bangalore – 560 001

[2]Department of Zoology, Vijaya College, Basavanagudi, Bangalore – 560 004

Received: 15 March; 2012; Revised: 25 March 2012; Accepted: 29 March 2012

ABSTRACT

Multifloral honey of giant honey bee, A. dorsata (wild) and Indian hivebee, A. cerana indica (apiary) was collected from the Udupi, Dakshina Kannada and Uttara Kannada districts of Karnataka and six amino acids characteristics were determined during April 2011 to March 2012. Six amino acids viz., proline (P), lysine (K), glutamic acid (E), aspartic acid (D), valine (V) and histidine (H) were analyzed in honey of wild and apiary honeybees. Proline (89.10 µ gms) was maximal from Dakshina Kannada in A. cerana honey while minimal of (83.01 µ gms) proline was found in A.dorsata honey in Udupi. Similarly A.dorsata honey had lowest of histidine (5.97 µ gms) and highest of (7.24 µ gms) in A.cerana from Udupi and Dakshina Kannada respectively. Lysine (45.12 µ gms) was maximum from Dakshina Kannada in A. cerana honey while minimum of (38.70 µ gms) lysine was found in A.dorsata honey in Udupi. Similarly A.dorsata honey had lowest of (17.16 µ gms, 15.95 µ gms and 8.52 µ gms) and highest of (20.95 µ gms,.18.13 µ gms and 10.90 µ gms) glutamic acid, aspartic acid and valine in A.cerana from Udupi and Dakshina Kannada respectively. Proline, lysine and glutamic acid of wild and apiary honey was significant at 1% (p<0.01) level, while aspartic acid, valine and histidine of honey of both species of honeybee was not significant at 1% (p<0.01) in Udupi, Dakshina Kannada and Uttara Kannada. All the six amino acids tested in A.dorsata and A.cerana displayed quantitative fluctuations in different geographical areas which are discussed in ensuing paper.

Keywords: *A.cerana*, *A.dorsata*, apiary, aspartic acid, glutamic acid, histidine, lysine, proline, valine, honey, Dakshina Kannada Udupi, Uttara Kannada, wild, Karnataka.

INTRODUCTION

Honeybees and flowers are classical examples of mutualism and co-evolution. Honeybees are eusocial hymenopterans which are reliant on floral wealth like nectar and pollen. Honey is delectable sweet

product, which essentially consists of simple sugars, predominantly laevulose and dextrose [1]. The amount of honey produced from the floral nectaries depends on the total quantity of nectar secreted and the sugar concentration of the nectar [2]. Nectar consists of ions, organic acids, terpenes, alkaloids, flavonoids, carotenoids, xanthophylls, glycosides, amino acids, volatile oils, pinocembrin, galagin, polyphenols, tocopherols, lycopene and amino acids which are obviously found in honey. Because of this unique, complex and typical quality, honey finds place in antiseptic, laxative, antibiotic, pacifier, anti-oxidant and ingredient of variety of pharmaceutical, bakery, cosmetics, confectionary, and tobacco industry. Since times immemorial honey and milk are considered as symbol of prosperity and sanctity. Honey besides milk, curd, sugar and ghee are requisite constituents of panchamrutha, food offerings to God and religious ceremonies [3].

Hitherto the quality of temperate honey of *A.mellifera* including its composition and physico-chemical properties has been well-known. On the contrary, information on composition of tropical honeybee *A.cerana* honey is limited [4, 5, and 6]. Interestingly, no information is available on minor constituents of honey like amino acids in wild and apiary honeybees which are essential as precursors and energy metabolites. Therefore, primary aim of the current study is to provide comprehensive information on the six amino acids in *A.dorsata* and *A.cerana* honey from Udupi, Dakshina Kannada and Uttara Kannada districts spreading over the western ghats of Karnataka.

MATERIALS AND METHODS

Karnataka state extends from 11^{o} 5 N to 19 ^{o} NL and from 74 ^{o} E 78 ^{o} EL. It lies in Deccan plateau with three major physical divisions' viz., coast, malnad and maidan. The total geographical area of the State is 1, 91,791 sq. kms, of which 54.70 % as net sown area, 16.14% forests, 10.66% not available for cultivation, 9.55% uncultivated land and 8.96% fallow land. The flora of three districts of Karnataka is rich and diversified, which includes agricultural, plantation, commercial, horticultural and forest flora. The temperature varied from 11 ^{o} C to 41 ^{o} C and the humidity ranges from 27.7% to 83.45%. Udupi is coastal district which extends from 13^{o} 20' NL and 74 ^{o} E 45' EL. Dakshina Kannada is also coastal district that extends from 12^{o} 52' NL and 74 ^{o} E 53' EL.

Uttara Kannada is towards the north-western part of Karnataka with extension from 14^{o} 48' NL and from 74 ^{o} E 11' EL. All these districts form an integral part of western ghats popularly well-known as Sahyadri hills are formed by the Malabar plains and succession of mountains running parallel to Indian west coast. Western ghats covers a large area from southern region (Agastyamalay range to Kalakkad Mundantorai Hill ranges) to Gujarat (Surat Dings) in the North. The entire hill range is divided into three regions namely southern western ghats (Kalakkad Mundantorai to Palghat),

Central western ghats (Nilgiri- Wyanaad to Goa) and Northern western ghats (Northern Goa, Rathnagiri, Amboli to Dings in Gujarat). The rainfall is mainly due to South-west monsoon and the rainfall drastically reduces from south to north. The western ghats regarded as one of the twelve global hotspots of biodiversity and one of the two biodiversity hotspots in India with huge capacity of endemic species of flora and fauna. Unique floral and faunal assemblages characterize the biodiversity of the Western Ghats. Western ghats does support a significant diversity of endemic species, with nearly fifty species and one endemic genus of bat along with lion tailed macaques, nilgiri martin and brown palm. In addition, these districts of western ghats support innumerable genera of Arthropoda including wild and domesticated honeybee species due to variety of forest, plantation, horticultural and agricultural bee flora which yield pollen and nectar throughout the year which are pre-requisite for survival, propagation and honey production in these areas.

Honey collection: Three districts namely Udupi, Dakshina Kannada and Uttara Kannada districts from western ghats were selected for the present study. From each district centres, twenty honey samples for each honeybee species were collected and analyzed from mineral characteristics. Honey samples of domesticated hive bee, *A. cerana* were collected from the beekeepers and that of the rock bee, *A. dorsata*

was procured from tribals and honey hunters. The honey of *A. cerana* was extracted by honey extractor and that of *A.dorsata* was obtained by squeezing and filteration. All honey samples were raw and unprocessed.

Preparation of honey samples: The honey samples were collected in sterilized polythene bottles from the place of honey extraction. The honey was filtered through single thickness fine cloth to remove suspended particles like dirt, beeswax and other impurities. Later it was stored in airtight container at room temperature under hygienic conditions.

ANALYSIS OF HONEY SAMPLES

Floral analysis of honey: All honey samples were prepared according to the method described by [7] for the identification of their floral source on the basis of pollen grains.

Determination of Amino acids in honey: Six amino acids *viz*., proline (P), lysine (K), glutamic acid (E), aspartic acid (D), valine (V) and histidine (H) were analyzed in honey of wild and apiary honeybees. Determination of amino acids was done according to method followed by [8]. All amino acids are measured in micrograms/ 100 gms. of honey.

Statistical analysis of Data: Data of the six amino acids of honey samples from Udupi, Dakshina Kannada and Uttara Kannada from western ghats were analyzed by F-test. The analysis of variance (ANOVA) along the F-test was calculated and significant levels were determined using F-table ($p<0.01$and $p<0.05$).

RESULTS AND DISCUSSION

Melissopalynological studies of honey samples from the study area revealed the occurrence of plantation, (*Cocus nucifera, Coffea arabica, Tectona grandis*), forest flora, (*Syzygium caryophyllatum, Borassus flabellifera, Sapindus emarginatus*) and commercial crops (*Musa paradisiaca, Ricinus communis Anacardium occindentale*) along with many other minor floral resources **(Table 1, 2 and 3)**. Abundant floral resources coupled with suitable environmental factors are mainly responsible for copious honey production in these regions. Further, bee flora was found throughout the year particularly in Western Ghats as when compared to plains and hills of Karnataka.

The amino acids characteristics of honey from wild and apiary honeybee species showed considerable variations both with the species and also from different geographical areas. In general, of the six amino acids identified, proline was found maximum in honey from two honeybee species, while histidine was least in concentration in all the different samples of honey tested. Proline (89.10 µ gms) was maximal from Dakshina Kannada in *A. cerana* honey while minimal of (83.01 µ gms) proline was found in *A.dorsata* honey in Udupi (Fig. 1 and 2).

Similarly *A.dorsata* honey had lowest of lysine (38.70 µ gms) and highest of (45.12 µ gms) in *A.cerana* from Udupi and Dakshina Kannada respectively (1 and 2). Glutamic acid (20.95 µ gms) was maximum from Dakshina Kannada while minimum of (17.16 µ gms) glutamic acid in *A.dorsata* honey in Uttara Kannada (2 and 3). Aspartic acid (18.13 µ gms) was maximal from Dakshina Kannada in *A. cerana* honey while minimal of (15.95 µ gms) aspartic acid was found in *A.dorsata* honey in Uttara Kannada (Fig. 2 and 3). Similarly *A.dorsata* honey had lowest of valine (8.52 µ gms) and highest of (10.90 µ gms) in *A.cerana* from Udupi and Dakshina Kannada respectively (1 and 2). Histidine (7.24 µ gms) was maximum from Dakshina Kannada while minimum of (5.97 µ gms) histidine in *A.dorsata* honey in Udupi (1 and 2). . Proline, lysine and glutamic acid of wild and apiary honey was significant at 1% ($p<0.01$) level, while aspartic acid, valine and histidine of honey of both species of honeybee was not significant at 1% ($p<0.01$) in Udupi, Dakshina Kannada and Uttara Kannada.

During the path of formation of honey, amino acids are also steadily augmenting to optimal levels found in comb honey [8]. Functionally amino acids play very vital roles like defense, transport, storage, vision, toxin, respiratory and structural activity in addition form muscle mass and form significant proportion in

enzymes, hormones, antibodies and serum. The amino acids are the simple compounds obtained after hydrolysis of proteins by enzymatic process. They are also referred to as building blocks of the proteins. Amino acids are biologically important nitrogen containing compounds, notably heme, physiologically active amines, nucleotide and nucleotide precursors. Two groups of amino acids are distinguished, essential and non-essential amino acids. Essential amino acids necessitate in diet as many mammals cannot generate them, while non-essential amino acids are synthesized by man through other metabolic precursors. Proline, glutamic acid and aspartic acid belong to non-essential amino acids, while lysine, valine and histidine are essential amino acids. Although the ultimate source of nitrogen for amino acid biosynthesis is atmospheric N_2, this inert gas must be reduced to metabolically useful form, NH_3 by nitrogen fixation [10]. Amino acids are known to react slowly or more rapidly while heating, with sugars to produce yellow or brown colour. Darkening of honey with age and heating is due to this reaction. The presence of proteins causes honey to have a lower surface tension that it would have otherwise, which produces a marked tendency to form foam and scum which encourages the formation of fine air bubbles [11].

CONCLUSION

The multitude properties of honey are exclusively reliant on chemical composition of floral nectaries. Like to any other component, amino acids of honey are also derived from floral nectar. The amino acids analyzed in honey of both wild and apiary honeybees were *viz*., proline (P), lysine (K), glutamic acid (E), aspartic acid (D), valine (V) and histidine (H). Proline was found maximum in honey from two honeybee species, while histidine was least in concentration in all the different samples of honey tested. The transformation of nectar to honey involves quantitative physical, chemical behavioral (fanning activity and thermoregulatory) and biochemical changes.

Table- 1: Major bee flora of Udupi, Karnataka.

Sl. No	Family	Botanical name	Source
1	*Anacardiaceae*	*Mangifera indica*	N + P
2	*Compositae*	*Helianthus annus*	N + P
3	*Compositae*	*Tridax procumbens*	N + P
4	*Cruciferae*	*Brassica juncea*	N + P
5	*Cruciferae*	*Brassica nigra*	N + P
6	*Fabaceae*	*Tamarindus indica*	N + P
7	*Fabaceae*	*Pongamia pinnata*	N + P
8	*Fabaceae*	*Peltophorum pterocarpum*	N + P
9	*Meliaceae*	*Azadirachta indica*	N + P
10	*Umbelliferae*	*Coriandrum sativum*	N + P

N= Nectar. **P= Pollen.**

Table- 2: Major bee flora of Dakshina Kannada, Karnataka.

Sl. No	Family	Botanical name	Source
1	*Aracaceae*	*Cocus nucifera*	N + P
2	*Anacardiaceae*	*Anacardium occidentale*	N+ P
3	*Fabaceae*	*Pterocarpus santalinus*	N + P
4	*Fabaceae*	*Tamarindus indica*	N + P
5	*Myrtaceae*	*Eucalyptus species*	N + P
6	*Polygonaceae*	*Antigonon leptopus*	N + P
7	*Rutaceae*	*Citrus species*	N + P
8	*Verbenaceae*	*Tectona grandis*	N + P

N= Nectar. **P= Pollen.**

Table- 3: Major bee flora of Uttara Kannada, Karnataka.

Sl. No	Family	Botanical name	Source
1	*Aracaceae*	*Borassus flabelliformis*	N + P
2	*Aracaceae*	*Cocus nucifera*	N + P
3	*Bignoniaceae*	*Tectoma stans*	N +P
4	*Compositae e*	*Ageratum conyzoides*	N + P
5	*Euphorbiaceae*	*Ricinus communis*	N + P
6	*Musaceae*	*Musa paradisiaca*	N
7	*Myrtaceae*	*Syzygium caryophyllatum*	N + P
8	*Rubiaceae*	*Coffea arabica*	N
9	*Sapindaceae*	*Sapindus emarginatus*	N + P
10	*Verbenaceae*	*Tectona grandis*	N + P
11	*Zygophyllacea*	*Tribulus terrestris*	N + P

N=Nectar. **P=Pollen.**

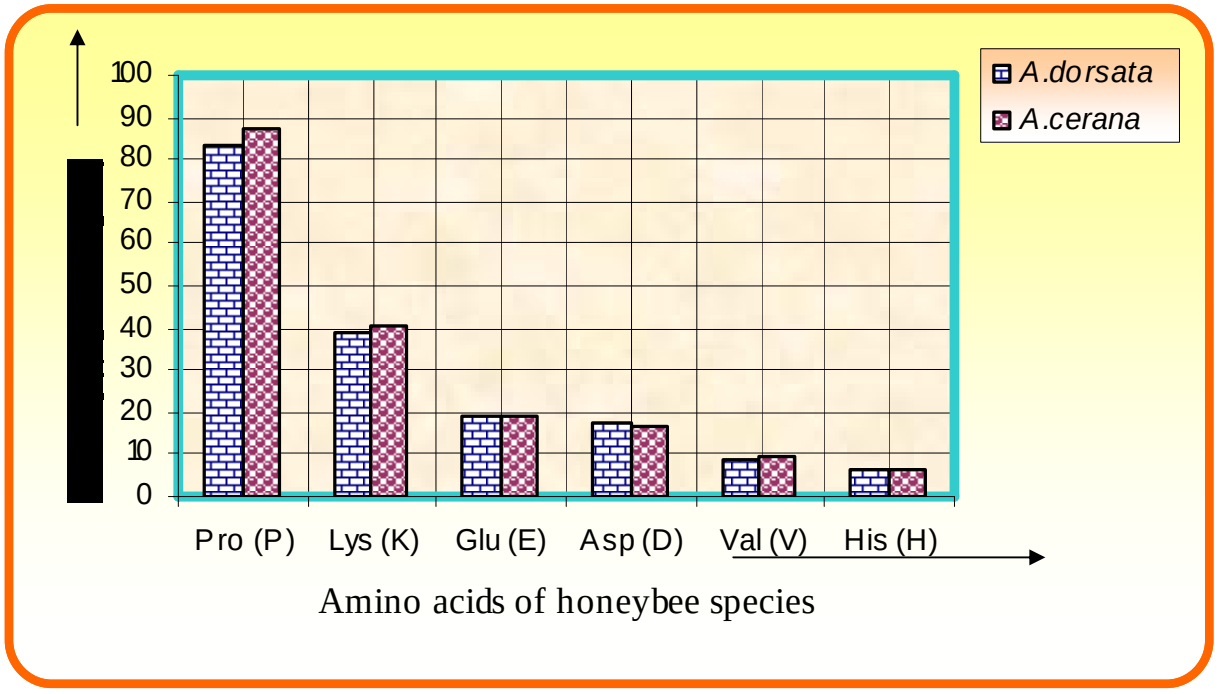

Fig.1: Amino acids of *A.dorsata* and *A.cerana* honey from Udupi, Karnataka in 2011-12.

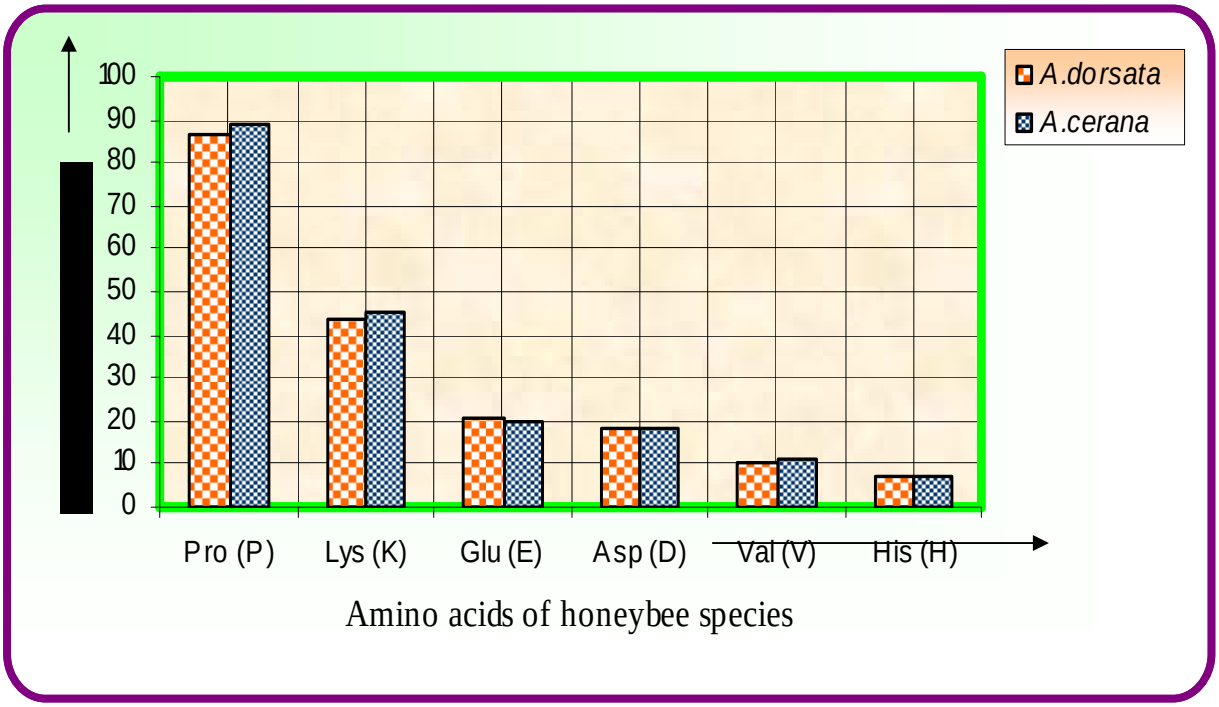

Fig.2: **Amino acids of *A.dorsata* and *A.cerana* honey from Dakshina Kannada, Karnataka in 2011-12.**

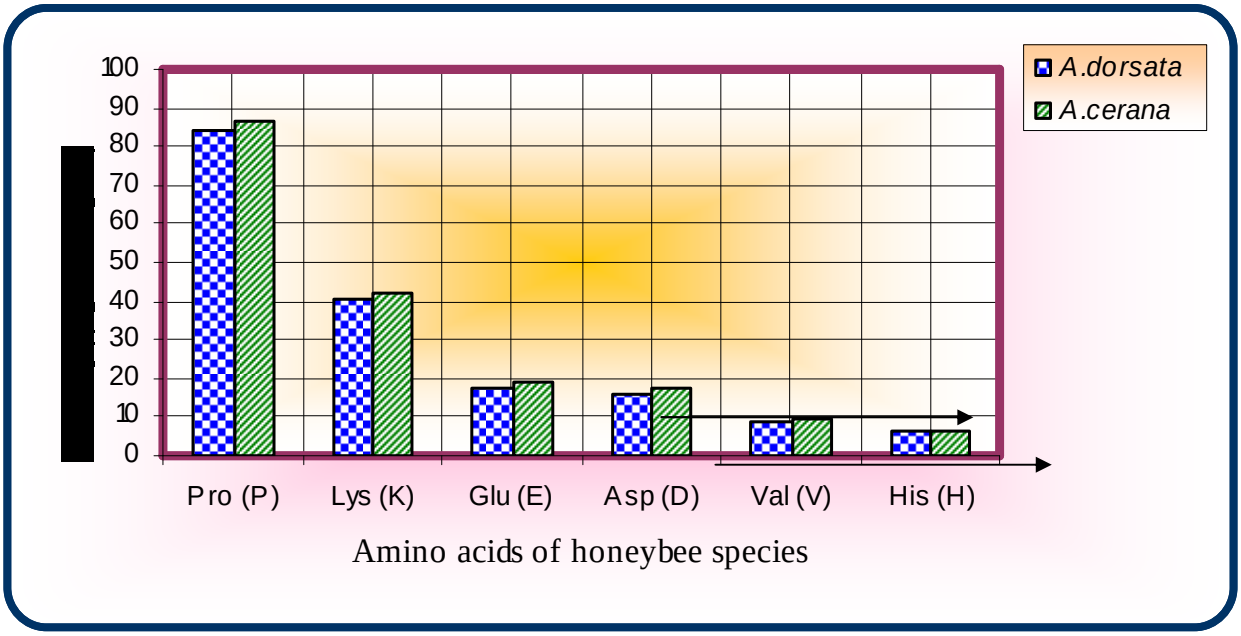

Fig.3.Amino acids of *A.dorsata* and *A.cerana* honey from Uttara Kannada, Karnataka in 2011-12.

ACKNOWLEDGEMENTS

Author acknowledges Dr.C.Chandrasekhara Reddy, Fulbright and Commonwealth Fellow, Former Chairman, Department of Zoology and currently working as Emeritus Professor, Department of Applied Genetics, Bangalore University for his constant encouragement and valuable suggestions in preparing the manuscript. Author also acknowledges wife Mrs. M. Bharathi, Associate Professor, Department of Computer Science, S.J.C.I.T, Chickaballapur for moral support and computation of data, beloved daughter

Ms. B. Hemashravya for her help in preparing manuscript and University Grants Commission, South Western Regional Office, Bangalore for funding my Minor Research Project.

REFERENCES

[1]. T.P.Kalpana and C.G.K. Ramanajum, Sugar cane honeys its significance. *Curr. Sci.* 1998, **70**(4) : 261-262.

[2]. Seema Singh Thakur and Sudha Kanaujia 2003. Influence of day hours, temperature and relative humidity on nectar-sugar secretion pattern and honeybee visit in neem, *Azadirachta indica* (*Meliaceae*). *Indian Bee J*. ,2003, **65** (3-4): 100- 104.

[3]. M.V.Balasubramanyam, Quantitative Chemical variations in ripening of honey of indigenous hive bee *Apis cerana indica*. *Intern. J. of Appl.Bio and Pharm. Tech.*, July- Sept.- 2011. **2**(**3**): 391-397.

[4]. E. Crane, Bees and Beekeeping, Science, Practices and World Resources. *Heinmann*, London, 1990, 388- 451.

[5].M.V. Balasubramanyam and C.C. Reddy Physical characteristics of multifloral wild and apiary honey from plains, hills and Western Ghats of Karnataka. *Indian Bee J*.2003, 65(3&4): 113-117.

[6]. N. Shripad Agashe and B.F Rangaswamy, Chemical characterization of *Apis cerana F* and *Apis dorsata F*. honey from Dakshina Kannada, Karnataka (India), *Indian Bee J*. , 2001, **63**(3&4): 15-20.

[7].J. Louveaux, A. Maurizio and G. Vorwohl Methods of Melissopalynology, *Bee Wld,1978*, **59:**139-157.

[8]. Joint Association of Analytical Chemists 2001. pp 594 - 606.

[9]. M.V. Balasubramanyam, Factors influencing the ripening of honey. *Ph.D Thesis*, Bangalore University, Bangalore ,2006, 43-48.

[10]. D. Voet and J.G. Voet, *Principles of Biochemistry*, Second Edition, John Wiley and Sons, Inc. New York.1994, 345-370.

[11].F.F. Bolander, Amino acids: not just for enzymes, *Curr. Opin. Investig. Drugs*. ,2006, **7** (10) : 912- 915.

***Correspondence Author: Balasubramanyam M.V.;** Department of Zoology, Maharani's Science College for Women,Palace Road, Bangalore – 560 001

.