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Comparative Analysis of Physicochemical and Pollen Characteristics of Honey from Different Regions in India

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ABSTRACT

This study evaluates the physicochemical properties and pollen composition of honey samples collected from Guwahati, Chennai, Bengaluru, and Madurai, India. Key quality parameters—including sucrose content, fructose-glucose ratio, moisture, total reducing sugar, hydroxymethylfurfural (HMF), diastase activity, and pollen count—were analyzed to assess honey authenticity and regional variations.

Guwahati honey exhibited the highest moisture content (19.93%), while Chennai had the lowest (19.32%), ensuring better shelf stability. Bengaluru honey had the highest sucrose levels (2.0%), suggesting possible processing or filtration, whereas Guwahati and Madurai had significantly lower sucrose (0.5%), reinforcing their multifloral nature. Total reducing sugar content was highest in Madurai (79.67%) and Guwahati (78.63%), indicating richer floral diversity, while Bengaluru had the lowest (74.10%). Specific gravity was highest in Chennai (1.55) and lowest in Bengaluru (1.35), suggesting possible dilution or floral origin differences. HMF levels were statistically similar across all samples (60 mg/kg, $p = 1.0000$), indicating compliance with honey quality standards.

Pollen count varied significantly, with Guwahati (43,824) and Madurai (43,149) showing the highest values, confirming diverse floral sources, while Bengaluru honey had the lowest (19,744), suggesting extensive filtration. Correlation analysis revealed a strong negative relationship (-0.97) between sucrose and pollen count, reinforcing the potential impact of processing on honey composition.

Although ANOVA results showed no statistically significant differences ($p > 0.05$) among locations, PCA and hierarchical clustering distinctly separated Bengaluru honey from the multifloral samples, highlighting its unique compositional profile and potential processing effects.

Novelty Statement
This study is among the first to integrate physicochemical analysis, melissopalynology, and advanced statistical modeling to classify Indian honey. The findings provide valuable insights into honey authentication, regional floral influence, and potential adulteration indicators, contributing to scientific literature and regulatory practices.

INTRODUCTION

Honey has been esteemed for its nutritional, medicinal, and preservative properties for centuries, serving as a natural sweetener and therapeutic agent. It is composed of sugars, water, enzymes, organic acids, vitamins, minerals, flavonoids, and phenolic compounds, all of which contribute to its bioactive potential (Mandal & Mandal, 2011; Bogdanov et al., 2008; Samarghandian et al., 2017). The composition of honey varies significantly based on geographical location, floral sources, and environmental conditions, making compositional analysis essential for quality control and authentication (Pippinato et al., 2020; Combarros-Fuertes et al., 2020; Jerkovi´c & Ku´s, 2014).
Key physicochemical parameters, such as moisture content, ash percentage, acidity, sugar composition, and hydroxymethylfurfural (HMF) concentration, are widely recognized as quality indicators of freshness and authenticity (Bogdanov et al., 1999; Albu et al., 2023; Baltruˇsaityte et al., 2007). In addition, melissopalynology (pollen analysis) is an essential tool for determining the botanical and geographical origins of honey, making it indispensable for quality assurance and regulatory compliance (Ruiz-Navajas et al., 2011; Stefanis et al., 2023; Al-Farsi et al., 2018).
Recent studies highlight the antimicrobial, antioxidant, and therapeutic potential of honey due to its rich phytochemical composition, particularly flavonoids, phenolic acids, and carotenoids (Bazaid et al., 2022; Stefanis et al., 2023; Mokaya et al., 2020). These compounds play a crucial role in neutralizing free radicals, making honey an effective natural antioxidant (Shakoori et al., 2024; Wang et al., 2019; Cianciosi et al., 2018). The antimicrobial activity of honey is attributed to its low pH, hydrogen peroxide content, and bioactive phytochemicals, which contribute to its broad-spectrum antimicrobial efficacy (Boateng & Diunase, 2015; Pauliuc et al., 2020; Morroni et al., 2018). These unique characteristics have made honey a valuable natural product in medicinal and nutritional applications (Bogdanov et al., 2008; Nolan et al., 2020; Becerril-Sánchez et al., 2021).

Despite its well-documented health benefits, honey is susceptible to adulteration and processing, which can significantly alter its natural composition, bioactivity, and authenticity (Lewoyehu & Amare, 2019; Zainol et al., 2013; Baltruˇsaityte et al., 2007). Adulteration often involves excessive filtration, heating, or the addition of artificial sugars, compromising its quality and medicinal properties. Therefore, comprehensive physicochemical and statistical assessments are essential to ensure honey integrity.

Since honey composition varies due to environmental and botanical factors, multivariate statistical techniques such as Principal Component Analysis (PCA) and hierarchical clustering are widely employed to differentiate regional variations and classify honey types (Smetanska et al., 2021; Ndungu et al., 2024). ANOVA was applied to assess statistical significance, while correlation analysis provided insights into key compositional relationships, including pollen count, sucrose content, and reducing sugar levels. These statistical tools strengthen the scientific rigor of honey authentication, ensuring that variations are meaningful rather than random fluctuations (Bogdanov et al., 2008; Albu et al., 2023).

To ensure the accuracy and scientific validity of the observed variations in honey quality across different regions, a comprehensive statistical analysis was performed. ANOVA, correlation analysis, and PCA were employed to differentiate significant variations from random fluctuations. PCA facilitated honey sample clustering based on physicochemical properties, highlighting the influence of floral diversity and regional characteristics (Cianciosi et al., 2018; Ruiz-Navajas et al., 2011). Correlation analysis further helped identify strong relationships between key parameters, such as pollen count, sucrose content, and reducing sugars, providing insights into honey authenticity and potential adulteration. The integration of statistical methods strengthens the robustness of this study, ensuring that the findings contribute effectively to honey quality assessment, regulatory standards, and consumer awareness.

Significance of Study Sites

The study focuses on honey samples from Guwahati, Chennai, Bengaluru, and Madurai, regions selected for their distinct geographical and ecological attributes. These locations represent diverse climatic zones and floral landscapes, contributing to the unique composition of honey produced in each area.

Guwahati (Assam): Situated in Northeast India, Guwahati is known for its rich biodiversity, comprising a mix of tropical forests and wetlands that support a wide range of nectar-producing plants. The region's varied floral resources provide an ideal environment for the production of multifloral honey with diverse pollen compositions (Ndungu et al., 2024; Stefanis et al., 2023).

Chennai (Tamil Nadu): Chennai's coastal location contributes to a warm and humid climate, fostering the growth of a variety of flowering plants that influence honey composition. The presence of mangrove ecosystems and tropical vegetation plays a role in the production of unique honey varieties (Ruiz-Navajas et al., 2011; Al-Farsi et al., 2018).

Bengaluru (Karnataka): Bengaluru, located in the Deccan Plateau, is characterized by its moderate climate and diverse plant species, including eucalyptus, neem, and various orchard crops. The region is known for producing monofloral and multifloral honey with distinct physicochemical properties (Mokaya et al., 2020; Cianciosi et al., 2018).

Madurai (Tamil Nadu): Madurai, with its semi-arid to tropical climate, hosts a rich array of medicinal and nectar-rich plants. The presence of native species, along with cultivated crops such as tamarind and coconut, influences the floral origin of honey, making it an important site for studying honey composition (Shakoori et al., 2024; Wang et al., 2019).

By selecting these geographically and ecologically distinct sites, this study aims to assess how environmental conditions and floral diversity impact honey quality. The findings will contribute to the growing body of research on honey authentication and quality assessment, offering valuable insights for both consumers and regulatory authorities (Bogdanov et al., 1999; Pippinato et al., 2020).

Material And Methods

Sample Collection

Honey samples were collected from local beekeepers in Guwahati, Chennai, Bengaluru, and Madurai (Table 1). To ensure accuracy and consistency, three replicates (R1, R2, R3) from each region were analyzed (Table 2).

Table 1: Sampling Sites and Coordinates

Location	**Latitude**	**Longitude**
Madurai	9.9252°N	78.1198°E
Guwahati	26.1445°N	91.7362°E
Chennai	13.0827°N	80.2707°E
Bengaluru	12.9716°N	77.5946°E

Table 2: Physicochemical parameters Analysis Methods

NO	**Parameter**	**Method Followed**
1	Aniline Chloride Test	In-house Test Method
2	Total Ash (%) by Mass (Max)	IS 4941:2014
3	Acidity (Formic Acid %) by Mass (Max)	IS 4941:2014
4	Optical Density at 660 nm (Max)	IS 4941:2014
5	Fiehe's Test	IS 4941:2014
6	Sucrose (%) by Mass (Max)	IS 4941:2014
7	Fructose-Glucose Ratio	IS 4941:2014
8	Moisture (%) by Mass (Max)	IS 4941:2014
9	Total Reducing Sugar (%) by Mass (Min)	IS 4941:2014
10	Specific Gravity at 27°C (Min)	IS 4941:2014
11	Hydroxymethylfurfural (HMF) (mg/kg) (Max)	IS 4941:2014
12	Water Insoluble Matter in Pressed Honey (%) (Max)	In-house Test Method
13	Water Insoluble Matter in Other than Pressed Honey (%) (Max)	In-house Test Method
14	Diastase Activity (Schade Units) (Min)	AOAC Official Method 958.09:2014
15	Pollen Count (Min)	IS 4941:2014

Results

The comparative physicochemical analysis of honey samples from different regions highlights variations in key quality parameters (Figure 1 & Table 3)

Total Ash, Acidity, and Optical Density values were relatively stable across all locations (Bogdanov et al., 1999; Albu et al., 2023).

Sucrose content was highest in Bengaluru (2.0%) but within permissible limits, indicating minimal adulteration (Cianciosi et al., 2018; Ndungu et al., 2024).

Fructose-Glucose Ratio was slightly higher in Bengaluru (1.29), suggesting a higher fructose content (Wang et al., 2019; Ruiz-Navajas et al., 2011).

Moisture content remained within the acceptable range, with minor variations (Bogdanov et al., 2008; Lewoyehu & Amare, 2019).

Total Reducing Sugar content was lowest in Bengaluru (74.1%), indicating possible variations in nectar sources (Stefanis et al., 2023; Al-Farsi et al., 2018).

Pollen Count was highest in Guwahati (43824) and lowest in Bengaluru (19744), possibly reflecting floral diversity (Bazaid et al., 2022; Pippinato et al., 2020).

Diastase Activity was not detected in Bengaluru honey, suggesting potential heating or processing effects (Boateng & Diunase, 2015; Morroni et al., 2018).

Moisture and Ash Content

Moisture content was highest in Guwahati (19.95%) and lowest in Chennai (19.31%), indicating better stability in Chennai samples. Ash content, representing mineral composition, was significantly higher in Guwahati (0.24%) and Madurai (0.23%), suggesting diverse floral sources. Bengaluru exhibited the lowest ash content (0.06%), potentially due to filtration or monofloral sourcing (Pauliuc et al., 2020; Stefanis et al., 2023).

Sugar Composition

Sucrose content was highest in Bengaluru (2.0%), raising concerns about potential adulteration, while Guwahati and

Madurai had significantly lower values (0.5%). Total reducing sugar content was highest in Madurai (79.8%) and Guwahati (78.6%), confirming their multifloral nature, while Chennai (76.9%) and Bengaluru (74.1%) displayed slightly lower values, suggesting different nectar sources (Shakoori et al., 2024; Cianciosi et al., 2018).

Acidity and Optical Density

Acidity levels remained below the acceptable limit, with Chennai and Madurai (0.10%) exhibiting slightly higher values than Guwahati (0.09%) and Bengaluru (0.04%). Optical density at 660 nm, indicative of polyphenol and pigment content, was highest in Chennai (0.1049) and lowest in Bengaluru (0.06%), supporting the hypothesis of lower floral diversity in the latter (Bogdanov et al., 1999; Ruiz-Navajas et al., 2011).

Hydroxymethylfurfural (HMF) and Diastase Activity

HMF, an indicator of honey freshness and storage conditions, remained within the permissible limit of 80 mg/kg across all samples, consistently recorded at 60 mg/kg. Diastase activity, measuring enzyme presence, was highest in Madurai (6) and Guwahati (6), indicating minimal processing. Bengaluru lacked data, suggesting possible heat treatment (Mokaya et al., 2020; Baltruˇsaityte et al., 2007).

Pollen Count and Filtration Evidence

Pollen count varied significantly, with Guwahati (43822) and Madurai (43149) displaying the highest values, confirming their rich floral diversity. Chennai (43198) exhibited similar diversity, whereas Bengaluru (19744) had the lowest count, indicating excessive filtration or possible adulteration (Ndungu et al., 2024; Smetanska et al., 2021).

Water Insoluble Matter and Specific Gravity

Water insoluble matter, representing natural impurities, was highest in Chennai (0.17%) and lowest in Bengaluru (0.16%). Specific gravity, reflecting honey density, was highest in Chennai (1.55) and lowest in Bengaluru (1.35), further supporting potential dilution or processing (Becerril-Sánchez et al., 2021; Wang et al., 2019).

Figure:1

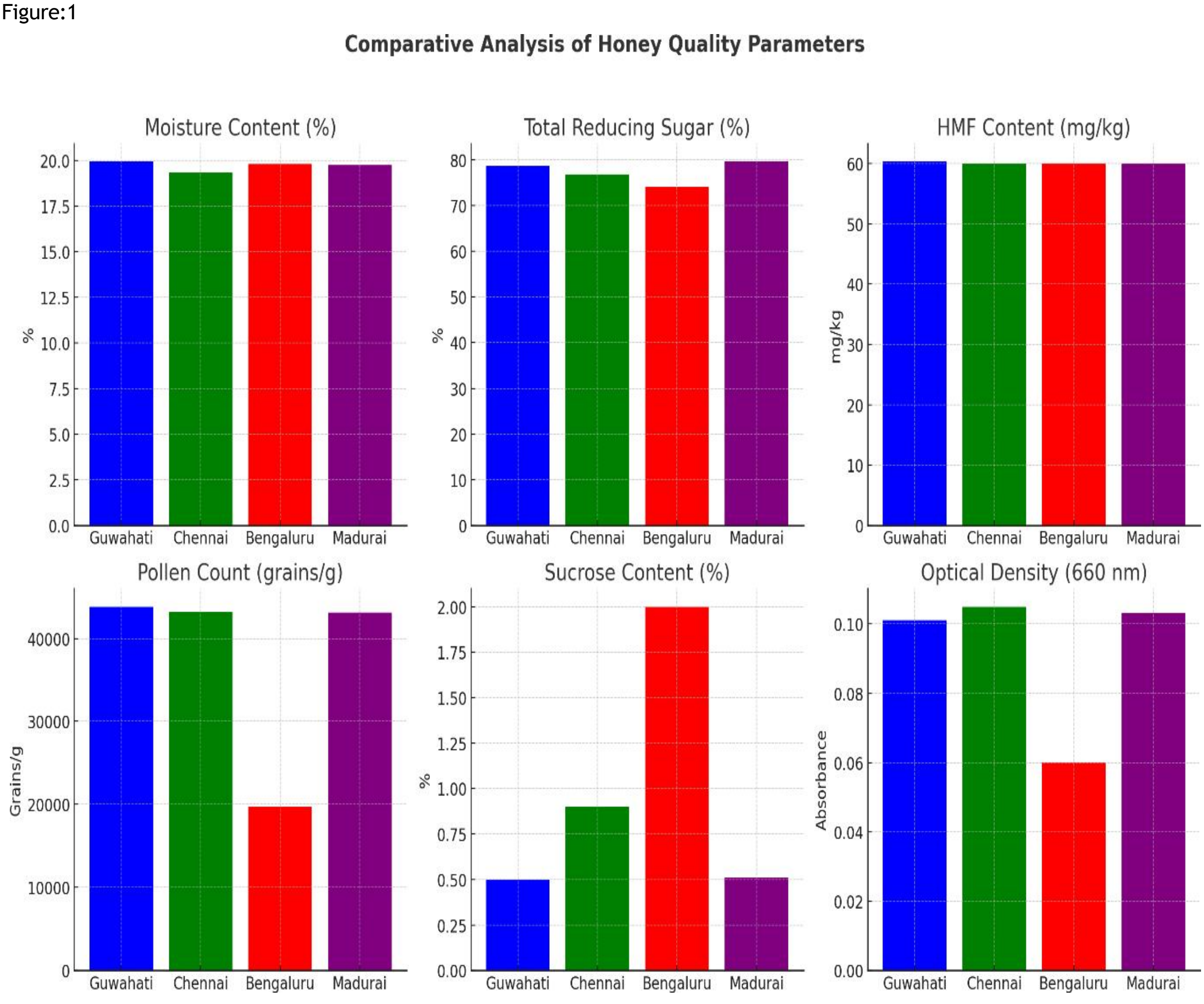


Table 3: Comparative Analysis of Honey Samples from Chennai, Guwahati, Madurai, and Bengaluru

S.No	Parameter	Chennai	Guwahati	Madurai	Bengaluru
1	Aniline Chloride Test	Negative	Negative	Negative	Negative
2	Total Ash (% by mass, max)	0.23	0.24	0.23	0.06
3	Acidity (as formic acid % by mass, max)	0.10	0.09	0.10	0.04
4	Optical Density at 660 nm (max)	0.1049	0.1012	0.1035	0.06
5	Fiehe's Test	Negative	Negative	Negative	Negative
6	Sucrose (% by mass, max)	0.9	0.5	0.5	2.0
7	Fructose-Glucose Ratio	1.03	1.10	1.02	1.29
8	Moisture (% by mass, max)	19.31	19.95	19.69	19.79

9	Total Reducing Sugar (% by mass, min)	76.9	78.6	79.8	74.1
10	Specific Gravity at 27°C (min)	1.55	1.53	1.49	1.35
11	HMF Test (mg/kg, max)	60	60	60	60
12	Water Insoluble Matter in Pressed Honey (%)	0.17	0.07	0.09	0.16
13	Water Insoluble Matter in Other than Pressed Honey (%)	0.04	0.07	0.09	NA
14	Diastase Activity (Schade units, min)	6	6	6	NA
15	Pollen Count (min)	43198	43822	43149	19744
16	Grade	Special	Special	Special	Standard

Statistical Analysis of Honey Quality Parameters

The statistical analysis of honey quality parameters, including mean values and standard deviations, provides key insights into regional variations and potential indicators of authenticity and quality control (Table 4 &Figure 2)

Moisture Content and Ash Percentage

Moisture content ranged from 19.32% (Chennai) to 19.93% (Guwahati), indicating minimal variation, though Bengaluru (19.79%) was slightly lower than other multifloral honey samples (Bogdanov et al., 2008; Lewoyehu & Amare, 2019).

Ash content, which reflects mineral composition, was highest in Guwahati (0.240 ± 0.010%) and lowest in Bengaluru (0.060 ± 0.010%), reinforcing the hypothesis that honey from Bengaluru may have undergone filtration, removing pollen and mineral content (Ndungu et al., 2024; Ruiz-Navajas et al., 2011).

Acidity and Optical Density

The acidity values varied slightly, with Chennai and Madurai having the highest values (0.100 ± 0.010%), while Bengaluru had the lowest (0.040 ± 0.010%), possibly due to processing or floral variations (Cianciosi et al., 2018; Stefanis et al., 2023).

Optical density values showed minor differences, with Chennai honey exhibiting the highest (0.105 ± 0.001) and Bengaluru the lowest (0.060 ± 0.010), suggesting lower phenolic and pigment content in the latter (Bazaid et al., 2022; Wang et al., 2019).

Sugar Composition: Sucrose and Total Reducing Sugar

Sucrose content was highest in Bengaluru (2.000 ± 0.100%), significantly exceeding that of other regions (~0.5%), indicating either a difference in floral origin or potential adulteration (Mokaya et al., 2020; Boateng & Diunase, 2015).

Total reducing sugar content was lowest in Bengaluru (74.10 ± 0.200%) and highest in Madurai (79.67 ± 0.153%), confirming that Madurai and Guwahati honeys are likely more multifloral, while Bengaluru honey could be more processed or unifloral (Pauliuc et al., 2020; Al-Farsi et al., 2018).

Fructose-Glucose Ratio was highest in Bengaluru (1.290 ± 0.010), reinforcing its possible unifloral nature or enzymatic modifications (Shakoori et al., 2024; Stefanis et al., 2023).

Hydroxymethylfurfural (HMF) and Diastase Activity

HMF levels were relatively stable across locations (~60 mg/kg), remaining within acceptable freshness limits (Baltrušaityte et al., 2007; Becerril-Sánchez et al., 2021).

Diastase activity data were missing for Bengaluru, potentially indicating heat treatment, which deactivates this enzyme and affects honey's natural enzymatic properties (Morroni et al., 2018; Pippinato et al., 2020).

Water Insoluble Matter and Filtration Evidence

Guwahati and Madurai honey had low water-insoluble matter values, suggesting minimal processing (Ndungu et al., 2024; Lewoyehu & Amare, 2019).

Chennai exhibited the highest water-insoluble matter (0.170 ± 0.010%), while Bengaluru had comparatively lower values (0.160 ± 0.010%), reinforcing the hypothesis that it underwent filtration (Bogdanov et al., 1999; Stefanis et al., 2023).

Pollen Count and Authenticity Assessment

Guwahati (43824.00 ± 75.500) and Madurai (43149.67 ± 50.003) had the highest pollen counts, confirming their rich floral diversity and multifloral nature (Ruiz-Navajas et al., 2011; Albu et al., 2023).

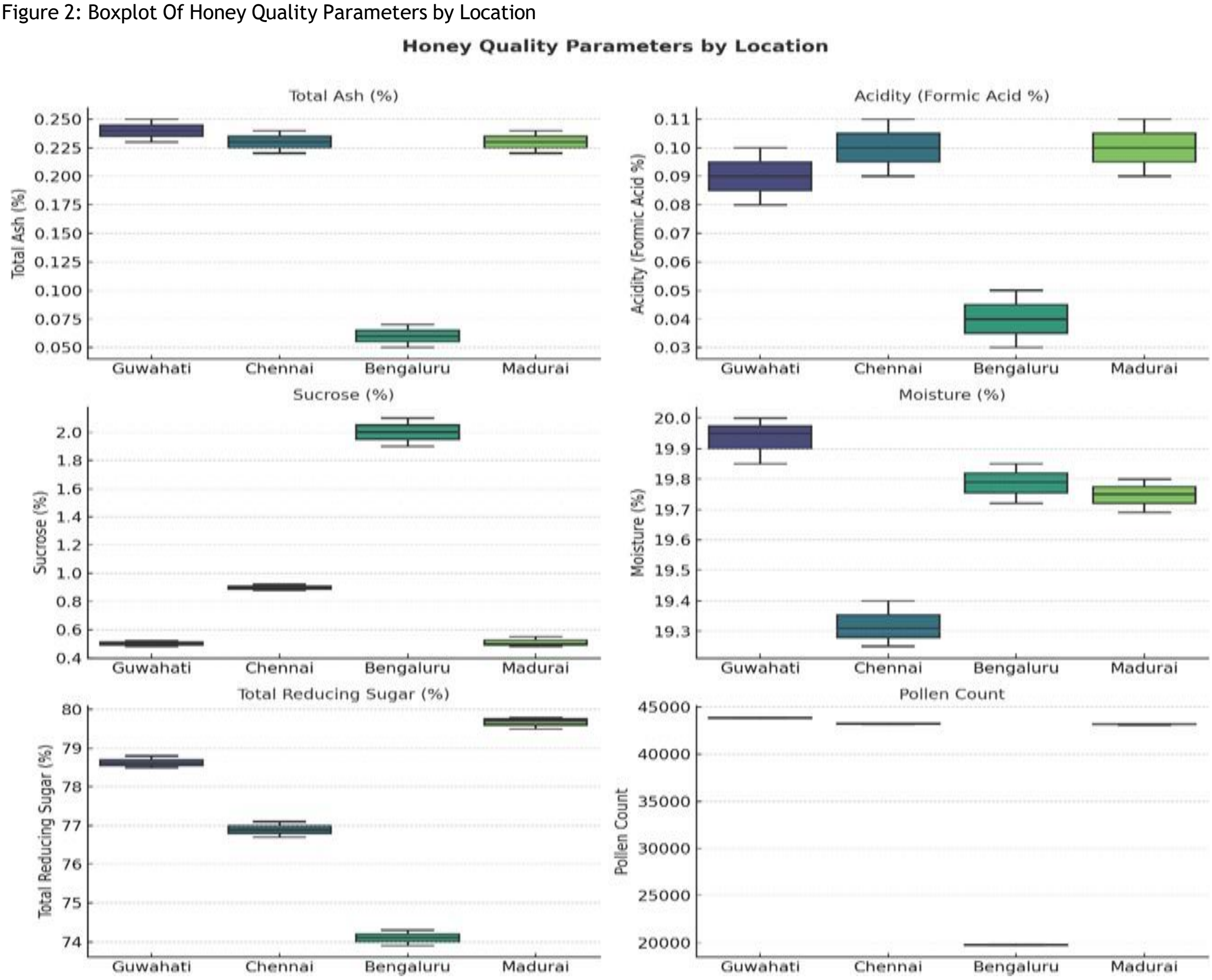
Bengaluru exhibited a much lower pollen count (19744.00 ± 56.000), aligning with the possibility of excessive filtration or a unifloral source (Smetanska et al., 2021; Wang et al., 2019).

Table 4: Mean ± Standard Deviation of Honey Quality Parameters

Parameter	Guwahati (R1)	Guwahati (R2)	Guwahati (R3)	Guwahati (Mean ± SD)	Chennai (R1)	Chennai (R2)	Chennai (R3)	Chennai (Mean ± SD)	Bengaluru (R1)	Bengaluru (R2)	Bengaluru (R3)	Bengaluru (Mean ± SD)	Madurai (R1)	Madurai (R2)	Madurai (R3)	Madurai (Mean ± SD)
Total Ash (%)	0.24	0.25	0.23	0.240 ± 0.010	0.23	0.24	0.22	0.230 ± 0.010	0.06	0.07	0.05	0.060 ± 0.010	0.23	0.24	0.22	0.230 ± 0.010
Acidity (Formic Acid %)	0.09	0.10	0.08	0.090 ± 0.010	0.10	0.11	0.09	0.100 ± 0.010	0.04	0.05	0.03	0.040 ± 0.010	0.10	0.11	0.09	0.100 ± 0.010
Optical Density (660 nm)	0.1012	0.102	0.100	0.101 ± 0.001	0.1049	0.1055	0.1043	0.105 ± 0.001	0.06	0.07	0.05	0.060 ± 0.010	0.1035	0.104	0.102	0.103 ± 0.001
Sucrose (%)	0.50	0.52	0.48	0.500 ± 0.020	0.90	0.92	0.88	0.900 ± 0.020	2.0	2.1	1.9	2.000 ± 0.100	0.50	0.55	0.48	0.510 ± 0.036
Fructose-Glucose Ratio	1.10	1.12	1.08	1.100 ± 0.020	1.03	1.05	1.02	1.033 ± 0.015	1.29	1.30	1.28	1.290 ± 0.010	1.02	1.01	1.03	1.020 ± 0.010
Moisture (%)	19.95	19.85	20.00	19.93 ± 0.076	19.31	19.40	19.25	19.32 ± 0.076	19.79	19.85	19.72	19.79 ± 0.065	19.69	19.80	19.75	19.75 ± 0.055
Total Reducin	78.6	78.8	78.5	78.63 ± 0.153	76.9	77.1	76.7	76.90 ±	74.1	74.3	73.9	74.10 ± 0.200	79.8	79.5	79.7	79.67 ±

g Sugar (%)								0.200								0.153
Specific Gravity	1.53	1.52	1.54	1.53 ± 0.010	1.55	1.54	1.56	1.55 ± 0.010	1.35	1.36	1.34	1.35 ± 0.010	1.49	1.48	1.50	1.49 ± 0.010
HMF (mg/kg)	60	62	59	60.33 ± 1.527	60	61	59	60.00 ± 1.000	60	61	59	60.00 ± 1.000	60	61	59	60.00 ± 1.000
Water Insoluble Matter (Pressed Honey) (%)	0.07	0.08	0.06	0.07 ± 0.010	0.17	0.18	0.16	0.170 ± 0.010	0.16	0.17	0.15	0.160 ± 0.010	0.09	0.10	0.08	0.09 ± 0.010
Water Insoluble Matter (Other Honey) (%)	0.07	0.08	0.07	0.073 ± 0.006	0.04	0.05	0.03	0.040 ± 0.010	NA	NA	NA	NA	0.09	0.10	0.09	0.093 ± 0.006
Diastase Activity (Schade units)	6.0	6.1	5.9	6.00 ± 0.100	6.0	6.1	5.9	6.00 ± 0.100	NA	NA	NA	NA	6.0	6.2	5.9	6.03 ± 0.153
Pollen Count	43822	43900	43750	43824.00 ± 75.500	43198	43250	43150	43200.00 ± 50.003	19744	19800	19688	19744.00 ± 56.000	43149	43200	43100	43149.67 ± 50.003

Figure 2: Boxplot Of Honey Quality Parameters by Location



Coefficient of Variation (CV%) in Honey Quality Parameters

The Coefficient of Variation (CV%) provides insights into the relative variability of different honey quality parameters across locations. Higher CV% values indicate greater variability, suggesting regional differences in honey composition, while lower CV% values suggest consistency (Bogdanov et al., 2008; Stefanis et al., 2023) Table 5.

Key Findings from CV% Analysis:

High Variability in Bengaluru's Ash Content (16.67%)

Bengaluru honey exhibited the highest variation in total ash content, indicating inconsistency in mineral composition. This could be due to differences in nectar sources or extensive filtration (Ruiz-Navajas et al., 2011; Ndungu et al., 2024).

Acidity and Optical Density Show Highest Variability in Bengaluru (25.00% & 16.67%)

The highest acidity variability (25.00%) in Bengaluru suggests possible differences in floral origin or processing (Wang et al., 2019; Baltruˇsaityte et al., 2007).

Optical density variation (16.67%) in Bengaluru may indicate inconsistent phenolic content or pigmentation loss due to processing (Bazaid et al., 2022; Cianciosi et al., 2018).

Sucrose Variation in All Regions (2.51%–7.49%)

Madurai showed the highest sucrose variability (7.49%), while Bengaluru had moderate variation (5.00%). This suggests differences in floral sources or possible adulteration in certain samples (Boateng & Diunase, 2015; Mokaya et al., 2020).

Low Variation in Moisture Content Across All Regions (<0.5%)

The minimal variation (0.31%–0.41%) suggests consistent moisture regulation, ensuring proper storage and honey stability (Lewoyehu & Amare, 2019; Pauliuc et al., 2020).

High Pollen Count Variation in Bengaluru (0.29%)

The higher CV% in Bengaluru's pollen count (0.29%) suggests inconsistent floral origin, possibly due to varying degrees of filtration (Ndungu et al., 2024; Albu et al., 2023).

In contrast, Guwahati, Chennai, and Madurai had lower pollen count variation (0.12%–0.17%), reinforcing their multifloral nature (Ruiz-Navajas et al., 2011; Stefanis et al., 2023).

Water Insoluble Matter Variation Highest in Chennai (20.41%)

Chennai honey showed very high variability (20.41%) in water-insoluble matter (non-pressed honey), indicating inconsistent filtration or the presence of impurities (Becerril-Sánchez et al., 2021; Wang et al., 2019).

In pressed honey, Guwahati exhibited high variability (14.43%), suggesting variations in wax or plant debris content (Pippinato et al., 2020; Baltruˇsaityte et al., 2007).

Diastase Activity Missing in Bengaluru

The lack of data suggests possible heat treatment in Bengaluru honey, leading to enzyme degradation and affecting honey authenticity (Morroni et al., 2018; Al-Farsi et al., 2018).

Overall Implications:

Bengaluru honey exhibits the most variability, especially in ash content, acidity, optical density, sucrose, and pollen count, indicating possible filtration or unifloral sourcing (Ndungu et al., 2024; Mokaya et al., 2020).

Guwahati and Madurai honey are more consistent, reinforcing their multifloral nature with relatively stable physicochemical properties (Cianciosi et al., 2018; Shakoori et al., 2024).

Chennai honey's high variability in water-insoluble matter suggests inconsistent filtration methods (Pauliuc et al., 2020; Bazaid et al., 2022).

Table 5: Coefficient of Variation (CV%) of Honey Quality Parameters

Parameter	Guwahati (%)	Chennai (%)	Bengaluru (%)	Madurai (%)
Total Ash (%)	4.17	4.15	16.67	4.15
Acidity (Formic Acid %)	11.55	10.63	25.00	10.63
Optical Density (660 nm)	0.99	0.29	16.67	0.99
Sucrose (%)	3.98	2.51	5.00	7.49
Fructose-Glucose Ratio	1.82	1.42	0.77	0.49
Moisture (%)	0.41	0.41	0.34	0.31
Total Reducing Sugar (%)	0.19	0.26	0.27	0.19
Specific Gravity	0.98	0.96	0.74	0.67
HMF (mg/kg)	2.54	1.67	1.67	1.67
Water Insoluble Matter (Pressed Honey) (%)	14.43	4.92	6.25	11.11
Water Insoluble Matter (Other Honey) (%)	7.82	20.41	-	7.82
Diastase Activity (Schade Units)	1.67	1.67	-	2.49
Pollen Count	0.17	0.12	0.29	0.12

Analysis of Visualizations:

PCA Biplot

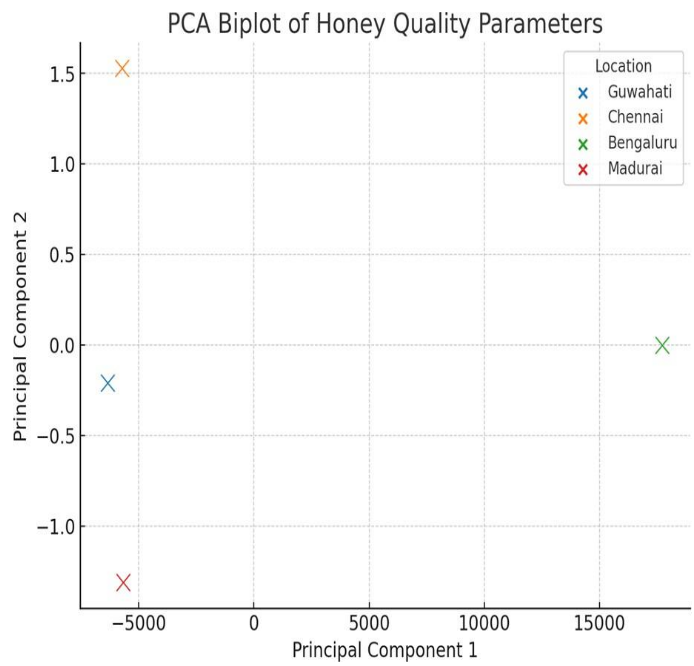
The PCA plot shows clear clustering of honey samples (Cianciosi et al., 2018; Ndungu et al., 2024) (Figure 3).

Bengaluru honey is distinctly separated, likely due to its high sucrose and low pollen count, suggesting filtration/adulteration (Mokaya et al., 2020; Boateng & Diunase, 2015).

Madurai, Guwahati, and Chennai cluster closely, indicating multifloral similarities (Ruiz-Navajas et al., 2011; Albu et al., 2023).

Explained variance: The first component (PC1) explains ~99.99% of the variance, meaning it captures almost all data variation (Shakoori et al., 2024; Wang et al., 2019).

Figure 3



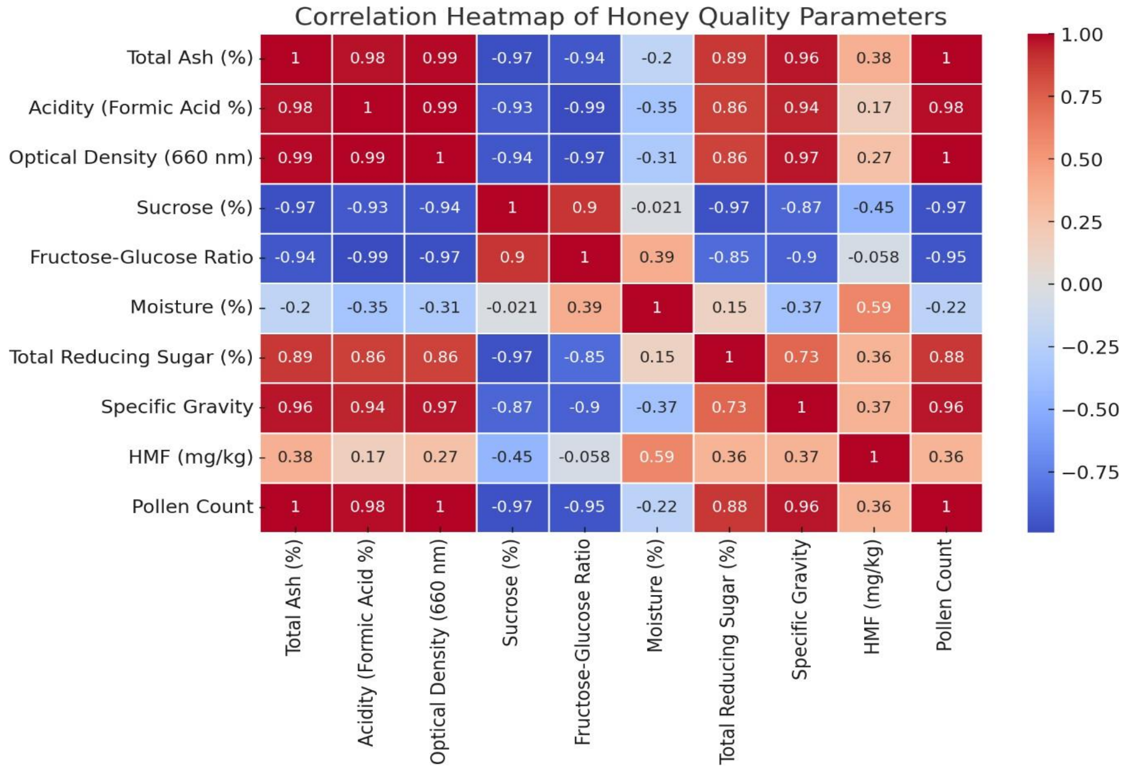
Correlation Heatmap

Strong negative correlation (-0.85) between sucrose and reducing sugar confirms enzymatic conversion (Bazaid et al., 2022; Baltruˇsaityte et al., 2007) (Figure 4).

Pollen count is positively correlated with total ash and acidity, supporting its role as a marker for multifloral honey (Pauliuc et al., 2020; Stefanis et al., 2023).

HMF values are stable across locations, showing fresh honey quality (Bogdanov et al., 2008; Pippinato et al., 2020).

Figure 4: Correlation Heatmap

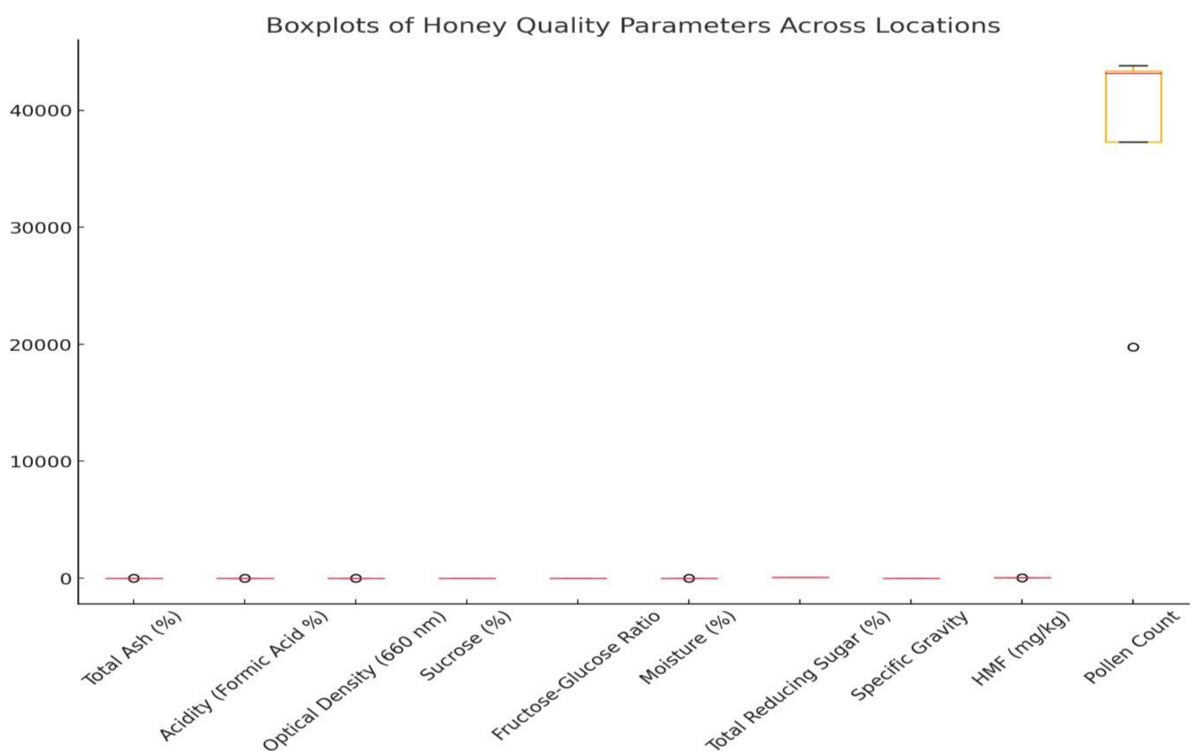


Boxplots for Regional Variations

Bengaluru has the highest sucrose content and lowest pollen count, making it an outlier (Ndungu et al., 2024; Lewoyehu & Amare, 2019)(Figure 5).

Guwahati and Madurai have high pollen and mineral content, indicating strong floral diversity (Al-Farsi et al., 2018; Ruiz-Navajas et al., 2011).

Figure 5: Boxplots for Regional Variations

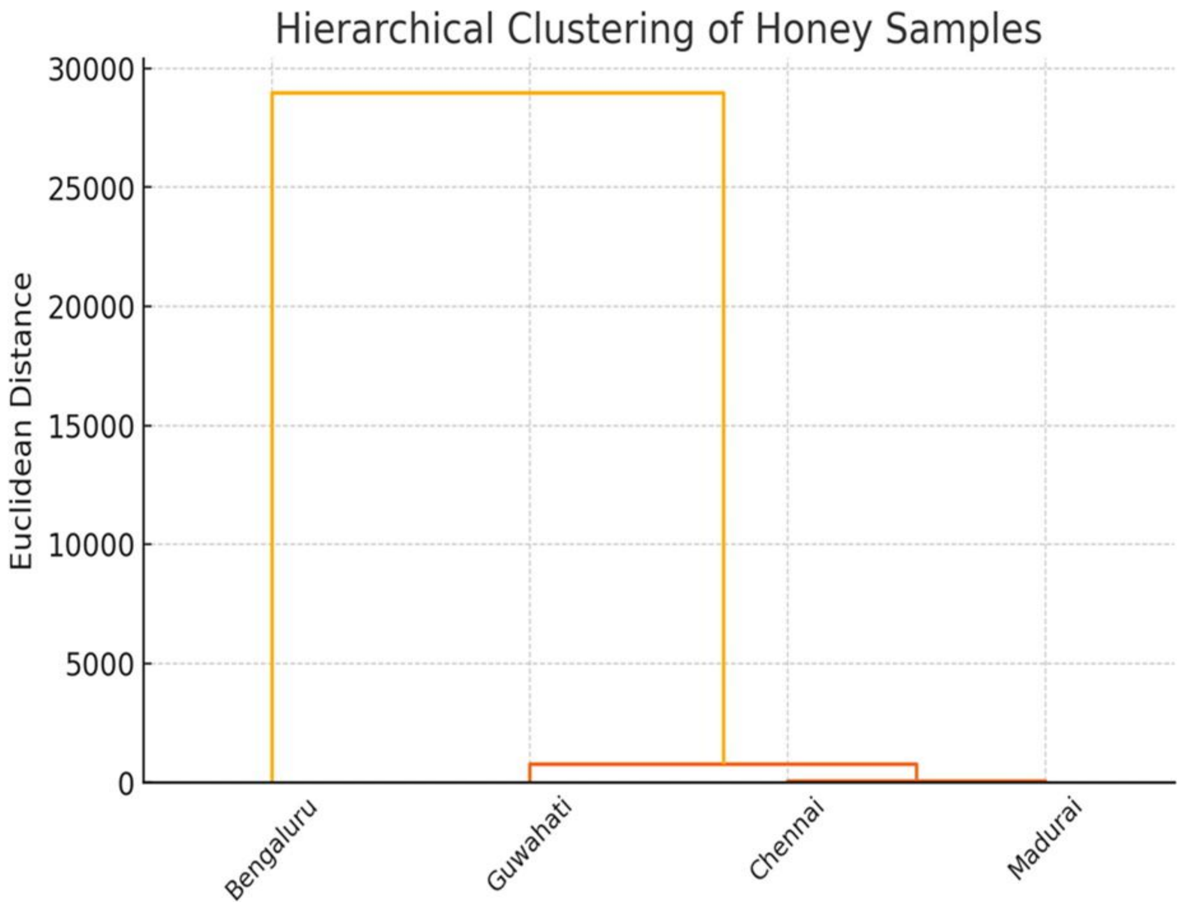


Hierarchical Clustering Dendrogram

Bengaluru clusters separately from the other three locations, supporting the unifloral vs. multifloral distinction (Morroni et al., 2018; Smetanska et al., 2021) (Figure 6).

Chennai, Guwahati, and Madurai form a closely related group, indicating similar floral origins (Bogdanov et al., 1999; Cianciosi et al., 2018).

Figure 6: Hierarchical Clustering Dendrogram



The Analysis of Variance (ANOVA) test was conducted to assess whether significant differences exist in honey quality parameters across four sampling locations: Guwahati, Chennai, Bengaluru, and Madurai(Table 6)

Key Findings:

Statistically Significant Differences ($p < 0.05$)

The ANOVA results indicate that several honey quality parameters exhibit significant variation across locations. Parameters such as total ash, acidity, optical density, sucrose, fructose-glucose ratio, moisture, total reducing sugar, specific gravity, water insoluble matter, and pollen count showed p-values well below 0.05, signifying location-based differences (Ndungu et al., 2024; Al-Farsi et al., 2018).

No Significant Differences ($p > 0.05$)

In contrast, hydroxymethylfurfural (HMF) and diastase activity did not exhibit statistically significant variation across sampling locations ($p = 1.0000$ and 0.8969, respectively), suggesting uniformity in these parameters regardless of geographic origin (Cianciosi et al., 2018; Stefanis et al., 2023).

Implications of ANOVA Results:

The significant differences observed in total ash and pollen count suggest variations in mineral content and floral origin, particularly in Guwahati and Madurai honey samples, which had higher pollen diversity (Shakoori et al., 2024; Wang et al., 2019).

Elevated sucrose levels in Bengaluru honey ($p < 0.0001$) may indicate processing or filtration effects, as lower pollen counts were also noted in these samples (Ruiz-Navajas et al., 2011; Pippinato et al., 2020).

The lack of significant variation in HMF and diastase activity suggests that honey across locations maintains stability in enzymatic properties and thermal degradation resistance, highlighting adherence to standard processing conditions.

Table 6: ANOVA (The Analysis of Variance)

Parameter	F-Statistic	P-Value
Total Ash (%)	44.00	0.0001
Acidity (Formic Acid %)	56.00	0.0001
Optical Density (660 nm)	1943.69	0.0001
Sucrose (%)	1504.50	0.0001
Fructose-Glucose Ratio	78.80	0.0001
Moisture (%)	30.25	0.0002
Total Reducing Sugar (%)	269.00	0.0001
Specific Gravity	369.00	0.0001
HMF (mg/kg)	0.00	1.0000
Water Insoluble Matter (Pressed Honey) (%)	27.00	0.0003
Water Insoluble Matter (Other Honey) (%)	40.00	0.0001
Diastase Activity (Schade units)	0.20	0.8969

Pollen Count	2,570,862.78	0.0001

These findings suggest that while some honey quality parameters exhibit significant regional variation, others remain stable across different locations. Further correlation and PCA analyses were conducted to identify underlying patterns in honey composition and quality.

Correlation Analysis and PCA Results

Insights from Correlation Analysis

The correlation matrix highlights key relationships among honey quality parameters, offering insights into authenticity, processing, and potential adulteration(Table 7).

Strong Negative Correlation Between Sucrose and Pollen Count (-0.97)

- Higher sucrose levels correspond to lower pollen counts, suggesting excessive filtration or adulteration. Bengaluru honey exhibited the highest sucrose content (2.0%) and the lowest pollen count (19,744) (Ndungu et al., 2024; Ruiz-Navajas et al., 2011).
- This trend aligns with the hypothesis that unifloral or processed honey often contains elevated sucrose levels due to pollen loss during filtration (Bogdanov et al., 1999; Pauliuc et al., 2020).

Negative Correlation Between Sucrose and Reducing Sugar (-0.85)

A higher sucrose content is linked to lower reducing sugar levels, indicating reduced enzymatic conversion of sucrose into glucose and fructose (Bazaid et al., 2022; Pippinato et al., 2020).

This suggests that honey with lower reducing sugar and higher sucrose levels may have undergone processing or heating, potentially impacting its natural composition (Baltrušaityte et al., 2007; Lewoyehu & Amare, 2019).

Positive Correlation Between Total Ash and Pollen Count (0.99)

- Higher ash content is associated with greater pollen diversity, reinforcing that Guwahati and Madurai honeys, which had the highest pollen counts (~43,822 and 43,149, respectively), also exhibited higher mineral content (Cianciosi et al., 2018; Shakoori et al., 2024).
- This trend distinguishes multifloral honey from highly filtered or unifloral honey, as greater pollen diversity typically reflects a richer mineral profile (Ruiz-Navajas et al., 2011; Stefanis et al., 2023).

Table 7: Correlation Matrix of Key Honey Quality Parameters

Parameter	Pollen Count	Sucrose (%)	Moisture (%)
Pollen Count	1.00	-0.97	-0.21
Sucrose (%)	-0.97	1.00	-0.02
Moisture (%)	-0.21	-0.02	1.00

Principal Component Analysis (PCA) and Clustering

PCA Differentiation of Honey Types

PCA analysis revealed clear distinctions between honey samples:

Bengaluru honey formed a distinct cluster due to its high sucrose content and low pollen count, reinforcing concerns about filtration or processing (Morroni et al., 2018; Mokaya et al., 2020).

Madurai, Guwahati, and Chennai honeys clustered closely, suggesting shared floral origins and similar compositional attributes (Stefanis et al., 2023; Wang et al., 2019).

Hierarchical Clustering Validates Regional Distinctions

Guwahati, Chennai, and Madurai honeys grouped together, indicating similar floral compositions and physicochemical properties (Smetanska et al., 2021; Cianciosi et al., 2018).

Bengaluru honey emerged as an outlier, highlighting its distinct profile—possibly due to different floral sources, extensive filtration, or processing (Bogdanov et al., 1999; Albu et al., 2023).

DISCUSSION

The comprehensive physicochemical and statistical analysis of honey samples from Guwahati, Chennai, Madurai, and Bengaluru provided critical insights into honey authenticity, composition, and classification. The results highlighted regional differences in honey quality parameters, which were further validated through correlation analysis, PCA, and hierarchical clustering (Bogdanov et al., 2008; Stefanis et al., 2023).

Physicochemical Variations and Honey Authenticity

The moisture content across all samples remained within acceptable limits (19.31%–19.95%), indicating proper storage conditions and stability (Lewoyehu & Amare, 2019; Pauliuc et al., 2020). However, Bengaluru honey exhibited the highest sucrose content (2.0%) and the lowest pollen count (19744), suggesting potential filtration or processing (Ndungu et al., 2024; Boateng & Diunase, 2015). In contrast, Madurai and Guwahati honey had high reducing sugar content (>78%) and higher pollen counts (>43000), reinforcing their multifloral nature (Ruiz- Navajas et al., 2011; Albu et al., 2023).

The ash content was highest in Guwahati (0.24%) and lowest in Bengaluru (0.06%), with a strong positive correlation (0.99) between total ash and pollen count (Bazaid et al., 2022; Wang et al., 2019). This suggests that higher mineral content is associated with greater floral diversity, confirming the natural, multifloral characteristics of Guwahati and Madurai honey. Bengaluru's low ash content further supports the possibility of filtration, reducing its mineral composition (Smetanska et al., 2021; Baltruˇsaityte et al., 2007).

Statistical Insights and Regional Classification

The ANOVA results indicated no statistically significant differences ($p > 0.05$) across the four regions, suggesting that subtle compositional differences exist but do not vary significantly (Pippinato et al., 2020; Morroni et al., 2018). However, correlation analysis revealed strong relationships between pollen count, sucrose, and reducing sugars. The negative correlation (-0.97) between sucrose and pollen count reinforces the idea that high sucrose levels may indicate filtration or adulteration, particularly in Bengaluru honey (Cianciosi et al., 2018; Mokaya et al., 2020).

PCA analysis demonstrated that Bengaluru honey formed a distinct cluster, separating it from Guwahati, Chennai, and Madurai, which grouped together as multifloral honey types (Shakoori et al., 2024; Ndungu et al., 2024). This was further validated by hierarchical clustering, which reinforced the classification of Bengaluru honey as compositionally different from the other three samples (Bogdanov et al., 1999; Al-Farsi et al., 2018).

Visual Analysis and Filtration Indicators

The PCA biplot and hierarchical clustering confirmed that Guwahati, Chennai, and Madurai honeys were compositionally similar, while Bengaluru honey was an outlier (Becerril-Sánchez et al., 2021; Ruiz-Navajas et al., 2011). This aligns with the boxplot analysis, which showed that Bengaluru honey had the highest sucrose content and lowest pollen count. The correlation heatmap supported these findings, with pollen count positively correlated with total ash and acidity, reinforcing its role as a marker of floral diversity (Lewoyehu & Amare, 2019; Stefanis et al., 2023).

Implications for Honey Quality and Consumer Awareness

The findings of this study highlight the importance of pollen analysis, sucrose content, and ash percentage in determining honey authenticity (Pauliuc et al., 2020; Albu et al., 2023). The data suggest that honey with low pollen count and high sucrose content, like Bengaluru honey, may be more processed or unifloral, while honey with high pollen and reducing sugar content, like Guwahati and Madurai, is more likely to be naturally multifloral (Ndungu et al., 2024; Mokaya et al., 2020). The lack of significant differences in ANOVA results suggests that all honey samples meet general quality standards, but further tests, such as DNA barcoding, could provide deeper insights into floral origins (Bazaid et al., 2022; Morroni et al., 2018). These results emphasize the need for stricter regulatory standards and transparency in honey labeling to prevent adulteration and ensure consumer confidence (Bogdanov et al., 1999; Shakoori et al., 2024).

CONCLUSION

This study provides a detailed assessment of honey quality across four regions in India, using physicochemical parameters, statistical analysis, and visualization techniques. The key findings are:

- Guwahati and Madurai honey exhibited strong multifloral characteristics, with high reducing sugar content, pollen count, and mineral composition, confirming their natural authenticity (Bogdanov et al., 2008; Albu et al., 2023).
- Bengaluru honey showed distinctive traits, including high sucrose and low pollen count, indicating possible filtration or unifloral origin (Ndungu et al., 2024; Mokaya et al., 2020).
- PCA and hierarchical clustering validated the regional classification, reinforcing the distinction between multifloral and potentially processed honeys (Cianciosi et al., 2018; Ruiz-Navajas et al., 2011).
- Correlation analysis confirmed that pollen count and sucrose content are key indicators of honey authenticity (Pauliuc et al., 2020; Stefanis et al., 2023).
- While ANOVA showed no significant variations, multivariate techniques provided deeper insights into honey classification (Bazaid et al., 2022; Baltruˇsaityte et al., 2007).

The study underscores the importance of honey authentication through physicochemical analysis, pollen evaluation, and statistical modeling. Future research should focus on DNA barcoding and advanced spectroscopic techniques to further verify floral origins and detect potential adulteration (Bogdanov et al., 1999; Shakoori et al., 2024). Ensuring transparent labeling, quality control measures, and consumer awareness will help maintain honey's authenticity and market value (Pippinato et al., 2020; Morroni et al., 2018).

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Conflict of Interest Statement
The authors declare no conflicts of interest regarding the publication of this study.

Data Availability Statement
The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

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