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Studies on Selected Factors Influencing Honey Ripening of Apis Cerana F

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ABSTRACT
The moisture and sugars levels in five stages of honey formation were studied in Indian hive honeybee, *Apis cerana F*., from December 2022 to January 2023 in Chintamani, Chickaballapur district. The five stages (FNS, FCS, HCS, USC, and SHC) were analysed and determined during honey ripening. The moisture shows fading activity from 79.08% (FNS) to 18.7% (SHC). Sucrose presented regression from 20.24% in FNS to 4.2% in SHC. Further, laevulose and dextrose displayed a cumulative increase from 1.3% in FNS, 40.2% in SHC, 0.9% in FNS, and 35.4% in SHC, respectively. Maximum regression of moisture levels occurred in USC (65%), HCS, FCS, and SHC, and sucrose in HCS (72%), followed by FCS, USC, and SHC, respectively. Similarly, a concentration increase occurred in laevulose in USC (55%), followed by HCS, FCS, and SHC. The dextrose arises in USC (47%), followed by HCS, FCS, and SHC. In the present work, correlation dynamics signify a decline in moisture and sucrose content in honey with a corresponding rise in laevulose and dextrose. The study concludes that due to the utilization of moisture in the formation of laevulose and dextrose followed by amalgamation of enzymes invertase and diastase from hypopharyngeal and mandibular glands of foragers and house bees. The data obtained was subjected to CRD-factorial analysis with multiple replicates and analysed at 5% ($P<0.05$) significant levels.

Keywords: Moisture, sucrose, laevulose, dextrose, honey ripening.

INTRODUCTION

Honey is an intriguing sweetener that may have many health benefits and ecological advantages. The current surge in commercialization is an evidence of importance of honey in daily use (Pascual-Maté et al., 2018). The captivating colour and viscous texture of honey are the result of diligent worker bees by its instinctive nature. The polylectic worker bees work nonstop, day after day, trip by trip, from sunrise to dusk, gathering flower secretions drop by drop so the colony's members can survive (Balasubramanyam, 2006). Undeniably, nectar is found in nectaries of flowers, but amazing honeybees require sheer diligence to collect, elaborate, process, transform, and store honey in honeycombs. Nectar consists of terpenes, alkaloids, flavonoids, xanthophylls and carotenoids, moisture, sugars, ions, and vitamins. Honey is considered a symbol of purity, prosperity, and sanctity. The relationship between honeybees and flowers is perhaps the best illustration of mutualism and co-evolution. Honeybees communicate both phonetically and kinetically. The qualitative and quantitative variations, such as refractive index, optical density, and viscosity of honey, are studied and well-documented (Kalpana et al., 1996). The kind of honeybee species can have an impact on the physical and chemical properties of honey, along with bee vegetation, the seasons, and the geographic location (Gela et al., 2023; Balasubramanyam, 2011). In 2018, India produced four thousand metric tonnes of honey annually.

According to the Food Safety and Standards Authority of India (FSSAI) and the Bureau of Indian Standards (BIS), the physical and chemical components of honey, such as moisture content, reducing sugars, sucrose content, EC, pH value, ash content, diastase activity, free acidity, HMF content, etc., are the standards for quality parameters (Thakur et al., 2022). Honey composition has been frequently employed for its characterization in addition to quality analyses. The two main factors that determine how ripe honey is are its moisture content and sugar content.

The two primary sugars found in honey are glucose and fructose, the sum of which, along with its sucrose level, is utilized by the Indian Union to determine the honey's quality (Council EU, 2022). The complex blend of mono, di, tri, oligo, and polysaccharides that is honey makes up the substance (Bogdanov et al., 2004). Another set of intriguing metrics for identifying some honey adulterations are sugar isotope ratios and honey sugars (Cengiz et al., 2018). Various analytical techniques have been used to determine the sugar content of honey, and the results have been reviewed and summarized in various studies (Pita-Calvo et al., 2017; Siddiqui et al., 2017). The sugar composition of honey contributes to its antibacterial properties and preservation by providing low water activity and high osmolarity, hindering microorganism growth. Sugar composition in honey exhibits variability, influenced by botanical origin, climatic conditions, and geographical origin. Factors such as floral sources, environmental and geographic origin, season, storage and processing methods, and honey-making processes by different bee species impact the composition and quality of honey. Bees add enzymes during honey-making which play a crucial role in determining its quality. Additionally, honey harvested in the same region but at different seasons may exhibit varying qualities, suggesting the influence of climatic and seasonal conditions and pre- and post-harvest beekeeping practices.

Karnataka, with its rich biodiversity from the Western Ghats, provides a favourable environment for various honeybee species. Chintamani in the Chickaballapur district, chosen as a sampling station for honey collection, is characterized by a diverse range of bee plant species. Information on tropical honey is from rock honeybees is abundant (Vijayakumar et al., 2020). Thus, the development of tropical honey by the rock honeybee, *Apis dorsata*, has been widely recognized, as have its physicochemical and composition characteristics are well documented . Conversely, information on honey ripening of Indian hive bee honey is limited (Crane, 2019). Earlier researchers studied the pollen diversity and physicochemical properties of Karnataka honey (Vijayakumar et al., 2020). However, information is scanty about the composition, including moisture and sugar content in honey processing. In this context, this study addresses the existing gap in knowledge by providing comprehensive information on the moisture and sugar content in honey ripening by *Apis cerana F.* from different localities of Chintamani, Chickaballapur district.

MATERIALS AND METHODS

The study area, area, Chickaballapur district, is located between 12º 58' and 13º 65 NL and 77º 35' and 77º 40 EL with a topography of 128m. It has an area of about 4,244 km^2 (1,639 sq miles). The average rainfall is about 280cm, with June to November being the peak. The temperature varied from 15ºC to 37.9ºC, and humidity varied from 42.5% to 84.5%.

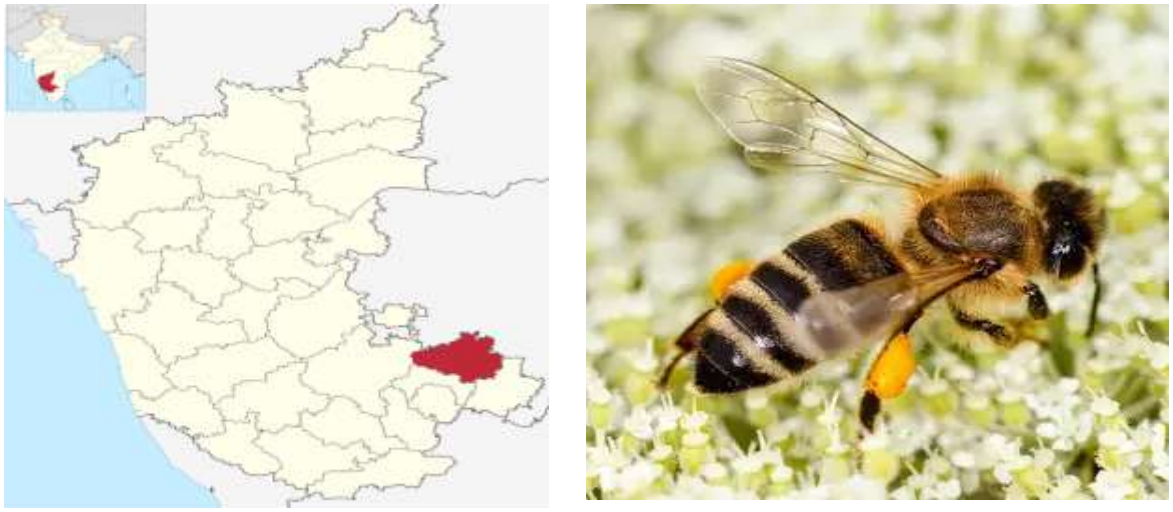

Fig 1: Map showing study site and honeybee species *Apis cerana*.

Latitude and longitude coordinates are: 13.401969 Long., and 78.055138 Lat., and Chintamani is a small town in eastern Karnataka bounded by Sidlaghatta on West, Bagepalli on the North west, Kolar in South west, Srinivasapura in South East and Anantapur in East. Chintamani taluk was selected for the sample collection of the present study. Based on the density of bee hives, three localities were chosen and from each location fifteen different samples of honey were extracted. A total of forty-five samples from three different locations were gathered and analysed their moisture and sugar contents in honey processing.

Fig 2: A: Tamarindus indica in flowering season; B: *Peltophorum pterocarpum* in full bloom ; C: Fresh honey samples of *A. mellifera* species; D: Sealed honey cells of Indian hive bee *A. mellifera*.

Estimation of sugars: Total sugars, sucrose, fructose, and glucose in different samples were analysed by the method of (Chunneja et al., 2016). Total sugar analysis: 1gm of honey was taken into a 250-mL

flask, diluted with water, and the volume to 250 mL. In a conical flask, 5 mL of Copper Sulphate solution, 5 mL of Potassium Sodium Tartrate solution, and 12 mL of honey solution from the burette were added. The mixture was heated to boiling over asbestos gauze. While the solution was boiling, 1 mL of methylene blue indicator was added, and the titration was finished. An indicator of the endpoint is a blue to red colour shift. The amount of honey solution (volume, H) needed for the titration was measured in mL, and the total sugar content was computed using the subsequent formula.

Total sugar, percent by mass =250x100xS/ H x M

where M= mass of honey in gms, H= volume of honey solution needed for titration, and S= strength of the copper sulphate solution.

Sucrose analysis: 1 mL of strong HCl was added to 100 mL of stock honey solution for the sucrose analysis. The mixture was then heated to almost boiling point and left overnight. As previously stated, sodium carbonate was used to neutralize the inverted honey solution, and the total reducing sugars were ascertained. This formula was used to determine the contents of sucrose, glucose, and fructose.

Sucrose content = [(reducing sugars after inversion, percent by mass) – (reducing sugars before inversion, percent by mass)] x 0.95

Approximate Glucose Content

(w) = (B – S) x 0.004502 x 100/a

B = Blank (volume of sodium thiosulphate solution), S = Sample (volume of sodium thiosulphate solution required for the sample), and a = mass of honey.

True glucose, percent by mass (y) = w – 0.012

Approximate fructose, percent by mass (x) =Approximate total reducing Sugar, percent – w/ 0.925

True fructose, percent by mass (z) =Approximate reducing sugars, percent – y/0.925

All the analyses were performed in triplicates, and data were presented as mean.

Detection of moisture*:* Total moisture in (FNS, FCS, HCS, USC, and SHC) was analysed with the method followed by BIS (2002). The moisture content was ascertained by use of the oven drying method. A precise weight of 1.5g of the sample was placed in the dry crucible (W_1). After 6–12 hours at 100–105°C in the oven, the crucible was taken out and its weight was kept constant. After that, the crucible was cooled for 30 minutes in the desiccator. It was weighed once again after cooling (W_2). The following formula was used to get the % moisture:

% Moisture = W_1-W_2 * 100 / Sample Weight.

Where: W_1 = crucible's initial weight plus sample; W_2 = crucible's final weight plus sample Note: For additional analysis, samples devoid of moisture were utilized. The data were given as mean, and all analyses were done in triplicate.

Statistical analysis: Data (FNS, FCS, HCS, USC, and SHC) was analyzed using CRD-factorial analysis. The data obtained from four parameters in honey amplification was subjected to CRD-factorial analysis, and multiple replicates analyzed were subjected to determine significant levels at 5% ($P<0.05\%$).

RESULTS AND DISCUSSION

The moisture content of honey is a critical factor influenced by various elements such as the origin of nectar, the maturity of honey, and environmental conditions like climate, weather, and storage practices. Typically, the moisture present in honey primarily stems from residual moisture left over after nectar ripening. However, improper storage conditions can lead honey to absorb moisture from its

surroundings, potentially affecting its quality. Honey with low moisture content tends to crystallize or granulate more rapidly but is otherwise trouble-free. In the present study, the moisture content in the floral nectaries (FNS) was found to be 79.4%, while the honey crop of foragers (FCS) was 76.64 and the honey crop of foragers (HCS) was 64.81%. The moisture content of unsealed honey cells (USC) and sealed honey cells (SHC) was 45.37% and 18.27%, respectively (fig.3).

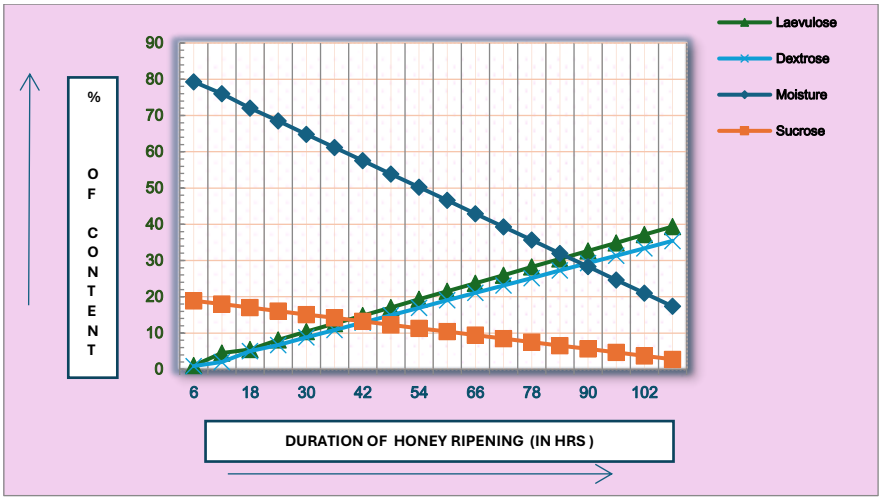

Fig 3: Correlation dynamics of Moisture, sucrose, Laevulose, and dextrose during honey ripening

This variance in moisture content is crucial as it directly impacts the honey quality and shelf stability. According to current regulations, honey should not exceed a maximum moisture value of 20%, except for ling heather (C*alluna vulgaris*) honey, which can reach up to 23% (Council EU, 2022). In the present study, moisture percentage ranged from 13.26% (SHC) to 79.4% (FNS) indicating that sealed honey cells maintain better quality in terms of moisture content compared to other varieties. The completed randomized design (CRD) factorial analysis of moisture in forager bee crop sac (FCS), house bee crop sac (HCS), unsealed honey cells (USC), and sealed honey cells (SHC) (table.1).

Table 1: Completed randomized design (CRD) factorial analysis of moisture, sucrose, fructose, and dextrose in Forager Bee Crop Sac, House Bee Crop Sac, Unsealed, and sealed honey cells.

Sl. No.	Stages of FCS, HCS, USC, and SHC *	Moisture (%)	Sucrose (%)	Laevulose (%)	Dextrose (%)
1.	Forager Bee crop sac (A1)	78.08± 0.26	18.50± 0.32	1.32± 0.16	0.9± 0.11
2.	House Bee Crop Sac (A_2)	76.65± 0.36	14.90± 0.28	2.70± 0.22	3.14± 0.24
3.	Honey bees (B_1)	42.15± 0.26	7.52± 0.26	22.18± 0.26	21.28± 0.26
4.	Honey sealed cells (B_2)	28.40± 0.26	2.44± 0.26	98.20± 0.26	33.14± 0.26
5.	A_1 B_1	69.65±0.78	17.20±0.56	3.80±0.51	3.70±0.55
6.	B_2	65.63±0.51	14.40±0.39	20.41±0.31	12.08±0.36
7.	A_2 B_1	67.55±1.	12.60±1.01	5.60±1.77	5.21±1.82

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8.	B_1	21.04±0.51	2.40±0.39	40.45±0.31	36.20±0.36

Values are represented as Mean ± standard error at 0.05 % significance levels.
* Replicates of 45 samples each for FCS, HCS, USC, and SHC.

Overall, understanding the intricate relationship between moisture content and honey quality is essential for beekeepers and honey producers to maintain product integrity and meet regulatory standards.

Sucrose, serves as a vital energy source for honey bees, particularly for foragers engaged in strenuous flight and colony maintenance tasks. Foraging honey bees rely on a continuous supply of sugar, mainly carbohydrates, to fuel their energy-intensive flights and colony thermoregulation. Honey is a vital source of energy for adult honey bees, especially foragers, who have higher metabolic rates and require a constant supply of sugar to sustain their activities. Legislatively speaking, sucrose is a significant sugar that is often limited to 5% (Council EU, 2022). Certain types of honey, such as clover honey from the Medicago sativa genus, have a maximum content of 10%, while other types, like lavender honey, have a maximum content of 15%. In the present study, sucrose content in the floral nectaries (FNS) was 20.72% while the honey crop of foragers (FCS) was 18.24%, and the honey crop of foragers (HCS) was 11.56%. The sucrose content of unsealed honey cells (USC) and sealed honey cells (SHC) was 5.02% and 2%, respectively (Fig.1). This decline in sucrose content in sealed cells aligns with previous research indicating an advanced stage of honey ripening, where conversion into glucose and fructose predominates (Pascual-Maté et al., 2018). The completed randomized design (CRD) factorial analysis of sucrose in Forager Bee Crop Sac (FCS), House Bee Crop Sac (HCS), unsealed honey cells (USC), and sealed honey cells (SHC) (Table 1). The analysis of variance (ANOVA) using an F-test revealed significant differences in sucrose content ($P<0.05$), indicating that the variability observed was not due to random chance.

Understanding sucrose dynamics in honey production is crucial for beekeepers and researchers to ensure honey quality and compliance with regulatory standards. It underscores the intricate processes involved in honey ripening and the role of sucrose as a fundamental carbohydrate source for honey bee nutrition.

The content of laevulose in honey serves as a crucial indicator of its sweetness, energy provision, and potential health benefits. This component can vary depending on the botanical source of the nectar and the maturity of the honey. Laevulose, along with glucose and fructose, constitutes the primary sugars found in honey. Floral nectaries (FNS) exhibited a relatively low laevulose content of 1.3%, indicating the initial composition of nectar. However, as honey ripens, the conversion of sucrose into glucose and fructose leads to higher laevulose concentrations. The honey crop of foragers (FCS) contained 1.8% laevulose, while that of house bees (HCS) exhibited a higher content at 4.41%. Unsealed honey cells (USC) showed a significant increase in laevulose content, reaching 15.67%, while sealed honey cells (SHC) displayed the highest concentration at 40.23%. This progression suggests an advanced stage of honey ripening, where the conversion of sucrose into its constituent sugars, including laevulose, is pronounced (Fig. 3). Average Moisture levels for FNS, FCS, HCS, USC and SHC was found to be 79.4±1.0, 76.78±0.5, 64.8±0.6, 45.37±0.7 and 18.27±0.4 respectively.

In addition to laevulose, dextrose is one of the main sugars present alongside fructose and glucose. The final constituents of honey can vary depending on the nectar source. The dextrose in the floral nectaries (FNS) was 1.02%, while the honey crop of foragers (FCS) was 1.2%, and the honey crop of foragers

(HCS) was 2.5%. Dextrose content of unsealed honey cells (USC) and sealed honey cells (SHC) was 13.22% and 35.017% respectively (Fig 3). The completed randomized design (CRD) factorial analysis of dextrose in Forager Bee Crop Sac (FCS), House Bee Crop Sac (HCS), unsealed honey cells (USC) and sealed honey cells (SHC) are presented in Table 1.

Understanding the dynamics of laevulose and dextrose in honey production is essential for assessing honey quality and nutritional value, as well as for complying with regulatory standards. Previous studies have extensively analysed the physico-chemical properties of Indian honey, focusing on parameters such as ash content and acidity levels (Shripad et al., 2001). Researchers investigated honey samples from three bee species in Uttara Kannada, shedding light on their chemical composition and characteristics.

The two main and principal sugars in honey are glucose and fructose. The main variables pertaining to honey quality are the total amount of fructose, glucose, the fructose/glucose ratio, and the glucose/water ratio. The glucose-fructose ratio in honey plays a crucial role in determining its taste, nutritional value, authenticity, and potential health benefits. Understanding and monitoring this ratio can provide insights into the quality and composition of honey products. Honey's capacity to crystallise is indicated by its fructose/glucose ratio (Saha, 2003). Honey's sugar composition varies significantly due to factors like botanical and geographic origin, climate, processing methods, and storage conditions. It's well-documented that sugars undergo alterations during storage, further influencing honey's overall characteristics. Typically, high-quality Indian honey maintains an optimal composition, comprising approximately 29.9 to 40.3% glucose, 30.3 to 46.7% fructose, 15.0 to 22.2% sucrose, and a moisture content ranging from 17 to 21% (Gela et al., 2023).

In a study focusing on honey from various climatic zones of Himachal Pradesh, sucrose and fructose contents fell within the acceptable range of 4.91-6.94% and 30.94-36.62%, respectively (Thakur et al., 2022). These findings align with earlier research, confirming the consistency of sugar levels across different studies. Notably, the fructose-glucose ratio is indicative of honey's crystallization propensity, with a higher ratio correlating with a more liquid consistency (Rodrigreuz-Otero et al., 2024). The tendency of honey to crystallise is indicated by the fructose glucose ratio, where a larger ratio denotes a liquid state. Higher fructose values are observed in squeezed honey, according to the research. In the past, A. mellifera honey from the Chamba district of Himachal Pradesh was found to have sucrose, fructose, glucose, and an F: G ratio in the range of 3.17 to 5.14%, 29.71 to 33.28%, 29.20 to 32.93 %, and 0.97 to 1.41 %, respectively (Balasubramanyam et al., 2003). Moreover, fresh Himachal honey showcased substantial fructose (35.90%), glucose (32.43%), and total sugar (80.70%) levels, underscoring the diverse sugar profiles across honey samples. Sugar composition has also been instrumental in distinguishing honey samples based on their botanical or geographic origins (Balasubramanyam et al., 2015).

In conclusion, the intricate interplay of glucose and fructose content, alongside other sugar constituents, underscores the multifaceted nature of honey's chemical composition. By understanding these dynamics, researchers can better assess honey quality and authenticity, paving the way for improved honey production and classification methods. This study addresses the moisture and sugar content in honey ripening by *Apis cerana F.* from different localities of Chintamani, Chickaballapur district. The study findings conclude that due to the utilization of moisture in the formation of laevulose and dextrose followed by an amalgamation of enzymes invertase and diastase from the hypopharyngeal and mandibular glands of foragers and house bees. The findings of this study have implications for Ayurvedic and naturopathic therapies. Ayurveda may use the findings for targeted treatments, while

naturopaths can appreciate honey's natural complexity. The research enhances nutritional understanding, aiding informed choices about honey as a natural sweetener. Further exploration of environmental influences, diverse geographic regions, therapeutic potentials, and sustainable beekeeping practices will contribute to a comprehensive and impactful understanding of medicinal use of honey.

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AUTHOR CONTRIBUTION STATEMENT

Balasubramanyam, M.V., has designed & executed the work followed by data analysis and manuscript preparation.

CONFLICTS OF INTEREST / COMPETING INTERESTS

Authors claim No conflict of interest.

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