**Variation of Protein content and mellisopalynogical status in the raw and branded honey samples in Chalisgaon region, North Maharashtra, India**

**ABSTRACT**

The aim of this study was to compare the protein content and melisopalynological characteristics of raw and branded honey from the Chalisgaon region in Jalgaon district, North Maharashtra, India. Twelve honey samples were analyzed, consisting of seven raw and five branded honey samples. The protein content in raw honey ranged from 0.92 to 1.62 g/kg, while in branded honey, it ranged from 0.56 to 0.82 g/kg. The variability in the protein content of raw honey could be attributed to differences in floral sources in both agricultural and forested areas. Mellisopalynological analysis revealed that raw honey samples contained a significant amount of pollen, while branded honey samples exhibited little or no pollen, likely because of filtration processes before packaging. The predominant pollen types (more than 45%) in raw honey included *Helianthus annuus, Allium cepa, Punica granatum, Amaranthus hybridus, Parthenium hysterophorus, Launea procumbens, Terminalia arjuna, Euphorbia heterophylla, Mangifera indica, Triticum estivum, Acacia nilotica,* and *Zea mays*, all of which are associated with major agricultural crops in the region. In conclusion, raw honey demonstrated higher protein and pollen contents, which contributed to its higher nutritional and storage value compared to branded honey. Therefore, raw honey may be preferable for consumers seeking higher nutritional benefits.

***Keywords:*** *raw and branded honey, protein content, melisopalynological status, Chalisgaon region*

**1. INTRODUCTION**

Honey has been consumed for centuries due to natural sweetness and potential health benefits. Previous studies have shown that honey contains various bioactive compounds, including proteins, vitamins, and pollen, which contribute to its nutritional value (Khalil *et al.*, 2001; Saxena *et al.*, 2010). Many international regulatory bodies established harmonized methods for determining the physicochemical compounds in honey samples, which regulate the quality and safety control of honey. For the commercialization of honey, recently several quality control methods applied for the determination of physicochemical properties and helpful to categorize honey from different geographic origin, authenticate chemical features and detect adulteration (Bogdanov *et al.* (2004). Many physicochemical parameters such as moisture content, pH, electric conductivity, acidity, sugars, proline, vitamins, Hydroxymethyfurfural (HMF), antioxidants have been extensively studied in honey samples from different regions (Das *et al.,* 2015; Nanda *et al.,* 2009; Nayik and Nanda, 2015; Sawarkar, 2023a,b; Thakur *et al.,* 2023). The variations in physicochemical properties in different regions were influenced by geographical origin, floral source, seasonal variation and environmental factors (Nanda *et al.*, 2009; Sajid *et al.*, 2023; Sawarkar, 2023a).

Honey contains a relatively low content of proteins (0.20%), including enzymes and free amino acids, which have minor nutritional significance but are important for evaluating honey quality (Flanjak et al., 2016). These proteins mostly originate from flowers and are introduced by bees during the honey maturation process (Azeredo et al., 2002; Bogdanov et al., 2004; Lim et al, 2022). Despite their low content, honey proteins are becoming popular study objects and are used as markers of honey authenticity and quality (Bocian et al., 2019). The protein content and enzyme activities in honey vary depending on the botanical origin. Dark honey types, such as honeydew and chestnut honey, generally have higher proline content and enzyme activities compared to lighter honey types like sage and black locust honey (Flanjak et al., 2016). Honey proteins play essential roles as bee nutrients and antimicrobials, protecting honey from microbial spoilage (Lewkowski et al., 2019). According to Widianingrum (2008), protein plays a vital role in the development of young worker bees, affecting the maturation of flight muscles, maximizing the thoracic period, supporting queen ovary development, and extending the lifespan of bees to reach adulthood and enhance colony productivity.

Along with different physicochemical parameters, the pollen content is crucial for determining honey's origin, quality, and authenticity. Raw honey typically contains a higher pollen content compared to branded honey. A study of 3917 raw honey samples found pyrrolizidine alkaloids (PAs) in 66% of raw honeys, while 94% of retail honeys contained PAs (Dübecke et al., 2011). This suggests that raw honey retains more of its natural pollen content. The pollen content in honey is closely tied to its geographical origin and botanical sources, allowing for the characterization and classification of honey types (Escuredo et al., 2023).

However, there is limited information on the comparative analysis of protein and pollen content between raw and branded honey (Lim et al, 2022). Understanding these differences can influence consumer choices and promote the use of raw honey for its enhanced nutritional properties. Hence the aim of this study is to analyze and compare the protein and pollen content of raw and branded honey in Chalisgaon region, North Maharashtra, India. This research may provide valuable insights into the nutritional and botanical differences between raw and branded honey.

**2. MATERIALS AND METHODS**

**2.1 Honey sample collection sites**

Eight fresh raw honey and six branded honey samples were collected from tribal and market respectively. All the honey samples were stored in airtight plastic container at room temperature in laboratory for further analysis.

**2.2 Analysis of Protein**

The protein content of the honey samples was determined by Folin-Ciocalteau reagent (FCR) method (Lowry et al., 1951). The sample was prepared by mixing 1 mL of honey with 1 mL of distilled water (1:1 ratio). Then 0.2 ml of prepared sample was placed in separate test tubes, and 5 ml of alkaline copper sulfate reagent was added. After thorough mixing, the mixture was incubated at room temperature for 10 min. Subsequently, 0.5 ml FCR was added to each tube and incubated for 30 min. After incubation, absorbance was measured at 660 nm using a UV/VIS spectrophotometer and the results are expressed in mg/kg. A Bovine Serum Albumin (BSA) solution of known concentrations was used to generate a standard curve for protein content determination. The experiments were conducted in triplicate, and the results are presented as mean values and standard deviations (mean ± SD).

**2.3 Analysis of Pollen**

The botanical origins of the honey samples were confirmed by analyzing the pollens according to the recommended method (AOAC, 2012). 10g of Honey was dissolved in 20mL warm distilled water (400C) and centrifuged for 10 min at 2500 rpm. The solution was poured into a small tube and centrifuged again for 10 min. The sediment was placed on a slide, spread across an area of 20 mm², and dried by carefully heating to 40°C. Afterward, the sediment was mounted with glycerin gelatin and liquefied by heating in a water bath at 40°C. The pollen grains in the honey samples were identified from experts.

**3. RESULTS AND DISCUSSION**

**3.1 Protein content**

The variation of protein contents in raw and branded honey samples were noticed in Chalisgaon region. The protein content in the raw honey had high range between 0.92 to 1.62 g/kg of honey. The highest protein content were observed in honey sample RH7 (1.62 g/kg), followed by RH4 (1.30 g/kg), RH6 (1.16 g/kg), RH3 (1.08g/kg), RH1 (1.04 g/kg), RH5 (1.16 g/kg) and RH2 (0.92 g/kg) of honey (Fig. 1). The variation in protein content in raw honey samples may be due to differences in the geographical locations as well as floral sources which were also supported by Donkersley et al. (2017) and Schäfer et al. (2006). The branded honey samples had the range between 0.56 to 0.82 g/kg which shows lower protein content than raw honey samples. The highest protein content were observed in the branded honey samples BH3 (0.82 g/kg), BH2 (0.75 g/kg), BH5 (0.72 g/kg), BH4 (0.64 g/kg) and BH1 (0.56 g/kg) (Fig. 2). The lower protein content in branded honey samples may be influenced by the reduction in pollen content during processing and packageing of honey (Sajid et al., 2020).

Raw honey samples generally exhibit higher protein content compared to branded honey samples. In a study of Polish honeys, raw local honeys were found to be more abundant in soluble protein than imported honey blends (Miłek et al., 2021). Similarly, research on Pakistani honeys revealed that fresh honey samples had better quality parameters, including higher enzymatic activity, compared to branded honey samples (Sajid et al., 2020). Interestingly, the protein content in honey can vary significantly based on botanical and geographical origins. For instance, Malaysian stingless bee honey from different botanical origins showed protein content ranging from 0.20 to 0.80 g/100 g (Lim et al., 2019).

In Indian honey samples, the protein content was reported to be lower (0.48 to 2.29 g/kg) (Saxena et al., 2010) which show a similar range of the data presented in this study. In the six Tunisian unifloral honey samples, protein contents were noticed in a range of 0.13 to 0.16 g/100 g honey (Boussaid *et al.,* 2018) while the range between 0.724 to 1.121 g/kg honey from Ceará State, Northeastern Brazil (Liberato *et al.,* 2013). In Malaysian honeys, the protein content varied between 2.04 and 4.83 g/kg. (Moniruzzaman *et al.,* 2013). According to Flanjak et al. (2016), the total protein content in Acacia honey ranged from 21 to 43 mg/100 g, while honeydew honey ranged from 30 to 95 mg/100 g. Lim et al. (2022) examined honey samples from street vendors, contract beekeepers, and branded sources in Sabah, Malaysia, and found that street vendor honey had the highest protein content, followed by honey from contract beekeepers and branded honey.

The previous studies stated that honey contains about 0.20% protein which contains the major constituents in the form of enzymes such as diastase, a-amylase, invertase, glucose oxidase, catalase, and phosphatase. These protein contents are floral originated i.e. pollen based, added by the bees during the honey ripening process (Anklam, 1998; Bogdanov *et al.,* 2004). Lim et al. (2022) stated that the protein content in honey derives from both external sources collected during foraging and from salivary and the hypopharyngeal glands of worker bees. To fulfill the requirements of protein and essential amino acids, honey bees must visit all types of flowering plants (Franti, 2018). Protein is essential for young worker bees which influence the maturity of the flying muscles, maximizing the thoracic period, the development of the queen’s ovaries, and extending the life of the honey bee to reach adulthood and colony productivity (Widianingrum, 2008).

**Figure 1 Protein content in Raw honey samples (RH1-RH7)**

**Figure 2 Protein content in Branded honey samples (BH1-BH5)**

##### 3.2 Analysis of Pollen

##### Among 12 honey samples, frequency of pollen was very high in the raw honey samples (RH1 to RH7) as compared to the branded honey samples (BH1 to BH5). In the examined samples, the identified pollen grains were belongs to different flora originated from agriculture and forest sites. Total 20 species from 14 families of plants were recognized (Fig. 3). The identified species, which belong to various genera of native herbs, grasses, shrubs, and trees are presented in Table 1. During analysis, it was found that Asteraceae, Euphorbiaceae, Poaceae and Amaranthaceae families has highest species (Table 2). Earlier studies stated that the members of Fabaceae and Asteraceae were dominant families which had great importance in honey (Bhusari *et al.*, 2005; Terrab *et al.*, 2001; Song *et al.*, 2012). The mentioned results show strong similarity as the families Asteraceae and Fabaceae showed the highest presence in the research of Shakoori *et al.* (2023) from Iran, Rašić *et al.* (2018) from North-eastern Croatia, Saha *et al.* (2023) from West Bengal, India, Selvaraju et al. (2019) from West Coast of Malaysia and Puusepp and Koff (2014) from Estonia.

##### Pollen grains of a specific plant species are predominant if their presence in the honey sample is more than 45% of total pollen count (Bhusari *et al.*, 2005). Among the raw honey samples, 5 samples were unifloral and found the predominant pollen of *Helianthus annuus* (52.2%)*, Allium cepa* (56.8%)*, Punica granatum* (52.3%)*, Citrus sp.* (48.4%)and *Zea maize* (63.2%) and represent that main agriculture crops cultivated in Chalisgaon region. Fathy (2008) was recorded 26 pollen species in 15 families in *Apis mellifera* honey from Dakahlia, Egypt. Honey bees collect pollen and nectar on the basis of availability of botanical resources in their foraging range, which is influenced by environmental and seasonal factors (Fechner *et al.*, 2016). The pollen of wild plants represented by *Zizipus jujube, Vitex negundo, Parthenium hysterophorus, Xanthium strumarium, Mangifera indica, Tridax procumbens,* and *Acacia sp.* were frequently found in all honey samples and suggest that these plants were the foraging preference of honey bees. The variation of plant pollen in honey samples may be influenced by foraging ability of bees, variation in flowering seasons, geographical locations, foraging preference, floral arrangement and availability of floral sources.

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**Figure 3 Pollen in honey samples**

##### *1. Helianthus annuus, 2. Alium cepa, 3. Punica granatum, 4. Amaranthus hybridus, 5. Terminalia arjuna, 6. Launaea procuumbens, 7. Zea maize, 8. Parthenium hysterophorus, 9. Euphorbia heterophylla, 10. Mangifera indica, 11. Gossypium sp., 12. Acacia nilotica, 13. Euphorbia sp., 14. Xanthium strumarium, 15. Capsicum annuum, 16. Ipomoea triloba, 17. Zizipus jujube, 18. Triticum sativum, 19. Citrus sp., 20. Solanum sp.*

##### Table 1. Pollen of different flora in honey samples

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Latin name of plants** | **Family** | **Habit** | **Flowering period** | **Economic importance** |
|  | *Amaranthus hybridus* | Amaranthaceae | Herb | July- August | Weed |
|  | *Allium cepa* | Amaryllidaceae | Herb | Jan. –Feb. | Vegetable |
|  | *Mangifera indica* | Anacardiaceae | Tree | March-April | Fruit |
|  | *Helianthus annuus* | Asteraceae | Shrub | July-Oct. | Oil Yielding |
|  | *Launaea procuumbens* | Asteraceae | Herb | March-April &  Oct. –Nov. | Weed |
|  | *Parthenium hysterophorus* | Asteraceae | Herb | Jan. – Dec. | Weed |
|  | *Xanthium strumarium* | Combretaceae | Herb | Jan. – Dec. | Weed |
|  | *Terminalia arjuna* | Combretaceae | Tree | May-June | Medicinal |
|  | *Ipomoea triloba* | Convolvulaceae | Herb | Jan. –Dec. | Weed |
|  | *Euphorbia heterophylla* | Euphorbiaceae | Shrub | April-May | Medicinal |
|  | *Acacia nilotica* | Fabaceae | Tree | June-October | Medicinal |
|  | *Punica granatum* | Lythraceae | Shrub | June-July &  Jan.-Feb. | Agriculture |
|  | Zea maize | Poaceae | Shrub | July-August | Agriculture |
|  | *Triticum sativum* | Poaceae | Grass | Jan. –Feb. | Agriculture |
|  | *Zizipus jujube* | Rhamnaceae | Tree | July-Nov. | Fruit |
|  | [*Capsicum annuum*](http://en.wikipedia.org/wiki/Capsicum_annuum) | Solanaceae | Herb | May-June | Agriculture |
|  | *Citrus sp.* | Rutaceae | Tree | April-May | Fruit |
|  | *Gossypium sp.* | Malvaceae | Shrub | July-Aug. &  Feb.-March | Agriculture |
|  | *Solanum sp.* | Solanaceae | Herb | Aug. –Sept. | Agriculture |
|  | *Euphorbia sp.* | Euphorbiaceae | Shrub | April-May | Medicinal |

**4 CONCLUSIONS**

In conclusion, raw honey demonstrated higher protein and pollen contents, which contributed to its higher nutritional and storage value compared to branded honey. Therefore, raw honey may be preferable for consumers seeking higher nutritional benefits. This research is significant because it provides valuable insights into the nutritional and botanical differences between raw and branded honey. The study highlights the higher protein and pollen content in raw honey, which underscores its greater nutritional value and potential health benefits for consumers. Understanding these differences can influence consumer choices and promote the use of raw honey for its enhanced nutritional properties. Additionally, the study's melisopalynological analysis contributes to the broader scientific knowledge of honey's botanical origins, which can be crucial for beekeeping practices, honey authentication, and quality control in the honey industry.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

The authors are 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.

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