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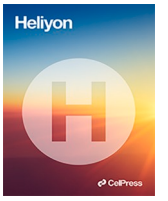

Research article

Comparative study of physiochemical properties in Iranian multi-floral honeys: Local vs. commercial varieties

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ABSTRACT

Iran, a leading honey-producing country, faces challenges in honey exports. This study aimed to evaluate the melissopalynological and physicochemical characteristics of local honeys belonging to Iranian flora, and compare them with Iranian commercial honeys. For this purpose, seven local honey samples were collected from Iran's renowned floristic regions, alongside seven commercial multi-floral honeys from a supermarket. Moisture content (MC), total solids (TS), pH, free acidity (FA), ash, electrical conductivity (EC), sugar profile, hydroxymethyl furfural (HMF), diastase number (DN), and proline were assessed. The sugar profile was analyzed by high-performance liquid chromatography with a refractive index detector (HPLC-RID). Pollen analysis classified local honeys as multi-floral. The results revealed that MC, TS, pH, FA, ash, and EC values in both local and commercial samples conformed to approved standards (Codex Alimentarius and European Union). Two local and one commercial sample exclusively satisfied the sucrose standard level. Of the local honeys, two samples complied with HMF standard, while five fulfilled DN criterion, and four had proline values within acceptable ranges. Conversely, HMF (56.32–228.11 mg/kg), DN (3.13–7.22 Schade units/g), and proline (109.84–173.86 mg/kg) levels in all commercial samples failed to meet the standard. A significant correlation was found between ash and EC ($r = 0.915$, $p < 0.01$) in local honeys, whereas no strong correlation ($r = 0.299$) existed in commercial samples. Hierarchical cluster analysis confirmed that Iranian honeys lacked clustering by botanical origin, possibly due to extensive sugar adulteration or thermal treatment. Overall, study findings confirmed the significantly inferior quality of Iranian commercial honeys compared to local varieties, albeit some local samples also exhibited quality concerns. Accordingly, it is recommended that regulatory bodies provide periodic training for beekeepers and establish monitoring programs to enhance honey quality, thereby boosting Iran's share in the global honey export market.

1. Introduction

Honey is considered a natural sugar-rich concentrated solution produced by different species of honeybees, either from the nectar of flowers and blossoms (called Nectar or Blossom Honey), or from the exudation of living parts of plants or secretions of sucking insects on the plants (called Honeydew Honey) [1,2]. The main component of honey comprises carbohydrates (75–80 %), which are primarily reducing sugars, namely glucose and fructose [3,4]. In addition, small quantities of mono-, di-, tri-saccharides, and

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oligosaccharides are also present in honey [5]. Water is quantitatively the second most abundant component of honey (15–20 %) [3]. Furthermore, honey encompasses various minor bioactive compounds, such as enzymes, amino acids, organic acids, polyphenols, pigments (carotenoids and anthocyanins), vitamins (B and C), and minerals [6–8]. A portion of them is derived from pollen or nectar, while others are produced by bees during the honey production process. These compounds give honey functional and biological properties, thus making it suitable for utilization as a functional food in the human diet [9]. Heating has a negative impact on these minor bioactive compounds, resulting in their degradation and loss [10,11].

The majority of food products are susceptible to intentional tampering; however, certain foods, such as honey, are at a higher risk of adulteration due to their high price, limited availability, the requirement for a laboratory setting to evaluate their authenticity, and their production under wide climatic fluctuations and harvesting circumstances [12]. Adulteration of honey is commonly conducted by adding industrial cheap sweeteners, namely, refined beet or cane sugar, high fructose corn syrup (HFCS), hydrolyzed inulin syrup (HIS), and inverted sugar syrup (ISS) [11,13]. Moreover, some profiteers deliberately expose bees to sugar solutions or grape and berry juices to divert their foraging activities from nectar collection, prompting them to feed on these alternatives [14]. This manipulation ultimately leads to increased honey production that benefits profiteers [3]. Nevertheless, it has an adverse effect on the health of consumers, including increased blood sugar, fatty liver, acute, and chronic kidney injury, among other potential consequences [15]. To solve this issue, various standards around the world established certain limits for physicochemical parameters to assess the quality of different types of honey and detect potential adulteration [2,16,17].

Iran is known as one of the largest honey-producing countries worldwide, with assorted sensory, organoleptic, and physicochemical characteristics due to the variety of climates, a wide range of floral sources, and the existence of different species of honeybees. According to facts and figures provided by the Food and Agriculture Organization of the United Nations (FAOSTAT), Iran ranked among the top ten honey-producing nations from 1993 to 2018 [18]. However, Iran stands as the sole country among them unable to export honey to Europe. This issue also endures in Iranian commercial honeys to the extent that Iran has not been able to become a successful exporter of commercial honeys. Although a substantial number of studies have been carried out worldwide, including in Iran, regarding the physicochemical parameters of mono-floral and multi-floral honeys to date [19–22], no literature has yet compared the quality of Iranian commercial honeys with local honeys produced by beekeepers. Therefore, the primary purpose of the current study is to evaluate and compare the physicochemical properties of some local honeys produced in different regions of Iran

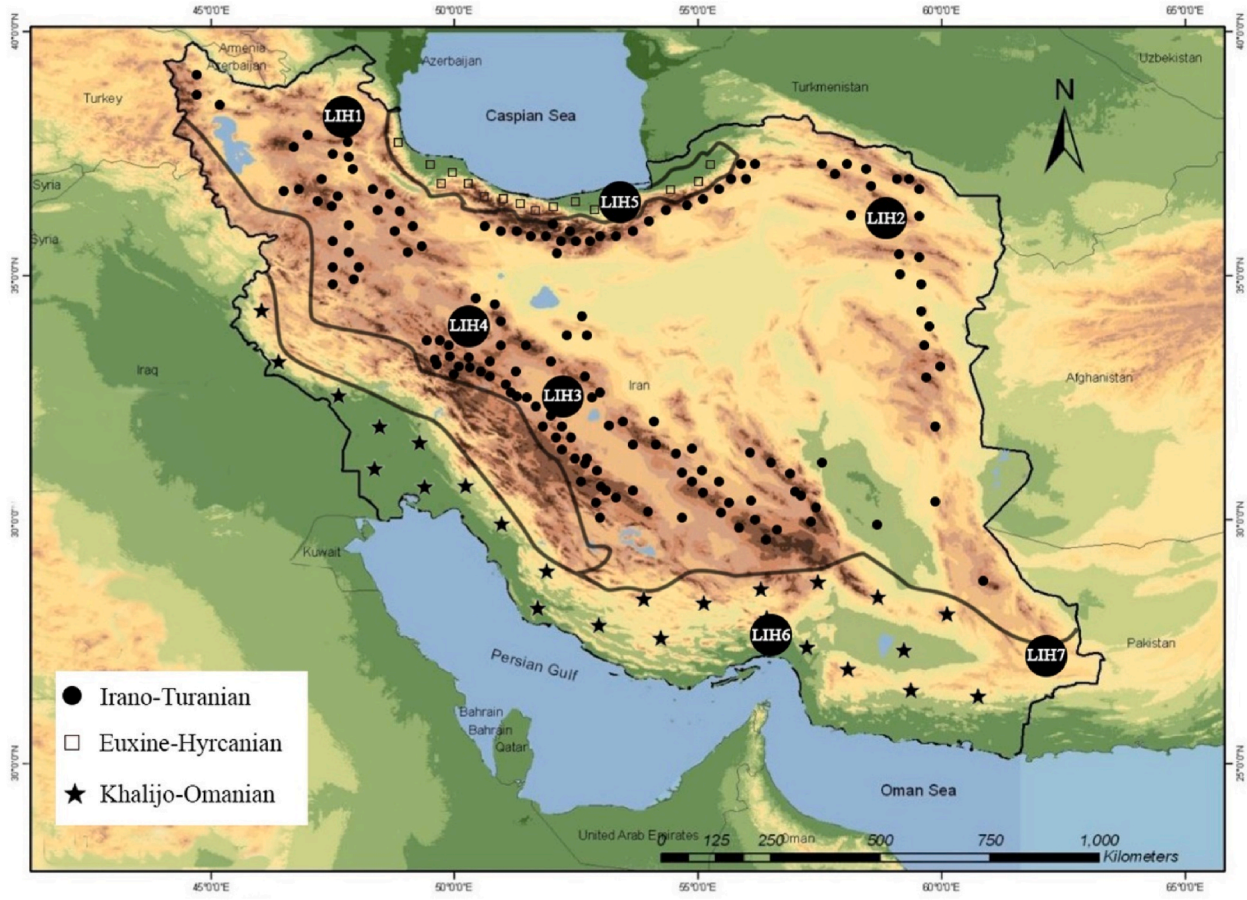

Fig. 1. Map of Iran indicating the regions where local multi-floral honey samples were collected.

with commercial honeys prevalent in the Iranian market, and verify their compliance with the Codex and European Union (EU) standards. Moisture content, total solids, pH, acidity, ash, electrical conductivity, sugar profile, hydroxymethyl furfural, diastase activity, and proline were assessed for physicochemical characterization. Besides, the analysis of melissopalynological characteristics, along with describing the correlation between these parameters using Pearson's correlation and classification of the parameters and Iranian honeys based on their similar properties, are subsequent objectives of this work.

2. Materials and methods

2.1. Honey samples and classification

During the winter season of 2022, a total of 14 honey samples (commercial honeys n = 7, local honeys n = 7) were purchased from supermarkets located in Sari City and local beekeepers within the most renowned floristic regions of Iran, including Irano-Turanian, Euxine-Hyrcanian, and Khalijo-Omanian. In accordance with the geographic extent of each floristic region, one or more cities were selected as representatives of that region, and local samples were collected from those cities, as illustrated in Fig. 1 and Table 1. These samples were placed in sealed plastic containers, labeled, and stored at room temperature (27 ± 2 °C) until completion of analysis. Prior to analysis, none of the honey samples displayed any visual signs of granulation or fermentation. There was initially a paucity of knowledge regarding the botanical origins of the locally prepared honeys, while the commercial honeys were categorized as multi-floral types based on the label information. For this purpose, melissopalynological classification was performed for at least 800 pollen grains in the samples using a microscope (Motic, Xiamen, China), as previously described by Louveaux et al. [23].

2.2. Chemicals and reagents

Potassium hexacyanoferrate (II), Zinc acetate, iodine, potassium iodide, sodium hydroxide, potassium chloride, phenolphthalein, and ethylene glycol monomethyl ether were supplied by Sigma-Aldrich (St Louis, MO., USA). Proline, formic acid, ninhydrin, 2-Propanol, sodium hydrogen sulfite, sodium acetate trihydrate, starch, glycerol-gelatine, and glacial acetic acid were all purchased from Merck (Darmstadt, Germany). All chemical standards used were HPLC-grade pure, and the sugar standards were sourced from Sigma–Aldrich (Munich, Germany). Acetonitrile was obtained from Sigma–Aldrich Co. (St. Louis, MO, USA) and methanol by Merck KGaA, (Darmstadt, Germany). The Milli-Q water purification system (Millipore, Molsheim, France) was employed to generate ultrapure water for mobile phase preparation.

2.3. Moisture and total solids

The moisture content was evaluated by measuring the refractive index of honey at 20 °C using a benchtop refractometer (RMI, Optech-Exacta, Germany), and deriving the relevant % moisture (g/100 of honey) by referring to the standard table provided by the International Honey Commission (IHC) [16]. Afterward, the total solids (TS) content was calculated by subtracting the moisture content from 100 %.

2.4. pH and free acidity

The pH measurements were undertaken at 20 °C using a pH-meter (3505, Jenway, UK) by dissolving 10 g of each sample in 75 mL of CO_2-free water. Thereafter, to measure free acidity in the same solution, a few drops of 1 % (w/v) alcoholic solution of phenolphthalein were added, and titration with 0.1 N NaOH ensued until the pH reached 8.3 within 2 min (V_s). For the blank sample, distilled water was used and titrated based on the aforementioned method (V_b). The free acidity was expressed in milliequivalents acid per kg of honey (meq/kg), as determined using equation (1), as described in IHC [16].

Table 1
Melissopalynological characterization of local honeys collected from different regions of Iran and commercial honeys.

Samples code	Honey type	Location	Principal pollen families	Floral type
LIH1	Local	Ardebil, (Irano-Turanian region)	*Fabaceae, Asteraceae, Brassicaceae*	multi-floral
LIH2	Local	Mashhad, (Irano-Turanian region)	*Asteraceae, Fabaceae, Caryophyllaceae*	multi-floral
LIH3	Local	Isfahan, (Irano-Turanian region)	*Fabaceae, Lamiaceae, Asteraceae, Brassicaceae*	multi-floral
LIH4	Local	Arak, (Irano-Turanian region)	*Fabaceae, Apiaceae, Asteraceae*	multi-floral
LIH5	Local	Sari, (Euxine-Hyrcanian region)	*Lamiaceae, Fabaceae, Rosaceae*	multi-floral
LIH6	Local	Bandar Abbas, (Khalijo-Omanian region)	*Fabaceae, Mimosaceae, Asteraceae*	multi-floral
LIH7	Local	Saravan, (Khalijo-Omanian region)	*Asteraceae, Mimosaceae, Fabaceae,*	multi-floral
CIH1	Commercial	Supermarket located in Sari city	No pollen observed	multi-floral
CIH2	Commercial	Supermarket located in Sari city	No pollen observed	multi-floral
CIH3	Commercial	Supermarket located in Sari city	*Fabaceae, Asteraceae, Rosaceae*	multi-floral
CIH4	Commercial	Supermarket located in Sari city	No pollen observed	multi-floral
CIH5	Commercial	Supermarket located in Sari city	No pollen observed	multi-floral
CIH6	Commercial	Supermarket located in Sari city	*Fabaceae, Lamiaceae, Caryophyllaceae*	multi-floral
CIH7	Commercial	Supermarket located in Sari city	No pollen observed	multi-floral

$$\text{Acidity} = (V_s - V_b) \times 10 \quad (1)$$

2.5. Ash

For the determination of ash content, vacant crucibles were initially weighed (M_1), and 10 g of honey samples were then transferred into each of them. Next, the crucibles were exposed to an open flame (105 °C) until the ash turned completely dark, followed by placement in an electric furnace (FM 20p, Iran khodsaz, Iran) at 600 °C for 5 h until whitened. After diminishing the furnace temperature to 70 °C, the crucibles were moved to a desiccator to achieve a constant weight, and then reweighed (M_2). Ultimately, the ash content in g/100 g of honey was obtained using equation (2), developed by the IHC [16].

$$\%\text{Ash} = (M_2 - M_1) \times 10 \quad (2)$$

2.6. Electrical conductivity (EC)

The EC was assessed for a 20 % (w/v) honey solution at 20 °C by a conductivity meter (CC-401, Elmetron, Poland) as per the method outlined in the IHC. Subsequently, the EC value was calculated in milli Siemens per centimeter (mS/cm) according to equation (3):

$$S_H = K \times G \quad (3)$$

where: S_H = electrical conductivity in mS/cm, K = cell constant in cm^{-1}, G = conductance in mS.

2.7. Sugar analysis

Sugar analysis was conducted using a high-performance liquid chromatography (Elite LaChrom, Hitachi, Japan) equipped with a refractive index detector (HPLC-RID), as described by Degirmenci et al. [22]. The analytical reverse phase-NH2 column (200 mm × 4.6 mm i.d., 5 μm particle; Nucleosil) was employed, and the mobile phase used for isocratic elution consisted of acetonitrile: water (79:21, v/v). The honey sample solutions mixed with ultrapure water (1:10 g/mL) were filtered through a 0.45 μm filter before being injected into the HPLC instrument. The operational parameters were set as follows: injection volume, 25 μL; column temperature, 80 °C; flow rate, 1.50 mL/min. The calibration curve was constructed for each sugar standard (fructose, glucose, sucrose, maltose, trehalose, melibiose, melezitose) using their solutions at various concentrations (0, 5, 10, 15, 20 and 25 mg/mL). For all types of sugars, the coefficient of determination (R^2) for the calibration curves was greater than 0.9961. Sugar quantification was performed through the comparison of peak areas obtained with the corresponding standard sugars. The results were expressed in g sugar per 100 g of honey (%).

2.8. Hydroxymethyl furfural (HMF)

The determination of HMF was conducted based on the White approach [24]. Briefly, 5 g of honey sample dissolved in 25 mL of distilled water was treated with Carrez I and Carrez II clarifying solutions (0.5 mL each). The final volume was then made up to 50 mL, followed by filtration, with one-fifth of the filtrate removed from the surface. Subsequently, a UV–visible spectrophotometer ($T80^+$, PG Instruments Ltd, UK) operating at 284 and 336 nm was employed to measure the absorbance of the filtrate against an aliquot of the same filtrate treated with $NaHSO_3$ solution (0.2 %). The solutions with absorbance above 0.6 were diluted with distilled water or $NaHSO_3$, until their absorbance reached or fell below 0.6. Finally, the results were quantified in mg/kg of honey using equation (4):

$$\text{HMF} = (A_{284} - A_{336}) \times 149.7 \times D \quad (4)$$

where: A_{284} = absorbance at 284 nm, A_{336} = absorbance at 336 nm, D = dilution factor (D = Final volume of sample solution/10).

2.9. Diastase activity

Diastase activity was determined following the enzymatic-spectrophotometric method [16], with buffered soluble starch and honey samples incubated within a thermostatic water bath (40 °C). Accordingly, 1 mL of aliquot from the mixture was withdrawn at 5 min intervals, and the absorbance of the sample was read at 660 nm. The absorbance versus time plot was then used to calculate the reaction time (tx) in min required to reach an absorbance value below 0.235 nm. The diastase value was determined by the following equation (5). The results were reported in Schade units/g of honey, representing the quantity of enzyme in 1 g of honey capable of hydrolyzing starch (1 %) during a 1 h incubation at 40 °C.

$$\text{DN} = 300/t_x \quad (5)$$

2.10. Proline

To measure the proline content, 5 g of each honey sample were dissolved in 50 mL of distilled water and transferred to a 100 mL

volumetric flask. Then, 0.5 mL of the sample solution, distilled water (blank test), and proline standard solution were dispensed into separate tubes. Next, 1 mL of formic acid and ninhydrin solution were introduced into each tube. The tubes were tightly sealed, shaken vigorously for approximately 15 min, and then immersed in a boiling water bath for 15 min, followed by a 10-min incubation in a 70 °C water bath. Following that, 5 mL of propanol-water solution (1:1) were promptly added to each tube, which was then sealed and incubated for 45 min. The tubes were removed from the water bath, and their absorbance was measured at 517 nm using a UV–visible spectrophotometer (T80^{+}, PG Instruments Ltd, UK). Finally, the proline content (WP) was quantified in mg/kg of honey, as per equation (6) [25].

$$W_P = \frac{E_P}{E_S} \times \frac{M_1}{M_2} \times 80 \tag{6}$$

where: E_p = absorbance of the honey sample, E_s = absorbance of the average proline standard solution, M_1 = proline content in the standard solution (in mg), M_2 = weight of honey sample (in g), 80 = dilution factor.

2.11. Statistical analysis

All measurements were run in a completely randomized design with triplicates, and the results were reported as mean ± standard deviation (SD). The significant differences were determined by a one-way analysis of variance (ANOVA), followed by Duncan's test ($p < 0.05$) with SPSS version 26.0. Correlations were obtained via Pearson's correlation coefficient (r) within bivariate linear correlation analysis. The strong negative or positive correlations were interpreted within the range of −1 to −0.7 or from 0.7 to 1, respectively. Hierarchical cluster analysis (HCA) was conducted on the standardized data using Ward's method as the amalgamation rule, with Pearson correlation used for variable classification and Euclidean distance for sample classification. The pie chart and heat-map plots were constructed in Origin Pro 2022 software.

3. Results and discussion

3.1. Melissopalynological characterization

The information regarding the principal pollen families identified in the studied honeys and the relative abundance of the 11 plant families most commonly foraged by honeybees in the Iranian flora is depicted in Table 1 and Fig. 2, respectively. The *Fabaceae* family was found as the principal pollen source in the majority of the honeys, followed by *Asteraceae* and *Lamiaceae* in the subsequent ranks. Of the analyzed honey samples, the highest number of pollen grains was found in the LIH3 and LIH5 samples, while markedly fewer were detected in the other samples. Notably, no discernible pollen types were observed in the commercial honeys, except for the CIH3 and CIH6 samples. This is likely due to adulteration with sugar, either directly or indirectly [26]. Furthermore, the pre-market filtration process employed by most Iranian producing companies through a mesh with pore sizes under 4.5 μm can be considered as another contributing factor to this issue [14]. According to melissopalynological examination results, all the investigated samples were classified as multi-floral since none of them contained a specific type of pollen exceeding 45 % of the total.

3.2. Moisture content and total solids

The determination of honey's moisture content characterizes the amount of water and total solids it contains. The moisture levels of the local and commercial honey samples ranged from 15.38 to 19.54 % and 14.86–16.17 %, respectively (Table 2), all falling within

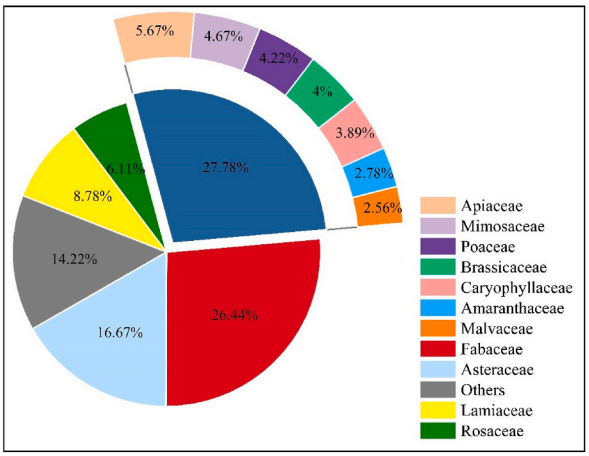

Fig. 2. Relative abundance of the most common pollen families found in the Iranian flora.

the permissible level (≤20 %) established by the Codex Alimentarius [2] and European Union (EU) legislation [17]. The local sample sourced from LIH6 demonstrated the highest moisture content, likely owing to the abnormally humid environmental conditions in the south of Iran. Higher water content provides a minimum water activity required for the growth of osmotolerant yeasts, leading to unfavorable fermentation, a sour taste, and ultimately spoilage of honey [27,28]. Hence, this sample possessed a higher probability of fermentation compared to the other samples examined. In contrast, the CIH3 sample was regarded as the most resistant to microbial growth among all the samples analyzed, resulting in an extended shelf life during storage by virtue of its low humidity. Additionally, the commercial honeys displayed a lower mean moisture content (15.53 ± 0.50 %) compared to local honeys (16.88 ± 1.38 %), most probably due to industrial thermal processing. The results align with those obtained by Silva et al. [29], with Portuguese honeys (13.53–19.70 %). Contrary to our findings, Hassan et al. [30] and Gela et al. [4] reported higher moisture content in Malaysian and Ethiopian honeys, with ranges of 15.1–35.9 % and 25.1–35.0 %, respectively. The discrepancies in honey samples moisture content across assorted studies could be attributed to several reasons, including geographical characteristics, harvest season, botanical origin, bee species, storage conditions, maturity degree, as well as the potential manipulation by beekeepers during the extraction and processing [1,3].

3.3. pH and free acidity

pH and acidity are considered as freshness and stability criteria in the evaluation of honey quality, which can influence the aroma, flavor, and texture of honey [6,29]. The pH values in the current study were found to be acidic, ranging from 3.95 to 4.31 and 3.91 to 4.41 for the local and commercial honeys, respectively (Table 2). This feature prevents microbial growth, as most microorganisms thrive within the environmental pH range of 7.2–7.4 [10]. According to Amir et al. [31], honey samples with a pH level below 4 decompose at a higher rate during storage, indicating that the investigated samples are relatively fresh. The pH results are in the range reported by Imtara et al. [28] (3.66–4.25) for Palestinian honeys and Saxena et al. [32] (3.70–4.40) for Indian honeys.

The free acidity values in the local and commercial honey samples varied between 14.93 (for LIH3) to 25.23 (for LIH6) meq/kg and 13.38 (for CIH7) to 26.83 (for CIH2) meq/kg, respectively (Table 2), which comply with the admissible limit set by the Codex (≤50

Table 2
Physicochemical parameters of local honeys collected from different floristic regions of Iran and commercial honeys.

	Samples code	Moisture (%)	TS (%)	pH	Free acidity (meq/kg)	Ash (%)	EC (mS. cm^{-1})	HMF (mg/kg)	DN (Schade units/g)	Proline (mg/kg)
Local Iranian honeys (LIH)	LIH1	15.70 ± 0.21^{fg}	84.29 ± 0.21^{cd}	4.18 ± 0.02^{de}	16.94 ± 0.14^{f}	0.09 ± 0.005^{fgh}	0.37 ± 0.004^{f}	113.32 ± 2.53^{f}	6.97 ± 0.19^{e}	168.39 ± 6.76^{e}
	LIH2	15.38 ± 0.09^{h}	84.62 ± 0.09^{b}	4.21 ± 0.04^{d}	15.95 ± 0.16^{gh}	0.04 ± 0.004^{i}	0.26 ± 0.007^{j}	70.45 ± 4.32^{h}	6.77 ± 0.14^{ef}	175.71 ± 4.71^{e}
	LIH3	16.08 ± 0.11^{e}	83.92 ± 0.11^{e}	4.31 ± 0.03^{bc}	14.93 ± 0.21^{i}	0.07 ± 0.005^{h}	0.28 ± 0.008^{i}	36.61 ± 2.07^{j}	12.22 ± 0.17^{b}	677.26 ± 8.85^{a}
	LIH4	16.42 ± 0.20^{d}	83.57 ± 0.20^{f}	4.30 ± 0.06^{c}	15.87 ± 0.30^{h}	0.18 ± 0.005^{b}	0.43 ± 0.008^{d}	116.66 ± 3.01^{f}	11.35 ± 0.15^{c}	210.05 ± 9.70^{d}
	LIH5	17.90 ± 0.23^{b}	82.10 ± 0.23^{h}	4.16 ± 0.04^{de}	17.93 ± 0.19^{e}	0.10 ± 0.006^{def}	0.32 ± 0.003^{g}	42.01 ± 2.01^{j}	15.25 ± 0.13^{a}	411.86 ± 7.44^{b}
	LIH6	19.54 ± 0.25^{a}	80.45 ± 0.25^{i}	4.01 ± 0.05^{gh}	25.23 ± 0.67^{b}	0.30 ± 0.011^{a}	0.79 ± 0.009^{a}	78.99 ± 2.68^{g}	9.75 ± 0.79^{d}	296.50 ± 12.47^{c}
	LIH7	17.15 ± 0.08^{c}	82.84 ± 0.08^{g}	3.95 ± 0.03^{hi}	20.64 ± 0.34^{c}	0.17 ± 0.006^{bc}	0.68 ± 0.005^{b}	57.79 ± 4.20^{i}	9.59 ± 0.67^{d}	172.54 ± 9.79^{e}
Mean ± SD		16.88 ± 1.38	83.11 ± 1.38	4.16 ± 0.13	18.21 ± 3.43	0.14 ± 0.082	0.45 ± 0.196	73.69 ± 30.32	10.27 ± 2.85	301.76 ± 178.45
Commercial Iranian honeys (CIH)	CIH1	16.09 ± 0.14^{e}	83.90 ± 0.14^{e}	4.11 ± 0.02^{ef}	17.97 ± 0.27^{e}	0.02 ± 0.004^{j}	0.11 ± 0.006^{m}	212.36 ± 7.82^{b}	3.22 ± 0.43^{h}	132.50 ± 11.34^{g}
	CIH2	16.17 ± 0.19^{de}	83.82 ± 0.19^{ef}	3.91 ± 0.03^{i}	26.83 ± 0.22^{a}	0.08 ± 0.002^{gh}	0.52 ± 0.007^{c}	228.11 ± 2.44^{a}	3.13 ± 0.62^{h}	109.84 ± 8.32^{h}
	CIH3	14.86 ± 0.16^{i}	85.13 ± 0.16^{a}	4.38 ± 0.04^{ab}	14.34 ± 0.31^{j}	0.08 ± 0.007^{fgh}	0.21 ± 0.005^{l}	56.32 ± 1.64^{i}	7.22 ± 0.10^{e}	173.86 ± 7.21^{e}
	CIH4	15.85 ± 0.30^{ef}	84.14 ± 0.30^{de}	4.16 ± 0.05^{de}	16.50 ± 0.45^{fg}	0.16 ± 0.004^{c}	0.31 ± 0.003^{h}	151.17 ± 1.39^{e}	4.26 ± 0.55^{g}	117.19 ± 4.92^{h}
	CIH5	15.34 ± 0.14^{h}	84.66 ± 0.14^{b}	4.07 ± 0.04^{fg}	19.82 ± 0.22^{d}	0.09 ± 0.009^{efg}	0.39 ± 0.005^{e}	206.26 ± 4.75^{c}	6.01 ± 0.51^{f}	148.32 ± 6.68^{f}
	CIH6	15.03 ± 0.09^{i}	84.96 ± 0.09^{a}	4.31 ± 0.03^{bc}	15.09 ± 0.42^{i}	0.10 ± 0.016^{de}	0.21 ± 0.008^{l}	155.84 ± 4.49^{e}	6.69 ± 0.60^{ef}	163.83 ± 4.39^{e}
	CIH7	15.41 ± 0.06^{gh}	84.59 ± 0.06^{bc}	4.41 ± 0.02^{a}	13.38 ± 0.40^{k}	0.11 ± 0.001^{d}	0.24 ± 0.004^{k}	182.18 ± 1.05^{d}	5.04 ± 0.83^{g}	169.02 ± 6.05^{e}
Mean ± SD		15.53 ± 0.50	84.46 ± 0.50	4.19 ± 0.17	17.70 ± 4.36	0.09 ± 0.039	0.29 ± 0.128	170.32 ± 55.02	5.08 ± 1.61	144.94 ± 25.07
Codex standard		≤20 %	–	–	≤50 meq/kg	≤0.6 %	≤0.8 mS/cm	≤40 mg/kg	≥8 Schade units/g	≥180 mg/kg

Results are reported as means ± standard deviation. The similar lowercase letters in the column indicate no statistically significant differences ($p < 0.05$). Note: TS, Total solids; EC, Electrical conductivity; HMF, hydroxymethyl furfural; DN, Diastase number.

meq/kg) [2] and EU (≤40 meq/kg) standards [17]. Such values are almost comparable to those of honeys from Cuba (16.8–27.7 meq/kg) [5]. Nevertheless, the findings differ from those previously reported for honeys originating from other countries, including Serbian honeys (10.12–45.75 meq/kg) [19], Romanian honeys (16.01–31.63 meq/kg) [33] and Omani honeys (21.5–54.0 meq/kg) [3]. The primary reason for these differences lies in the origin of the nectar fed by bees [1]. Due to the diversity of plants, honey contains multiple quantities and types of sugars and organic acids, which undergo transformation into a wide range of organic acids by yeasts present in honey [19]. By way of illustration, fructose and glucose are converted into CO_2 and ethyl alcohol during fermentation, followed by the subsequent oxidation of alcohol to acetic acid in the presence of oxygen [28]. The duration of honey storage is regarded as one of the most momentous factors in the conversion of sugars into organic acids. Cavia et al. [34] reported a consistent increase in the free acidity levels of Spanish honeys after 20 months of storage. The free acidity content is considerably correlated with the equilibrium between organic acids and their respective lactones or internal esters, as well as different inorganic ions found in honey, namely, phosphates, sulfates, and chlorides [1,20]. Moreover, numerous factors also contribute to differences in acidity values, including the harvesting season, bee species, and chemical components present in honey, such as proteins, phenolics, and vitamin C, which possess the capacity to donate H^+ atoms [10,27]. Beekeepers can prevent an increase in honey acidity by maintaining low moisture levels, as high moisture content stimulates microorganism activity.

3.4. Ash and EC

Honey naturally contains a scant amount of ash. The ash content results of the local samples (0.04–0.30 %) and commercial samples (0.02–0.16 %) conform to the allowed standard (≤0.6 %), as stipulated by the international Codex [2] and EU [17]. The LIH6 sample recorded the highest ash (0.30 %), while the CIH2 and LIH2 were found to have the lowest ash levels of 0.02 and 0.04 %, respectively. The determination of ash content provides a reliable means for measuring the mineral content within pure honey [19]. In essence, honey ash comprises the inorganic elements originating from the floral sources of nectar collected by bees during their foraging activities, and the concentration of which relies on the variety of soil where the nectar or plant is found [10]. Hence, ash content functions as a crucial chemical marker for discerning the geographic and botanical origins of honey. EC is intimately associated with the concentration of inorganic molecules (e.g., minerals and salts) as well as organic molecules, including proteins, polyols, organic

Table 3
The sugar profile of local honeys collected from different floristic regions of Iran and commercial honeys.

Samples code		Monosaccharide (%)		Disaccharide (%)				Trisaccharide (%)	F + G ratio	F/G ratio	G/W ratio
		Fructose	Glucose	Sucrose	Maltose	Trehalose	Melibiose	Melezitose			
Local Iranian honeys (LIH)	LIH1	29.41 ± 1.44	37.47 ± 0.60	5.44 ± 0.09	1.47 ± 0.09	N.D.	N.D.	N.D.	66.88	0.78	2.38
	LIH2	39.64 ± 0.89	23.55 ± 0.49	8.76 ± 0.13	N.D.	0.02 ± 0.001	0.17 ± 0.011	N.D.	63.19	1.68	1.53
	LIH3	34.79 ± 0.53	27.67 ± 0.86	3.26 ± 0.07	0.52 ± 0.04	0.19 ± 0.006	0.52 ± 0.014	N.D.	62.46	1.25	1.72
	LIH4	33.58 ± 0.33	19.74 ± 0.58	6.28 ± 0.06	N.D.	N.D.	0.27 ± 0.006	N.D.	53.32	1.70	1.20
	LIH5	32.49 ± 0.37	25.73 ± 1.10	3.29 ± 0.16	0.21 ± 0.03	0.04 ± 0.003	N.D.	0.35 ± 0.006	58.22	1.26	1.43
	LIH6	33.20 ± 0.96	39.28 ± 0.32	6.92 ± 0.13	N.D.	0.10 ± 0.005	N.D.	N.D.	72.48	0.84	2.01
	LIH7	31.57 ± 0.48	26.08 ± 0.77	7.30 ± 0.11	1.15 ± 0.05	0.02 ± 0.003	0.02 ± 0.001	N.D.	57.65	1.21	1.52
Mean ± SD		33.53 ± 3.09	28.50 ± 6.87	5.89 ± 1.95	0.48 ± 0.57	0.05 ± 0.067	0.14 ± 0.191	0.05 ± 0.126	62.02	1.24	1.68
Commercial Iranian honeys (CIH)	CIH1	38.51 ± 0.28	29.42 ± 0.57	8.80 ± 0.19	2.21 ± 0.09	N.D.	N.D.	N.D.	67.93	1.30	1.82
	CIH2	47.42 ± 0.42	31.33 ± 0.55	4.82 ± 0.05	0.92 ± 0.01	0.01 ± 0.006	N.D.	N.D.	78.75	1.51	1.93
	CIH3	42.91 ± 0.94	29.28 ± 0.30	12.39 ± 0.06	2.28 ± 0.05	0.05 ± 0.012	N.D.	N.D.	72.19	1.46	1.97
	CIH4	41.48 ± 1.29	28.21 ± 0.69	10.22 ± 0.11	0.68 ± 0.09	N.D.	N.D.	N.D.	69.69	1.47	1.77
	CIH5	46.26 ± 0.24	30.16 ± 0.37	8.15 ± 0.08	1.31 ± 0.08	0.06 ± 0.009	N.D.	N.D.	76.42	1.53	1.96
	CIH6	42.15 ± 0.76	29.74 ± 0.27	7.39 ± 0.06	0.44 ± 0.03	N.D.	N.D.	N.D.	71.89	1.41	1.97
	CIH7	40.19 ± 0.18	28.01 ± 0.70	10.75 ± 0.17	0.87 ± 0.02	0.02 ± 0.004	N.D.	N.D.	68.20	1.43	1.81
Mean ± SD		42.70 ± 3.07	29.45 ± 1.16	8.93 ± 2.35	1.24 ± 0.69	0.02 ± 0.027	–	–	72.15	1.44	1.89

Results are reported as means ± standard deviation. Ribose, arabinose, and galactose were not detected. Note: F/G ratio, Fructose/Glucose ratio; G/W ratio, Glucose/Water ratio; N.D., not detected.

acids, and certain complex sugars [1,21]. The EC values of the local honeys ranged between 0.26 and 0.79 mS/cm, whereas those in the commercial honeys varied from 0.11 to 0.52 mS/cm (Table 2). All of the explored samples meet the threshold (≤0.8 mS/cm) recommended by the Codex [2] and EU requirements [17]. As shown in Table 2, there was no significant difference ($P > 0.05$) between the EC values of the LIH3 and LIH6 samples, while other investigated samples exhibited significant differences ($P < 0.05$) in their EC content. Similar values for EC were found in Argentinean honeys (0.12–0.68 mS/cm) [35], whereas higher levels were reported in honeys from Tunisia (0.39–0.89 mS/cm) [36] and Algeria (0.30–1.20 mS/cm) [31].

3.5. Sugars

The sugar profile of the 14 Iranian honeys is summarized in Table 3. The overlaid HPLC chromatogram comparing standard sugars at a concentration of 25 mg/mL with the LIH1 sample is provided in Fig-S1 of the Supplementary File. The maltose and trehalose disaccharides were identified in certain honey samples. None of the commercial honeys contained melibiose, while it was observed in some local samples at levels of approximately 0.5 % or less. Melezitose was exclusively detected in the LIH5. Ribose, arabinose, and galactose were not found within either of the analyzed honeys. The mean F + G values in the local and commercial honeys were determined as 62.02 % and 66.87 %, respectively. The sucrose content observed in the majority of the commercial honeys (4.82–12.39 %) exceeded those found in the local honeys (3.26–8.76 %). Importantly, all of which are above the maximum limit (5 %) regulated by the Codex [2] and EU [17] guidelines, except for the LIH3 (3.26 %), LIH5 (3.29 %), and CIH2 (4.82 %) samples. This is because of various causes, such as feeding honeybees with sucrose syrup, direct sugar incorporation, and nectar source fed by bees [9,32]. Although early harvesting of honey is often cited as a reason for high sucrose levels [35], this factor most likely cannot account for the elevated sucrose content in the present study, as all samples illustrated moisture content below 20 %. Indeed, the sucrose values in honey are straightforwardly proportional to its maturation in the hive, during which bee invertase enzymes continuously break down sucrose molecules within the nectar into fructose and glucose [3]. Our results are markedly higher than those reported by Degirmenci et al. [22] for Azerbaijani honeys (0–2.57 %), Moniruzzaman et al. [6] for Malaysian honeys (1.66–4.51 %), and Smetanska et al. [9] for honeys from worldwide (2.15–6.38 %).

The Fructose/Glucose (F/G) ratio reflects honey's crystallization potential. When the F/G ratio surpasses 1.3, honey remains liquid owing to the higher solubility of fructose in water, slowing down crystallization. Conversely, when the F/G ratio falls below 1, crystallization accelerates. Nevertheless, the crystallization prediction rate based solely on the F/G ratio has not been conclusively proven due to the impact of other variables, such as sucrose, maltose, and insoluble substances (dextrin, colloid, and pollen) [31]. Thus, the Glucose/water (G/W) ratio is suggested as a more appropriate parameter to estimate honey crystallization rate [26]. The crystallization of honey is sluggish when the G/W ratio is beneath 1.3, whereas it occurs rapidly when the ratio exceeds 2 on account of the low water content promoting glucose crystal aggregation [31]. According to the findings obtained (Table 3), the F/G and G/W ratios of the local honeys exhibited a range of 0.78–1.70 and 1.20 to 2.38, respectively. Meanwhile, such ratios varied from 1.30 to 1.53 and 1.77 to 1.96 in the commercial honeys. In both parameters, the samples collected at LIH1 and LIH6 recorded $F/G < 1$ and $G/W > 2$, indicating a higher tendency to crystallize. Additionally, the F/G ratio can affect the honey flavor, as fructose is notably sweeter than glucose [36]. The F/G ratio results are in line with those found by Zerrouk and Bahloul [7], and El Sohaimy et al. [26], which ranged from 0.92 to 1.60 and 0.42 to 2.35, respectively.

3.6. HMF

HMF, a mutagenic and genotoxic substance, is produced as an intermediate during the dehydration of hexoses (mainly fructose) under an acidic medium like honey, and high-temperature conditions in the non-enzymatic caramelization process known as the Maillard reaction [21,37]. Hence, HMF is practically not present in freshly harvested honey or exists in trace amounts (less than 10 mg/kg). However, its concentration considerably increases during storage and handling, as well as a result of liquefaction and pasteurization to eliminate crystallization nuclei, ultimately reducing the honey quality [3,27]. Khalil et al. [37] reported the HMF levels in Malaysian honeys increased by over fiftyfold after two years of storage at ambient temperature. The assessed HMF contents were found to range from 36.61 to 116.66 mg/kg for the local honeys and 56.32–228.11 mg/kg for the commercial ones (Table 2). The samples sourced from LIH3 recorded the least HMF content (36.61 mg/kg), while the CIH2 and CIH1 samples displayed the most HMF values, at 228.11 and 212.36 mg/kg, respectively. With the exception of honey samples from the LIH3, and partially LIH5, the HMF levels in the other samples noticeably went beyond the maximum conventional limit (40 mg/kg) proposed by the Codex [2] and EU [17] standards. In this regard, Jalili [38] reported significant HMF values (17.33–834.46 mg/kg) in Iranian commercial honey samples, which is in agreement with the results of the current study. Therefore, it appears that adulteration of Iranian commercial honeys is a prevalent practice. Furthermore, the outcomes indicated remarkably greater HMF levels compared to those obtained by Addi et al. [1] for southwest Ethiopian honeys (1.1–2.6 mg/kg), Can et al. [21] for Turkish honeys (0.61–62.24 mg/kg) and Pham et al. [27] for Vietnamese honeys (33.4–75.4 mg/kg). This could be caused by a multitude of factors, including the processing method employed (thermal intensity and duration), age of honey, storage conditions, botanical source, geological location, and honey composition, such as water activity, pH, acidity, sucrose level, F/G ratio, and mineral content [9,20,28]. Consequently, it is insufficient to rely solely on the HMF content to demonstrate honey overheating. Other variables, as mentioned earlier, must also be taken into account. Typically, both HMF content and diastase activity parameters are jointly assessed to ascertain evidence of overheating in honey.

3.7. Diastase activity

Diastases, a mixture of α-amylase and β-amylase, are enzymes naturally derived from the salivary glands of honeybees, degrading starch to maltose and maltotriose through their amylolytic activity [10,11]. The local honey samples illustrated diastase values ranging from 6.77 to 15.25 Schade units/g with a mean value of 10.27 ± 2.85 Schade units/g, while the commercial samples varied from 3.13 to 7.22 Schade units/g (mean 5.08 ± 1.61 Schade units/g), as detailed in Table 2. The highest diastase level was observed in the LIH5 sample, and the lowest level was found in CIH2. Out of the investigated honey samples, the diastase activity in all the Iranian commercial honeys, LIH1 and LIH2 falls under the minimum allowable level (8 Schade units/g), according to the Codex Alimentarius [2] and EU [17] regulations. The high HMF values and low diastase activity observed in such samples could be attributed to overheating during processing or prolonged storage. On the other hand, the majority of the aforementioned samples are likely to be relatively fresh, as they revealed a pH exceeding 4. Hence, the primary factor responsible for the observed discrepancies is overheating, resulting in a decline in their overall quality. In addition, the LIH4, LIH6, and LIH7 samples showed elevated concentrations of HMF and moderately acceptable diastase activity. The high HMF levels in these samples may be explained by other factors, including honey composition, botanical source, and harvest season. Therefore, the presence of low HMF content (≤40 mg/kg) combined with high diastase activity (≥8 Schade units/g) are essential features of high-quality honey [3]. It seems that proper thermal treatment was not employed during the processing of the commercial Iranian honeys (particularly CIH2 and CIH1), leading to an increase in HMF and a decrease in diastase activity. Besides, despite the low F/G ratio (0.78) and high G/W ratio (2.39) of the LIH1 sample, it has shown no signs of crystallization even after one year of storage, suggesting that it was most likely subjected to heat treatment. The abnormally high HMF content and relatively low diastase activity offer evidence for this claim. Our findings are compatible with those reported by Afshari et al. [20] for honey samples sourced from the Khorasan province of Iran (1.00–15.95 Schade units/g), and lower than those reported by Alvarez-Suarez et al. [5] for Cuban honeys (13.4–33.4 Schade units/g).

3.8. Proline

Proline, a principal amino acid found in honey, is primarily secreted from bee salivary glands throughout the biochemical transformation of nectar into honey [10]. The quantity of proline depends somewhat on the type of nectar collected by honeybees, so it may vary based on the botanical source [6]. The measured proline contents in the commercial honeys (109.84–173.86 mg/kg) were noticeably below the minimum (180 mg/kg) threshold reported by the Codex Alimentarius [2] and EU [17], as compared to the explored local honeys (168.39–677.26 mg/kg) (Table 2). The proline level indicates honey ripeness and aids in distinguishing adulteration, since high proline content is assumed to decrease the possibility of sugar adulteration in honey [4,36]. Accordingly, the findings revealed that the highest levels of sugar adulteration occurred in all of the commercial samples and certain investigated local samples (LIH1, LIH2, and LIH7), while the local samples collected at LIH3 and LIH5 exhibited high quality and authenticity in this regard. Our results are consistent with those found for Indian honeys (133–674 mg/kg) [32], and lower than the Turkish and Azerbaijani honeys, with proline values ranged from 282 to 845 and 314.34–1077.21 mg/kg, respectively [21,22].

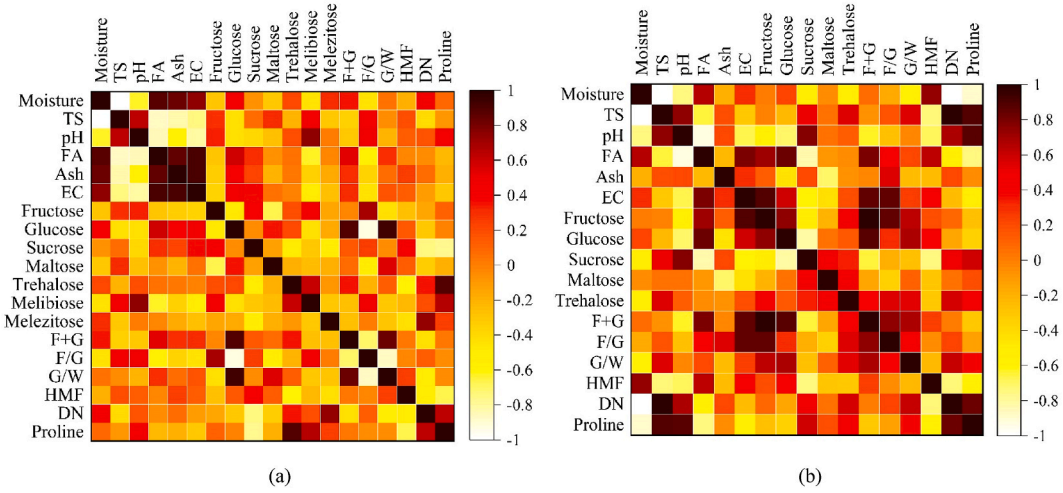

Fig. 3. Pearson's correlation matrix heat-map between investigated parameters. (a) Local Iranian honeys, and (b) commercial Iranian honeys. In the right-hand figure, the melibiose and melizitose parameters were excluded from the correlation plot analysis due to their absence in any of the commercial samples. The color scale on the right side of each figure denotes the correlation coefficients, ranging from +1 (complete direct covariations) to 0 (without covariations) and to −1 (complete inverse covariations).

3.9. Correlation between the physicochemical properties of Iranian honeys

The Pearson's correlation matrix heat-map between all examined parameters within the local and commercial honeys is shown in Fig. 3(a and b). The obtained results demonstrated a significant positive correlation ($r = 0.915$, $p < 0.01$) between EC and ash content in the local honeys, aligning with prior findings reported by Boussaid et al. [36] and Saxena et al. [32], with correlation coefficients of 0.97 and 0.98, respectively. In contrast, no strong correlation ($r = 0.299$) was found between those within the commercial honeys. The leading cause of this discrepancy is most likely the adulteration practices employed in the commercial honeys. Notably, the nature and magnitude of adulterants used in the commercial honeys can give rise to fluctuations in EC values. This was supported by Oroian et al. [39] that fructose adulteration results in decreased EC values, while adulteration with hydrolyzed inulin syrup (HIS) elevates EC by virtue of its acidic nature. In the current study, the potential HIS adulteration in the CIH2 sample is suggested due to its markedly higher EC than CIH3, despite both samples sharing an identical ash content (0.08 %) (Table 2). The high levels of fructose, glucose, and free acidity, along with low sucrose content in the CIH2 as compared to the other commercial samples, further support this assertion. In addition, there was a significant correlation ($r = 0.869$, $p < 0.01$) between EC and moisture content in the local honeys, reflecting the dependable nature of EC on honey moisture. Indeed, the addition of water to honey displays an increase in EC values when compared to pure honey [40]. This correlation finding is in agreement with those found by Addi et al. [1] ($r = 0.791$, $p < 0.01$) and Belay et al. [40] ($r = 0.762$, $p < 0.01$). Besides, moisture was negatively correlated with sugars (fructose and fructose), indicating that the high moisture content of honey leads to a decline in sugar levels through fermentation. The proline content displayed a strong negative correlation with the moisture levels in the commercial samples ($r = -0.840$, $p < 0.01$). Gzik [41] reported that rapid augmentation in proline concentration coincides with the onset of leaf water potential reduction under various stress conditions (osmotic, water, and salt). A significant positive correlation coefficient also existed between proline and diastase in both local ($r = 0.637$, $p < 0.01$) and commercial honeys ($r = 0.806$, $p < 0.01$), consistent with the outcomes of Flanjak et al. [42] ($r = 0.834$, $p < 0.05$). This is probably owing to the denaturation of proline [43], accompanied by a decrease in diastase numbers during thermal treatment applied to Iranian honeys.

3.10. Hierarchical cluster analysis (HCA)

The heat-map with two-dimensional dendrograms for the classification of the studied variables and samples is presented in Fig. 4. The multivariate HCA of variables was classified into four distinct clusters. The first cluster is composed of moisture, ash, EC, and free acidity, and the second cluster includes trehalose, proline, melibiose, melezitose, and diastase. The third cluster consists of total solids, F/G ratio, and pH, while the fourth cluster includes fructose, F + G, HMF, sucrose, maltose, glucose, and G/W ratio. The co-occurrence of monosaccharides and HMF within the same cluster once more supports the evidence of adulteration with sugar solutions and post-harvest thermal treatment in Iranian honey samples. The dendrogram analysis results indicated the presence of three discrete groups based on the similarities in the physicochemical properties of the analyzed samples. The first group encompasses samples from LIH1, CIH1, CIH3, CIH4, CIH6, and CIH7, while the second group is composed of CIH2 and CIH5. The third group comprises samples sourced from LIH2, LIH4, LIH7, LIH5, LIH3 and LIH6. The LIH1 sample revealed physicochemical characteristics more closely resembling those of the commercial honeys, as compared to other honeys collected from its native floristic region, Irano-Turanian. Indeed, noticeable adulterations in the honeys and limited knowledge among Iranian beekeepers most likely hindered the clustering of the investigated honeys based on their geographical and botanical origin across various floristic regions.

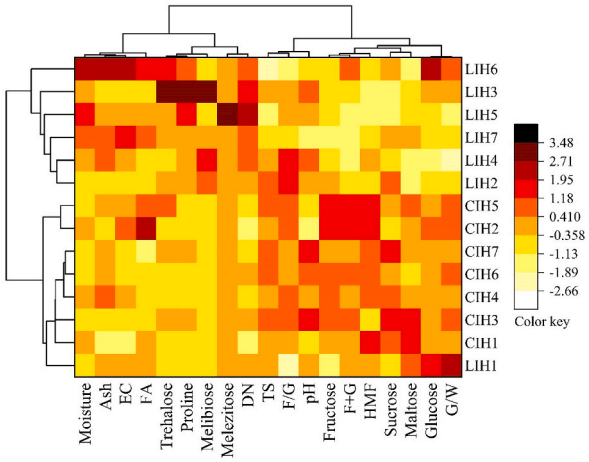

Fig. 4. The Z-score heat-map with two-dimensional dendrograms for classification of the investigated variables and honey samples using the Ward's method. The color key represents the scaled values of physicochemical parameters, with the yellow spectrum corresponding to low levels, and the red spectrum corresponding to high levels.

4. Conclusion

This is the first comparative study of the physicochemical properties of locally sourced honeys from various floristic regions of Iran and commercially available multi-floral honeys in the Iranian market. The melissopalynological analysis of the honeys demonstrated that the *Fabaceae*, *Asteraceae*, and *Lamiaceae* families were predominantly visited by bees within the Iranian flora, and the local samples were categorized as multi-floral honey. Among all the parameters investigated, low HMF values and high diastase activity are the two most crucial factors in the measurement of honey authenticity. Out of the tested honey samples, the LIH3 and partially LIH5 samples were found to comply with the Codex and EU standards. The Iranian commercial honeys exhibited exceedingly poor quality when compared to the local honeys, possibly due to the direct utilization of industrial sugar syrups and improper thermal treatment practices. The findings of HCA analysis and the correlation between certain parameters, particularly the significant absence of correlation between ash and EC in the commercial honeys, confirmed this issue. In general, Iranian multi-floral honeys were not of acceptable quality in comparison with honeys produced in other regions of the world. This is a result of the lack of periodic training for beekeepers and the absence of monitoring programs by the relevant institutions to protect consumer rights and public health, leading to challenges in exporting Iranian honeys.

Compliance with ethics requirements

The article does not contain any studies with human participants or animal subjects.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Adel Hajian-Tilaki: Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Reza Esmaeilzadeh Kenari:** Writing – original draft, Supervision, Software, Resources, Methodology, Funding acquisition. **Reza Farahmandfar:** Writing – original draft, Visualization, Formal analysis, Data curation. **Razie Razavi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37550.

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