Uttar Pradesh Journal of Zoology

Volume 46, Issue 2, Page 12-24, 2025; Article no.UPJOZ.4491
ISSN: 0256-971X (P)

Comparative Physicochemical Properties of Honey from *Apis cerana indica* and *Apis dorsata* Across Different Floral Origins in Khandesh, North Maharashtra, India

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2025/v46i24759

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4491

Original Research Article

Received: 10/11/2024
Accepted: 12/01/2025
Published: 16/01/2025

ABSTRACT

The aim of this study was to compare the physicochemical properties of honey produced by *Apis cerana indica* and *Apis dorsata* from three sites with distinct floral orgins (agriculture, forest, and urban) in Khandesh, North Maharashtra, India. Physicochemical analysis of honey samples was conducted using established methods described by the Association of Official Analytical Chemists (AOAC 2012), and the protein content was determined using the Folin-Ciocalteau reagent method. Among the different sources of honey, significant differences were found across honey samples in terms of moisture content, pH, electrical conductivity, ash content, free acidity, protein, hydroxymethylfurfural (HMF), proline, and diastase activity.

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Cite as: *Sawarkar, Arun Baburao. 2025. "Comparative Physicochemical Properties of Honey from Apis Cerana Indica and Apis Dorsata Across Different Floral Origins in Khandesh, North Maharashtra, India". UTTAR PRADESH JOURNAL OF ZOOLOGY 46 (2):12-24. https://doi.org/10.56557/upjoz/2025/v46i24759.*

The honey samples from both bee species with different geographical and floral origins had varing ranges of moisture content (15.84%-23.16%), pH values (3.31-4.84), electrical conductivity (0.52-0.96 mS/cm), ash content (0.18%-0.51%), free acidity (26.83-40.18 meq/kg), protein content (1.45-3.05 g/kg), HMF levels (19.38-35.69 mg/kg), proline (223.49- 849.26 mg/kg), and diastase activity (9.46-21.57 DN). Notably, physicochemical parameters were significantly higher in *A. dorsata* honey than in *A. c. indica* honey. These variations highlight the influence of floral and geographical origin-on the physicochemical properties of honey. Furthermore, honey collected from forest sites was superior to that collected from agricultural and urban sites. All these values fall within the criteria set by international standards, highlighting the quality and compliance of honey samples. Principal component analysis (PCA) revealed a strong separation between the measured parameters, and was mostly dominated by moisture, pH, EC, ash, free acidity, protein, proline, and diastase.

Keywords: Hone; Apis cerana indica; Apis dorsata; physico-chemical characteristics; Khandesh.

1. INTRODUCTION

Honey is a naturally occurring sweet substance produced by forager bees, either from the nectar of flowers or from honeydew secretions from certain hemipterans (Codex Alimentarius, 2001). It is highly popular in the market not only for its rich nutritional profile but also for its safety for human consumption. Its diverse composition makes it in great demand in the food and beverage industry, as well as in the pharmaceutical and cosmetic sectors, where its natural properties are widely utilized.

The sweetness of honey is primarily attributed to its high fructose and glucose content, which also reflects its nutritional characteristics (Khalil *et al.*, 2001). Along with the sugar, it contains proteins, enzymes, vitamins, aromatic substances, pigments, waxes, phenolic acids, flavonoids, minerals and pollen grains which give its specific aroma, flavor and even its biological activity to maintain the honey quality (Khalil *et al.*, 2001; Saxena *et al.*, 2010; Singh and Bath, 1997).

However, variations in the composition and physicochemical properties of honey are largely influenced by many factors such as its floral origin, geographical location, seasonal changes, and environmental conditions (Nanda *et al.*, 2009; Sajid *et al.*, 2023; Sawarkar, 2023a). The use of insufficient storage periods and processing of honey has also shown varied impacts on physicochemical parameters (Sohaimy *et al.*, 2015; Subramanian *et al.,* 2007). Among the parameters, hydroxy methyl furfural (HMF) and diastase level are used as indicators for the freshness and quality of honey (Al-Ghamdi *et al.*, 2019; Korkmaz and Küplülü, 2017). Freshly prepared honey contained very small amounts of HMF and moderate amounts of diastase level are present. However, during the heating and storage process of honey, the level of HMF increases and the level of diastase decreases (Korkmaz and Küplülü, 2017; Sajid *et al.*, 2019; Sawarkar, 2024a; Tosi *et al.*, 2002; Yilmaz and Küfrevioğlu, 2001). Furthermore, sugar, pH, ash content, electrical conductivity, and total acidity content may correlate with variations in HMF and diastase content (Kamboj *et al.,* 2013; Sawarkar, 2023b; Subramanian *et al.,* 2007).

For the commercialization of honey, several quality control methods has been applied to determine physicochemical properties and are helpful in categorizing honey from different geographic origins, authenticating chemical features, and detecting adulteration (Bogdanov *et al.,* 2004). Many international regulatory bodies impose specific regulations on honey quality and safety. They established the physicochemical parameters and chemical compounds to be determined in honey using harmonized methods for the quality and safety control of honey (AOAC, 2012). The physicochemical determination of honeys from different regions of India have been extensively studied (Balasubramanyam and Reddy, 2003; Das *et al.,* 2015; Gairola *et al.,* 2013; Nanda *et al.,* 2009; Nayik and Nanda, 2015; Thakur *et al.,* 2023).

In the Khandesh region of India, which is heavily covered by forests and agricultural land, farmers use bee colonies of *Apis cerana indica* and *Apis mellifera* to boost crop productivity. These crops include cotton, onions, pomegranates, lemons, and guava. In addition to domesticated bees, wild bees such as *Apis dorsata* and *Apis florea* also contribute to the pollination of both agricultural and forest flora. In addition to pollination, honey production from both domestic and wild bee species provides a moderate boost to the economies of farmers, beekeepers, and tribes. To a certain extent, honey from the North Maharashtra region has been examined for its

physicochemical properties, processing, and storage conditions to confirm its quality (Sawarkar, 2023a,b; Sawarkar, 2024a). In a recent study, Sawarkar (2024b) analyzed the significant antioxidant content of *A. c. indica* honey produced in North Maharashtra, India. In earlier comparative studies, it was noted that honey from *A. dorsata* and *A. c. indica* has better physicochemical properties than other honeys that meet international standards (Balasubramanyam, 2011; Balasubramanyam and Reddy, 2003; Joshi *et al.*, 2000).

There is limited information regarding the comparison of physicochemical properties of honey samples from different floral origins. However, owing to market demand, it is essential to verify the quality of honey from *A. c. indica* and *A. dorsata* collected from agricultural, forest, and urban sites according to international standards. Therefore, this study aimed to access the physicochemical properties of honey produced by *A. c. indica*, and *A. dorsata* in the Khandesh region.

2. MATERIALS AND METHODS

2.1 Honey Sample Collection Sites

To conduct the physicochemical analysis of honey samples, three distinct sites were chosen based on their floral and geographical origins: agricultural, forest, and urban locations from the Khandesh region in North Maharashtra, India (Fig. 1). The Khandesh region comprises the districts of Jalgaon, Dhule, and Nandurbar. During the summer, total of 42 honey samples of *Apis cerana indica* and *Apis dorsata* were collected from beekeepers, tribal communities and honey hunters. All the samples were fresh, unprocessed, unheated, and naturally pure. In the laboratory, honey samples were filtered through a fine muscline cloth to remove suspended particles such as dirt, beeswax, and other impurities. After filtration, the honey was transferred to airtight plastic containers and stored at room temperature to preserve its natural properties.

2.2 Physicochemical Analysis

For the analysis of physicochemical determination in honey, applied standard methods determined by the Association of Official Analytical Chemists (AOAC 2012). It includes the determination of moisture, pH, electrical conductivity, ash content, free acidity, proline, HMF and diastase number from the collected honey samples.

2.2.1 Moisture

The moisture content in the honey samples was determined using an Abbe refractometer. Each sample was measured in triplicate, at a temperature of 20^0C to ensure accuracy. The refractive index readings were then converted to moisture content percentage (g/100 g) using a Chataway table.

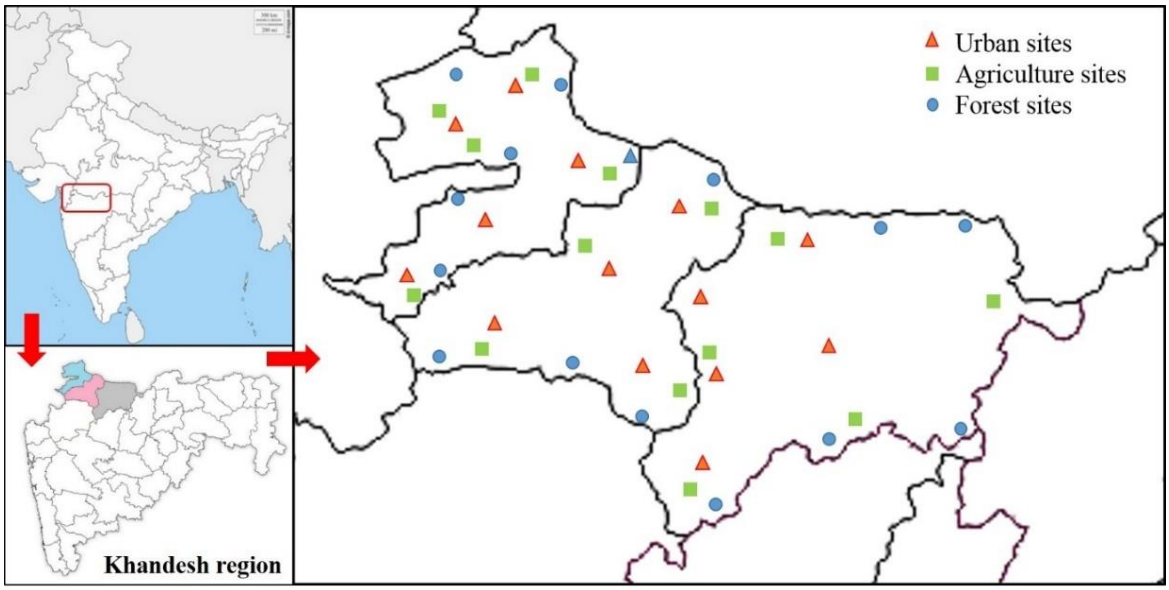

Fig. 1. Locations of collected honey samples from Khandesh region
(Source: https://www.mapsofindia.com/)

2.2.2 pH

The pH of honey samples was measured using a pH meter (Elico, India). To prepare the solution for pH measurement, a 10% (w/v) honey solution was prepared by dissolving 10 g of honey in 100 mL of CO_2-free distilled water.

2.2.3 Electrical conductivity

The electrical conductivities (EC) of the honey samples were measured at 20^0C using a conductivity meter (Elico, India). EC of each honey sample was analyzed in triplicate, and the mean value was expressed in Milli-Siemens per centimeter (mS/cm).

2.2.4 Ash

Ash content was determined by burning each honey sample at 600^0C in a muffle furnace for 6h. After cooling to room temperature, the resulting ash was weighed and expressed as a percentage (g/100 g).

2.2.5 Free acidity

The free acidity was determined by the titrimetric method. A 10g homogenized honey was dissolved in 75 mL distilled water and then titrated with 0.1 N NaOH to a pH of 8.30. Acidity was measured in milliequivalents of acid per kg (meq/kg) of honey.

2.2.6 Proline

Proline content was determined using the spectrophotometric method. A 0.5 mL honey solution (5% w/v with distilled water) was pipetted out in a test tube. Then added 1 mL each of formic acid and ninhydrin solution with vigorous shaking. The mixture was heated in a boiling water bath at 70°C for 15 minutes. Then added with 5 mL of isopropanol, leave to cool the mixture, and the absorbance was measured at 520 nm using a spectrophotometer against a blank. The concentration of proline in the honey samples was then calculated.

2.2.7 HMF

The HMF content of honey was determined using the spectrophotometric method describe by AOAC (2012). A 5 g honey sample was dissolved in 25 mL of distilled water in a 50 mL volumetric flask. To this, 0.5 mL of Carrez I solution and 0.5 mL of Carrez II solution were added, and the volume was made up to 50 mL with distilled water. The solution was filtered, discarding the first 5 mL of the filtrate. Subsequently, 5 mL of 0.2% sodium bisulfite solution was added to a test tube, while 5 mL of distilled water was used as a blank. The absorbance of both solutions was measured at 284 nm and 336 nm using a UV-visible spectrophotometer, and the HMF content was expressed in mg/kg.

2.2.8 Protein

The protein content of the honey was measured using the method described by Lowry et al. (1951). A 0.1 mL aliquot of the protein extract (50% w/v honey sample) was pipetted into a test tube, followed by the addition of 2 mL of alkaline copper sulfate reagent. The mixture was thoroughly mixed and incubated at room temperature for 10 minutes. Subsequently, 0.2 mL of Folin-Ciocalteau reagent was added to each tube, and the samples were incubated for an additional 30 minutes. The absorbance was measured at 660 nm using a spectrophotometer, with a BSA solution (1 mg/mL) used as the standard for protein estimation.

2.2.9 Diastase

Diastase activity in the honey samples was determined using the spectrophotometric method (AOAC, 2012). For the determination of diastase activity, a 50 mL beaker containing 10 g of honey samples was dissolved in 15 mL of distilled water and 5 mL of acetate buffer. After mixing, 3 mL NaCl was added and diluted to 50 mL with distilled water. The starch solution was calibrated using an iodine solution at 660 nm. Pipette out 10 mL of honey and starch solution into two separate 50 mL beakers. Both the solutions were heated at 40^0C. After 15 min, 5 mL of starch solution was added to the honey solution, mixed thoroughly, and the stopwatch was started. At 5-minute intervals, an aliquot was taken, and 5 mL of iodine solution was added rapidly. Subsequently, 11 mL of distilled water was added to each solution, mixed well, and the absorbance at 660 nm was recorded immediately. Diastase activity was expressed as the diastase number (DN).

2.3 Statistical Analysis

All physicochemical tests were performed in triplicate, and the results are presented as mean values with standard deviations (mean ± SD).

Differences were considered statistically significant at $p<0.05$. The comparison of means was conducted using ANOVA (one-way analysis of variance). Correlations between the evaluated parameters were assessed using Pearson's correlation coefficient (r). Additionally, the experimental data were analyzed using multivariate analysis in XLSTAT software. Among these analyses, principal component analysis (PCA) was applied to identify the dominant eigenvectors responsible for dimension reduction and variation.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties

A. c. indica and *A. dorsata* honeys were obtained from three distinct sites: agricultural, forest and urban. All honey samples were determined and showed variations in physicochemical parameters, as summarized in Table 1.

3.1.1 Moisture content

Moisture content is a crucial factor that determines the amount of water present in the honey samples (Moniruzzaman *et al.*, 2013). In the present study, the range of moisture content was between 18.3 to 21.3%, which is within the limit fixed by the international standards (Codex Alimentations, 2001; FSSAI, 2019). The range of moisture content in the honey samples was similar to honey from Morocco (14.55 to 20.99%) (Bouhlali *et al.*, 2019), Eastern Romania (15.20 to 20.77%) (Albu *et al.,* 2021), and Malaysia (11.59 to 19.06%) (Moniruzzaman *et al.*, 2013) and Ethiopia (17.07 to 25.0%) (Gela *et al.*, 2023).

The range of moisture content was higher in honey of *A. dorsata* (19.1 to 21.3%) than *A. c. indica* (18.3 to 19.5%). Significant differences were observed in the moisture content of honey samples from *A. c. indica* and *A. dorsata*. This indicates that *A. c. indica* honey has lower water content and is more resistant to microbial growth than *A. dorsata* honey. Similar variations in moisture content were observed in earlier fingings (Balasubramanyam, 2011; Balasubramanyam and Reddy 2003; Joshi *et al.*, 2000), When considering moisture content, Indian honey often has a much larger range of 20 to 25% than other honey (Singh and Bath, 1998) and is supported by (Das *et al.,* 2015; Kharkamni, 2021; Saxena *et al.*, 2010; Waykar and Joshi, 2021).

It was observed that the honey samples (both *A. c. indica* and *A. dorsata*) collected from the forest sites had higher moisture content than that of honey from Agriculture and Urban sites (Table 1). A number of factors, including harvested time, geographical location, climatic and seasonal changes, and the process of honey extraction, affect the moisture content of honey samples (Saxena *et al.*, 2010). However, the moisture content of all honey samples indicates good storage ability and quality, in accordance with international honey quality regulations (Codex Alimentarius, 2001).

3.1.2 pH

pH values of all honey samples varied in the range of 3.74 to 4.43. Honey samples collected from *A. dorsata* had the highest pH value (4.03 to 4.43) compared to *A. c. indica* (3.74 to 4.18). The pH of all honey samples was within the standard range (Codex Alimentarius, 2001; FSSAI, 2019), indicating the freshness of honey. The more acidic pH values observed in the honey samples of *A. dorsata, A. c. indica* and *A. mellifera* were 3.68, 3.62 and 3.52 respectively (Joshi *et al.,* 2000). The pH of honey samples was similar to those reported in the Western Ghats of Karnataka (Balasubramanyam, 2011; Balasubramanyam and Reddy, 2003), North India (Thakur *et al.*, 2023), J & K, Tamil Nadu (Manzoor *et al.*, 2013), Jammu and Kashmir (Nayik and Nanda, 2015), Meghalaya (Das *et al.,* 2015), Pakistan (Sajid *et al.*, 2023), and Nepal (Joshi *et al.*, 2000).

According to Bogdanov et al. (2004), the acidic pH of honey is influenced by the presence of organic acids, which contribute to its flavor and enhance stability against microbial spoilage. It also influences honey quality, stability, and shelf life (Sawarkar, 2023a,b; Terrab *et al.,* 2002).

3.1.3 Electric conductivity

Electrical conductivity is a crucial parameter for assessing the physical properties of honey. The range of EC values of all honey samples from the selected sites were 0.61 to 0.86 mS/cm (Table 1). The evaluated EC values of honey samples from different sites had shown similar results reported from Malaysia (0.35 to 0.76 mS/cm) Moniruzzaman *et al.* (2013); Tunisia (0.39 to 0.89 mS/cm) Boussaid *et al.* (2018); Bangladesh (0.2 to 0.8 mS/cm) Islam *et al.* (2012); Sohra (Meghalaya) India (0.51 to 0.61) mS/cm Kharkamni (2021); Saudi (0.53 mS/cm) Sohaimy

et al. (2015); North Maharashtra (0.49 to 0.67 mS/cm) Sawarkar (2023a); Jammu and Kashmir (0.25 to 0.79 mS/cm) Nayik and Nanda (2015); Southern Karnataka (0.53 to 0.8 mS/cm) Almasi and Sekarappa (2019).

The honey from *A. dorsata* had a higher EC value (0.66 ±0.27 to 0.86 ±0.39 mS/cm) which contained a high mineral content as compare to the EC in honey from *A. c. indica* (0.61 ±0.21 to 0.71 ±0.29 mS/cm). According to Joshi *et al.* (2000) analyzed the Nepalean honey, the highest EC was recorded in *A. dorsata* (0.96 mS/cm) honeys followed by *A. cerana* (0.65 mS/cm) and *A. mellifera* (0.31 mS/cm) honeys, respectively. Large variations in EC may depend on the floral origin and amount of plant pollen (Boussaid *et al.*, 2018). The EC value is influenced by ash content, organic acid, inorganic salts, proteins and minerals in honey, as honey with a higher acid content has a higher conductivity (Lullah-Deh *et al.*, 2018; Lim *et al*., 2022).

3.1.4 Ash

Ash content serves as a significant quality indicator, reflecting the mineral richness found in honey. In the honey samples, it was noticed that the ash content was in the ranged of 0.22 to 0.39%. Honey from *A. dorsata* has higher ash content (0.36 to 0.39%) than observed in *A. c. indica* (0.22 to 0.36%). Table 1 show that forest honey has high ash content, with a mean value of 0.4% followed by honey from agricultural sites (0.35%) and urban sites (0.3%). The ash content of all samples was significantly different ($p<0.05$). Similar results for ash content in honey samples have been reported previously (Balasubramanyam, 2011; Gela *et al.*, 2023; Manzoor *et al.*¸2013; Nanda *et al.*, 2009).

The ash content of honey is composed of a variety of major and trace elements, including calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), and sodium (Na), which are present in varying concentrations depending on floral and geographical origins (Khalil *et al*., 2012; Lanjwani and Channa, 2019). Other factors such as environmental pollution, soil composition, beekeeping practices, and processing and handling of honey affect the mineral contents of honey (Lim *et al*., 2022).

3.1.5 Free acidity

The free acidity between honey samples from three different sites was in the range of 30.74 to 37.64 meq/kg which was significantly different ($p<0.05$) from the free acidity value (Table 1). The honey samples of *A. dorsata* are more acidic than *A. c. indica* (Table 1), indicating that *A. dorsata* forages in many diverse and wild flora, which may produce nectar that can result in honey with higher acid content.

According to the results, the maximum free acidity was noted in forest honey, followed by agriculture honey and urban honey. A similar range of free acidity has also been reported in previous studies (Kumar *et al.*, 2018; Kamboj *et al.,* 2013; Kharkamni, 2021). Variations in free acidity can be caused by many factors, such as the harvesting period, honey ripeness, floral sources, storage conditions and climate (Ahmed *et al.*, 2016). The elevated acidity of honey samples is linked to the fermentation of sugars into organic acids, which contribute to the flavor of the honey and its stability against microbial spoilage (Bogdanov et al., 2008). However, all the values of free acidity were in accordance with international standards (lower than 50 meq/kg), indicating good quality of honey samples.

3.1.6 Protein

The variation in protein and amino acid concentrations in honey samples is influenced by their botanical and geographical origins, as well as the duration of storage (Robin *et al.*, 2022; Schäfer *et al.,* 2006). The protein content in the *A. dorsata* honey had a higher range (2.19 to 2.62 g/kg) than *A. c. indica* (1.64 to 2.29 g/kg). The mean range of protein content observed in the agriculture, forest and urban sites was 2.15, 2.46 and 1.9 g/kg respectively. The variation in protein content may be due to differences in geographical locations and floral sources.

In honey, the major constituents of the protein remain in the form of free amino acids and enzymes such as diastase, invertase and glucose oxidase, which are introduced by bees during the honey ripening process (Bogdanov *et al.,* 2004). According to international standards, the protein content of honey remains in the range of 2-5 g/kg, as noted by Codex Alimentarius (2001) and FSSAI (2019). A similar range of protein content in honey samples was noted in earlier studies from India (Kumar *et al.,* 2018); Tunisia (Boussaid *et al.,* 2018); Malaysia (Moniruzzaman *et al.,* 2013); and Bangladesh (Khalil *et al.,* 2001).

3.1.7 Hydroxymethylfurfural (HMF)

The mean level of HMF content was highest in honey samples collected from urban sites (29.98 mg/kg) followed by agriculture (25.53 mg/kg) and forest sites (22.73 mg/kg) (Table 1). Honey from *A. dorsata* had a higher HMF level than *A. c. indica* honey. The HMF content varied significantly ($p<0.05$) between the honeys produced from the selected sites. The variation in HMF values suggests that all honey samples were raw and unprocessed. Meanwhile, it was suggested that the tropical climatic factor, such as temperature ranging from 39 to 44^{0}C during the summer, may increase HMF content in honey samples from the selected sites. Previous studies reported similar HMF ranges in honey were noticed (Kharkamni, 2021; Waykar and Joshi, 2021; Korkmaz and Küplülü, 2017; Yilmaz and Küfrevioğlu, 2001). Many factors can affect the formation of HMF, including pH, storage conditions, heating, processing methods and floral sources (Al-Ghamdi *et al.*, 2019; Sawarkar, 2024a; Terrab *et al.*, 2002; Tosi *et al.*, 2002). All HMF values remained within the international standard (<40 mg/kg), and FSSAI (<80 mg/kg) confirmed the relative freshness of honey.

3.1.8 Proline

Proline is one of the most important amino acids in honey. Moderate proline content is an indication of honey ripeness and is free from adulteration (Bogdanov *et al*., 2008; Lim *et al.*, 2022). As shown in Table 1, proline content in honey produced from both *A. dorsata* (798.39±86.11 mg/kg) and *A. c. indica* (403.43±49.18 mg/kg) was higher in forest sites followed by agriculture and urban sites and varied significantly ($p<0.05$). According to international standards, the proline content was higher than 180 mg/kg indicating that all honey samples might be considered ripened, fresh, pure and unadulterated. The current results show that the proline content in honey samples aligns with findings from previous studies on Indian honey (Saxena *et. al.*, 2010); Malaysian honey (Moniruzzaman *et al.*, 2013); Nepalean honey (Joshi *et al.,* 2000); Tunisian honey (Boussaid *et al.*, 2018); Kashmirian honey (Nayik and Nanda, 2015) and Bangladeshi honey (Islam *et al.*, 2012).

3.1.9 Diastase

Diastase is one of the most dominant enzymes present in honey that can break down starch and transform it into maltose. This might help indicate the freshness and quality of honey because of its heat resistance feature (Bogdanov *et al*. 2008). According to the results shown in Table 1, the diastase number was highest in honey samples collected from forest sites, followed by those from agriculture and urban sites. A higher value of diastase number was observed in the honey collected from *A. dorsata* (16.23 to 19.69 DN) and *A. c. indica* (10.71 to 13.23 DN), as shown in Table 1. All examined samples herein conformed to the required standards that is greater than 8 DN (Codex Alimentarius, 2001; FSSAI, 2019) and determined that all honey samples were fresh and unprocessed. The diastase numbers of the analysed samples were similar to those reported by Nayik and Nanda (2015), Korkmaz and Küplülü (2017), Sawarkar (2024a) and Yilmaz and Küfrevioğlu (2001). The variation in diastase activity in honey samples may be influenced by prolonged honey storage and the duration of heating (Tosi *et al.*, 2008; Subramanian *et al.*, 2007).

3.2 Correlation between the Physicochemical Properties of Honey

Spearman's correlation coefficients for various honey parameters were found in this study, as shown in Table 2. In this study, a positive and highly significant correlation ($p<0.05$) was found between moisture, pH, free acidity, EC, ash, protein, proline, and diastase, indicating a moderate association among them. A negative correlation was observed with HMF. The differences in the correlation between honey parameters might be attributed to the impact of variations in the foraging preference of bee species, floral sources, geographical origins, soil composition, honey harvesting process, and storage time. Earlier findings also stated that these factors are interrelated with each other and their correlation may depend on the floral and geographical origin, soil composition, mineral contents, ripeness and storage of honey (Boussaid *et al*., 2018; Joshi *et al*., 2000; Kharkamni, 2021; Nayik and Nanda, 2015; Lim *et al*., 2022; Thakur *et al*., 2023).

3.3 Principal Component Analysis

Principal component analysis (PCA) was used to analyze and identify similarities between 42 honey samples collected from different sites in the Khandesh region, North Maharashtra, India. The first three components were considered with eigenvalues greater than 1, which explained 73.33% of the data variation in the honey samples analyzed (Tables 3, 4). The first, second, and third principal components (PC1, PC2, PC3) explained 45.41%,

Table 1. Physicochemical parameters of honey sampels from Khandesh region

Physicochemical paramenters	Agriculture		Forest		Urban	
	A. c. indica	***A. dorsata***	***A. c. indica***	***A. dorsata***	***A. c. indica***	***A. dorsata***
Moisture (g/100g honey)	18.7±3.89	19.9±2.98	19.5±3.73	21.3±2.92	18.3±3.60	19.1±2.67
pH	3.96±0.31	4.21±0.31	4.18±0.34	4.43±0.29	3.74±0.24	4.03±0.22
Electric conductivity (mS/cm)	0.64±0.08	0.71±0.05	0.71±0.05	0.86±0.06	0.61±0.05	0.66±0.07
Ash content (g/100 g)	0.31±0.05	0.39±0.05	0.36±0.05	0.43±0.04	0.22±0.03	0.37±0.05
Free acidity (meq/kg)	30.42±2.25	33.87±2.63	33.21±2.18	36.77±2.08	30.02±2.40	32.42±2.65
Protein (g/kg)	1.91±0.19	2.38±0.25	2.29±0.24	2.62±0.25	1.64±0.10	2.19±0.24
HMF (mg/kg)	24.77±0.75	26.29±1.04	22.09±1.59	23.37±1.54	28.34±3.87	31.61±1.39
Proline (mg/kg)	337.78±32.70	719.73±35.03	403.43±31.04	798.39±33.15	264.19±26.15	620.32±48.69
Diastase (DN)	11.93±0.60	18.10±0.66	13.23±1.02	19.69±1.14	10.71±0.69	16.23±0.95

Table 2. Correlation matrix showing the interrelation among physicochemical parameters (Pearson correlation, p< 0.05)

Variables	Moisture	pH	EC	Ash	Free Acidity	Protein	HMF	Proline	Diastase
Moisture	**1**								
pH	**0.640**	**1**							
EC	**0.603**	**0.588**	**1**						
Ash	**0.440**	**0.450**	**0.545**	**1**					
Free Acidity	**0.341**	**0.349**	**0.488**	**0.472**	**1**				
Protein	**0.427**	**0.558**	**0.604**	**0.613**	**0.611**	**1**			
HMF	-0.271	-0.223	**-0.344**	-0.247	-0.299	-0.250	**1**		
Proline	**0.487**	**0.527**	**0.622**	**0.725**	**0.641**	**0.687**	-0.006	**1**	
Diastase	**0.487**	**0.535**	**0.688**	**0.708**	**0.642**	**0.713**	-0.052	**0.959**	**1**

Notes: *EC: Electric Conductivity; HMF: Hydroxymethylfurfural*

16.85%, and 11.07% of the variance, respectively (Fig. 2). Based on PCA, the first component explained 45.41% of the variance, except HMF was mostly dominated by moisture, pH, EC, ash, free acidity, protein, proline, and diastase.

Fig. 2 shows a clear separation of honey samples from three different sources as agriculture (Ag), forest (Fo), and urban (Ur) in the Khandesh Region. The honey samples from Fo and Ag were located in the upper quarter and had the positive values, indicating high geographic similarity between these two sources. The honey samples from Ur showed negative PC1 values for free acidity, diastase, and proline.

The loading plot of PC1 and PC2, specifies the relationship between the variables and the PC (Fig. 3). All variables except HMF played a significant role in determining PC1 in this study.

Table 3. Principal component analysis (PCA)

	PC1	PC2	PC3
Initial Eigenvalue	5.449	2.022	1.328
(%) of Variance	45.411	16.850	11.066
Cumulative%	45.411	62.260	73.326

(Eignvalues, explained and cumulative variance for the first three components.)

Table 4. Principal component analysis. Loading of the first three components

Factor loading	Principal components		
	1	2	3
Moisture	0.651	0.354	-0.028
PH	0.714	0.185	0.174
EC	0.796	0.270	0.081
Ash	0.776	0.118	0.121
Free Acidity	0.720	-0.054	-0.413
Protein	0.813	0.117	-0.003
HMF	-0.253	-0.624	0.565
Proline	0.913	-0.254	0.073
Diastase	0.925	-0.211	0.074

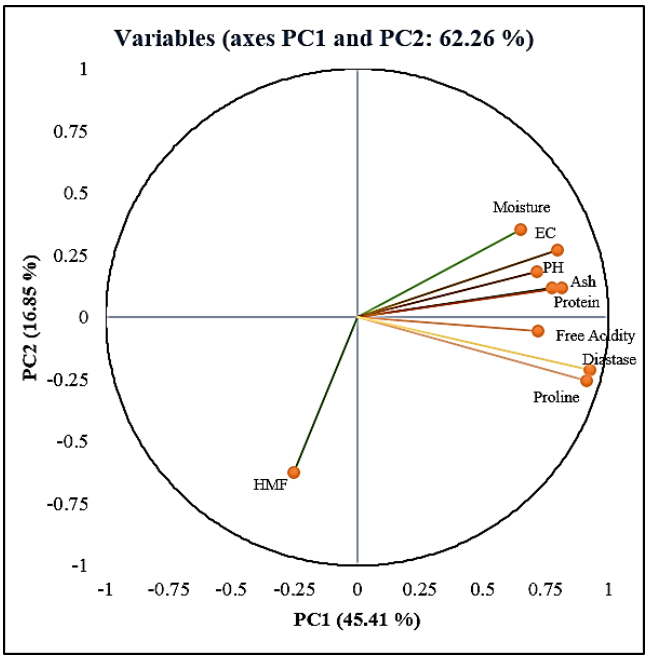

Fig. 2. Principal component analysis. Distribution of honey samples on scores plot

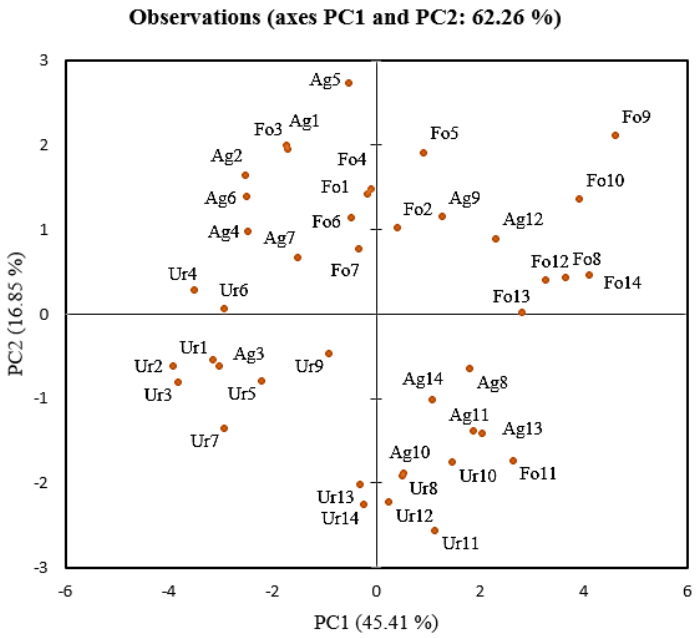

Fig. 3. Principal component analysis. Distribution of honey samples of different origin on scores plot. (Geographical: Ag: Agriculture, Fo: Forest, Ur: Urban)

4. CONCLUSION

This study is the first to present the physicochemical characteristics of honey from *Apis cerana indica* and *Apis dorsata* collected from three distinct locations in the Khandesh region: agriculture, forest, and urban. The physicochemical properties of *A. dorsata* honey were found to be significantly greater than those of *A. c. indica* honey. Furthermore, honey collected from forest sites exhibited superior quality compared to samples from agricultural and urban sites, suggesting that both the bee species and the geographical origin play key roles in determining honey quality.

A highly significant correlation was found among the physicochemical parameters, demonstrating a moderate association. Principal component analysis revealed a strong separation between the measured parameters, with three components accounting for up to 73.33% of the total explained variation. It also indicated a higher geographic similarity between the forest and agricultural sources compared to the urban sources. The observed variations in the physicochemical properties of honey samples from these sites are primarily influenced by geographical origin, floral sources, and the harvesting process. Additionally, differences in physicochemical properties between *A. dorsata* and *A. c. indica* honey may be attributed to their distinct nesting and foraging behaviors. All honey sourced from the Khandesh region met international quality standards, and they represent an excellent option for consumers seeking high-quality honey.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author is hereby declared that no any propagative AI-technology as Large Language Models and Text-to-image generators have been used throughout preparing and editing this article.

DECLARATION

The material for research i.e. honey was collected directly from beekeepers and tribes for further analysis in the laboratory. This is an observational study. The Institutional Research Ethics Committee, BP Arts, SMA Science & KKC Commerce College, Chalisgaon Dist- Jalgaon (India) has confirmed that no ethical approval is required.

ACKNOWLEDGEMENT

The author gratefully acknowledge the financial support provided by North Maharashtra University, Jalgaon, Maharashtra, India, through

the Vice-Chancellor Research Motivation Scheme (VCRMS) under the grant number KBCNMU/11A/VCRMS/Budget 21-22/Sci. & Tech./159/22, dated May 17, 2022.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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