



# Sensory Characterization of Iranian Honey Samples Using the IHC Sensory Wheel

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| ARTICLE INFO                                                                            | ABSTRACT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
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| <b>Article type:</b><br>Research Paper                                                  | Honey is a complex natural product whose sensory characteristics are strongly influenced by botanical origin. In this study, the sensory profiles of 28 Iranian honey samples (25 monofloral and 3 multifloral) collected from different geographical regions of Iran were evaluated using the standardized International Honey Commission (IHC) sensory protocol and Honey Sensory Wheel. A trained panel of 20 assessors performed descriptive sensory analysis of aroma, flavor, taste, mouthfeel, and aftertaste using a 10-point intensity scale. Descriptive statistics were complemented by principal component analysis (PCA) and permutational multivariate analysis of variance (PERMANOVA). Prior to PCA, sampling adequacy was confirmed by a Kaiser–Meyer–Olkin (KMO) value of 0.579 and a significant Bartlett’s test of sphericity ( $\chi^2 = 169.51$ , $df = 66$ , $p < 0.001$ ). The first two principal components explained 42.45% of the total variance (PC1 = 25.91%; PC2 = 16.54%). PC1 was primarily associated with taste-related attributes, including bitterness, warmth, saltiness, and vegetal notes, whereas PC2 was positively associated with aroma-related descriptors such as olfactory intensity, floral notes, and aromatic perception. Radar plots and heat map visualization further highlighted both inter- and intra-botanical variability among honey samples. PERMANOVA confirmed a significant effect of botanical origin on the overall sensory profile of honeys ( $p = 0.001$ ). These findings demonstrate that sensory profiling contributes to the differentiation of Iranian honeys according to botanical origin and may serve as a complementary tool alongside physicochemical and melissopalynological approaches for honey characterization and authenticity assessment. |
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| <b>Keywords:</b><br>Sensory profiling<br>Honey<br>IHC sensory wheel<br>Botanical origin |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

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## Introduction

Honey is a complex natural product whose composition and sensory characteristics are strongly influenced by botanical origin, environmental conditions, and post-harvest handling. Although physicochemical, chromatographic, and spectroscopic techniques have greatly improved honey quality assessment and authentication, sensory evaluation remains indispensable for characterizing organoleptic properties and discriminating botanical and geographical origins (1, 2). The standardized methodology introduced by the International Honey Commission (IHC), including the Honey Sensory Wheel, has established a common sensory lexicon and improved the comparability of honey sensory studies worldwide (3, 4).

Sensory characteristics are closely associated with honey composition, particularly volatile organic compounds (VOCs), organic acids, and phytochemicals, making sensory profiling a valuable complementary tool for botanical

authentication (5, 6). Furthermore, several quality-related attributes, including bitterness, floral and herbaceous notes, and sensory defects such as smoky or metallic off-flavors, can only be reliably detected through trained sensory evaluation, emphasizing its complementary role alongside physicochemical and melissopalynological analyses (7, 8). The identification of more than 600 volatile constituents in honey, including well-established botanical markers such as methyl anthranilate in citrus honeys, further highlights the diagnostic potential of sensory and volatile profiling (3, 9). Iran possesses exceptional botanical diversity and a long tradition of beekeeping, providing favorable conditions for the production of numerous monofloral honeys. Despite this diversity, standardized sensory characterization of Iranian honeys based on the IHC protocol remains scarce, as previous studies have mainly focused on physicochemical properties and pollen analysis (9).

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Therefore, this study aimed to (i) characterize the sensory profiles of 28 Iranian honey samples representing diverse botanical and geographical origins using the IHC sensory methodology; (ii) evaluate the influence of botanical origin on sensory characteristics; and (iii) assess the discriminatory ability of descriptive sensory analysis combined with multivariate statistical approaches, including principal component analysis (PCA), hierarchical clustering, and PERMANOVA. The findings provide standardized sensory reference data to support honey quality evaluation and botanical authenticity assessment.

## Materials & Methods

### Honey Samples

All honey samples (25 monofloral and 3 multifloral) were randomly collected directly from local beekeepers as bulk honey between November 2021 and August 2022 from 11 provinces representing different climatic regions of Iran (Supplementary Table S1). According to beekeeper questionnaires, most samples had been harvested within the previous three months, although a few originated from the late 2021 harvest. None of the samples had undergone pasteurization or thermal treatment before collection. Samples were transferred into sealed glass jars and stored at  $20 \pm 2$  °C in the dark until sensory evaluation to preserve volatile compounds. The containers remained unopened until sensory analysis (10-14).

The geographical distribution of sampling locations is shown in Figure 1, and detailed information on sample codes, botanical origins, and locations is provided in Supplementary Table S1.

### Sensory Panel

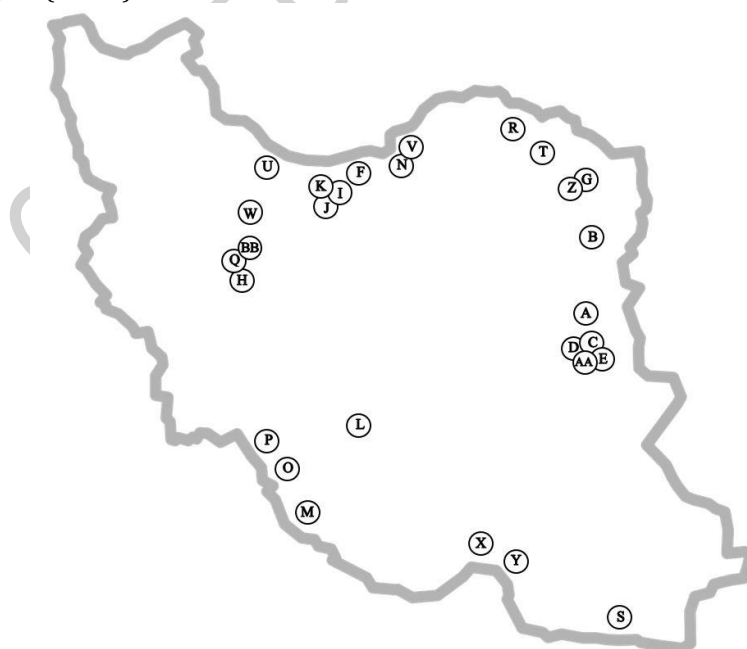
The sensory panel consisted of 20 trained assessors (25–45 years, 11 men and 9 women) selected according to IHC recommendations (3):

- Good general health
- Non-smokers
- No allergies to honey
- Previous training in distinguishing basic taste and aroma attributes

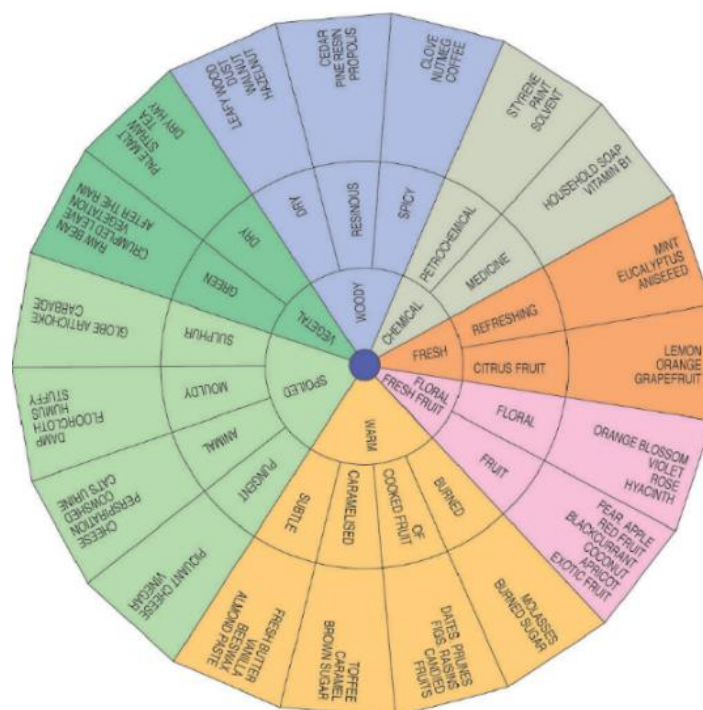
Panelists completed a two-week training program (six 2-hour sessions) covering the IHC Honey Sensory Wheel, reference standards, and intensity scoring. Panel performance was verified through replicate evaluations of a reference honey to ensure consistency.

### Sample Preparation and Presentation

Samples were equilibrated to 20–22 °C before analysis. Approximately 10 g of honey was presented in coded transparent glass containers. Sensory evaluation was performed under controlled laboratory conditions ( $21 \pm 1$  °C; 45–60% relative humidity). Samples were presented in randomized order using a balanced Latin square design. Panelists rinsed their mouths with room-temperature water between samples to minimize carry-over effects (3, 15).



**Figure 1.** Geographic distribution of honey sampling locations across different regions of Iran. Sample codes and corresponding botanical origins are described in Supplementary Table S1.



**Figure 1.** Sensory Attributes and Definitions (Honey Aroma-Taste Wheel) (16)

### Sensory Evaluation

Based on the IHC Honey Sensory Wheel, sensory evaluation included aroma, flavor, taste, mouthfeel, and aftertaste attributes. Each attribute was scored on a 10-point intensity scale (0 = not perceivable; 10 = extremely intense).

Descriptors included the following categories following IHC guidelines IHC (3, 16):

- Aroma: floral, fruity, herbal, caramel, spicy, woody
- Flavor: sweet, sour, bitter, pungent
- Mouthfeel: viscosity, granularity, smoothness
- Aftertaste: persistence of aroma, astringency

Sensory attributes and descriptors were selected based on the "Italian Honey Sensory Wheel" proposed by Marazzan et al. (2018), with slight adaptations to fit the characteristics of the samples analyzed. The evaluation sheet included 12 sensory attributes: olfactory intensity, floral, fruity, warm, aromatic, chemical, vegetal, animal, sweetness, sourness, bitterness, and saltiness. Figure 2 provides a complete list of attributes and their definitions based on IHC (3, 16). Minor modifications were made to the descriptor sheet to better match the floral origins of the monofloral samples.

### Statistical Analysis

Descriptive statistics were calculated as the mean, standard deviation, and range of panel scores averaged across replicate evaluations. Data normality was assessed using the Shapiro-

Wilk test. Because most variables were non-normally distributed, differences among botanical origins were evaluated using the Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction.

The suitability of the dataset for principal component analysis (PCA) was assessed using the Kaiser-Meyer-Olkin (KMO) measure and Bartlett's test of sphericity. PCA was performed on standardized (Z-score) sensory data. PERMANOVA (999 permutations) was applied using Euclidean distance to evaluate the effect of botanical origin on overall sensory profiles. Hierarchical cluster analysis (Ward's method, Euclidean distance) and heat map visualization were used to explore sample grouping. Statistical analyses were performed in R version 4.3.1 using the factoextra, vegan, corrplot, pheatmap, and ggplot2 packages (3, 15, 17).

## Results

### Descriptive Sensory Characteristics

Marked variability was observed among the 28 honey samples in aroma persistence, viscosity, color, and visual appearance (Supplementary Table S2). Long aroma persistence (>5 min) was mainly observed in *Ziziphus spina-christi*, *Berberis vulgaris*, and *Tilia begoniifolia* honeys, whereas short persistence (<30 s) was recorded for *Alhagi maurorum*, *Eucalyptus*, and *Astragalus spp.* Viscosity ranged from very fluid (*Citrus aurantium* and *Eucalyptus*) to highly viscous (*Ziziphus*, *Berberis*, *Coriandrum*, *Thymus*, and *Foeniculum*). Honey color varied from very light

(*Astragalus spp.* and *Robinia pseudoacacia*) to very dark (*Berberis vulgaris* and *Oliveria decumbens*). Most samples appeared visually clear, although minor impurities or air bubbles were observed in several samples (Supplementary Table S2).

### Differences in Sensory Attributes among Botanical Origins

The Kruskal–Wallis test revealed significant differences among botanical origins for olfactory intensity, floral, warm, aromatic, vegetal, animal,

sweetness, and bitterness (Table 1, supplementary table S3). Bitterness showed the strongest discrimination ( $\chi^2 = 35.62$ ,  $p < 0.001$ ), with *Berberis vulgaris* and *Tilia begoniifolia* exhibiting the highest scores, whereas *Robinia pseudoacacia* and *Astragalus spp.* showed negligible bitterness. Floral and olfactory intensity were greatest in *Citrus aurantium*, *Coriandrum sativum*, and *Ziziphus jujuba*, while sweetness was highest in *Robinia pseudoacacia* and *Astragalus spp.*

**Table 1.** Kruskal–Wallis test results for sensory attributes across botanical origins.

| Sensory Attribute   | $\chi^2$ | df | p-value | Significant pairwise differences ( $p < 0.05$ )            |
|---------------------|----------|----|---------|------------------------------------------------------------|
| Olfactory intensity | 24.87    | 13 | 0.009   | C. aurantium > R. pseudoacacia, Astragalus                 |
| Floral              | 29.14    | 13 | 0.001   | Z. jujuba, C. sativum > Eucalyptus, R. pseudoacacia        |
| Fruity              | 18.32    | 13 | 0.082   | NS                                                         |
| Warm                | 31.56    | 13 | <0.001  | B. vulgaris, T. begoniifolia > R. pseudoacacia, Astragalus |
| Aromatic            | 22.14    | 13 | 0.028   | C. aurantium > Eucalyptus, R. pseudoacacia                 |
| Chemical            | 15.76    | 13 | 0.112   | NS                                                         |
| Vegetal             | 27.43    | 13 | 0.004   | B. vulgaris, T. begoniifolia > Astragalus                  |
| Animal              | 20.15    | 13 | 0.045   | T. begoniifolia > R. pseudoacacia                          |
| Sweetness           | 28.45    | 13 | 0.002   | R. pseudoacacia, Astragalus > T. begoniifolia              |
| Sourness            | 19.87    | 13 | 0.064   | NS                                                         |
| Bitterness          | 35.62    | 13 | <0.001  | B. vulgaris, T. begoniifolia > all others                  |
| Saltiness           | 16.43    | 13 | 0.096   | NS                                                         |

NS = Not significant ( $p > 0.05$ )

### Multivariate Analysis of Sensory Data

The suitability of the dataset for principal component analysis (PCA) was confirmed by a Kaiser–Meyer–Olkin (KMO) value of 0.579 and a significant Bartlett's test of sphericity ( $\chi^2 = 169.51$ ,  $df = 66$ ,  $p < 0.001$ ). The first two principal components explained 42.45% of the total variance (PC1 = 25.91%; PC2 = 16.54%), whereas the first four components accounted for 73.76% of the variance, indicating that sensory variability was distributed across multiple dimensions. Although the explained variance of the first two PCs is moderate, this is not uncommon in descriptive sensory datasets due to the inherent complexity of sensory perception and sample variability.

PC1 was primarily associated with taste-related attributes, showing strong positive loadings for bitterness, warmth, saltiness, and vegetal perception, while sweetness exhibited a moderate negative association. PC2 was characterized by aroma-related attributes, including olfactory intensity, floral notes, and aromatic perception (Table 2). The PCA score plot (Supplementary figure S1) revealed a heterogeneous distribution of honey samples according to botanical origin, with partial overlap among groups reflecting the multidimensional nature of honey sensory characteristics. Samples from *Citrus*

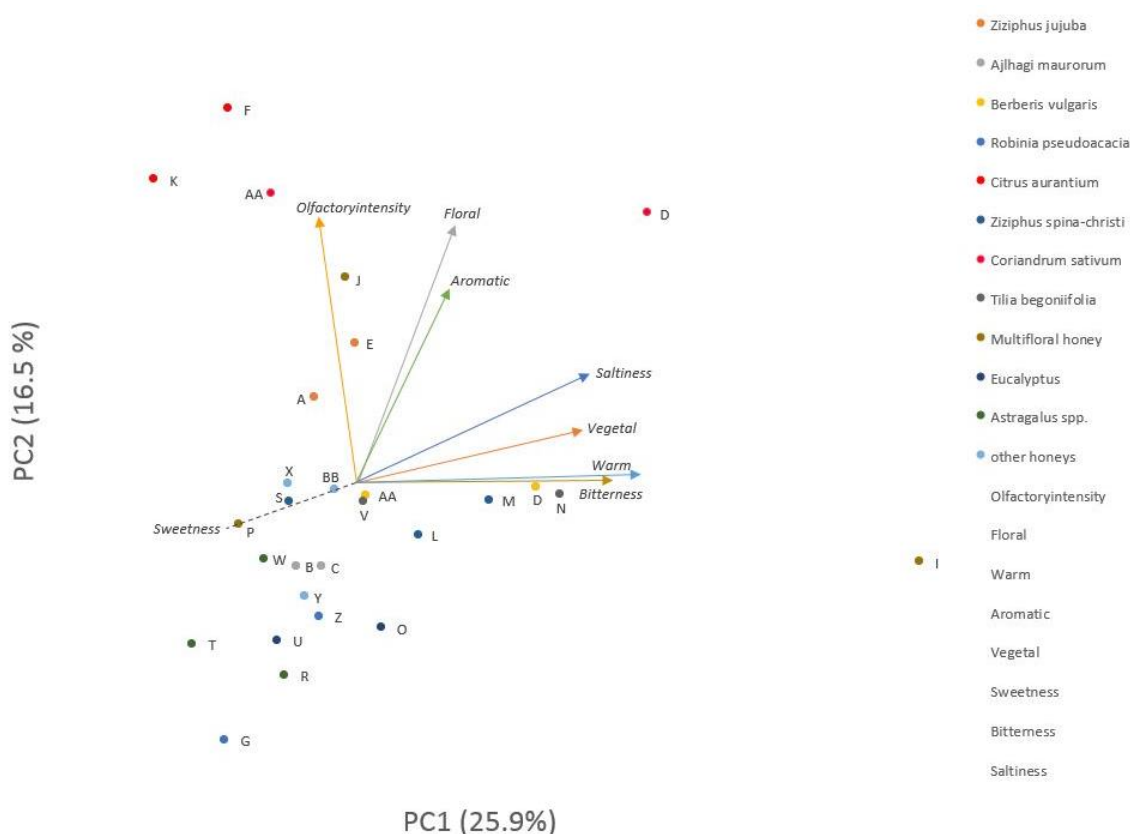
*aurantium* and *Ziziphus jujuba* were located on the positive side of PC2, consistent with their high olfactory intensity and floral notes. In contrast, *Berberis vulgaris* and *Tilia begoniifolia* honeys were positioned on the positive side of PC1, corresponding to their elevated bitterness, warmth, and vegetal attributes.

*Robinia pseudoacacia* and *Astragalus spp.* honeys clustered on the negative side of PC1, characterized by high sweetness and low bitterness.

**Table 2.** Principal component loadings for sensory attributes.

| Sensory Attribute   | PC1   | PC2   |
|---------------------|-------|-------|
| Olfactory intensity | 0.18  | 0.79  |
| Floral              | 0.05  | 0.74  |
| Fruity              | 0.32  | 0.41  |
| Warm                | 0.72  | -0.10 |
| Aromatic            | 0.28  | 0.63  |
| Chemical            | 0.35  | 0.22  |
| Vegetal             | 0.59  | -0.08 |
| Animal              | 0.44  | 0.18  |
| Sweetness           | -0.45 | 0.32  |
| Sourness            | 0.38  | 0.15  |
| Bitterness          | 0.68  | -0.12 |
| Saltiness           | 0.61  | -0.05 |

Bold values indicate loadings > |0.50|



**Figure 3.** Biplot of principal component analysis (PCA) based on sensory attributes of honey samples. Vectors represent sensory descriptors, while points correspond to individual honey samples (color-coded by botanical origin).

The PCA biplot (Figure 3) further illustrated the relationships between samples and sensory descriptors. Vectors represent sensory attributes, while points correspond to individual honey samples. The orientation and length of vectors indicate the contribution of each descriptor to the principal components. Taste-related attributes (bitterness, warm notes, vegetal perception, and saltiness) were mainly associated with PC1, whereas aroma-related descriptors (olfactory intensity, floral, and aromatic notes) were primarily aligned with PC2. Samples positioned closer to specific vectors exhibited higher intensities of the corresponding sensory attributes.

Permutational multivariate analysis of variance (PERMANOVA) confirmed a significant effect of botanical origin on the overall sensory profile of the analyzed honeys ( $p = 0.001$ ), indicating that the combined sensory descriptors differed significantly among floral sources.

#### Heat Map and Hierarchical Clustering Analysis

Heat map visualization combined with hierarchical clustering (Supplementary Figure S2) confirmed the PCA results, revealing a clear division between aroma-related (olfactory intensity, floral, aromatic, and fruity) and taste-

related (bitterness, warmth, vegetal, saltiness) attributes. Samples from *Berberis vulgaris* and *Tilia begoniifolia* formed a distinct cluster characterized by high bitterness, warmth, and vegetal notes, while *Citrus aurantium* and *Ziziphus jujuba* clustered together based on their strong floral and aromatic characteristics. *Robinia pseudoacacia* and *Astragalus spp.* were grouped by high sweetness and low bitterness. Multifloral honeys (I, J, P) exhibited broader and more heterogeneous profiles than most monofloral honeys, reflecting the diversity of their floral sources.

#### Sensory Profiles According to Botanical Origin

Radar plot visualization (Supplementary Figure S3) revealed distinct sensory profiles among botanical groups. Monofloral honeys exhibited characteristic patterns, while multifloral honeys showed broader, more heterogeneous profiles. *Berberis vulgaris* and *Tilia begoniifolia* were characterized by high bitterness, warmth, and vegetal notes, whereas *Citrus aurantium* and *Ziziphus jujuba* displayed pronounced olfactory intensity and floral attributes. *Robinia pseudoacacia* and *Astragalus spp.* were

distinguished by high sweetness and low bitterness.

Intra-botanical variability was also observed, particularly in *Z. jujuba* (samples A vs. E) and *C. sativum* (samples H vs. Q), suggesting influences of geographical origin and harvesting time on sensory expression. These patterns were consistent with the PCA results.

### PERMANOVA Results

Permutational multivariate analysis of variance (PERMANOVA) demonstrated a significant effect of botanical origin on the overall sensory profile of honey samples ( $F = 2.84$ ,  $R^2 = 0.62$ ,  $p = 0.001$ ), indicating that the combined sensory descriptors differed significantly among floral sources. Pairwise PERMANOVA comparisons (with Bonferroni correction) revealed that *Berberis vulgaris* and *Tilia begoniifolia* honeys were significantly different from *Robinia pseudoacacia*, *Astragalus* spp., and *Citrus aurantium* honeys ( $p < 0.05$ ).

### Discussion

The results of this study demonstrate that botanical origin significantly influences the sensory characteristics of Iranian honeys, as supported by descriptive analysis and multivariate statistics. PCA revealed two major dimensions: a taste-related dimension (PC1) dominated by bitterness, warmth, vegetal notes, and saltiness, and an aroma-related dimension (PC2) characterized by olfactory intensity and floral-aromatic notes, consistent with previous honey sensory studies (15, 17).

The pronounced bitterness and warmth of *Berberis vulgaris* and *Tilia begoniifolia* honeys likely reflect elevated phenolic compounds and secondary metabolites such as berberine in *B. vulgaris* (7) and volatile terpenes in *T. begoniifolia* (4). Conversely, the floral and aromatic character of *Citrus aurantium* and *Ziziphus jujuba* honeys is consistent with volatile markers like methyl anthranilate and limonene derivatives (2, 5, 6, 9). Kruskal–Wallis results confirmed significant differences among botanical origins, particularly in bitterness, floral, and sweetness. The high sweetness of *Robinia pseudoacacia* and *Astragalus* spp. (8.40 and 7.80) reflects their high fructose-to-glucose ratio and low acidity, typical of these honeys (10).

Radar plots and heat map clustering (Supplementary Figures S2, S3) corroborated the PCA findings. *B. vulgaris* and *T. begoniifolia* clustered based on bitterness and vegetal notes, while *C. aurantium* and *Z. jujuba* grouped by floral and aromatic characteristics. Multifloral honeys showed

broader profiles, reflecting diverse floral sources. Intra-botanical variability, observed for *Z. jujuba* (samples A vs. E) and *C. sativum* (H vs. Q), suggests that geographical origin and harvesting time influence sensory expression, likely due to variations in climate, soil, or flowering phenology (9).

PERMANOVA confirmed a significant effect of botanical origin ( $p = 0.001$ ,  $R^2 = 0.62$ ), indicating that sensory profiling can effectively differentiate honey samples. The moderate explained variance of PCA (42.45% for PC1–PC2) is typical for sensory data, where variability arises from panelist differences and sample heterogeneity; four PCs explained 73.76% of total variance, suggesting additional dimensions contribute to differentiation (15).

Direct comparisons with previous studies are limited by differences in botanical coverage and geography (9, 15), but the observed trends—floral honeys with higher aroma intensity and darker honeys with stronger bitterness—are consistent with reports for citrus and jujube honeys from other regions (2, 4, 9). Minor variations may reflect differences in local varieties or evaluation protocols. For example, European citrus honeys have been reported to exhibit higher floral intensity compared to Iranian citrus honeys, possibly due to differences in citrus varieties or climatic conditions (4).

The heat map and hierarchical clustering analysis provided additional insights into the grouping of samples based on their sensory profiles. The distinct clustering of *Berberis vulgaris* and *Tilia begoniifolia* honeys, characterized by high bitterness and vegetal notes, suggests that these honeys share similar sensory characteristics despite their different botanical origins. This clustering may reflect similarities in phenolic composition or volatile profiles rather than botanical origin alone. Conversely, the clustering of *Citrus aurantium* and *Ziziphus jujuba* honeys based on high floral and aromatic notes indicates that these honeys share aroma-related sensory characteristics, likely due to the presence of similar volatile compounds.

The moderate explained variance of the PCA (42.45% for PC1 and PC2) warrants discussion. While this value is lower than typically observed in physicochemical datasets, it is well within the expected range for descriptive sensory analysis, where substantial variability is inherent due to panelist differences, sample heterogeneity, and the multidimensional nature of sensory perception (15). The first four principal components explained 73.76% of the total variance, indicating that additional dimensions

contribute to sensory differentiation. Future studies incorporating larger sample sizes and additional sensory attributes may improve the explanatory power of PCA.

A limitation of the present study is the relatively small number of samples for certain botanical origins (*Thymus daenensis*, n=1; *Oliveria decumbens*, n=1; *Foeniculum vulgare*, n=1), which may restrict the generalization of the observed sensory patterns. Additionally, the KMO value of 0.579, while acceptable, indicates that the dataset is not ideally suited for PCA, suggesting that future studies should include more samples per botanical origin to improve sampling adequacy. The presence of impurities or air bubbles in several samples may have influenced visual and textural perceptions, although panelists were instructed to focus on aroma, flavor, and taste attributes rather than visual appearance. Future studies should standardize sample preparation to minimize these confounders.

The findings of this study have several practical implications for the Iranian honey industry. First, the sensory reference data generated for 10 distinct botanical origins can be used by beekeepers and honey processors for quality control and product differentiation. Second, the identification of characteristic sensory fingerprints for each botanical origin can support authenticity assessment and fraud detection, particularly for high-value monofloral honeys. Third, the integration of sensory profiling with physicochemical and melissopalynological analyses can enhance the robustness of honey authentication frameworks. Finally, the standardized IHC protocol used in this study facilitates cross-study and cross-country comparisons, contributing to the global harmonization of honey sensory data.

## Conclusion

This study provides the first standardized sensory reference database for Iranian monofloral and multifloral honeys using the IHC protocol. Our findings demonstrate that:

- 1- Botanical origin significantly influences sensory profiles (PERMANOVA:  $p = 0.001$ ), with taste-related (PC1) and aroma-related (PC2) dimensions explaining 42.45% of variance.
- 2- Specific sensory fingerprints were identified for key honeys, including high bitterness and warmth in *B. vulgaris* and *T. begoniifolia*, high floral intensity and olfactory notes in *C. aurantium* and *Z. jujuba*, and high sweetness in *R. pseudoacacia* and *Astragalus spp.*
- 3- Intra-botanical variability was observed, highlighting the influence of geographical origin,

harvesting time, and environmental conditions on sensory expression.

4- Multivariate statistical tools (PCA, PERMANOVA, hierarchical clustering) effectively discriminated honey samples based on their sensory profiles.

Practical implications: Sensory profiling, when combined with physicochemical and melissopalynological analyses, can serve as a cost-effective, rapid, and non-destructive tool for honey authenticity assessment. The generated dataset can be used by Iranian beekeepers, quality control laboratories, and regulatory bodies for product differentiation and fraud detection.

Limitations: The relatively small number of samples for certain botanical origins (n=1 for *T. daenensis*, *O. decumbens*, *F. vulgare*) and the moderate KMO value (0.579) suggest that the dataset is not ideally suited for PCA, and results should be interpreted with caution. The presence of impurities in several samples may have influenced sensory perceptions.

Future directions: Studies with larger sample sizes ( $\geq 5$  per botanical origin) and integrated chemical analyses (volatile profiling, phenolic composition) are recommended to validate the observed sensory patterns and establish robust authentication models. Additionally, consumer acceptance studies could explore the relationship between sensory profiles and consumer preferences to support marketing strategies for Iranian honeys. Future studies integrating sensory, colorimetric, and chemical data may further improve the predictive power of sensory profiling for honey quality assessment.

## Declaration

### Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

### Author Contributions

All authors contributed to the study conception and design, data collection and analysis, interpretation of the results, and preparation and revision of the manuscript. All authors have read and approved the final version of the manuscript.

### Ethical Considerations

This study did not involve human participants or experimental procedures involving live animals. Therefore, ethical approval was not required.

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### **Conflict of Interest**

The authors declare that there is no conflict of interest regarding the publication of this article.

### **Data Availability**

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

### **AI Statement**

The authors declare that no artificial intelligence tools were used in the preparation, analysis, or writing of this manuscript.

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