Short Research Article

EVALUATION OF ENZYMATIC ACTIVITY IN THE TRANSFORMATION OF NECTAR INTO HONEY IN INDIGENOUS ROCKBEE, *Apis Dorsata* F.

ABSTRACT

Enzymes form integral part and play decisive role in biological Systems and metabolic network. The present studies delve on the role of enzymes *viz*., invertase, amylase, glucose oxidase and catalase in nectar-honey transformation during May 2019 to April 2020. Data on enzymatic activity in all the five stages related to honey formation from nectar i.e., floral nectar (fn), honey crop of foragers (hf), honey crop of house bees (hh), unsealed honey cells (uh) and sealed honey cells (sh) was evaluated. Invertase in fn and sh cells was found minimum of 0.00ml and maximum of (42.40 ml), respectively. Similarly, amylase in the fn was least (0.00ml) and highest in sh (16.01 ml) and catalase was observed to be 0.001ml in floral nector and highest in sealed honey cells i.e., 16.01ml and 4.96 ml,respectively. Accordingly, glucose oxidase in fn and sh cells was minimum of (0.00ml) and maximum of (6.68 ml) respectively. Correspondingly, catalase in the fn was least (0.00ml) and highest in sh (4.96 ml). The analysis of variance of invertase and amylase was significant at 1 % level ($p<0.01$), whereas glucose oxidase and catalase Were statistically non-significant at 1 % level ($p<0.01$). Results have confirmed that the sources of all enzymes are instigated from hypopharyngeal, post-cerebral, thoracic, labial and mandibular glands of foragers and house bees which are accountable for honey formation. Further, each enzyme is substrates specific which are discussed in ensuing paper.

Keywords: *A.dorsata indica*, amylase, catalase, glucose oxidase, invertase, honey ripening**.**

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. Bees produce honey which is delectable sweet product, essentially consists of simple sugars, predominantly laevulose and dextrose [1]. Nectar is secreted from mass of cells within the flower called nectaries [2]. The amount of honey produced from the floral nectaries depends on the total quantity of nectar secreted and the sugar concentration of the nectar [3]. 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 [4].

Hitherto, various emphasis has been given to quality of honey of European honeybee *Apis mellifera* including its composition, physico-chemical and biochemical properties. On the contrary, information on the role of enzymes in nectar-honey transformation of indigenous rock bee *A.dorsata* is limited [5, 6 and 7]. Interestingly, no information is available on the various enzymes which play a fundamental role in formation of honey from nectar in *A.dorsata*. Hence, the current study aims to provide comprehensive information on the enzymatic involvement in ripening of honey of *A.dorsata*.

MATERIALS AND METHODS

The study was carried out in Shivakote,North district of Bangalore,Karnataka during May 2019 to April 2020.which is situated at 12^{o} 58’ to 13 ^{o}65 NL to 77^{o} 35’ to 77^{o} 40 EL with an elevation of 928m. It has an area of about 998 sq. km. The average rainfall is about 780 cms. with June to October as peak rainfall period. The temperature varied from 15.7^{o} C to 37.9 ^{o} C and humidity varied 23.3% to 84.5%. The flora of Bangalore district consists of diversified and consisting of Agriculture,Horticulture,Plantation and food cropsincludes ornamental, plantation, agricultural, horticultural and food crops. Further, the honeybee species are very well distributed in the study area, *A.cerana* exist both as wild and domesticated species, whereas *A. dorsata* and *A.florea* exists as only feral species. The nectar samples were taken from floral nectaries. Honey crop of Apis dorsata foragers and house bees as well as from sealed and unsealed honey cells.

Collection of samples

The forager bees with swollen abdomen and without pollen pellet in corbiculae were captured near the hive entrance by using sweep net. Then they were anesthized, the contents were drawn into micropipette by squeezing the abdomen. To distinguish the nectar from water of honey crop of foragers, filter paper test was conducted. The nectar deposited by house bees into empty cells of honeycomb remains for 4-5 days depending upon the rate of inflow of nectar. The nectar from unsealed cells was collected after 2 days of deposition. And finally the sealed honey with thin layer of wax was taken to determine the enzymatic activity levels.

Invertase activity: The invertase is measured as gms. of sucrose hydrolyzed per 100gms of honey / hr at 40^0C. The IN indicates the amount of sucrose per gram hydrolyzed in one hour by the enzymes contained in 100 g. of nectar / honey under test conditions. Sucrose was determined according to method [8].

Sucrose by mass = [(Reducing sugars after inversion; percent by mass) – (Reducing sugars before inversion; percent by mass)] X 0.95 [8].

Amylase activity: The amylase is measured as gms. of starch hydrolyzed per 100gms of honey / hr at 40^0C. The diastase activity of nectar and honey as ml 1% starch solution hydrolyzed by the enzyme of 100 gm of nectar/ or honey in 1hr at 40^oC. This diastase number corresponds with the Gothe scale number.

Amylase activity (AN) = ml starch solution (percent)/ gms of nectar/ honey /hour at 40^oC [9].

Glucose oxidase activity: The glucose oxidase is measured as gms. of glucose hydrolyzed per 100gms of honey / hr at 40^0C. Glucose is enzymatically oxidized with glucose oxidase to form hydrogen peroxide, which reacts with dye in presence of peroxidase to give stable colored product proportional to glucose [10].

Catalase activity: The catalase is measured as gms. of hydrogen peroxide oxidized per 100gms of honey / hr at 40^0C.

Catalase activity per g. , Kf =1/t Inxo/x D/W;

where xo is initial substrate at time t, D is the dilution and W is sample weight in g. (hydrogen peroxide hydrolyzed/ 100 g. sample/hour at 40^{o}C BS 2006 [11].

Statistical analysis of data

Data related to all the studied enzymes viz., invertase, amylase, glucose oxidase and catalase from five stages of honey ripening was analyzed by using 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

Honey has enticed color and viscous substance contrived by indefatigable worker bees which are driven by sheer intuitive culture. The worker bees are polylectic in nature and operate in perpetuity, days after days, from dawn to dusk, trip by trip for collecting the floral secretions drop by drop to provide food for members of the colony [12].The transformation of floral nectar into sealed honey is progressive and definitive process. Honey ripening duration fluctuates within species, colony size, climatic, floral and seasonal and geographical conditions. Generally honey ripening process occurs between 126.5 ± 1.43 hrs to 138.5 ± 3.65 hrs.

Invertase in honey

*The invertase activity in fn and hf was 0.00 and 2.30 ml respectively, while hh and uh was 3.80 and 12.30ml respectively. The invertase activity of sh cells was found highest, 42.40 ml (Fig 1). The analysis of variance of invertase activity of honey during different stages of ripening was significant at 1% ($p<0.01$) level (Tab. 1). Foragers after filling nectar in their honey crop secrete enzymes from hypopharyngeal, post-cerebral, thoracic, labial and mandibular glands. The enzymes are mixed thoroughly with nectar by foragers and passed to housebees through trophallaxis. Housebees after receiving nectar from foragers instantaneously secrete enzymes and associated with nectar. Invertase/Sucrase hydrolysis sucrose into fructose and glucose, hence these monosaccharide units are

abundantly found in ripened honey. The activity of invertase enzyme significantly increased at each stage of honey ripening [13].

$$\underset{\text{Sucrose}}{C_{12}H_{22}0_{11}} \xrightarrow[+\text{ Hydrolysis}]{\text{Invertase / Sucrase}} \underset{\text{Fructose}}{C_6H_{12}0_6} + \underset{\text{Glucose}}{C_6H_{12}0_6}.$$

Table 1. ANOVA of invertase of honey in different stages of ripening.

Source of Variation	Degrees of Freedom	S.S	M.S.S	F-ratio
Bees	1	275.28	275.28	03.70*
Storage	1	307.32	307.32	08.79*
Bees and Storage	1	257.76	257.76	05.92*
Error	20	1.00	0.06	
Total	23	841.37	44.28	

* Significant at 1% level (P<0.01).

* Invertase activity: g. Sucrose hydrolyzed/ 100 g. sample/hour at 40^{o}C.

Fig 1. Invertase activity of honey in different hours of ripening.

Amylase in honey

*Amylase in the floral nectar (fn) and honey crop of foragers (hf) was observed 0.00ml and 0.58 ml respectively. Further, the levels of amylase in honey crop of house bees (hh) and sealed honey cells (sh) were 0.79 ml and 16.01 ml respectively (Fig. 2). The analysis of variance of amylase activity of honey during different stages of ripening was significant at 1% ($p<0.01$) level (Tab.2).The pre-dominant substrate of amylase is starch existing in the floral nectar and hence diastase/amylase hydrolyzes starch into maltose and dextrins.

$$\underset{\text{Starch}}{[C_6H_{12}O_6]_n} \xrightarrow[\text{Diastase}]{\text{Amylase}} \underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + \text{Dextrins}$$

Table 2. ANOVA of amylase activity of honey in different stages of ripening.

Source of Variation	Degrees of Freedom	S.S	M.S.S	F-ratio
Bees	1	1711.25	1711.25	22.81*
Storage	1	1901.25	1901.25	25.34*
Bees and Storage	1	1394.45	1394.45	18.59*
Error	20	12.00	0.75	
Total	23	5018.95	264.15	

* Significant at 1% level ($P<0.01$).

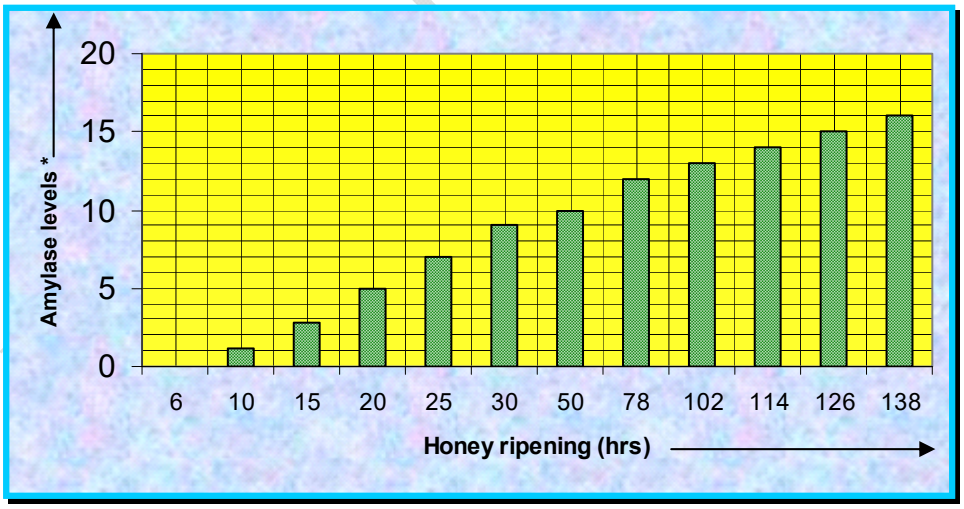


Fig.2. Amylase activity of honey in different hours of ripening.
* Amylase activity: gms. starch hydrolyzed/ 100 g. of honey / hour at 40°C.

Glucose oxidase in honey

*Glucose oxidase in fn and hh was 0.00 ml and 1.24 ml respectively. The glucose oxidase of honey of uh and sh was 2.59 and 6.68 ml respectively (Fig.3). The analysis of variance of glucose oxidase of honey during different stages of ripening was not significant at 5% levels (Tab.3). Glucose oxidase converts dextrose into gluconic acid and hydrogen peroxide. Studies have indicated that hydrogen peroxide formed increase upon stored honeys and hence the anti-microbial properties of these honeys are more interesting. The presence of glucose oxidase in honey is partially responsible for the increase of acidity of stored honey with gradual decrease in levels of dextrose [14].

$$\text{Dextrose} \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + \text{Hydrogen peroxide}$$

Catalase in honey

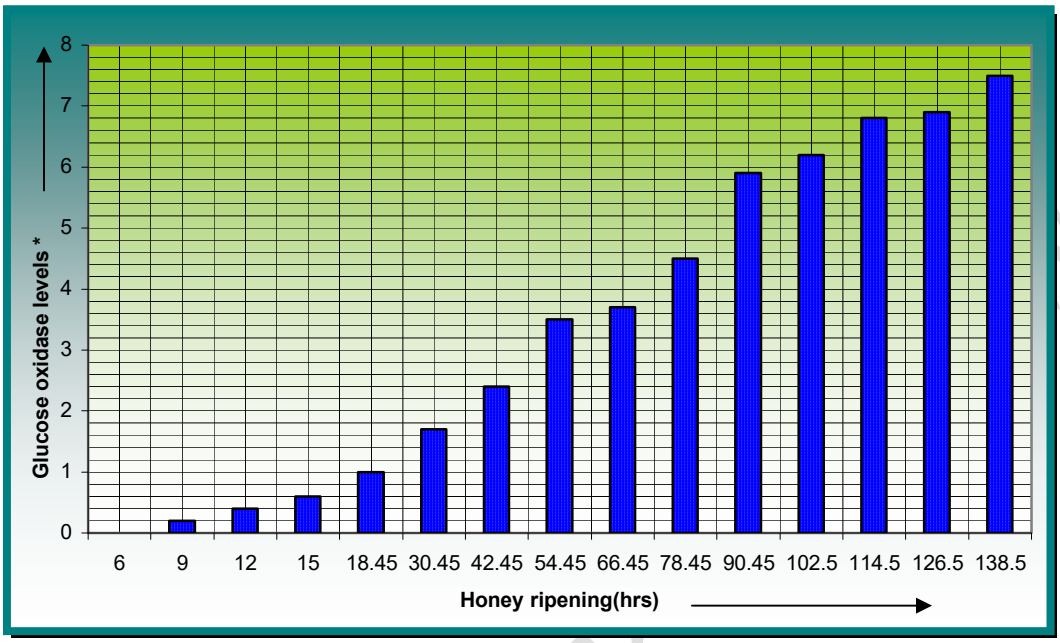
*Catalase in fn and hh was 0.00 ml and 0.63 ml respectively. The catalase of honey of uh and sh was 1.73 and 4.96 ml respectively (Fig.4). The analysis of variance of catalase of honey during different stages of ripening was not significant at 5% levels (Tab.4). Catalase acts on hydrogen peroxide and splits it into water and oxygen. [15] showed that most of the antibacterial activity / inhibine in honey is due to the production of hydrogen peroxide (H_2O_2). He also reported evident antibacterial activity in honey even in absence of glucose oxidase. Catalase was known to reduce antibacterial activity as it decomposes hydrogen peroxide into water and oxygen [16]. There was no correlation between catalase and diastase in honey.

$$\text{Hydrogen peroxide} \xrightarrow{\text{Catalase}} \text{Water} + \text{Oxygen}$$

CONCLUSIONS

The present studies successfully determined the activity of enzymes *viz*., invertase, amylase, glucose oxidase and catalase in nectar-honey transformation in honey from hive bee *A.cerana*. Invertase produced by foragers and housebees hypopharyngeal, post-cerebral, thoracic, labial and mandibular glands accomplish hydrolysis of sucrose into

laevulose and dextrose. Similarly, amylase acts on starch present in nectar to form maltose and dextrins in honey.



* Glucose oxidase activity: g. glucose hydrolyzed/ 100 g. sample/hour at 40°C

Fig.3. Glucose oxidase activity of honey during different hours of ripening

Table 3. ANOVA of glucose oxidase of honey in different stages of ripening.

Source of Variation	Degrees of Freedom	S.S	M.S.S	F-ratio
Bees	1	43.51	43.51	26.77**
Storage	1	59.51	59.51	36.62**
Bees and Storage	1	29.04	29.04	17.87**
Error	20	0.26	0.01	
Total	23	132.32	6.96	

** Non significant at 1 % level ($p<0.01$).

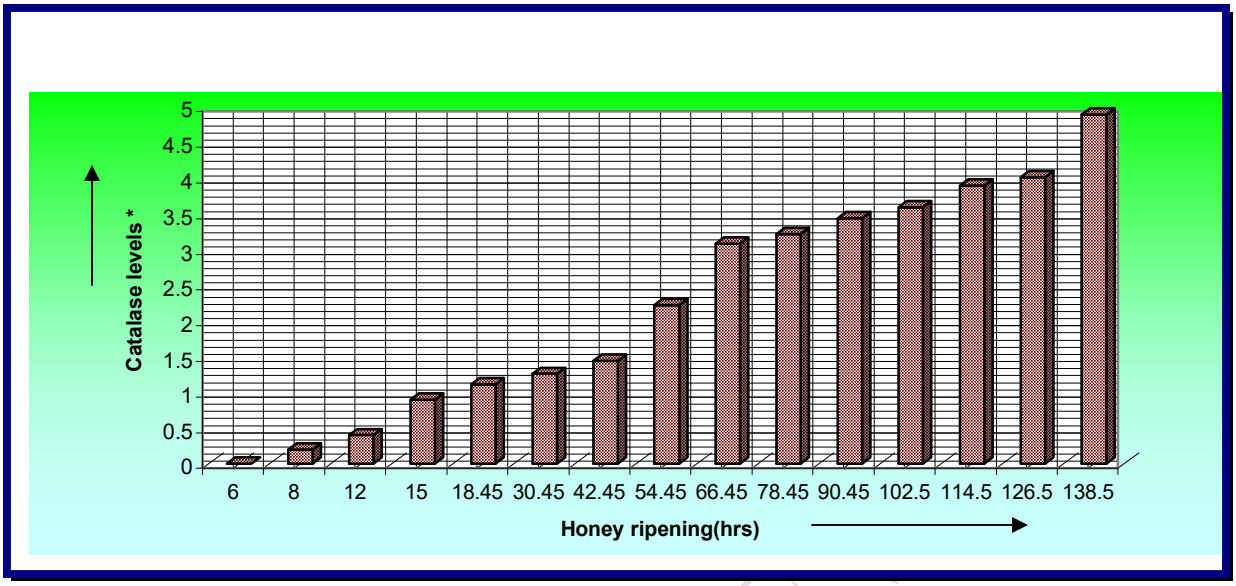
Glucose oxidase converts glucose into gluconic acid and hydrogen peroxide. Catalase acts on hydrogen peroxide and splits into water and oxygen. Obviously, enzymes play an important role in formation of monosaccharides found in honey (65%-75%) which form the bulk of honey composition. Correspondingly, The change of nectar to honey involves

physical, chemical, biochemical, physiological, behavioral and ecological factors. Honeybees by their active trophallaxis and thermoregulatory behavior gradually transform the nectar collected from flowers into comb honey by bringing spontaneous quantitative changes. 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.

Table 4. ANOVA of catalase of honey in different stages of ripening.

Source of Variation	Degrees of Freedom	S.S	M.S.S	F-ratio
Bees	1	25.99	25.99	12.37**
Storage	1	30.25	30.25	14.40**
Bees and Storage	1	22.47	22.47	10.70**
Error	20	0.33	0.02	
Total	23	79.05	4.16	

** Non significant at 1% level ($p<0.01$).



* Catalase activity per g. , Kf =1/t Inxo/x D/W; where xo is initial substrate at time t, D is the dilution and W is sample weight in grams (hydrogen peroxide hydrolyzed/ 100 g. sample/hour at 40°C

Fig. 4. Catalase activity of honey during different hours of ripening.

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