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Physicochemical and ecological profiling of Iranian Sidr (Ziziphus spp.) honey: A chemometric approach toward authenticity and medical-grade standardization

Akbar Nemati Parashkouh
Shahid Beheshti University
Ahmad Reza Mehrabian
mehrabian.pe@gmail.com
Shahid Beheshti University
Parastoo Saniee
Shahid Beheshti University
Mohammad Reza Faghihi
Shahid Beheshti University

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Physicochemical and ecological profiling of Iranian Sidr (*Ziziphus* spp.) honey: A chemometric approach toward authenticity and medical-grade standardization

Nemati Parashkouh, Akbar[1]., Mehrabian, Ahmad Reza [1,3]., Saniee, Parastoo [1,3]., Faghihi, Mohammad Reza [3]

1*Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran*

2 Faculty of Mathematical Sciences, Shahid Beheshti University, Tehran, Iran

3 Bee Products Research Centre, Shahid Beheshti University, Tehran, Iran

*Corresponding author: Ahmadreza Mehrabian 19839-63113, Iran Tel: +98 912 344 7544

Email :a_mehrabian@sbu.ac.ir

Abstract

Monofloral honeys, particularly Iranian Sidr honey, are highly valued for their unique sensory and medicinal properties; however, they are also prone to fraud. To address this issue, it is essential to create reference databases documenting the physicochemical and pollinological profiles of these honeys. This study analyzed Sidr honey samples from various regions in Iran to document their physicochemical and pollinological characteristics and to understand how ecological factors impact these properties. The analysis of 25 honey samples revealed that pollen from *Ziziphus* spp. was predominant, indicating a consistent botanical origin. A significant relationship was found between sugar composition, pH levels, and environmental factors, all of which considerably affect honey quality. Principal Component Analysis (PCA) showed that the first two components explained 50.6% of the total variance, highlighting distinct differences in physicochemical and ecological traits. In the first principal component (PCA1), strong positive correlations were identified among erlose, sucrose, pH, rainfall, and altitude. The second component indicated positive relationships between diastatic activity, phenolic compounds, moisture content, and the fructose-to-glucose ratio. Both PCA and hierarchical cluster analysis revealed that honey samples from the same counties did not cluster together, suggesting that environmental factors create unique physicochemical profiles, influencing both the quantity and quality of honey. These findings could enhance the authenticity and quality control of Iranian Sidr honey, improving its commercial value, competitiveness in international markets, and potential for grading.

Keywords ziziphus spp., physicochemical properties, melissopalynology, ecological factors, chemometrics, principal component analysis, medical-grade honey, authenticity, standardization

1. Introduction

The global honey market has expanded significantly in recent years, driven by growing consumer awareness of honey's nutritional and medicinal properties, rising demand for natural and organic products, and its diverse applications in the food and beverage industry. The global honey trade was valued at USD 3.9 billion in 2023, with projections reaching USD 10.3 billion in 2025, USD 10.8 billion in 2026, and USD 11.4 billion in 2027. This rapid growth has unfortunately incentivized widespread honey adulteration [1]. Monofloral honeys, highly esteemed for their distinctive aromatic and sensory characteristics, are particularly vulnerable to fraud. Their botanical origin plays a critical role in determining authenticity, quality, and market value. Iran, producing approximately 129 thousand tons of honey annually, is ranked as the third largest honey-producing country worldwide [2]. The country's diverse climate and rich floral biodiversity support the production of numerous monofloral and polyfloral honeys, including Sidr, thyme, and astragalus varieties. According to national agricultural data, Iran produced approximately 3.5 thousand tons of honey annually as of 2012, with production continuing to grow due to increasing domestic demand and improved beekeeping practices [2]. Iran's climatic diversity, ecological isolation, complex geological history [3], and unique soils [4], combined with its connection to multiple phytogeographical zones, make it a global center of plant diversity [5, 6]. The country hosts about 9,500 plant taxa [7].These ecological and botanical resources, together with high purity, organic production conditions, medicinal properties, and distinctive taste, have made Iranian honey particularly Sidr honey one of the most attractive varieties worldwide. Sidr honey, a tropical monofloral honey produced in southern provinces such as Khuzistan, Hormozgan, and Bushehr, is especially valued for its medicinal properties and high global price. However, its commercial significance also makes it susceptible to adulteration, often through blending with lower-quality polyfloral honeys. Authentication methods have traditionally relied on melissopalynology[8], phytochemical profiling [9-11], and integrated physicochemical and mineral analyses [12-15], often supported by ordination and discriminant statistical methods [9, 16]. One of the most important steps in preventing fraud is the creation of reference databases for

each honey type from its geographical regions of production. Without region-specific sampling, it is impossible to establish reliable physicochemical and pollinological profiles, which are essential for verifying authenticity. Representative samples serve as the foundation for such databases, enabling researchers to detect adulteration and classify honey types {Nowak, 2023 #70]. Accordingly, ecological representatives must be evaluated using physicochemical, melissopalynological, phytochemical, organoleptic, and toxicological methods to prepare comprehensive biological data banks. These databases provide variation ranges for each parameter, supporting both authentication and grading, and contribute to the global puzzle of honey biological information. Given the increasing demand for high-quality and traceable monofloral honeys, it is essential to conduct a thorough evaluation of the physicochemical and pollinological characteristics of ecological representatives of Iranian Sidr honey (*Ziziphus spina-christii*, *Z. numularia*). These assessments serve as critical references for verifying authenticity and distinguishing Sidr honey from adulterated or blended products. Despite its commercial and medicinal significance, Iranian Sidr honey has not been systematically studied across its diverse ecological zones. Therefore, the present study aims to analyze the physicochemical properties and pollen profiles of Sidr honey samples collected from various regions in Iran. The findings will contribute to the development of a regional reference database, supporting both authentication and grading of this valuable monofloral honey.

2. Materials and methods

2.1 Study area

The study area covers the tropical lowlands of southern Iran, a region characterized by diverse soils, complex geology, and distinctive climatic and geomorphological features. Southern Iran lies within the Zagros fold-thrust belt, part of the Arabian Plate margin. The area is dominated by thick sequences of sedimentary rocks (7–14 km), including limestone, marl, and sandstone, which provide the parent material for soil formation [17]. Soils in these tropical zones are typically calcareous, alluvial, and shallow, with variable fertility depending on geomorphic position. In coastal plains, Entisols and Inceptisols are common, while upland areas show more developed soils influenced by slope and erosion [18]. The region experiences a tropical to subtropical climate, with high summer temperatures often exceeding 40 °C and mild winters. Rainfall is seasonal and irregular, concentrated in winter and early spring, averaging 200–400 mm annually . High evapotranspiration rates contribute to soil salinity and limit moisture availability, shaping vegetation and agricultural potential. Geomorphologically, southern Iran is marked by coastal plains, river terraces, and foothills of the Zagros Mountains. Active tectonics and erosion processes have produced alluvial fans, karstic features, and dissected plateaus [18]. These landforms strongly influence soil distribution and water retention. Altitudinal gradients from coastal lowlands to upland valleys create ecological niches that affect honey production, vegetation, and land use. In addition, prominent vegetation types the area including Mangroves in tidal zones, crucial for

coastal ecology, Halophytes (salt-tolerant plants), Shrubs and grasses and *Prosopis juliflora*, *Acacia*, and drought-resistant grasses cover arid plains.

2.2 Sampling Methods

Twenty-five honey samples were collected from different regions of Iran during October–November 2021(Fig 1).

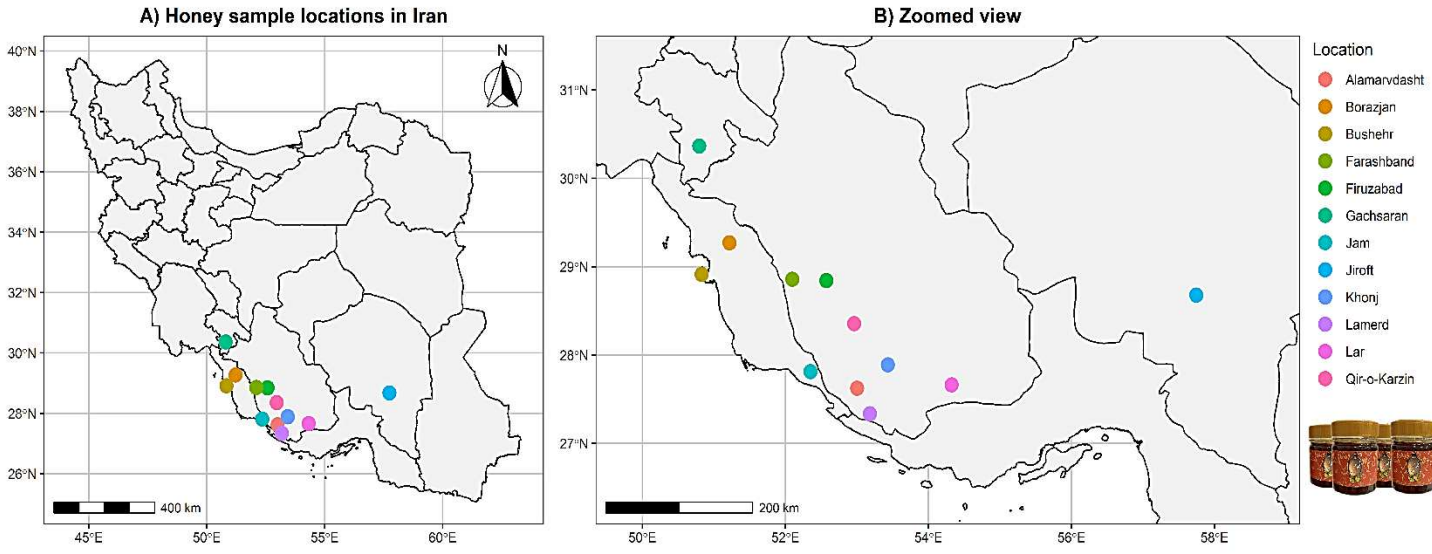


Fig. 1 Map of Iran showing the collection sites for the Sidr honey samples studied

2.3 Physicochemical and Biochemical Analyses

The physicochemical and biochemical properties of honey samples were determined using validated analytical procedures routinely applied for honey characterization and quality assessment. All analyses were conducted under controlled laboratory conditions using calibrated instruments. Moisture content was measured using a digital refractometer (OPTIKA, Italy), pH was determined with a calibrated pH meter (AZ 86502, Taiwan), spectrophotometric analyses were performed using a UV–Visible spectrophotometer (Bio-Rad 3000, USA), and sugar composition was analyzed using a high-performance liquid chromatography (HPLC) system equipped with a refractive index detector (RID) (Agilent 1100, USA). All chemicals and reagents were of analytical grade. Certified sugar standards, including fructose, glucose, sucrose, maltose, isomaltose, turanose, nigerose, erlose, and melezitose, as well as other, analytical standards, and solvents used throughout the study were supplied by Merck (Darmstadt, Germany) and were of analytical or HPLC grade as appropriate.

Moisture content, pH, diastase activity, and hydroxymethylfurfural (HMF) content were determined according to internationally accepted methods for honey quality evaluation. Moisture content was measured by refractometry, pH was determined potentiometrically, diastase activity was expressed as diastase number (DN), and HMF concentration was quantified spectrophotometrically following established procedures for honey characterization [19, 20].

Proline content was determined using a ninhydrin-based spectrophotometric method. Honey solutions were reacted with ninhydrin reagent under controlled heating conditions to form a colored complex, and absorbance was subsequently measured using a UV–Visible spectrophotometer. Proline concentration was calculated from calibration standards and expressed as mg kg^{-1} honey. Proline content was used as an indicator of honey maturity and authenticity [19].

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric assay. Gallic acid (Merck, Darmstadt, Germany) was used as the calibration standard, and the results were expressed as milligrams of gallic acid equivalents (GAE) per kilogram of honey. The analysis was performed according to the method described by Singleton refined [21].

The carbohydrate profile, including fructose, glucose, sucrose, maltose, isomaltose, turanose, nigerose, erlose, and melezitose, was determined using HPLC-RID. Individual sugars were identified by comparing their retention times with those of certified reference standards obtained from Merck (Darmstadt, Germany) and quantified using external calibration curves. Sugar concentrations were expressed as g 100 g^{-1} honey. Chromatographic analyses were performed following internationally recognized procedures for honey carbohydrate characterization [19, 20].

All measurements were performed in triplicate. Appropriate quality assurance and quality control procedures were applied throughout the analytical process to ensure the accuracy, precision, and reproducibility of the results.

2.4 Melissopalynological Analysis

To investigate the pollen composition of honey samples, a melissopalynological method based on scanning electron microscopy (SEM) was applied, following the protocol described by Louveaux et al. and the standard method recommended by the International Commission for Bee Botany (ICBB)[22].

In this procedure, 10 g of honey was first dissolved in approximately 20 mL of distilled water. Dissolution was carried out on a hot plate at a temperature below 40 °C to prevent damage to pollen grains. The resulting solution was then filtered to remove coarse honey wax particles, and the filtrate was transferred into 30 mL conical centrifuge tubes.

The samples were centrifuged in several steps at room temperature (21–25 °C) at a speed of 2500–3000 rpm. In the first step, centrifugation was performed for 10 minutes, after which the supernatant was carefully discarded. This process was repeated until a suitable pollen sediment was obtained. Finally, the collected pollen sediment was used for further analyses.

After preparation, the extracted pollen grains were examined and identified using scanning electron microscopy (SEM). Pollen identification was performed based on morphological characteristics such as shape, size, aperture type, and surface ornamentation, and by comparison with standard palynological reference materials.

2.5 Statistical Analysis

All physicochemical and ecological parameters were analyzed and presented as mean ± standard deviation (SD). The normality of the data was assessed using the Shapiro-Wilk test prior to conducting any statistical analyses. Variables that exhibited skewed distributions, such as HMF, the F/G ratio, sucrose, and melezitose, were log-transformed to better approximate normality[[23]. Additionally, moisture and temperature, which displayed moderate skewness, were square-root transformed [23]. To evaluate the relationships between the physicochemical and ecological properties of the 25 honey samples, Pearson's correlation coefficients (r) were calculated at a 99% confidence level ($p \leq 0.01$). This analysis identified significant correlations among key physicochemical and ecological variables. Principal Component Analysis (PCA) was performed on mean-centered and scaled Sidr honey data to reduce dimensionality and explore physicochemical and ecological patterns. Using the *factoMineR* and *factoextra* packages in R, we obtained sample scores, variable loadings, and variance percentages for each principal component. Visualization was enhanced with biplots from *factoextra* and *ggplot2*, which included sample labels and color coding based on variable importance (cos^2) to facilitate the interpretation of sample clustering and correlations. Finally, hierarchical clustering of the Sidr honey samples was conducted using Ward's method (ward. D2) on the PCA-transformed data. The dendrogram featured color-coded branches to clearly illustrate the relationships among the samples.

3. Results and Discussion

3. Results and Discussion

3.1 Melissopalynological characteristics of honey samples

The results of the melissopalynological analysis of the 25 honey samples are presented in Fig. 1. In all samples, pollen grains belonging to the family Rhamnaceae (Ziziphus spp.) were identified as predominant pollen (PP; >45%), indicating a uniform and consistent botanical origin. This persistent dominance of Ziziphus pollen across all samples clearly supports the classification of the investigated honeys as monofloral Ziziphus honey, despite variations in the composition and abundance of accompanying pollen types. Non-predominant pollen taxa mainly belonged to families such as Amaranthaceae, Fabaceae, Resedaceae, Asteraceae, Poaceae, and several other minor families. Depending on the sample, these pollen types were recorded as secondary pollen (SP), important minor pollen (IP), or minor pollen (MP). None of the accompanying pollen taxa reached the level of predominance. Their generally low to moderate frequencies and irregular distribution among samples indicate incidental or secondary pollen input during foraging activity rather than a contribution of multiple nectar sources. In several samples, particularly those exhibiting higher total pollen counts, an increased number of IP and MP taxa was observed. However, even in these samples, Ziziphus pollen remained clearly dominant, and the overall pollen spectra continued to conform to the criteria for monofloral honey as defined by Louveaux et al. (1978) [22]. The elevated total pollen concentrations in these samples therefore reflect a higher intensity of pollen incorporation from the dominant floral source rather than an increase in botanical diversity of nectar sources. Overall, the melissopalynological data demonstrate that all 25 honey samples share a common and dominant botanical origin associated with Ziziphus spp. The findings align with Alsharhi's 2021 study, which indicated that Sidr honey from Shabwah, Yemen, is classified as monofloral and has a high concentration of Sidr pollen grains [24]. Additionally, a study of 794 Sidr honey samples collected from the Saudi market, which had been imported from 12 different countries, showed that monofloral honey derived from the nectar of Ziziphus spp. across various geographical origins contained more than 70% of a specific type of pollen [25]. This global perspective is further supported by the extensive characterization work of Hegazi et al. (2022) [25], who confirmed that despite minor regional variations across 12 countries, genuine Sidr honeys maintain a distinct palynological threshold of Ziziphus dominance, verifying their botanical authenticity. The presence of accompanying pollen types does not alter their classification as monofloral honey and is interpreted as the result of secondary pollen introduction. These findings are fully consistent with the physicochemical analyses and collectively confirm the monofloral nature of the investigated Ziziphus honeys (Fig. 2).

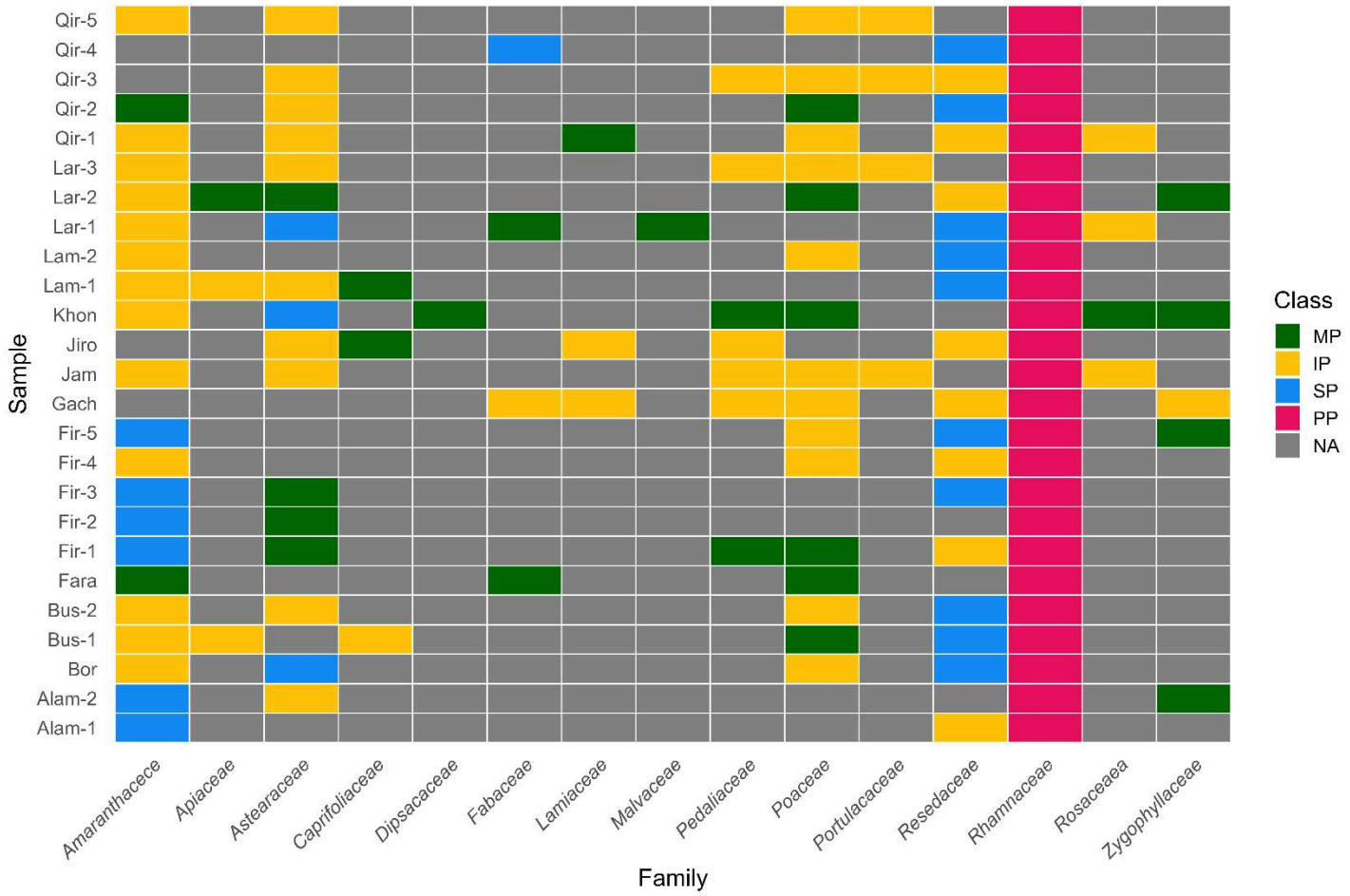


Fig. 2 Quantitative evaluation of honey pollen. PP = predominant pollen grains (>45% of total); SP = secondary pollen grains (16–45% of total); IP = important minor pollen (3–15% of total); and MP = minor pollen grains (<3% of total). Louveaux et al. (1978)

.2 Descriptive statistics of the physicochemical and ecological parameters

Descriptive statistics of the physicochemical and ecological parameters measured in Iranian Sidr honey samples are presented in Table 1. HMF, sucrose, and melezitose displayed greater variability among samples, while moisture content and the F/G ratio were relatively consistent. A study by Zhuk et al. (2022) on honey samples from various regions of Ukraine reveals variability in HMF, melezitose, and other components, while indicating relative stability in moisture content and the fructose-to-glucose ratio[26]. Recent studies of honey samples from different regions, including Saudi Arabia and Kuwait, have yielded similar results [27, 28]. However, research conducted by El-Meihy (2025) shows that while the ratio of fructose to glucose remained relatively stable, the levels of hydroxymethylfurfural (HMF) were also consistently stable, which contrasts with the findings of our study [29]. This variability in quality parameters across different environmental settings is a common feature in geographical profiling. As Tomczyk et al. (2019) demonstrated for central European honeys (Polish and Slovak), local microclimates can induce

notable fluctuations in minor carbohydrate fractions and enzyme profiles, which supports the variations in sucrose and HMF levels observed within our Iranian samples[30].

Table 1. Physicochemical and ecological parameters of 25 Iranian Sidr honey samples

Parameter(unit)	Abbreviation	Mean ± SD	Min	Max
Altitude (m)	Alt	708.92 ± 384.85	80	1320
Diastatic	Dias	11.76 ± 3.08	5.07	18.56
Erlose (%)	Erl	3.67 ± 1.18	1.7	5.7
F/G ratio	FG	1.39 ± 0.09	1.1	1.5
Fructose (%)	Fruc	34.09 ± 2.46	28.4	38
Glucose (%)	Glu	24.6 ± 2.1	21	28.1
HMF (mg/kg)	HMF	2.52 ± 4.78	0.15	21.11
Isomaltose (%)	Iso	1.62 ± 0.38	0.9	2.3
Maltose (%)	Malt	1.33 ± 0.27	0.9	1.9
Melezitose (%)	Mele	0.63 ± 0.54	0	2.2
Moisture (%)	Mois	16.13 ± 0.67	14.2	16.8
pH	Ph	5.71 ± 0.54	4.52	6.44
Phenolic content (%)	Phen	1.04 ± 0.27	0.6	1.53
Proline (mg/kg)	Pro	426.43 ± 111.27	231.34	662.67
Rainfall (mm)	Rain	279.73 ± 75.31	123	445.5
Sucrose (%)	Sucr	4.61 ± 4.33	0.5	15.6
Temperature (°)	Tem	24.17 ± 2.1	20.7	27.2
Turanose (%)	tura	2.24 ± 0.42	1.2	2.9

3.3 Descriptive statistics of physicochemical and environmental parameters

3.3.1 Chemical properties

Carbohydrate content

Honey is composed of 82% carbohydrates, primarily fructose and glucose, which together account for about 70-75% of its total volume. In Iranian honey samples, the average fructose content is 34.09 ± 2.46 g/100 g, ranging from 28.4 g/100 g in sample Fir-5 to 38 g/100 g in sample Lam-1

(Supplementary 1 and Fig. 3). The average glucose content in these samples is 24.6 ± 2.1 g/100 g, with a minimum of 21 g/100 g in sample Fir-5 and a maximum of 28.1 g/100 g in sample Lam-1. Sample Lam-1 exhibited the closest values to the natural concentrations of these two monosaccharides, while sample Fir-5 showed the lowest values. The sugar content of honey is vital for evaluating its authenticity and quality [31]. Adulteration with cheaper syrups is common, making sugar analysis essential to determine if honeybees were fed natural nectar or sugar solutions [32]. A higher glucose level than fructose suggests artificial feeding [33]]. A higher glucose content in honey indicates a tendency for quicker crystallization, while higher fructose content suggests slower crystallization. Ideally, the fructose-to-glucose ratio should fall between 0.9 and 1.35 [34]. For Iranian honey samples, the average fructose-to-glucose ratio is 1.39 ± 0.09. The highest ratio of 1.5 is found in samples Alam-1, Qir-3, Gach, Fir-2, Lar-3, and Lar-2, whereas sample Fara has the lowest ratio (Supplementary 1). Fructose generally appears in higher proportions than glucose in honey, though concentrations vary by origin. The fructose and glucose levels in all honey samples studied were within the standard normal range, confirming their authenticity and high quality. This finding aligns with the authenticity standards noted by [35-38]. These carbohydrate baselines are consistent with the findings of [39] who evaluated national and international honeys in Saudi Arabia, noting that authentic Ziziphus honeys generally display higher fructose-to-glucose ratios compared to other floral types, serving as an indicator of quality.
The average sucrose content was 4.61 ± 4.33 percent. Some samples, such as Fir-3, Fir-5, and Qir-4, exhibited the highest sucrose levels, with values of 15.6, 15, and 11.4 percent, respectively, all of which are above the normal range. In contrast, the samples Bus-2, Khon, and Fara contained only 0.5 percent sucrose, which is below the normal range. A study by AlFarsi et al. (2018) revealed considerable variation in sucrose levels in Sidr honey from Oman, ranging from 0.1% to 17.5% [40]. Besharati et al. (2025) similarly noted that while the average sucrose content in some areas of East Azerbaijan Province met Codex standards, elevated levels were found in specific regions of the province [41]. Although sucrose is not a primary component of honey, its presence can indicate potential adulteration and may provide insights into the honey's botanical origin [32]. High sucrose levels in samples could suggest that bees were fed concentrated sucrose solutions, which may have prevented them from collecting nectar [42]. Alternatively, it could indicate the direct addition of commercial sugar syrup to the honey [43, 44]. The variation in sucrose content among honey samples can be attributed to the enzyme invertase, which breaks down sucrose into glucose and fructose during the honey ripening process [45, 46]. Furthermore, factors such as harvesting methods, handling procedures, and the types of flora available can also contribute to differences in sucrose levels [47, 48]. The sugar profile, particularly the oligosaccharides, plays a crucial role in determining the quality and authenticity of honey [49]. This includes the ability to identify syrup adulteration [50], distinguish between honeydew and blossom [51], and recognize the botanical origins of honey [52]. In this study, other carbohydrates such as erlose, isomaltose, melezitose, maltose, and turanose were found to be within the normal range for honey, indicating a natural sugar composition. In general, as illustrated in Fig. 3, different honey samples exhibit unique sugar content profiles. This suggests that multivariate profiling of these sugar configurations can reliably differentiate geographic origins, matching the chemometric approach used by Roshan et al. (2016) to successfully discriminate the floral origin of Middle Eastern Ziziphus honeys from other local varieties [53].

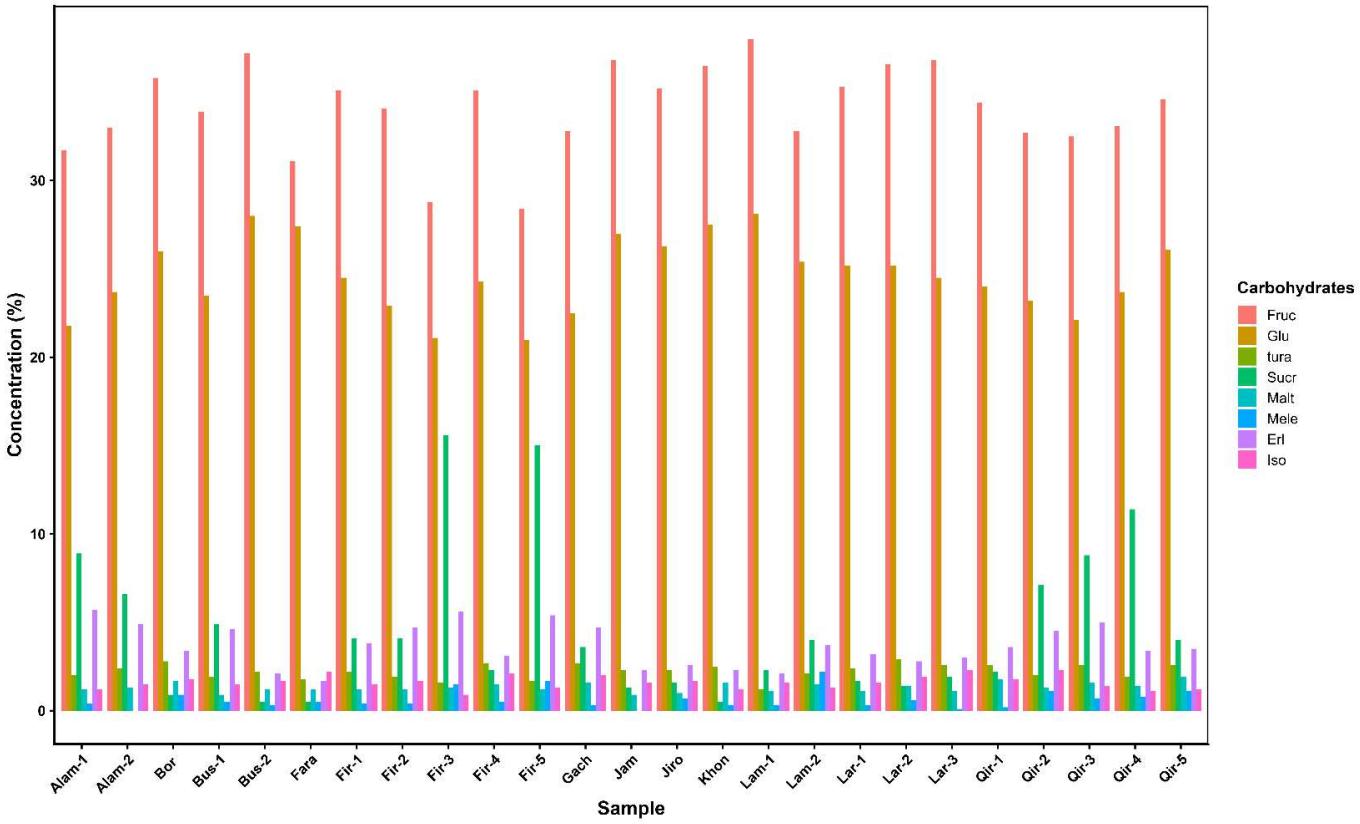


Fig. 3 Carbohydrate content of Iranian Sidr honey samples

3.3.2 pH and acidity

pH is a parameter that indirectly influences the shelf life and stability of bee pollen [24]. It is affected by factors such as storage duration, adulteration with sugar, and botanical origin[54]. The pH measurements ranged from 4.52 (in Bus-2) to 6.44 (in Alam-1), with an average of 5.71 ± 0.54. These results confirm the acidic nature of bee pollen (Table 1, Supplementary 1).

3.3.3 Physical properties

Moisture content is a crucial physical property for determining the shelf life of honey [27, 55]. Higher moisture levels can accelerate fermentation due to the activity of osmotolerant yeast, which can affect the storage stability and flavor of honey [48, 56]. The honey directive 2001/110/EC (Council of the European Union, 2001) specifies that moisture should not exceed 20 grams per 100 grams. In various honey samples, moisture content ranged from 14.2 g/100 g in honey Qir-5 to 16.8 g/100 g in honey Bus-1, with a mean of 16.13 ± 0.67 (Table 1, Supplementary 1). These values are below the limit, indicating good stability and a low risk of fermentation. Moisture levels are influenced by geographical and environmental conditions, including temperature and relative humidity during honey production, as well as factors such as the honey's maturity level in the hive, nectar composition, harvest time, processing methods, storage conditions, and apiary management [39]. Al-Madani et al. (2026) study found that the moisture content of Kuwaiti cedar honey is lower than the maximum permissible moisture content set by the standard amount, supporting the

stability and high quality of locally produced honey [27]. Additionally, several studies have reported the normal moisture content of other monofloral and polyfloral honeys [28, 33].3.3.4 Parameters related to honey quality and freshness

3.3.4 Parameters related to honey quality and freshness

The concentration of HMF (hydroxymethylfurfural) is a crucial indicator of honey quality and freshness [19]. Fresh honey typically contains little to no HMF. However, its concentration increases during processing and as honey ages. According to the Codex Alimentarius, the permissible limit for HMF is a maximum of 40 mg/kg. In this study, most samples fell below this limit, indicating that they were fresh and had not been subjected to high heat or prolonged storage. The mean HMF content observed was 2.52 ± 4.78 mg/kg, with values ranging from 0.15 mg/kg in sample Lar-3 to 21.11 mg/kg in sample Qir-3. These results suggest that most samples were fresh, with sample Lar-3 being the freshest and of the highest quality among those studied (Fig. 4, Supplementary 1). Our HMF content, in accordance with international standards, closely resembles that reported for various honey types around the world. This includes honey brands distributed in Tehran City, Iran [25], as well as multiflower, jujube, and carob honeys from the Fez-Meknes region in Morocco [27], and Sidr honey samples from Egypt and Saudi Arabia [28].

Diastase enzyme activity is another important indicator of honey quality [29]. According to the International Honey Commission, diastase activity should be greater than or equal to 8 [31]. In this study, the mean diastase activity was 11.76 ± 3.08, ranging from 5.07 in sample Qir-4 to 18.56 in sample Gach. Most samples demonstrated acceptable diastase activity values, indicating that they were natural honeys (Fig. 4Supplementary 1). In 2023, Mohsen et al. demonstrated that the Diastase enzyme levels in Sidr honeys from the Saudi Arabian region fell within the international standard range [32]. Additionally, Ali and colleagues found that southern Saudi Sidr honey exhibited greater diastase enzyme activity compared to other tested varieties, including Anab Sidr honey, Pakistani (Kashmiri) Sidr honey, and Yemeni Sidr honey [33].

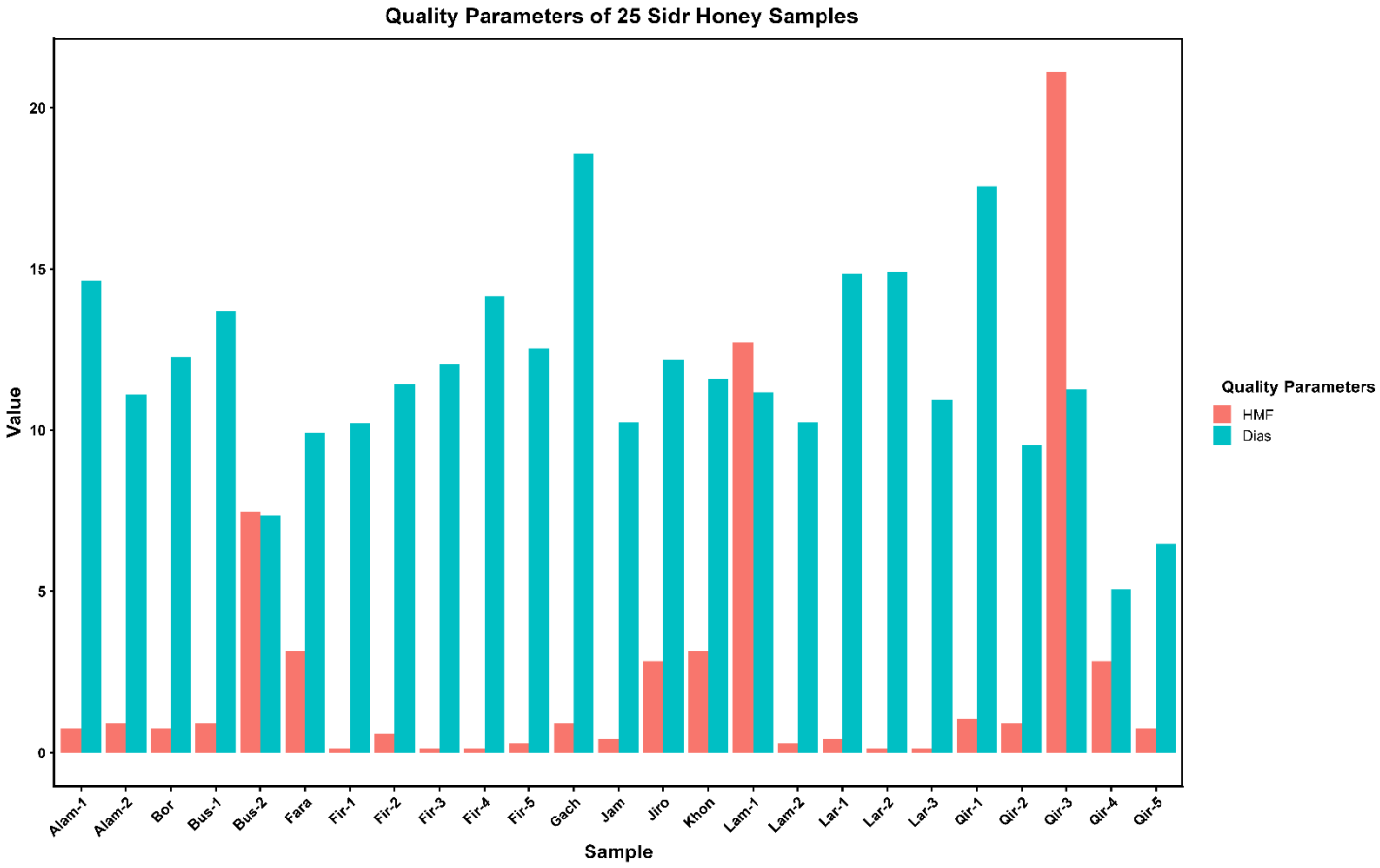


Fig. 4 Honey quality parameters of Iranian Sidr honey samples

3.3.5 Parameters related to biological quality

Proline is the primary free amino acid in honeys, useful for determining the botanical source and indicating proper pollen ripening [57]. Its levels decrease with added artificial sugars, ranging from 231.34 to 662.67 mg/kg, with an average of 426.43 ± 111.27 (Table 1, Supplementary 1). Honeys from Lam-1 showed higher proline contents compared to others analyzed. The acceptable standard level for proline in honey is over 180 mg/kg, according to the Iranian Standard Organization (ISO No. 92, 2013). The mean proline value for 25 samples of Iranian Sidr honey is higher than the values reported by Afshari et al. (2022), who found an average of 316.1 mg/kg among 25 different honey brands available in Khorasan Province [58]. Additionally, Parviz et al. (2014) reported an average of 20.96 ± 11.6 mg/kg for 60 samples of Iranian honey from 10 different provinces [59]. Similarly, the proline content of honeys from Para was found to be higher than that of Tunisian honey [60, 61]. Proline serves as an indicator of honey ripening and potential adulteration. It is also used to identify floral sources of honey [57]. However, proline alone cannot be used as the sole criterion for comparing the quality of honey from different regions, as each area has its own environmental conditions such as varying climates, topography, and vegetation which all influence the rate of proline production. The total phenolic content in honey holds significant potential for

health-oriented products due to its antioxidant, anti-inflammatory, antimicrobial, antihypertensive, anticancer, and antiatherosclerotic effects [55]. In this study, the phenolic content ranged from 0.6 g/100 g in sample Qir-4 to 1.53 g/100 g in sample Alam-1, with a mean value of 1.04 ± 0.27 g/100 g. This high phenolic load positions Iranian Sidr honey as a candidate for medical applications. According to the refined standards outlined by Peters et al. (2025), medical-grade honey (MGH) requires strict parameters of physicochemical consistency and bioactive standardization. The raw phenolic content and high purity observed in our samples suggest that with proper standardization, Iranian Sidr honey could meet these clinical benchmarks [62].

3.4 Ecological factors

This study reveals that honey samples were collected from a diverse range of altitudes, spanning from 80 to 1,320 meters, with an average altitude of 708.92 ± 384.85 meters. The sample labeled Bus-2 was collected from the lowest altitude (80 meters), while samples Fir-1 to Fir-3 were taken from higher altitudes at 1,320 meters (Fig. 5, Supplementary 1). The average annual rainfall in the study areas was 279.73 ± 75.31 mm, ranging from 123 to 445.5 mm (Fig. 4, Supplementary 1). The highest rainfall was recorded in the Gach (Gachsaran) sample area, while the lowest was from Jiro (Jiroft). Gachsaran, located in western Iran, receives relatively high annual rainfall compared to the southern regions of the country. The average temperature across the sampling areas was 24.17 ± 2.1 degrees Celsius, with variations between 20.7 and 27.2 degrees (Fig. 5, Supplementary 1). Notably, the highest temperature was associated with the Jam sample, while the lowest was related to the Fir-4 sample. Environmental conditions, such as climate and altitude, play a crucial role in honey production rates and physicochemical properties [63].

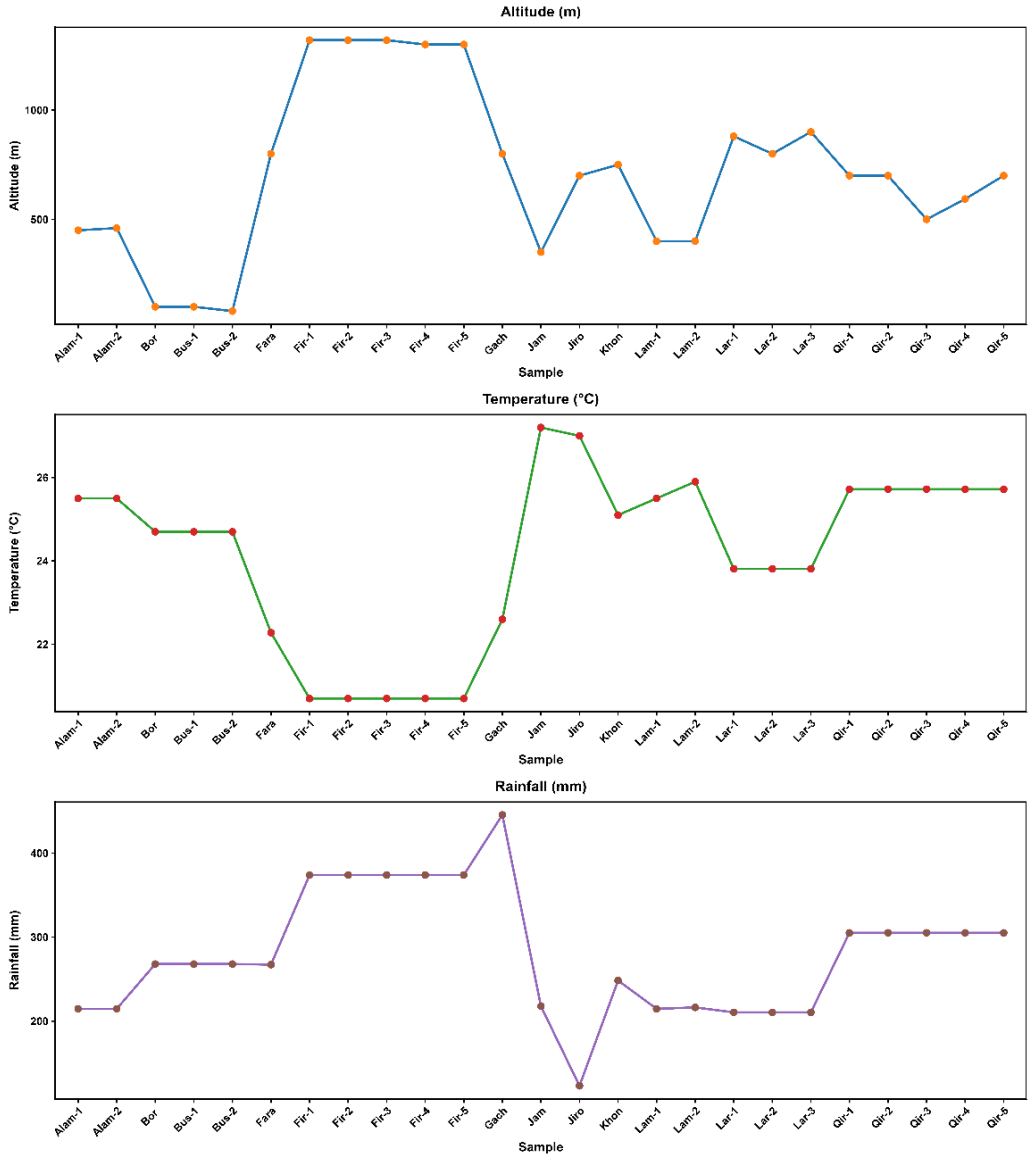


Fig. 5 Environmental factors of Iranian Sidr honey samples

3.5 Statistical analysis

The study investigated the relationships among 15 physicochemical parameters and 3 ecological parameters in 25 Sidr honey samples from Iran (Fig. 6) .

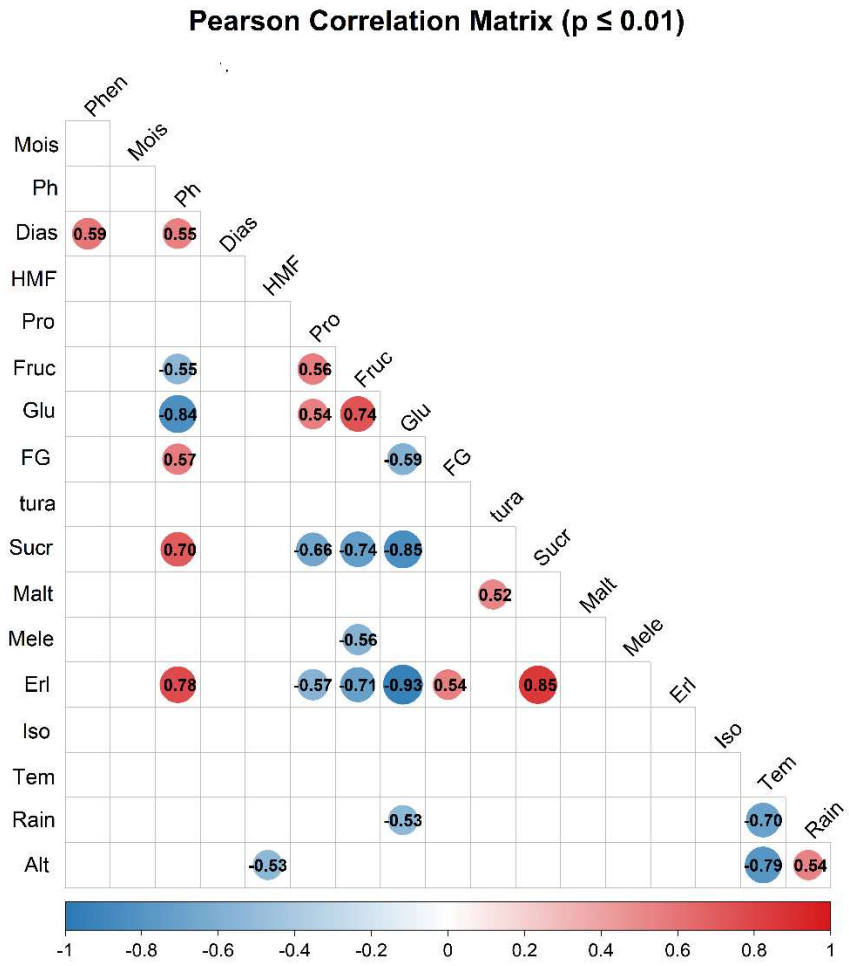


Fig 6. Correlogram showing the Pearson correlation coefficients (p≤0.01) for physicochemical and ecological parameters in 25 Sidr honey samples from Iran.

Several significant correlations were found ($p \leq 0.01$). A notably strong negative correlation was observed between erlose and glucose percentage ($r = -0.93$), indicating that higher erlose content is associated with lower glucose levels. Similarly, sucrose percentage was strongly negatively correlated with glucose percentage ($r = -0.85$), and glucose percentage showed a negative correlation with pH ($r = -0.84$). Other notable negative correlations included erlose with fructose percentage ($r = -0.71$) and altitude with temperature ($r = -0.79$). Conversely, significant positive correlations were identified among several sugar and chemical components. For instance, erlose was positively correlated with sucrose percentage ($r = 0.85$) and with pH ($r = 0.78$). Additionally, glucose percentage had a positive correlation with fructose percentage ($r = 0.74$), and sucrose percentage showed a positive correlation with pH ($r = 0.70$).Figure 7 illustrates the regression analysis performed on the highly correlated

parameters. The findings highlight a strong relationship between the sugar composition, pH, and environmental factors in Iranian Sidr honey samples. This indicates that both the natural composition and ecological conditions have a significant impact on honey quality. For example, as the glucose content increases, the percentages of sucrose and erlose decrease, while fructose levels subsequently rise.

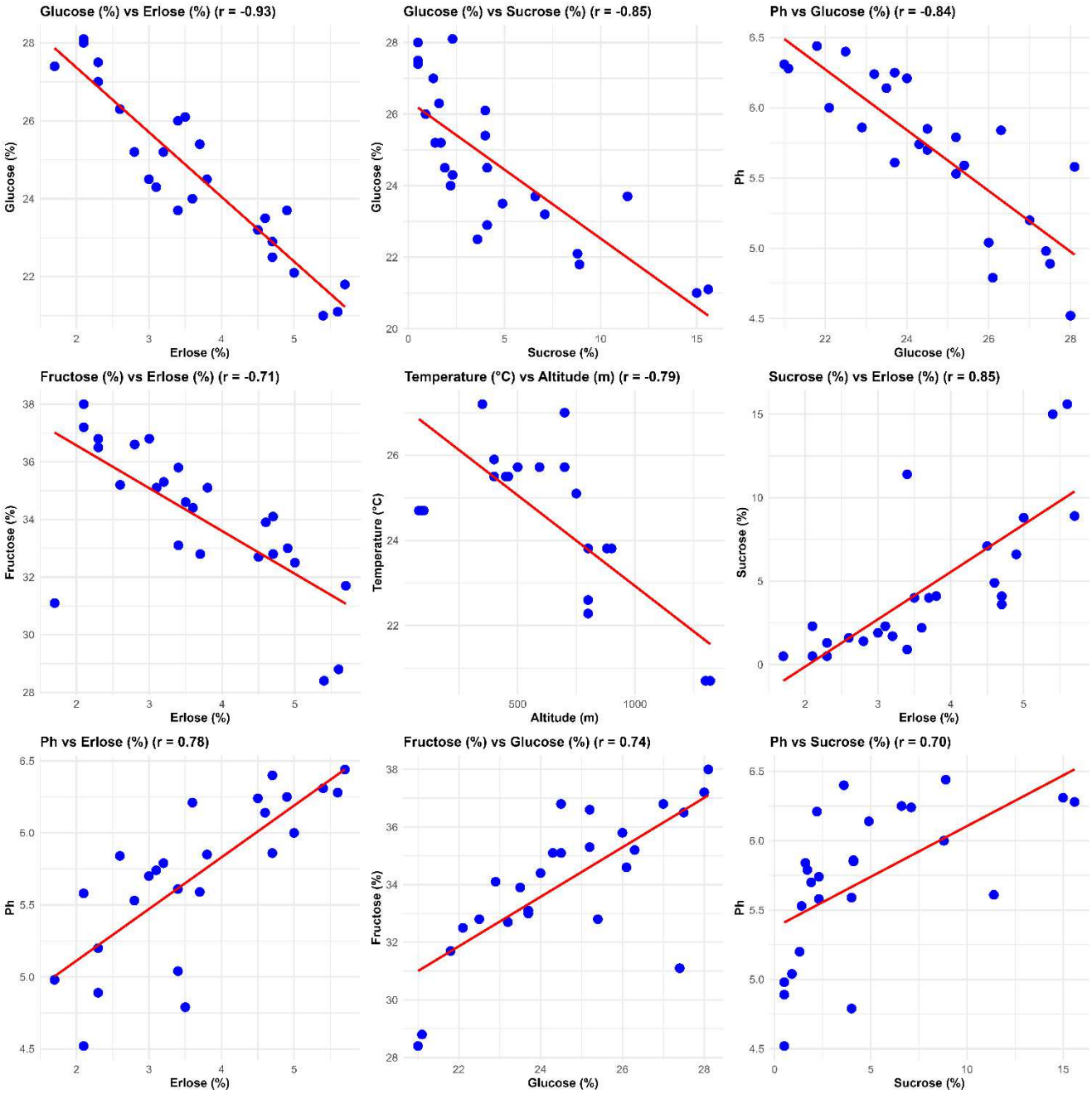


Fig 7. Scatter plots and linear regression models showing relationships between selected physicochemical and ecological variables in 25 samples of Iranian Sidr honey.

Additionally, higher fructose levels correspond to lower erlose levels. Conversely, an increase in sucrose leads to a rise in erlose. Moreover, increasing acidity is associated with a decrease in glucose levels, alongside an increase in both erlose and sucrose.The correlation between sucrose concentration and invertase activity during honey ripening is significant, indicating an interaction between these two parameters [64]. Invertase is a key enzyme that breaks down sucrose into fructose and glucose, playing a crucial role in converting nectar into honey [65]. As honey matures, an increase in glucose and fructose and a decrease in sucrose typically signal full ripening and normal invertase activity [66]. This change can serve as a reliable indicator of honey maturity and quality. In line with the findings of this study, Zhang et al. (2021) observed that, as rapeseed honey matured, sucrose levels gradually decreased while glucose, fructose, and total protein levels increased [67]. Honey contains oligosaccharides like erlose, which are crucial for assessing honey quality. Sucrose serves as a glucosyl donor, forming erlose through glycosyl transferase and α-glucosidase [46]. Glucose also combines with sucrose to create erlose. When glucose levels are excessively high, sucrose decreases, disrupting enzymatic balance and reducing erlose production as the enzyme shifts toward

hydrolysis instead of transglycosylation [68]. Higher erlose levels in honey from bees fed sucrose indicate that erlose could be a reliable marker for detecting adulterated honey [49]. In 2022, Tedesco and colleagues found that among the honeys studied, rhododendron honey had a high level of erlose, which was positively correlated with its sucrose content [69]. As the pH increases, the rate at which glucose undergoes reduction through the Maillard reaction also increases [70]. At higher pH levels, glucose decomposes more rapidly. Furthermore, in more alkaline conditions, glucose becomes less stable. This instability leads to an increased rate of enolization and isomerization of sugars, which can significantly reduce the amount of glucose, as it enters more complex reaction pathways [71]. The analysis also reveals that temperature decreases with increasing altitude. This reduction in temperature can indirectly affect the composition of sugars, acidity, or enzyme activity, thereby impacting the overall quality of honey. Ambient temperature is one of the most significant factors affecting nectar production, bee activity, enzymatic processes, and the diversity and abundance of vegetation. For instance, a study by Alaerjani et al. (2025) demonstrated that ambient temperature significantly influences various parameters of honey, including moisture content, conductivity, sucrose levels, diastase activity, fructose, glucose, the fructose/glucose ratio, and invertase activity in both multifloral and monofloral honey [72]. These parameters vary significantly between honey sourced from cold-climate regions and that from warm-climate regions. Altitude also plays an indirect yet important role in the physicochemical properties of honey [19, 73, 74]. In mountainous areas, lower temperatures and adequate rainfall contribute to better flowering and increased nectar production, leading to higher honey yields. Cooler temperatures help preserve the enzymatic quality of honey [75]. The study by Erdoğan et al. (2022) found statistically significant differences in the physicochemical structures of honey produced at various altitudes [76]. Environmental factors significantly influence the characteristics of the honeys studied. For instance, the Gach honey sample showed the highest levels of diastase enzyme activity. This mountainous region, which boasts favorable temperatures and rainfall, is believed to enhance diastase levels compared to other areas. Since diastase, like all enzymes, is sensitive to heat and humidity, its activity decreases when temperatures and humidity exceed the optimal range [77]. The principal component analysis (PCA) of the physicochemical and ecological parameters of Iranian Sidr honey samples revealed distinct groupings based on these characteristics. The first principal component (Dim1) explained 32.4% of the total variance, while the second principal component (Dim2) contributed an additional 12.9%. Together, these two components accounted for 50.6% of the overall variation in the dataset (Table 2). This highlights the importance of PCA1 and PCA2 in illustrating the physicochemical and ecological variations of Iranian honey samples. The explained variance aligns with findings from previous studies [78]. These findings support the use of chemometrics for quality and origin classification, as shown by Tarapatskyy et al. (2021), where PCA models resolved Polish honeys into clear botanical groups by isolating key variables like sugar distribution, acidity, and bioactive markers [79].

Parameters	PCA 1	PCA 2
Variance Explained (%)	32.37863	18.20867
Phen	0.341322	0.660168
Mois	-0.19483	0.585979

Ph	0.761651	0.395639
Dias	0.204609	0.844837
HMF	-0.17681	-0.23389
Pro	-0.5945	0.432447
Fruc	-0.83341	0.264848
Glu	-0.95424	-0.18589
F.G	0.419113	0.548874
tura	-0.24544	0.488894
Sucr	0.868782	-0.37492
Malt	0.089499	0.013362
Mele	0.448799	-0.5114
Erl	0.91697	0.108577
Iso	-0.33586	0.529379
Tem	-0.50323	-0.16644
Rain	0.62332	0.098421
Alt	0.51609	0.09851

Table 2. Component loadings of physicochemical and ecological parameters of 25 Iranian Sidr honey samples.

In the first principal component (PCA1), strong positive loadings were identified for erlose, sucrose, pH, rainfall, and altitude. In contrast, high negative loadings were noted for glucose (%), fructose (%), and proline, which also showed the highest Cos2 scores, indicating their significant contribution to PCA1 (Fig. 8). This contrast illustrates a trade-off: as erlose, sucrose, pH, rainfall, and altitude increase, there is a corresponding decrease in glucose (%), fructose (%), and proline. The second principal component (PCA2) revealed positive loadings, indicating a positive relationship between diastatic activity, phenolic compounds, moisture, and the fructose-to-glucose (F.G.) ratio. Conversely, it showed negative loadings for melezitose, sucrose, and hydroxymethylfurfural (HMF), suggesting an inverse association between diastatic activity, phenolics, moisture, and the F.G. ratio with these variables (Fig. 8). In the PCA plot, the lower right quadrant includes honey samples Fir-3, Qir-3, Qir-2, Qir-4, Fir-1, and Fir-5. These samples are distinct from others in the study due to their higher sucrose and melezitose content. The upper right quadrant contains samples Fir-4, Gach, Alam-1, Fir-2, Bus-1, and Alam-2, which are separated from other samples because of their higher levels of diastatic activity, phenolics, F.G. ratio, erlose, pH, rainfall, and altitude. Most of these samples were collected from higher altitudes and areas with greater rainfall compared to the other honey samples. The upper left quadrant features samples Lar-2, Lar-3, Qir-1, Fir-4, Lar-1, Jam, and Bor. The primary variables that separate them from the other samples include fructose, isomaltose, proline, turanose, and moisture. These samples generally have higher total amounts of these compounds than the others. Finally, the lower left quadrant includes samples Bus-2, Fara, Lam-2, Khon, Qir-5, Jiro, and Lam-1, which are distinguished by higher levels of glucose, temperature, and HMF. Most of these samples were

collected from warmer areas with higher temperatures and tend to have greater amounts of HMF and glucose than the others, except for samples Lam-2 and Qir-5, which have high glucose levels but low HMF. This indicates that ambient temperature is positively correlated with sugar compounds like glucose and overall honey quality. In summary, environmental factors such as altitude, temperature, and humidity significantly impact the presence of chemical compounds in honey, including sugars and acidity, as well as enzyme activity. This was demonstrated through PCA analysis. The reliability of these environmental segregations in PCA maps matches the findings of Machado et al. (2021) in Portuguese monofloral honeys, where environmental variables played a dominant role in shaping chemical clustering[80].

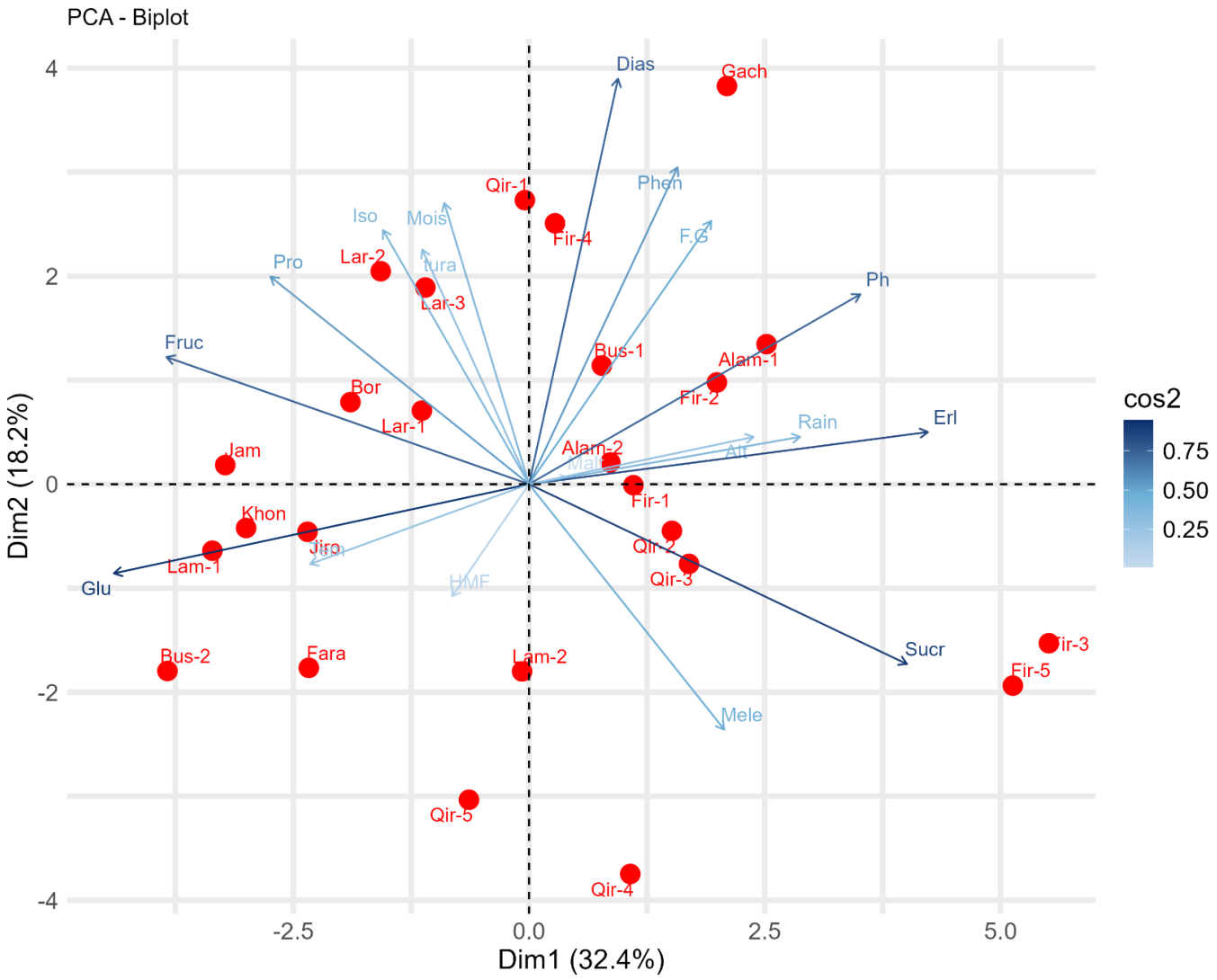


Fig 8. Principal component analysis of physicochemical and ecological parameters in 25 samples of Sidr honey from Iran.

A hierarchical clustering analysis was conducted to assess the similarities and differences among different honey samples based on their physicochemical and ecological profiles (Fig. 9). The samples were classified into three main clusters, referred to as groups I, II, and III, according to these parameters. By comparing these clusters to the results of principal component analysis (PCA), it was found that the groups identified in PCA largely overlap with the clusters from the hierarchical analysis, with some exceptions. Specifically, group I in the hierarchical analysis corresponds to the honey samples located in the lower left of the PCA plot. The samples in the upper left and upper right corners of the plot are associated with group II, while the honey samples in the lower right corner correspond to group III. These three main clusters can be further divided into eight subclusters. Samples within the same subclusters, indicated by the same colors, show the highest similarity in their physicochemical and ecological profiles. Notably, honey samples collected from the same counties did not all belong to the same cluster. This suggests that environmental factors affect the honey samples in various ways, leading to unique physicochemical profiles that influence the quantity and quality of the honey produced. The characteristics of honey are significantly influenced by biogeography, which accounts for the differences in the physicochemical properties of monofloral honeys derived from the same plant species. For example, monofloral chestnut honeys found in the Canary Islands and Croatia exhibit variations due to the distinct soil, climate, and plant communities present in each region [81, 82]. The study by El-Meihy et al. (2025) found that Sidr honeys from various biogeographical regions of Saudi Arabia contain distinct bioactive compounds and antioxidants [29]. Pena Júnior et al. (2022) conducted a study on 15 monofloral honey samples collected from various geographic regions of Minas Gerais, Brazil [83]. Their findings indicated that honeys sourced from the same type of flowering but from different geographic areas exhibit distinct

chemical compositions.

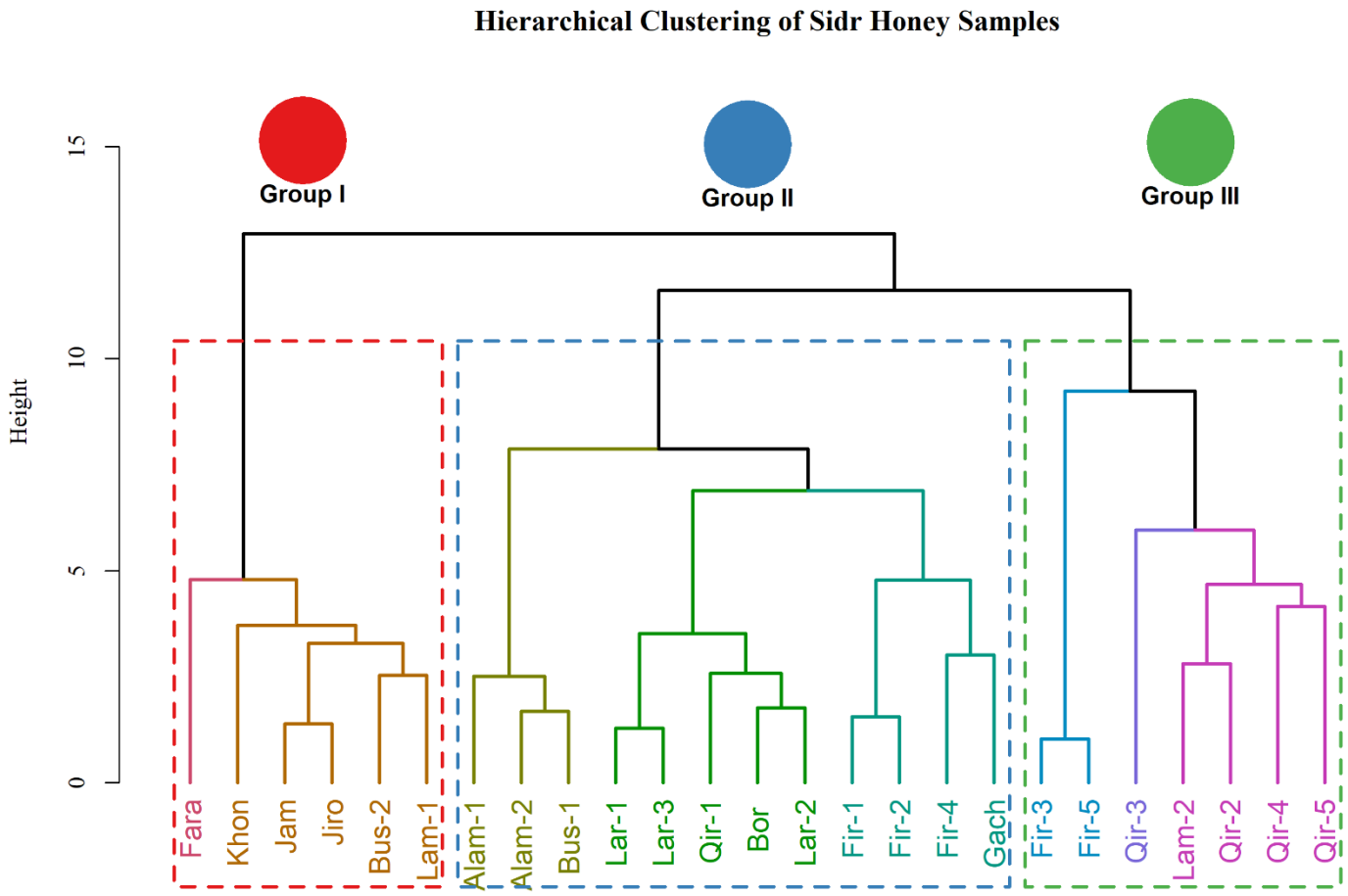


Fig 9. Hierarchical clustering from the multivariate analysis of physicochemical and ecological parameters in 25 Sidr honey samples from Iran, utilizing Ward's linkage with rescaled distance clustering.

Honey authenticity cannot be reliably determined using a single analytical parameter. Milojković Opsenica et al. (2015) demonstrated that integrating physicochemical characteristics, sugar profiling, melissopalynological analysis, and chemometric approaches significantly improves the accuracy of botanical origin determination and honey authenticity assessment. This concept is consistent with the findings of the present study, in which the combined application of these complementary analytical tools provided a more reliable evaluation of the authenticity of Iranian Ziziphus honey[84].

4. Conclusion

This study offers a comprehensive assessment of the physicochemical properties of Iranian honey and analyzes how ecological factors such as temperature, precipitation, and altitude affect these

properties. The findings reveal that geographical origin significantly influences the quality, chemical composition, and shelf life of honey due to the unique ecological conditions of each region. It is recommended that the International Honey Commission incorporate geographical and ecological factors, alongside plant origin, when reviewing standards. The study found significant variations in levels of hydroxymethylfurfural (HMF), sucrose, and melezitose among samples, while fructose and glucose levels remained within standard ranges, indicating high honey quality. Some samples had sucrose content above or below acceptable limits, potentially due to early harvesting or added sugars, which requires further investigation. The research highlights the importance of assessing the majority of physicochemical parameters to authenticate honey and notes that deviations from standard ranges do not necessarily indicate adulteration. All samples showed compliant moisture content, suggesting stability and minimal fermentation risk. HMF levels were mostly within acceptable limits, reflecting freshness and proper storage conditions. In summary, the study underscores the high quality of Iranian honey and the need for updated standards to enhance competitiveness in domestic and international markets.

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