INVESTIGATIONS ON MINERAL, VITAMINS AND PROTIENS OF INDIGENOUS *APIS DORSATA* AND *APIS CERANA INDICA* HONEY OF UDUPI DISTRICT, KARNATAKA.

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ABSTRACT

Multifloral honey of indigenous giant honeybee *A.dorsata* (wild) and Indian hivebee, *A. cerana indica* (apiary) were collected from Udupi, Karnataka and its minerals, vitamins and proteins were determined during December 2017 to November 2018.. The potassium content of honey *of A.dorsata* was 70.12 ppm while honey of *A.cerana* had 68.50ppm. The potassium content of honey of *A .dorsata* was highest (70.12 ppm), and honey of *A. cerana* was least (68.50ppm). The chromium content of honey of *A.dorsata* was maximum (0.027ppm) while, honey of *A.cerana* was minimum (0.025ppm). The thiamine (B_1) content of honey of *A.dorsata* was 0.09 µ gms., while honey of *A.cerana* was highest of 0.08 µ gms. The pyridoxine (B_6) content of honey of *A.dorsata* was maximum of 1.80 µ gms. and minimum of 1.44 µ gms. from *A cerana* honey. The protein content of honey of *A.dorsata* was highest (0.67%) and least (0.59%) from honey of *A.cerana*. The potassium of honey from all two honeybee species was statistically significant at 1 % ($p<0.01$) level and chromium of honey from all two honeybee species was not statistically significant at 1% ($p<0.01$) level. The thiamine (B_1) content of honey from all two honeybee species was not statistically significant at 1 %($p<0.01$) level and pyridoxine (B_6) content of honey of wild and apiary honeybee species was not statistically significant at 1% level ($p<0.01$). The protein content of honey from two honeybee species was not statistically significant at 1 % level ($p<0.01$). Results clearly exemplify honey of *A.dorsata* had relatively higher minor constituents than that of *A.cerana*. Further, studies obviously indicate that quality of honey of wild and apiary honeybee species are equally good with subtle variations which are discussed in ensuing paper.

Key words: *A.cerana*, *A.dorsata* honey, minor constituents, Udupi, 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, vitamins, volatile oils, pinocembrin, galagin, polyphenols, tocopherols, lycopene and amino acids which are obviously found in honey. Because of this unique, complex and distinctive 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 minor constituents of tropical honeybees, *A.dorsata* and *A.cerana* honey is limited [4, 5, and 6]. Interestingly, no information is available on the minerals, vitamins and proteins in honey of *A .dorsata* and *A.cerana*. Therefore, primary aim of the current study is to provide comprehensive information on the minerals, vitamins and proteins levels in two indigenous honeybees of Udupi, Karnataka, India.

MATERIALS AND METHODS

Karnataka state extends from 11º 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. Udupi located in the south-western part of Karnataka, belongs to Malnad region and largely forest hilly area with average rainfall of 1886 mm. Udupi district occupy a total area of 7,201 sq. km. out the total geographical area of the Karnataka state 1, 91,791 sq. kms. Udupi extends between 12 º 54' 42" and 13 º 53' 53" NL and 75 º 04' 46" and 76 º 21' 50" EL. The flora of Udupi district is rich and diversified, which includes agricultural crops like paddy and sugarcane, commercial crops like coffee and black pepper, horticultural crops like coconut, cashew, banana and potato and forest flora like teak, hone, salwood and sandal wood. The temperature varied from 11 º C to 37 º C and the humidity ranges from 23.5 % to 89.58 %. Agriculture and tourism are main sources of economy to district in addition to beekeeping with *A.cerana* and occurrence of feral colonies of *A.cerana* and *A.dorsata* are additional source of economy.

Honey collection

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. Twenty-six study centers from Udupi district spreading over the Western Ghats were selected for honey samples collection. The sub-station centres include Mudigere and Sakrepatna from Udupi spreading over prime locality of Western Ghats of Karnataka. Abundant floral resources coupled with suitable environmental factors are mainly responsible for copious honey production in these regions. 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 sealed and uncapped before extracted by honey extractor (Plate 1 and 2) and that of *A .dorsata* honey was obtained by squeezing and filteration (Plate 3). All honey samples were raw and unprocessed. The honey sample size from each study centre was 13.

Quantification of minerals in honey

The detection of potassium, calcium, sodium, magnesium, iron, manganese, copper, chromium and zinc was done by Atomic Absorption Spectroscopy (AAS). The phosphorous was identified by colorimeter method [7].

Table 1. Wavelength and fuel required for different mineral constituents.

Sl. No	Element	Wave length (nm)	Fuel
1	Potassium	766.6	Air/ Acetylene.
2	Calcium	228.85	Air/ Acetylene.
3	Sodium	166.6	Air/ Acetylene.
4	Magnesium	285.20	Air/ Acetylene.
5	Iron	248.37	Air/ Acetylene.
6	Manganese	279.9	Air/ Acetylene.
7	Copper	324.80	Air/ Acetylene.
8	Zinc	213.90	Air/ Acetylene.
9	Chromium	357.90	Air/ Acetylene.

Lamp current: 3.5 m A.

Determination of Vitamins in honey

Raw honey samples collected from *A.dorsata* and *A.cerana* were used for analysis of vitamins levels. B_1, B_2, B_3, B_5, B_6 and C vitamins in honey were determined by the method followed [8].

Detection of Proteins in honey.

The product is digested with concentrated sulphuric acid, using copper sulphate as a catalyst, to convert organic nitrogen to ammonium ions. Alkali is added and the liberated ammonia distilled into an excess of boric acid solution. The distillate is titrated with hydrochloric acid to determine the ammonia absorbed in the boric acid [9]. .

Statistical analysis of data

The data obtained from mineral, vitamins and proteins of honey was subjected to complete randomized design factorial analysis [CRD-Factorial analysis]. Further the data obtained from wild and apiary honey was used determine the significant levels at 1% level ($p<0.01$).

RESULTS AND DISCUSSION

The mineral, vitamin and protein characteristics of honey characteristics from wild and apiary honeybee species showed considerable variations both with the species and also from different areas of the district. In general, of the ten mineral components identified, potassium was found maximum in honey from all the three honeybee species, while chromium was least in concentration in all the different samples of honey tested.

Mineral characteristics of wild and apiary honey

The potassium content of honey of *A.dorsata* was 70.12 ppm, while honey of *A.cerana* was 68.50 ppm and least of 67.13 ppm from *A.dorsata* honey. The potassium content of honey from all three honeybee species was statistically significant at 1 % levels. The chromium content of honey of *A.dorsata* was least of 0.027 ppm, while honey of *A.cerana* was 0.025 ppm and maximum of 0.030 ppm from *A.dorsata* honey. The chromium content of honey from all three honeybee species was not statistically significant at 5 % levels. (Table.3). [10] analyzed honey samples and reported average content of phosphorous, potassium, calcium, magnesium, iron and zinc in range of 8-10ppm, 65-75ppm, 6-7ppm, 2-4 ppm, 0.25-0.52 ppm and 0.12 – 0.29ppm respectively in North-Western Himalayas. [11] reported positive correlation of most of the minerals such as potassium, iron, manganese and magnesium content of honey with electrical conductivity. Honey contains less sodium than potassium and hence it is suggested to use Na/K ratio to detect honey adulteration. Various parameters such as color, aroma, flavour, texture, medicinal properties of honey are largely dependent on mineral content which eventually are derived from floral resources [12]. Mineral content of honey from all three honeybee species varied seasonally due to diversity of floral resources and nectar composition. Raw honeys are fresh, light, mild flavors, aroma and have less mineral content in honeys from three honeybee species. Raw honeys are susceptible to fermentation and granulation faster than processed honey since these honeys have osmophilic sugar-tolerant bacteria and yeast that act on sugars in honey leading to partial breakdown to form acids. On the contrary processed honeys are less likely to get fermented due to obliteration of bacteria and yeast. Granulation is another major problem faced by honey traders and consumers as deposition of dextrose sugar in bottom of the container. Dextrose being heavier than water and laevulose settles readily at floor of the container causing ambiguity among consumers who believe as adulteration. Honeys are rich in ash content and highly variable with species of honeybee. Honey may be also dark which are related to the plant source of honey, which include a high content of tyrosine, tryptophan and the presence of polyphenolic compounds [13, 14].

Vitamins of honey of wild and apiary honeybee species.

The thiamine content of honey of *A.dorsata* was 0.09 µ gms., while honey of *A.cerana* was highest of 0.08 µ gms. The thiamine content of honey from all three honeybee species was not statistically significant at 1 % levels. The pyridoxine content of honey of *A.cerana* was least of 1.44 µ gms. and maximum of 1.80 µ gms. for *A dorsata* honey. The pyridoxine content of honey of wild and apiary honeybee species was not statistically significant at 5 % levels (Fig. 1). In the present study maximum quantity of B_6 (1.80 µ gms) was observed, followed by C (1.55 µ gms) and least amount of B_1 (0.08µ gms). Qualitative variations of vitamins do not occur, but there is gradual significant quantitative augmentation of vitamins in each stage of honey ripening [15]. Although vitamins in honey are found in diminutive quantities, deficiency may cause some disorders, *viz*., B_1 (beriberi), B_2 (dermatitis), B_3 (pellagra), B_5 (nervous problems), B_6 (anemia) and C (scurvy). They are also essential for metabolic reactions and as coenzymes [16, 17]. All Vitamin of B are commonly referred as B complex, in general indispensable for healthy

skin and nervous system and C for prevention of internal hemorrhage, bleeding gums, loosening of teeth and swollen tender joints. [18] reported that C is a powerful activator of the glucose oxidase system. [19] have found high C values ranging from 1.18-2.40 µ gms for three samples of honey of unknown source from mountains of Damavand area in Iran. They have suggested the possibility of encouraging the use of honey from this region as means of helping to relieve the marginal vitamin C deficiency often found in Iran leading to scurvy and bleeding problems. [20] reported an average vitamin C requirement of 3-6 µ g/day for animals used and concluded that honey actually contained 7.5 -15.0 µ g of C.

Plate: 1. Sealed honey cells of Indian hivebee *A. cerana.*

Proteins of honey of wild and apiary honeybee species.

The protein content of honey in *A.dorsata was* highest of 0.67% and least of 0.57 % from honey of *A.cerana*. The protein content of honey from two honeybee species was not statistically significant at 1 % levels (Fig. 2). Proteins form major portion of muscle mass and form significant proportion in enzymes, hormones, antibodies and serum. The amino acids are the simple compounds obtained when protein are broken down by chemical or digestive process. They are also referred to as building blocks of the proteins. 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. Several of them are essential to life and must be obtained through the diet. 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 [21].

Plate 2: Fresh honey samples of *A. cerana* species of Bantwal, Udupi.

Plate 3: Fresh honey samples of *A.dorsata* species of Belthangady, Udupi.

Table 3. **Mineral characteristics of wild and apiary honey [#] from Udupi, Karnataka.**

Sl. No.	Parameters	Honeybee species		F-ratio
		A.dorsata	*A. cerana*	
1.	Potassium (ppm)	70.12	68.50	5.34 *
2.	Phosphorous (ppm)	13.47	12.09	8.45 *
3	Calcium (ppm)	7.52	6.94	2.15 *
4.	Sodium (ppm)	3.85	3.70	1.78 *
5.	Magnesium (ppm)	4.15	3.92	2.73 *
6.	Iron (ppm)	0.71	0.68	0.08 +
7.	Manganese (ppm)	0.25	0.17	0.75 +
8.	Copper (ppm)	0.08	0.09	0.001 +
9.	Chromium (ppm)	0.027	0.025	0.32 +
10	Zinc (ppm)	0.19	0.17	0.04 +

[#] Sample size = 26

* Significant at P<0.01 [+]Non-significant at P<0.01

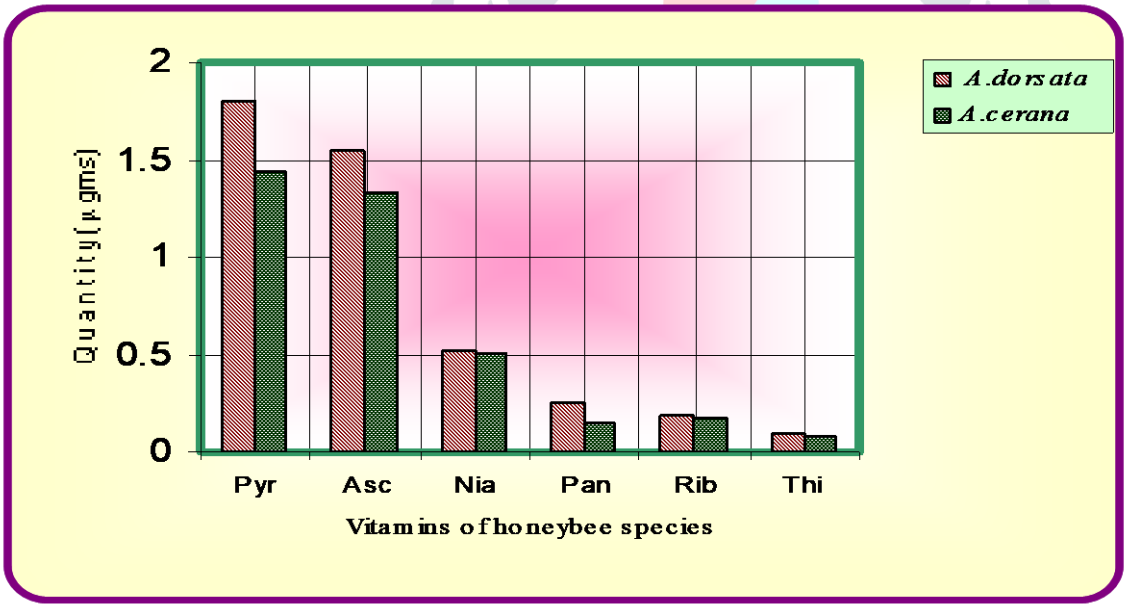

Fig 1. Vitamin characteristics of wild and apiary honey, Udupi, Karnataka.

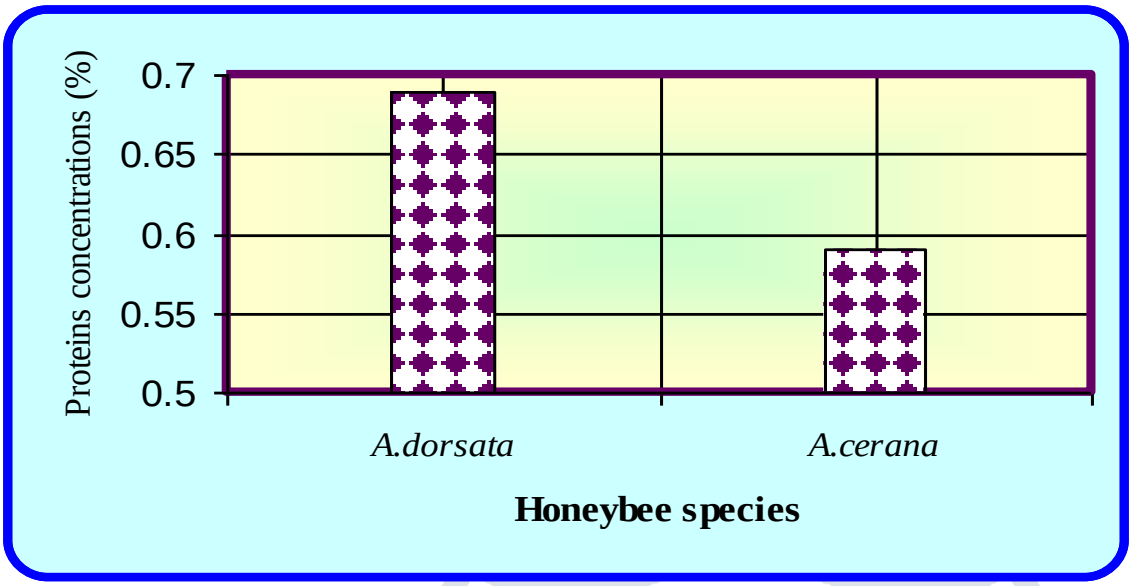

Fig 2. Protein characteristics of wild and apiary honey, Udupi, Karnataka.

CONCLUSIONS

The multitude properties of honey are exclusively reliant on chemical composition of floral nectaries. Like to any other component, minerals, vitamins and proteins of honey are also derived from floral nectar. The minerals form major and minor category and their proportions vary significantly within the two indigenous honeybee species with potassium (wild, 70.12 ppm) and (apiary, 68.50ppm). Vitamins analyzed in honey of both wild and apiary honeybees was B_1, B_2, B_3, B_5, B_6 and C. B_6 in honey was highest (1.80 µ gms) and B_1 (0.07 µ gms) of *A.dorsata* honey was minimum and maximum. The protein content of honey of Udupi district in *A.dorsata was* highest of 0.67% and least of 0.57 % from honey of *A.cerana*. The transformation of nectar to honey involves quantitative physical, chemical behavioral (fanning activity and thermoregulatory) and biochemical changes. During the path of formation of honey; minerals, vitamins and proteins steadily augment to the optimal quantities found in ripened honey.

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