



## FACTORS INFLUENCING THE TRANSFORMATION OF NECTAR TO HONEY IN *APIS CERANA INDICA*

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**Abstract:** The factors like TRS, NRS, ash, acidity and pH were detected in five stages of honey ripening process of indigenous hive honeybee *Apis cerana indica*. Five stages of nectar to honey transformation comprise floral nectar (fn), honey crop of foragers (hf), honey crop of house bees (hh), unsealed honey cells (uh) and sealed honey cells (sh). The TRS of fn and sh cells was 2.12% and 73.01% respectively. Correspondingly NRS of hh and uh cells was 14.90% and 7.50%. The ash content of hf and sh cells was 0.20% and 1.49% respectively, whereas the acidity of sh cells and hf was 0.503 and 0.06 respectively. Similarly pH of hh and uh cells had a value of 1.03 and 2.61. All the five parameters viz., TRS, NRS, ash, acidity and pH tested apart from NRS were less in floral nectaries and maximum in sealed honey cells. The analysis of variance (ANOVA) of TRS of ripening of honey was significant at  $P < 0.01\%$  levels, while NRS, ash, acidity and pH in honey formation was not found significant at  $P < 0.01\%$  levels.

**Keywords:** *Apis cerana indica*, Chemical changes, Honey ripening.

### INTRODUCTION

Honeybees and flowers are classical examples of mutualism and co-evolution. The bees belong to class Insecta, the largest class of animals (Verma and Prakash, 2020). Honeybees are eusocial hymenopterans, true natural bio indicators and entirely dependent on floral resources like nectar and pollen. Honey is truly a remarkable product of high-density and high-calorific food (Crane, 1975). The honey and milk are considered as symbol of prosperity and sanctity since ancient times. Honey together with milk, curd, sugar and butter are essential ingredients of panchamrutha, which is offered to God during religious ceremonies. Honey is as nectar gathered, modified, stored and sealed by honeybee in well planned and architected hexagonal comb cells.

Honey has enticed flavour, color, aroma and texture mainly due to the presence of volatile oils, flavonoids, aromatic acids, carotenoids and polyphenols.

Due to the unique and complex nature, honey gets place as an antiseptic, laxative, antibiotic, pacifier, anti-oxidant and ingredient of variety of pharmaceutical, confectionary, cosmetics, bakery and tobacco industry. Nectar is dilute sugar-solution secreted by floral glands termed as nectaries. The quantity of honey that produced from the nectar of single flower depends on the total amount of nectar secreted and the sugar concentration of the nectar (Thakur and Kanaujia, 2003).

Physico-chemical properties and composition of honey of European honeybee, *A. mellifera* are well documented. On contrary, information on nectar to honey transformation of Asiatic hivebee, *A. cerana indica* available is not extensive (Balasubramanyam and Reddy, 2003). Interestingly, little information is available on the chemical factors influencing transformation of nectar to honey of indigenous honeybee species. The primary objective of the present study is to characterize the chemical factors involved in the ripening of honey.

## MATERIALS AND METHODS

### Study Area

The study area, Bangalore district is situated at 12° 58' to 13° 65' NL to 77° 35' to 77° 40' EL with an elevation of 928 m. It has an area of about 1098

sq. km. The average rainfall is about 780 cm. from June to October as peak rainfall period. The temperature and humidity varied from 15.7° C to 37.9° C and 23.3% to 84.5%, respectively. The diversified flora of Bangalore district includes ornamental, plantation, agricultural, horticultural and food crops. Further, the honeybee species are very well distributed in the study area, *A. cerana* subsists both as wild and domesticated species, whereas *A. dorsata* and *A. florea* exist as only feral species. The nectar samples from floral nectaries namely *Pongamia glabra*, *Tamarindus indica* and *Peltophorum pterocarpum* (Fig. 1-2), honey crop of foragers, honey crop of house bees of *A. cerana*, honey from unsealed honey cells and honey from sealed honey cells were collected from Chintamani, Chickaballapur district, Karnataka during May 2020 to April 2021.



Fig. 1: *Peltophorum pterocarpum* in flowering season.

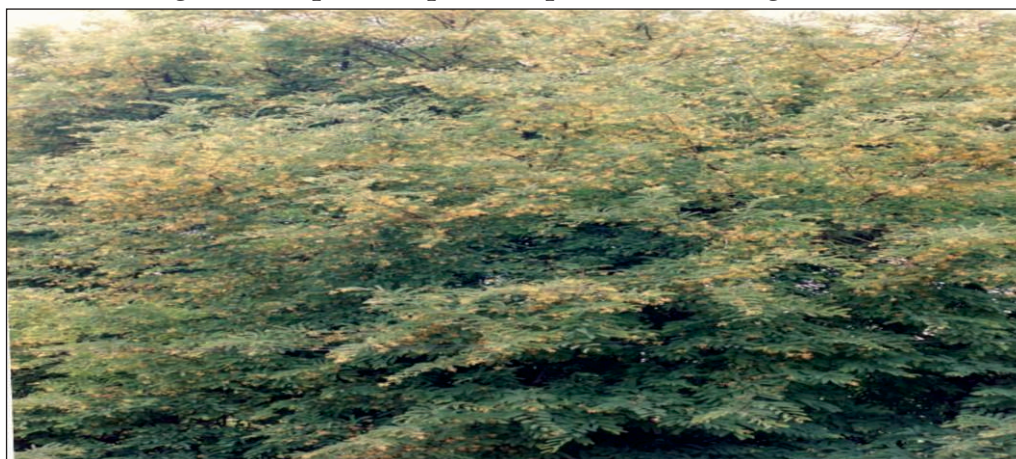


Fig. 2: *Tamarindus indica* in full bloom.

### Collection of nectar and honey

The floral nectar was collected in morning hours (07.30 hrs. - 08.30 hrs.) and late afternoon (03.00 hrs. to 04.30 hrs.) by using micropipette and immediately stored in vials of 0.5 ml. The forager bees with swollen abdomen and without pollen pellets in corbiculae were captured near the hive entrance by using sweep net. Then they were anesthetized and abdomen was squeezed, the contents were drawn into micropipette. To detect whether the foragers brought nectar or water, a filter paper test was conducted. Similar tests were conducted for house bees inside the hive. The nectar deposited by house bees into empty cells of honeycomb remains for 4-5 days depending upon the inflow of nectar. The nectar from unsealed honey cells was collected after 1-2 days of deposition. About 0.5 ml. of unripened honey was collected from each unsealed honey cell. The ripened honey covered with thin layer of wax was used for analysis after removing thin layer of wax.

### Determination of total reducing sugar and non-reducing sugar

Total reducing sugars (TRS) and non-reducing sugars (NRS) of nectar, nectar of foragers, house bees, unsealed honey and sealed honey was measured with the help of Abbe's refractometer.

### Detection of ash and acidity

Optical density of nectar, nectar of foragers, house bees, unsealed honey and sealed honey was determined by colorimeter.

### Measurement of pH

pH of nectar, nectar of foragers, house bees, unsealed honey and sealed honey was determined by pH meter.

### Statistical analysis of data

Data of the five chemical characteristics of stages of honey ripening was 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

The transformation of floral nectar into sealed honey is a progressive and definitive process. Honey ripening duration fluctuates with the species, climate, colony size, floral and seasonal

conditions. Honey ripening process duration is in between  $126.5 \pm 1.43$  hrs. and  $138.5 \pm 3.65$  hrs.

TRS content of floral nectar (fn) and of honey crop of foragers (hf) was 2.12 % and 3.14 % respectively. The TRS of nectar of hh was of 12.13%. The TRS content of honey of uh cells and sh cells were 42.05% and 73.10% respectively (Fig. 3). The analysis of variance of TRS of honey during different stages of ripening was significant at 1% levels. Total reducing sugars includes monosaccharide units in form of laevulose, ketose, dextrose and aldose sugar. Popek (2002) recorded TRS ranging between  $69.07 \pm 2.73\%$  and  $79.69 \pm 3.40\%$  in unifloral melliferous honey from Punjab. The minimum level of TRS is prescribed as 65% (BIS, 1994). Khatija and Ramanujan (1993) reported 72.66 % to 75.30 % of TRS from honey from Hyderabad. In the present study, the laevulose level is more than that of dextrose which is characteristic feature of all non-granulating, non-fermenting and authentic honey (Terrab *et al.*, 2002).

The NRS levels in fn were found 19.5 %, with crop of house bees (hh) 14.90% and unsealed honey cells (uh) 7.50% (Fig. 4). The NRS levels in nectar of honey crop of foragers (hf) and sealed honey cells (sh) cells were 19.1% and 2.40%, respectively. The analysis of variance of NRS of honey during different stages of ripening was not significant at 1% levels. The content of sucrose (2-5%) is an excellent indicator of non-adulterated/genuine honey.

Honeybees after filling their honey crop with nectar secrete many enzymes from hypopharyngeal, post cerebral and thoracic glands, therefore, NRS, disaccharide molecule is hydrolyzed into laevulose and dextrose monosaccharide units by action of invertase enzyme and water molecules in nectar are utilized for hydrolysis of sucrose into monosaccharide units like fructose and glucose. Hence, water molecules in the nectar decreased from 76.75 % to 19% in ripened honey. The non-reducing sugar concentration usually ranges from 2-5% in all special, standard grades and Agmark honeys.

The ash level in nf was 0.05 %, hh 0.70 % and uh cells was 1.02 % (Fig. 5). The ash levels in nectar

of hf and sh cells were 0.20 % and 1.49%, respectively. The analysis of variance of ash of honey during different stages of ripening was not significant at 1% levels. The ash content is a measure of mineral content of honey. Though the quantities of minerals are less, they play a vital role in determining the color, medicinal and nutritional value of honey. Rodrigreuz-Otero *et al.* (1994) found that mineral content of honey

from Spain varied from 0.06 % to 1.34 % in *A. mellifera* species. Variations such as aroma, flavor, medicinal value and keeping qualities of honey are largely dependent on the mineral content of honey (Wakhle,1997). Mineral content of honey was highly variable with the species of honeybee, seasons, color, geographical zones and storage conditions (Balasubramanyam and Reddy, 2011).

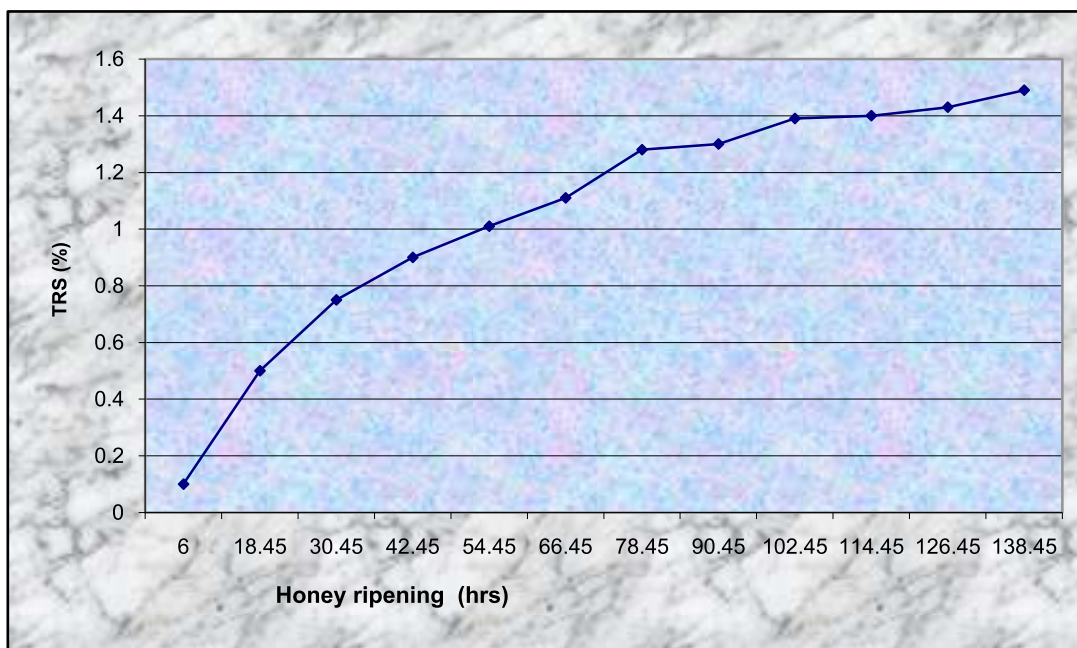


Fig.3: Total reducing sugars of honey in different hours of ripening.

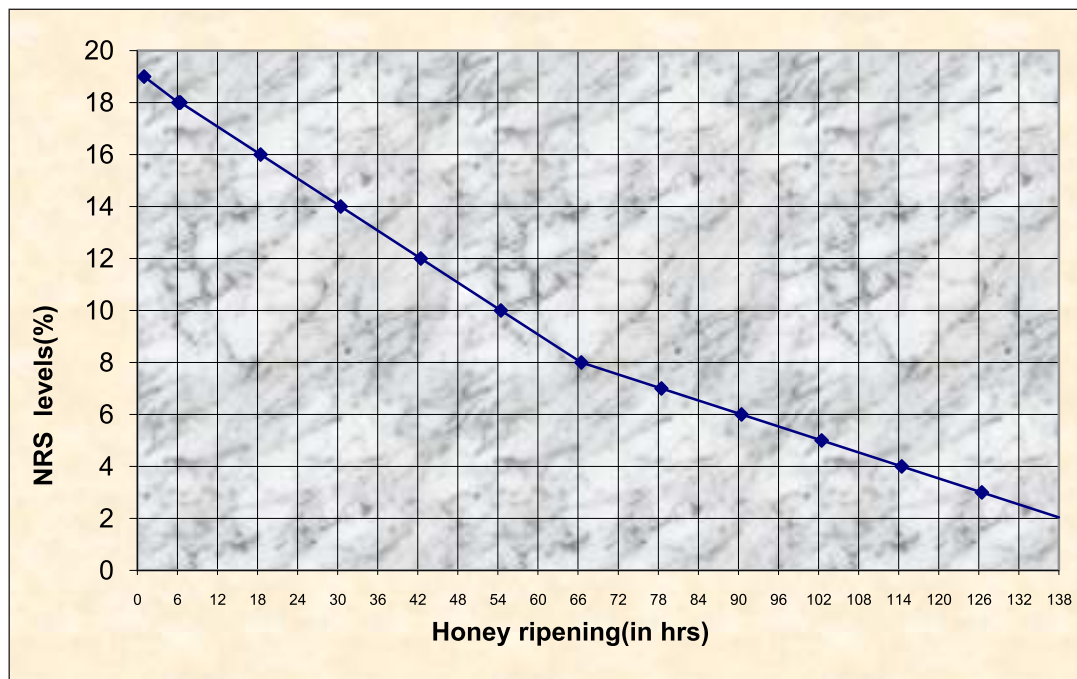


Fig. 4: NRS of honey in different hours of ripening.

The acidity levels in nf was recorded 0.02, hh 0.13 and uh cells 0.27 (Fig. 6). The acidity levels in nectar of hf and sh cells were 0.06 and 0.503, respectively. The analysis of variance of acidity of ripening of honey was not significant at 1% level. According to Agmark specifications, the maximum limit of acidity is 0.3%. Generally, Indian honeys possess higher acidity as compared to foreign honey samples due to

tropical climatic environments. The acidic nature of honey is due to the action of osmophilic yeasts and sugar-tolerant bacteria which readily act on honey sugars. The formation of acids like acetic, propionic, butyric and malic acids in stored honeys are more than fresh and processed honeys. The presence of acidic components beyond the prescribed limits (0.3%) in honey samples is referred as fermentation.

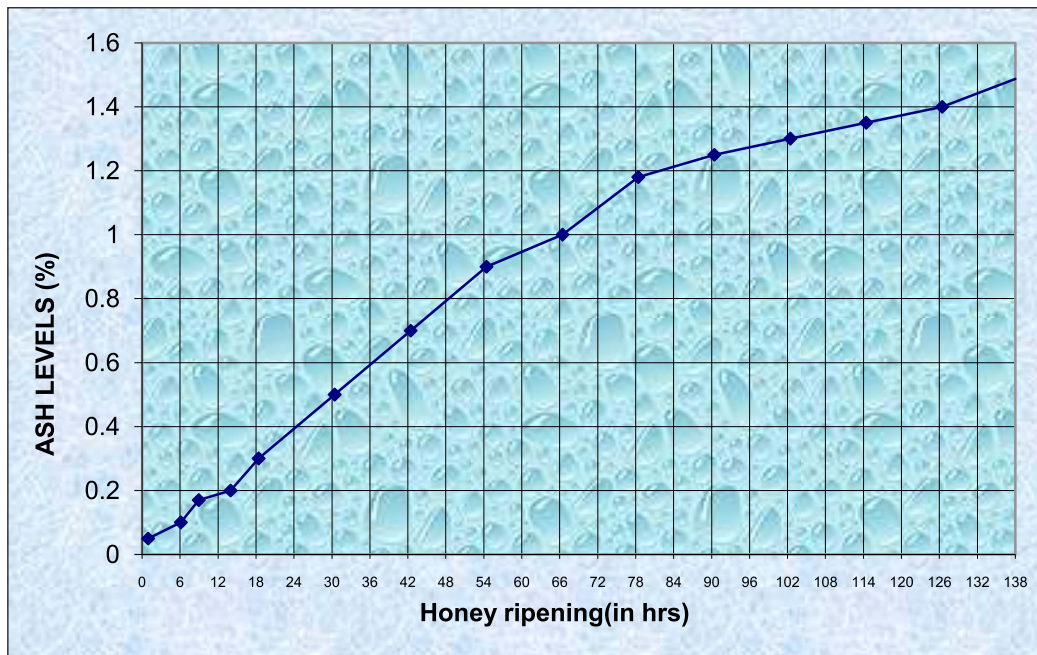


Fig.5: Ash of honey in different hours of ripening.

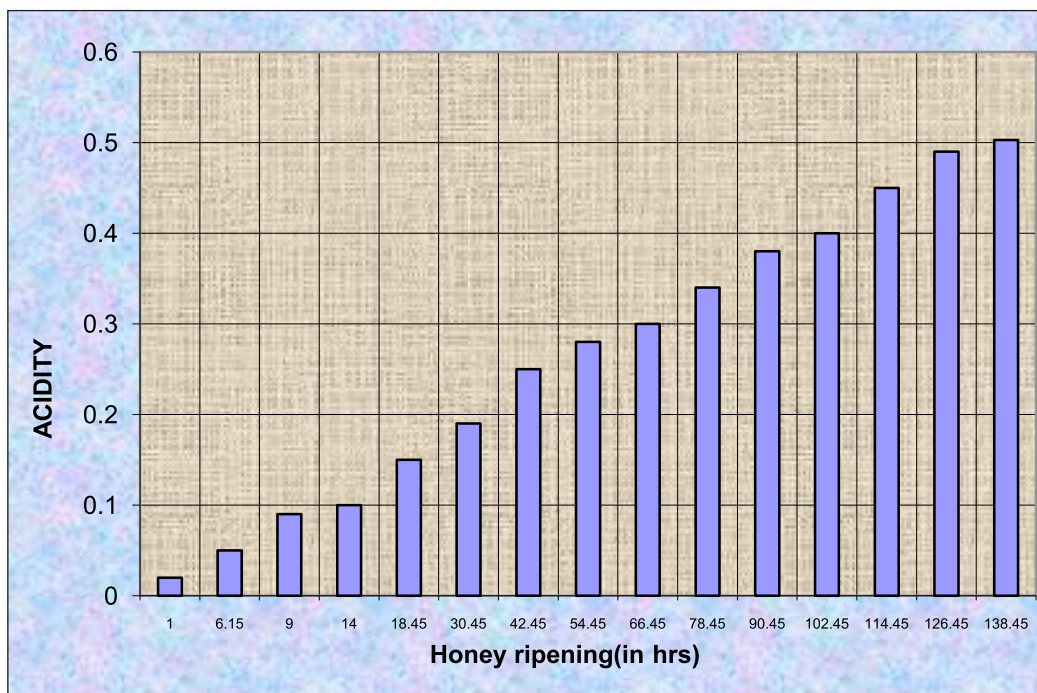


Fig.6: Acidity of honey in different hours of ripening.

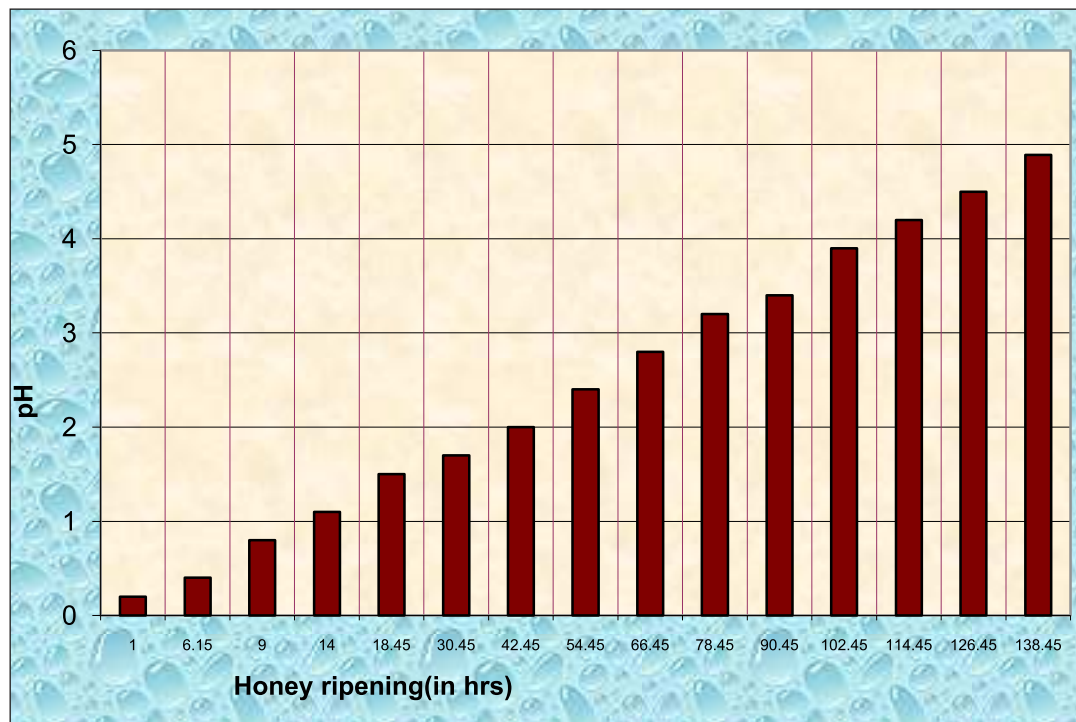


Fig. 7: pH of honey in different hours of ripening.

The pH of nf was 0.20 and that of hh and uh cells was 1.03 and 2.61 respectively (Fig. 7). The pH of nectar of hf and sh cells were 0.78 and 4.85 respectively. The analysis of variance of pH of honey during different stages of ripening was not significant at 1% levels. Esti. *et al.* (1997) reported pH in the range of 3.05-4.50, Gurel *et al.* (1998) reported pH range from 3.61 to 4.97 while Karbournioti and Drimjias (1997) mentioned a mean value of pH between 2.2 and 5.2 for Greek monofloral honeys. The pH of *A. dorsata* and *A. cerana* honey was recorded  $3.68 \pm 0.36$  and  $3.62 \pm 0.4$ , respectively from Nepal (Joshi *et al.*, 2000). The pH of a fresh honey sample is always lesser than stored and unripe honey. However, pH of processed honeys tends to remain fairly constant and then fluctuates depending upon the storage conditions.

## CONCLUSIONS

Nectar is thin, dilute sugary solution, technically termed as unripened honey. The unripened honey is different from ripened honey in aroma, flavor, color, texture, surface tension, density including other physical and chemical characteristics. The TRS, NRS, ash, acidity and pH demonstrated variations in the successive stages of honey

ripening process. The conversion of nectar to honey is innate behavior and pre-requisite to hoard honey for future generations. Obviously, honey though a plant origin but definitely is as much a primary product of honeybees. Well ripened honey has an important role in preparation of Ayurvedic and related other naturopathic therapies. Further, ripened honey is natural food that has vital essential nutrients in proper proportions and easily absorbed through the blood stream.

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