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Physicochemical Profiling of Unifloral Tulasi and Hibiscus Honeys from *Apis cerana F.* Insights into Unifloral and Multifloral Honey Quality

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Abstract

Honey is a natural product with significant nutritional and medicinal value, whose physicochemical characteristics are influenced by floral and geographical origin. This study evaluated and compared the physicochemical properties of unifloral Tulasi (Ocimum sanctum) and unifloral Hibiscus (Hibiscus rosa-sinensis) honeys collected from Apis cerana F. colonies in Madurai, Tamil Nadu, and India. Parameters analyzed included colour, moisture content, specific gravity, pH, ash (mineral content), hydroxymethylfurfural (HMF), reducing sugars, total carbohydrates, protein, and pollen content using standard analytical methods. Both honey types complied with FSSAI and BIS standards. Hibiscus honey exhibited slightly higher density, protein, ash, and carbohydrate content, suggesting superior nutritional value, while Tulasi honey showed lower moisture, indicating better storage stability. Pollen analysis confirmed processed honey characteristics, as pollen grains were not detected. The findings provide insight into the quality and compositional differences of these unifloral and multifloral honeys and support their classification under national and international honey standards.

Key Words:

Tulasi honey, Hibiscus honey, physicochemical analysis, Apis cerana, moisture content, HMF, ash content, reducing sugars, honey quality.

Introduction

Honey is one of nature's most valued natural products, renowned for its nutritional, medicinal, and therapeutic properties since ancient times. It is a natural sweetener produced by honeybees (Apis spp.) from the nectar of flowers, composed primarily of sugars, water, organic acids, minerals, amino acids, enzymes, and bioactive compounds (Bogdanov et al., 2008). The physicochemical properties of honey depend largely on its botanical origin, geographical source, floral diversity, and processing methods (Albu et al., 2025). India, being a tropical

country with rich floral diversity, offers a variety of unifloral and multifloral honeys, each differing in flavor, aroma, and composition. Among the cultivated flora, unifloral Tulasi (Ocimum sanctum) and unifloral Hibiscus (Hibiscus rosa-sinensis) are important nectar sources known for their medicinal value in traditional Ayurvedic systems.

Honey derived from these sources is gaining attention for its potential therapeutic benefits, including antioxidant, antimicrobial, and anti-inflammatory properties. Physicochemical characterization serves as a reliable approach for assessing the quality, purity, and authenticity of honey (FSSAI, 2018; IHC, 2009). Parameters such as moisture, pH, specific gravity, ash, hydroxymethylfurfural (HMF), and reducing sugar content provide insights into its freshness, storage stability, and processing history (Abera et al., 2023). The presence of pollen grains further confirms the botanical origin and helps in classifying unifloral honeys (Louveaux et al., 1987). The present study aims to evaluate and compare the physicochemical characteristics of Tulasi and Hibiscus honeys collected from Apis cerana F. colonies in Madurai, Tamil Nadu, and India. The results will contribute to understanding the compositional differences between these honeys and their compliance with national and international honey quality standards.

Materials and methods

Study Area and Sample Collection

Physicochemical analyses were conducted on Tulasi and Hibiscus honeys produced by Apis cerana F. colonies. Samples were collected from Vibis Natural Bee Farm, Kadachanendhal, Madurai, Tamil Nadu, during January 2023. The honeys were stored at room temperature (20–25 °C) until analysis.

Colour Determination

Honey colour was visually assessed after liquefying samples in a 40 °C water bath. Colours were compared with standard colour charts (Kornerup and Wancher, 1978; Soni, 1993).

Moisture Content (Refractive Index Method)

Moisture content was determined using a Portable Brix Refractometer with Automatic Temperature Compensation (ATC), following FSSAI (2018) guidelines. The refractive index values were converted to percentage moisture using standard tables.

Specific Gravity

Specific gravity was determined using a 50 mL specific gravity bottle as per BIS standards (IS 4941: 1994). The ratio of the mass of honey to that of distilled water at 27 ± 1 °C was used for calculation.

Calculation

$$Specific\ gravity\ at\ 27℃ = \frac{C - A}{B - A}$$

Where, C = mass, in g. of the specific gravity bottle with the honey sample; A = mass, in g. of the empty specific gravity bottle; and B = mass, in g. of the specific gravity bottle with water.

pH Measurement

Ten grams of honey were dissolved in 75 mL of distilled water, and pH was measured using a calibrated digital pH meter (buffers pH 4.0 and 7.0) according to the method of De Moraes and Teixeira.

Ash Content (Mineral Content)

Ash percentage was determined by charring 20 mL of honey and ashing in a muffle furnace at 600 °C until constant weight (AOAC, 2000).

% Ash = (Weight of crucible + ash) – (Weight of empty crucible) x 100/ sample weight

(OR)

$$Ash\ prevent\ by\ mass = 100\frac{(M2-M)}{M1-M}$$

Where, M1 = mass, in g, of the dish with the ash, M = mass, in g, of the empty dish and, M1 = mass, in g, of the dish with the material taken for test.

Hydroxymethylfurfural (HMF) Content

HMF was estimated by Winkler's method (IHC, Bogdanov, 1999). The absorbance of the reaction mixture was read at 550 nm using a UV-1900 spectrophotometer.

$$HMF\ in\ mg = \frac{192 \times A \times 10}{Weight\ of\ honey\ in\ grams}$$

Where, A = Absorbance, 192 = Factor for dilution and extinction coefficient express results in mg to 1 decimal place.

Reducing Sugars (Fehling's Test)

Equal volumes (1 mL each) of Fehling's A and B solutions were mixed, and 2 mL of honey solution was added. Formation of a brick-red cuprous oxide precipitate indicated the presence of reducing sugars.

Carbohydrate Estimation (Anthrone Method)

Carbohydrate content was estimated using the Anthrone method. Two mL of honey solution was treated with 2 mL of 0.2 % anthrone in concentrated H2SO4, boiled, and the optical density (OD) was read at 620 nm.

Protein Estimation (Biuret Method)

Protein content was determined using the Biuret method. Three mL of honey were mixed with NaOH and CuSO4 reagents; the resulting purple colour was measured spectrophotometrically at 540 nm.

Pollen Analysis

Pollen analysis followed Louveaux et al. (1987) and Suryanarayana et al. (1981). One g of honey was diluted with 2 mL of water, centrifuged at 5000 rpm for 10 min, washed, and the sediment mounted on slides for microscopic identification.

Results and Discussions

Physicochemical Characteristics of Tulasi and Hibiscus Honeys

The physicochemical parameters of Tulasi and Hibiscus honeys are presented in Table 1. Both samples fall within acceptable ranges recommended by the Food Safety and Standards Authority of India (FSSAI, 2018) and the Bureau of Indian Standards (IS 4941: 1994) for natural honey.

Results of various physico-chemical properties of honey samples studied are summarized as follows :(Table 1)

S.NO	QUALITY PARAMETERS	HIBISCUS	THLASI
1	Color	Light amber	White
2	Moisture	17.0 %	16.1 %
3	Specific gravity	1.4095	1.3890
4	pH	3.5 - 4.5	3.5 – 4.5
5	Ash	0.22%	0.16%
6	HMF	60 mg	60 mg
7	Sugar (Fehling's test)	Blue change to orange color	Blue change to brick red precipitate
8	Carbohydrates	2,466.5	1,266.5
9	Protein	56.1	51.5
10	Lipid	0	0
11	Pollen	No pollen	No pollen

Table 1: comparative analysis of quality parameters in hibiscus and tulasi honey

Colour

The colour of Tulasi honey was white, whereas Hibiscus honey exhibited a light amber tone. Colour variation in honey largely depends on the floral source and mineral content (Albu et al., 2025). Darker honeys are usually richer in phenolic compounds and minerals (Puścion-Jakubik et al., 2020). Thus, the light amber colour of Hibiscus honey suggests relatively higher mineral and phytochemical content than Tulasi honey.

Moisture Content

The moisture content of Tulasi honey (16.1%) and Hibiscus honey (17%) were both within the FSSAI permissible limit of 20% and Codex Alimentarius standard (≤ 20%). Moisture content below 18% is indicative of well-ripened honey with a lower tendency for fermentation (Abera et al., 2023). Hence, both honeys demonstrated good maturity, with Tulasi honey showing slightly superior storage potential due to its lower moisture value.

Specific Gravity

Specific gravity values were 1.38 for Tulasi honey and 1.40 for Hibiscus honey, both satisfying the BIS requirement (≥ 1.35). Higher specific gravity reflects greater concentration of dissolved solids (mainly sugars) and lower water content (Albu et al., 2025). The slightly higher value in Hibiscus honey indicates a denser sugar matrix.

pH measurement

Both samples recorded pH values between 3.5 and 4.5, conforming to the International Honey Commission (IHC, 2009) range (3.2–4.5). The moderate acidity supports microbiological stability and contributes to honey's flavor profile (IHC, 2009).

Ash Content

Ash values were 0.16% (Tulasi) and 0.22% (Hibiscus), both well below the FSSAI limit of 0.5%. Ash content reflects total mineral composition; the higher value in Hibiscus honey suggests slightly richer mineral content, aligning with its deeper colour (Puścion-Jakubik et al., 2020).

Hydroxymethylfurfural (HMF)

Both honeys contained 60 mg/kg HMF, which is below the FSSAI maximum limit of 80 mg/kg for tropical honeys. Although acceptable, these levels are higher than those typical for freshly extracted honey (< 40 mg/kg) (Albu et al., 2025). This indicates possible mild heating or storage for extended periods, as HMF increases with heat and time (Puścion-Jakubik et al., 2020).

Reducing Sugars and Carbohydrate Content

Fehling's test produced a brick-red precipitate for Tulasi honey and an orange precipitate for Hibiscus honey, confirming the presence of reducing sugars (mainly glucose and fructose). Quantitatively, Tulasi honey contained 1.3 g carbohydrates, whereas Hibiscus honey had 2.5 g in the tested aliquot. Although absolute values appear low due to sample dilution, the relative difference indicates higher sugar concentration in Hibiscus honey. According to standard honey composition, carbohydrates constitute approximately 80% of honey's dry matter (IHC, 2009).

Protein Content

Protein content was 51.5 mg/100 g in Tulasi honey and 56.1 mg/100 g in Hibiscus honey. These values are comparable to reported protein levels in floral honeys (20–80 mg/100 g) (Albu et al., 2025). The slightly higher value in Hibiscus honey may be attributed to trace pollen proteins or enzyme residues, despite both samples showing no detectable pollen microscopically.

Lipid Content

Both samples recorded zero lipid content, consistent with the known biochemical composition of honey, which is virtually fat-free (IHC, 2009).

Pollen Analysis

Pollen was not detected in either sample, suggesting filtration or processing. While this improves clarity and shelf appeal, it also removes floral pollen grains that aid in confirming botanical origin (Puścion-Jakubik et al., 2020). Therefore, these honeys can be classified as processed or filtered honeys rather than raw floral honeys.

Overall Evaluation

Both Tulasi and Hibiscus honeys conformed to major physicochemical standards for natural honey. Hibiscus honey displayed marginally higher density, protein, ash, and carbohydrate content, indicating richer nutritional potential. Tulasi honey showed lower moisture content, beneficial for shelf stability. Slightly elevated HMF values in both samples point toward mild heating or prolonged storage. The absence of pollen grains indicates processing, which may affect botanical authentication.

Conclusion

The present study demonstrates that both Tulasi and Hibiscus honeys are of acceptable physicochemical quality under FSSAI and BIS standards. Hibiscus honey is nutritionally superior, while Tulasi honey shows better stability characteristics. Future work involving antioxidant profiling and floral origin analysis through melissopalynology and spectroscopy could further validate their unique properties.

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