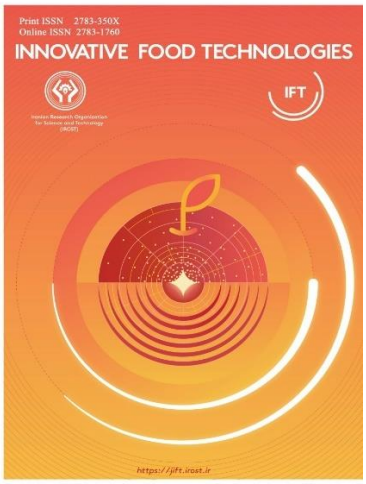
Journal Pre-proofs



Physicochemical Characterization and Compliance Assessment of Commercial Honey with International Standards

Vahid Mofid, Sama Barzegar-bafrouei, Behrouz Tajdar-Oranj, Parisa Sadighara, Amirhossein Abedini, Solmaz Abedinzadeh, Anosheh Rahmani,

DOI: https://doi.org/ 10.22104/ift.2026.8404.2315

To appear in: Innovative Food Technologies (IFT)

Received Date: 8 July 2026
Revised Date: 5 August 2026
Accepted Date: 22 August 2026

Please cite this article as: Vahid Mofid, Sama Barzegar-bafrouei, Behrouz Tajdar-Oranj, Parisa Sadighara, Amirhossein Abedini, Solmaz Abedinzadeh, Anosheh Rahmani, Physicochemical Characterization and Compliance Assessment of Commercial Honey with International Standards, *Innovative Food Technologies (2026)*, doi: https://doi.org/ 10.22104/ift.2026.8404.2315

Physicochemical Characterization and Compliance Assessment of Commercial Honey with International Standards

Vahid Mofid[1], Sama Barzegar-bafrouei[2], Behrouz Tajdar-Oranj[3], Parisa Sadighara[4], Amirhossein Abedini[5], Solmaz Abedinzadeh[6,*], Anosheh Rahmani[7,*]

[1.] Department of Food Science and Technology, Faculty of Nutrition and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2.] Department of Food Safety and Hygiene, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[3.] Research Center for Environmental Determinants of Health (RCEDH), Kermanshah University of Medical Sciences, Kermanshah, Iran.
[4.] Department of Environmental Health, Food Safety Division, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
[5.] Student Research Committee, Department of Food Science and Technology, Faculty of Nutrition and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[6.] Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
[7.] Department of Food, Halal and Agricultural Products, Food Technology and Agricultural Products Research Center, Standard Research Institute (SRI), Karaj, Iran.

* **Corresponding authors*:*** S. Abedinzadeh; **(**Email: s.abedinzadeh92@gmail.com), A. Rahmani; (Email: a.rahmani@standard.ac.ir)

Abstract

Honey is a natural food product widely valued for its nutritional and functional properties, yet its high economic value makes it vulnerable to quality deterioration and fraudulent practices. This study evaluated the physicochemical quality of 76 commercially available honey samples collected from different regions of Iran, in accordance with national and international standards. Proline, sucrose, reducing sugars, diastase activity, hydroxymethylfurfural (HMF), fructose-to-glucose (F/G) ratio, and free acidity were determined using methods recommended by the International Honey Commission and Iranian National Standardization Organization (INSO). Proline ranged from 51.20 to 480.00 mg/kg, sucrose from 0.70 to 15.21 g/100 g, reducing sugars from 30.80 to 83.70 g/100 g, diastase activity from 0.50 to 30.00 DN, HMF from 7.10 to 126.80 mg/kg, F/G ratio from 0.10 to 2.50, and free acidity from 1.00 to 28.10 mEq/kg. Notable regional variations were observed: samples from Ahvaz and Shiraz showed higher average proline contents, while Isfahan samples exhibited the highest mean HMF levels. In contrast, samples from Zahedan and Tehran generally presented lower proline values. Overall, most samples

complied with the established standard limits, indicating acceptable physicochemical quality of commercial honeys marketed in Iran. These findings provide useful information for quality control and regulatory monitoring programs.
Keywords: Honey quality, Physicochemical parameters, Commercial honey, Iran

1. Introduction

Honey is a wholesome, natural, and nutritional food made by honey bees from the nectar of various flowers (Fairchild, Capps Jr, & Nichols, 2000). Honey is mainly composed of carbohydrates, predominantly fructose and glucose, whose concentrations may vary depending on botanical and geographical origin, typically ranging between 30–44% for fructose and 25–40% for glucose (Santos-Buelga & González-Paramás, 2025). In addition, this product encompasses minerals, proteins, vitamins (niacin, riboflavin, pantothenic acid, folate, pyridoxine, and vitamin C), enzymes (such as catalase, reduced glutathione, and superoxide dismutase), flavonoids, and phenolic acids (Ahmed, 2020). Weather conditions, environmental factors, honey beekeeping procedures, and most importantly, the type of plants from which bees collect their nectar, determine the composition of honey (Se, Wahab, Yaacob, & Ghoshal, 2019).

The therapeutic potential of honey is complex, as the concentrations of its various compounds vary across different types of honey. Several studies have reported biological activities of honey, including antioxidant and antimicrobial effects, which are influenced by its chemical composition(Abu-Farich et al., 2024; Nweze, Olovo, Nweze, John, & Paul, 2019).

Natural honey's price is much higher than that of other sweeteners such as refined cane sugar, beet sugar, or corn syrup due to its high nutritional value and unparalleled flavor. The reasons for food product fraud are mainly economic. In honey, the high price and high consumption are among the reasons for fraud (Kurt, Palabiyik, Gunes, Konar, & Toker, 2020). Honey adulteration has been increasingly reported since the introduction of inexpensive sugar syrups into the food industry, making quality monitoring an important regulatory concern (Mehryar & Esmaiili, 2011; Nhlapho, 2023). Adulteration may reduce the quality of honey and sometimes threaten the product's safety. For example, when honey is adulterated with chemicals and artificial substances, consumer health is affected, and the resulting disease can be identified over the long term. Also, in medicinal products, fraud can significantly reduce their medicinal value (Naila, Flint, Sulaiman, Ajit, & Weeds, 2018). Cheating has two categories: direct and indirect. Direct methods include the addition of foreign materials to honey, and indirect methods include the use of synthetic and industrial sugars (Zábrodská & Vorlová, 2015).

In addition to sweeteners, feeding bees during the nectar flow or extracting combs containing bee feed is considered fraud. Sugar syrups were considered an important source of fraud in honey (Bogdanov, 2007). Methods of identifying fraud in food products are used traditionally and innovatively. These methods are mainly based on quantitative and qualitative identification of fraud (CABANero, Recio, & Ruperez, 2006). In recent years, research has focused on instrumental analysis techniques, including isotopic ratio, chromatography, nuclear magnetic resonance (NMR), and spectroscopic methods. Traditional methods have disadvantages such as high cost and longer time. With the spread of food fraud and considering the disadvantages of traditional methods, new methods with high accuracy and sensitivity can be considered as one of the solutions (Kurt et al., 2020).

Despite the availability of advanced analytical techniques for honey authentication, routine market surveillance studies based on physicochemical quality indicators remain essential, particularly in countries with diverse climatic and botanical conditions such as Iran. Previous

studies on Iranian honey have mainly focused on limited geographical areas or samples collected directly from apiaries, and comprehensive data on the quality of commercially available honeys across multiple regions are still scarce (Zielińska, Wesołowska, Bilek, Kaniuczak, & Dżugan).

Therefore, the objective of the present study was to evaluate the physicochemical quality of 76 commercially available honey samples collected from 12 cities in different regions of Iran and to assess their compliance with national (INSO) and international (Codex Alimentarius) standards. The key parameters examined included proline, diastase activity, hydroxymethylfurfural (HMF), reducing sugars, sucrose, fructose-to-glucose (F/G) ratio, and free acidity. By providing a multi-regional, market-based assessment, this study aims to generate updated baseline data that can support quality control, regulatory monitoring, and consumer protection efforts in the Iranian honey market.

2. Materials and methods

2.1. Sampling

A total of 76 commercially available honey samples were collected from retail hypermarkets and local markets in 12 cities across different geographical regions of Iran, including Mashhad, Sari, Tabriz, Tehran, Isfahan, Zanjan, Karaj, Ahvaz, Bandar Abbas, Kerman, Zahedan, and Shiraz. These cities were selected to represent major honey-producing and consuming areas with diverse climatic, altitude, and environmental conditions.

Approximately equal numbers of samples (6–7) were obtained from each city based on market availability. The inclusion criteria were commercially packaged honeys labeled as pure honey and offered for retail sale to consumers. Samples were purchased in their original commercial packaging. The sampling was based on convenience sampling according to market availability in each city at the time of collection.

Prior to laboratory analysis, the samples were stored under refrigerated conditions (4 °C) and analyzed within two weeks to minimize physicochemical changes. Sample handling and transportation followed standard laboratory practices to ensure sample integrity.

2.2. Determination of Proline

Proline content was determined using a ninhydrin-based colorimetric method, following standard procedures, and expressed relative to the weight of honey. A honey solution (0.05 g/mL) was reacted with formic acid (80%) and 3% ninhydrin in ethylene glycol monomethyl ether, heated according to the standard protocol, and after cooling, absorbance was measured at 510 nm. Proline standards and distilled water were used for calibration and blanks, respectively. Proline concentration (mg/kg) was calculated using the standard calibration Equation 1(Meda, Lamien, Romito, Millogo, & Nacoulma, 2005).

Equation 1. $\text{Proline} = (E_s/E_a) \times (E_1/E_2) \times 80$

where Es represents the absorbance of the sample solution, Ea the absorbance of the proline standard solution, E1 the amount of proline (mg) in the standard solution, and E2 the weight of honey in grams.

2.3. Determination of Sucrose

Sucrose content was estimated indirectly by measuring the total soluble solids (°Brix) of honey using a digital refractometer (D-22297, KRUESS Optronic, Germany) and calculating water content from the refractive index according to standard protocols (Sapri & Jamaludin).

2.4. Determination of Reducing Sugars

Reducing sugars and apparent sucrose were determined as described by Sant'ana et al. (2020). Honey samples were appropriately diluted and titrated with Fehling's reagent, with acid

hydrolysis used to estimate sucrose content. All procedures were conducted in accordance with the referenced standard method (Sant'ana, de Carvalho, OdaSouza, Souza, & Dias, 2020).

2.5. Determination of Diastase

The diastatic activity of honeys was estimated by the procedure of the International Honey Commission (IHC), based on the Phadebas method. In brief, 1.00 g of honey was dissolved in 100 mL of acetate buffer (pH 5.2). One Phadebas tablet was then introduced into 5 mL of honey solution and the solution was incubated for 30 min at 40 °C. The reaction was halted by addition of 1 mL of 0.5 M NaOH, and after filtration of the sample, the absorbance of filtrates was measured at 620 nm. DN was estimated from the standard calibration curves of α-amylase and expressed in Shade units (Sak-Bosnar & Sakač, 2012).

2.6. Determination of HMF

The content of HMF was determined using the spectrophotometric method of White (IHC) (Parviz et al., 2015).

5 g of honey was diluted with 25 mL of distilled water, placed in a 50 mL volumetric flask, and filtered using 0.5 mL each of Carrez I and Carrez II solutions. The absorption value of the filtrate was measured at wavelengths 284 nm and 336 nm for the solution, which contained 0.2% sodium bisulfite. The content of HMF was calculated as follows:

$$\text{HMF (mg/kg)} = (A_{284} - A_{336}) \times 149.7 \times 5/ \text{ sample weight (g)}$$

2.7. Determination of F/G Ratio

F/G ratio was calculated by means of the iodometric titration method based on the standard procedure recommended by Khandelwal, Paliwal, and Zade (2020). Firstly, a certain quantity of the honey sample was mixed with distilled water. To measure glucose concentration, the test solution was subjected to the reaction with the solution of iodine in an alkaline medium (iodometric oxidation) followed by the titration with the standardized sodium thiosulfate solution (with starch being the indicator). Then fructose content was estimated by the difference from the total content of reducing sugars measured using any reducing sugar method (e.g., Lane-Eynon or its equivalent).

2.8. Determination of Free Acidity

Free acidity was determined by titrating a honey solution with 0.1 M NaOH to pH 8.30, and results were expressed in milliequivalents per kilogram (mEq/kg) following standard procedures (Yadata, 2014; Živkov-Baloš et al., 2018).

2.9. Statistical Analyses

Each experiment was performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS software version 24 (IBM, Armonk, NY, USA). The normality of data distribution was checked. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to compare means among different cities. Differences were considered statistically significant at $p \leq 0.05$.

Each experiment was performed in triplicate, and results were represented as the mean and standard deviation (SD). Statistical analysis was done using SPSS software version 24 (SPSS, IBM, Armonk, NY). One-way ANOVA, followed by Duncan's test, was used to compare means and assess statistical significance at $p \leq 0.05$.

3. Results and Discussion

Physicochemical properties of the 76 samples of honey taken from different parts of Iran are given in Tables 1 and 2. Significant variability is noted among all measured variables, which

include proline, diastase activity, hydroxymethyl furfural, reducing sugars, sucrose, fuctose/glucose ratio, and free acidity (Danieli & Lazzari, 2022). The quality properties of honey samples were tested using Codex Alimentarius Standard for Honey (CXS 12-1981, revised edition) and also based on the standard criteria laid down by the INSO, which are usually considered standard guidelines for controlling honey quality.

3.1 Proline content

The mean values, standard deviations, and ranges of the physicochemical parameters analyzed in the honey samples are summarized in **Tables 1** and **2**. In the present study, proline content ranged from 51.20 to 480.00 mg/kg among the analyzed honey samples. The mean proline content was 215.60 ± 90.17 mg/kg, with approximately 70% of samples meeting the minimum levels established by CAC (>200 mg/kg) and INSO. Conversely, about 30% of the samples exhibited proline levels below the recommended CAC threshold. A higher frequency of samples with proline values below the standard limit was observed in samples marketed in Zahedan, Sari, Kerman, Isfahan, and Tehran. The distribution of proline content across different regions is illustrated in **Figure 1**. The highest mean proline contents were recorded in samples from Ahvaz (314.00 mg/kg) and Shiraz (301.83 mg/kg), whereas the lowest means were observed in Zahedan (143.89 mg/kg) and Tehran (161.36 mg/kg). These variations may be related to differences in botanical origin and environmental conditions across the sampling regions.

Proline is the predominant free amino acid in honey and is widely used as an indicator of amino acid content and overall honey quality. Proline content has also been associated with antioxidant capacity and freshness of honey (Truzzi, Annibaldi, Illuminati, Finale, & Scarponi, 2014). According to international standards, proline concentrations below 200 mg/kg may indicate immature or adulterated honey (Bogdanov, 2015). The proline levels observed in the present study were generally comparable to values reported in previous studies, although substantial variability was evident. Previous studies have demonstrated wide variability in proline content across floral origins, botanical diversity, and geographical locations. For example, markedly higher proline levels have been reported in monofloral honeys such as coriander and chestnut, whereas lower levels are typically associated with acacia and multifloral honeys (Czipa, Borbély, & Győri, 2012; Meda et al., 2005; Széles, Borbély, Hovánszki, Tisza, & Győri, 2007). In the present study, approximately 70% of samples exhibited proline levels within the acceptable range, supporting the notion that a substantial proportion of marketed honeys meet basic quality requirements. Recent studies have emphasized that proline variability in market honey samples should be interpreted with caution, as post-harvest handling, storage duration, and blending practices may influence amino acid profiles without necessarily indicating intentional adulteration (Cilavdaroğlu, 2026; Zhang et al., 2023).

3.2 Sucrose content

According to CAC standards, the sucrose content of honey should not exceed 5 g/100 g (CAC, 2001). No statistically significant differences in sucrose content were observed among regions ($p > 0.05$). In this study, sucrose content ranged from 0.7% to 15.21%. The mean sucrose content was 5.47 ± 3.33 g/100 g, with 63.15% of samples complying with both CAC and INSO requirements. Also, the results showed that Higher proportions of non-compliant samples were observed among honeys marketed in Sari, Kerman, Shiraz, Mashhad, Isfahan, and Karaj. The sucrose content of samples from different areas is illustrated in **Figure 2**.

Sucrose content was evaluated as an important quality parameter that may reflect production practices and harvesting conditions. Elevated sucrose levels may result from practices

such as early harvesting, supplementary feeding, or technological interventions during production. Moreover, due to early harvesting, sucrose is not fully converted into glucose and fructose, which may be another reason for high sucrose levels. Previous studies have shown that prolonged feeding of bees with sugar syrups may also contribute to increased sucrose levels in honey.

Recent evidence suggests that elevated sucrose levels in commercial honeys are not exclusively associated with fraudulent practices but may also arise from seasonal harvesting strategies, nectar composition, and regional feeding management, particularly in large-scale production systems (Awulachew, 2025).

Comparative studies from neighboring regions, such as Southeastern Anatolia and Ethiopia, have reported physicochemical parameters largely within regulatory limits, although variations among regions were evident (Gürbüz et al., 2020). Gebremariam and Brhane (2014) evaluated honey samples from northern Ethiopia. The reported cases include HMF, sucrose, reducing sugars, and free acidity, which were observed at 8.32-45.26 mg/kg of honey, 2.24-12.21%, 50.31-79.56%, and 3.99-45.17 mEq/kg, respectively. They reported that the quality of honey and the limits of national and international standards showed that the collected honey products had good quality in terms of standard parameters (Gebremariam & Brhane, 2014). The sucrose values found in this study were higher than Zehra Can et al. (2015) study in Turkey, with the highest amount being 3.4 g/100 g, which means all of the Turkish samples comply with CAC (less than 5%) (Can et al., 2015).

3.3 Sum of reducing sugars

According to the EC Directive, the sum of reducing sugars (fructose and glucose) in honey should exceed 60 g/100 g (EC, 2002). Reducing sugar content ranged from 30.80% to 83.70% among the analyzed samples. The mean reducing sugar determined for honey samples was 68.05±7.70 g/100 g; approximately 15.78% of samples exhibited reducing sugar levels below the EC minimum requirement. Moreover, the results showed that the cities of Kerman (65.06±2.49), Zahedan (65.18±3.79), and Tehran (65.29±4.60) had the highest numbers of samples with values below the maximum permitted by INSO.

The observed mean reducing sugar content of 68.05 ± 7.70 g/100 g in the analyzed Iranian honey samples aligns closely with values reported in other studies, such as 71.2 ± 1.3% in local Omani honeys and 70.84% (range 65.01–76.61%) in honeys from Khorasan Province, Iran. However, the wide range (30.80–83.70 g/100 g) and the fact that approximately 15.78% of samples fell below the EC Directive minimum of 60 g/100 g (fructose + glucose) indicate notable variability in quality. This non-compliance is consistent with findings from other Iranian surveys, including those from Ardabil Province, where 6.11% of samples were below standards, and may stem from factors such as botanical origin, geographical and climatic variations, adulteration with sugars or syrups, or improper harvesting/processing practices that dilute natural invert sugars.

Higher proportions of substandard samples in cities like Kerman, Zahedan, and Tehran could reflect regional differences in floral sources, environmental stressors (e.g., arid conditions that affect nectar composition), or supply chain issues, including greater exposure to adulteration in urban markets. Similar regional variations have been documented in studies of stingless bee and Apis mellifera honeys, where reducing sugar levels are influenced by climate, processing, and botanical diversity. These results underscore the need for stricter adherence to EC (2002) and INSO standards, enhanced traceability, and routine monitoring to ensure consumer safety and maintain the authenticity of Iranian honey. Further investigations into specific floral origins

and production practices in these regions would help mitigate such quality deviations (Raweh, Badjah-Hadj-Ahmed, Iqbal, & Alqarni, 2023).

3.4 Diastase number

Diastase number (DN), expressed in Göthe units, reflects the enzymatic activity of α- and β-amylases naturally present in honey. This number is related to the hydrolysis of starch in 1 h at 40°C by the enzyme activity in 1 gram of honey (CAC, 2001). In this study, the range of diastases was 0.50 to 30.00 DN. The mean diastase content determined for honey samples was 9.07±4.75 DN, of which 64.47% of samples exceeded the 8 DN threshold set by CAC and INSO. Lower diastase activity values were more frequently observed in samples marketed in Shiraz, Kerman, Tehran, Tabriz, Isfahan, and Karaj. **Figure 3** compares various areas of Iran in terms of the diastase activity of honey samples. Mean diastase activity was highest in samples from Zanjan (13.36 DN) and lowest in samples from Kerman (5.93 DN) and Mashhad (6.34 DN).

Diastase activity, primarily associated with α- and β-amylases, is a well-established indicator of honey freshness and thermal history. Diastase content is related to the floral and geographical origins of the honey. Because diastase enzymes are heat-sensitive, reduced activity may reflect excessive heating or prolonged storage (Beykaya, Samancı, Samancı, & Önder, 2026; da Silva, Gauche, Gonzaga, Costa, & Fett, 2016). Al-Farsi et al. (2018) observed that examining the samples against the criteria of the Gulf Standardization Organization (GSO) for fructose, glucose, sucrose, diastase, HMF, and acidity is not suitable. They reported that the HMF and acidity parameters of the samples were 29% and 30% higher, respectively (Al-Farsi et al., 2018). In Nigeria, Bako et al. (2019) reported 23.20±0.02 to 26.46±0.02 DN scale values for diastase activity in honey, and no significant differences were observed among the honey samples (Bako, Mamai, & Bature, 2019). Studies conducted in Iran and other countries have reported comparable ranges of diastase activity, suggesting that observed variations are influenced by multiple factors, including floral origin, climate, and storage conditions (Parviz et al., 2015). Low diastase levels and proline contents in some samples could be the consequences of overheating, prolonged storage, or possibly diluting with sugar syrups.

3.5 HMF concentration

According to the INSO, the HMF concentration in honey should not exceed 40 mg/kg. In the current study, HMF values ranged from 7.10 to 126.80 mg/kg. The mean HMF content was 36.05 ± 22.67 mg/kg. 71% of the samples had levels below 40 mg/kg. The cities of Isfahan (65.04±36.53 mg/kg), Ahvaz (45.50±26.36 mg/kg), Zanjan (45.50±34.03 mg/kg), Kerman (39.23±14.95 mg/kg), Tabriz (32.66±20.53 mg/kg), and Sari (33.28±19.21 mg/kg) have the highest number of samples that exceeded the maximum permitted value, respectively. Elevated HMF levels observed in some samples may indicate the influence of processing and storage conditions rather than botanical origin alone. The number of HMF-containing samples marketed in different cities is presented in **Figure 4**. The highest mean HMF concentration was found in samples from Isfahan (65.04 mg/kg), with several individual samples exceeding 100 mg/kg, while samples from Zahedan showed the lowest mean value (21.46 mg/kg).

HMF is widely used as an indicator of honey freshness, thermal processing, and storage conditions (Kukurova, Karovičová, Greif, Kohajdová, & Lehkoživová, 2006). Elevated HMF levels are commonly associated with excessive heating, prolonged storage, or exposure to high ambient temperatures (Saeed & Jayashankar, 2020). The investigation of HMF content in honey has been the focus of many researchers and legislators. Bely et al. (2013) investigated the physicochemical characteristics of honey in Ethiopia. The results depicted that reducing sugar (69.48±1.72 g/100g), sucrose (2.43±1.02 g/100g), free acidity (34.57±4.80 mEq/kg), and HMF

content (84±0.46 mg/kg) comply with standard levels by CAC, the European Union (EU), and Ethiopian standards (Belay, Solomon, Bultossa, Adgaba, & Melaku, 2013). Ghorbani et al. (2017) reported that HMF content differs between northwest and southwest Iran. They observed that the northwest yielded 16.9 mg/kg and the southwest yielded 2.85 mg/kg. The safety limit recommended by INSO was also checked, and 70% of commercial honey samples and 5% of natural honey samples exceeded the INSO limit. As mentioned, climatic and geographical conditions affect compound content. For example, HMF content can be significantly affected by this relationship. In addition, studies have shown that atmospheric and geographical effects on acetic acid are influenced by other intervening factors, such as the presence of microorganisms that use sugars as an energy source and the composition of nectar. These factors can ultimately affect the taste and aroma of the final product (Ghorbani, Saei-Dehkordi, Mohebbi, & Pak, 2017).

Several recent investigations have highlighted that climatic stress, transportation conditions, and prolonged exposure to elevated ambient temperatures can significantly affect HMF formation and enzyme stability, particularly in honeys marketed in warm regions (Bhure, Alam, Nanda, Pawar, & Saxena, 2025).

The HMF content of the commercial Iranian honeys in the present study (range: 7.10–126.80 mg/kg; mean: 36.05 ± 22.67 mg/kg) was considerably higher and more variable than the values reported for floral honeys from the Çoruh Valley of Türkiye (11.80–31.00 mg/kg; mean: 21.57 ± 6.30 mg/kg), where all samples remained well below the Codex limit of 40 mg/kg (Tosun, Cengiz, Erdoğan, & Turan, 2026). The higher mean and the presence of samples exceeding 40 mg/kg (and even 100 mg/kg) in the Iranian commercial samples may reflect differences in post-harvest handling, storage conditions, or thermal processing compared with the fresher, regionally produced honeys examined by Tosun et al. (2026). High HMF levels present in some samples, especially the ones extracted from Isfahan, might be caused by too much heating at the stage of processing or storing under higher temperatures.

3.6 Fructose to glucose ratio

According to INSO, the F/G ratio in honey should be greater than 0.9. In this study, the F/G ratio ranged from 0.10 to 2.50. The mean F/G ratio for honey samples was 1.05±0.34, and 18.42% of samples had lower levels than the INSO. As shown in **Figure 5**, lower F/G ratios were more frequently observed in samples marketed in Isfahan, Kerman, and Zanjan.

As shown in **Figure 6**, all analyzed samples exhibited free acidity values within the limits established by the EC Directive. The range of free acidity varied between 1.00 and 28.10 mEq/kg. Also, the mean free acidity determined for honey samples was 13.04±6.42 mEq/kg, with all the examined samples meeting the requirements set by the regulation, which establishes that the acidity should not exceed 50 mEq/kg, as well as INSO (EC, 2002).

The main sugars of honey are fructose and glucose, respectively. In most nectar honeys, fructose content tends to exceed glucose, although this ratio varies depending on botanical origin. In addition, the total amounts of fructose and glucose, the fructose/glucose ratio, and the glucose/water ratio are other determining factors indicating honey quality. The F/G ratio is a key parameter influencing honey crystallization behavior and texture (El Sohaimy, Masry, & Shehata, 2015). Kirs et al. (2011) reported the mean of free acidity (20.4±3.00 mmol/kg), diastase activity (23.1±3.89 DN), HMF (all analyzed honey was lower than 3.8 mg/kg), and fructose/glucose ratio (1.03±0.08), respectively, in Minas Gerais (Brazil), which their findings are to some extent similar to the findings of the current study (Kirs, Pall, Martverk, & Laos, 2011). In a study conducted in Brazil, the F/G ratio was reported to be on the order of 1 to 2:1.

This ratio can affect the flavor and granulation of honey and affect the physicochemical and technological parameters of honey (Maieves et al., 2020). Buba et al. (2013) reported that one of the important things to control and predict granulation tendencies in honey-based products is the F/G ratio and glucose/water ratio (Buba, Gidado, & Shugaba, 2013).

Khalafi et al. (2016) found that the HMF levels, diastase activity, and fructose/glucose ratio of the honey samples ranged between 2.2 and 39.4 mg/kg, 5.8 and 21.3 DN, and 1.1 and 1.5%, respectively. The result of this study showed that the diastase activity of honey samples ranged higher than that reported by the CAC (Khalafi, Goli, & Behjatian, 2016). In a study in the west Azerbaijan province, Iran, the range of HMF content, reducing sugar, F/G ratio, sucrose content, free acidity, and diastase activity of samples of honey were 0.04-17.2 mg/kg, 68.21-78.49 g/100g, 0.85-1.23, 1.73-5.47%, 12-25 mEq/kg, 9.41-25.20 DN, respectively. This study showed that all factors vary by regional and climatic conditions; similar values are also found in this study (Mehryar, Esmaiili, & Hassanzadeh, 2013).

From a technological perspective, recent studies have underlined the importance of the F/G ratio not only as a crystallization indicator but also as a parameter influencing consumer perception, shelf stability, and processing behavior of honey-based products (Tarapoulouzi, Mironescu, Drouza, Mironescu, & Agriopoulou, 2023).

Free acidity in honey primarily arises from the formation of organic acids, particularly gluconic acid, during enzymatic and fermentation processes. The conditions of this fermentation have been observed in balance with internal lactones or esters and inorganic ions such as phosphate and chloride (Parviz et al., 2015). Excessive fermentation leads to increased acidity (Iglesias et al., 2012). All analyzed samples exhibited free acidity values below the maximum limit of 50 mEq/kg, indicating acceptable freshness and fermentation status. The results of this study are in agreement with the study of Baloš et al. (2018), which worked on Fifty-five acacia, meadow, floral, linden, and dew honey samples in which acidity values ranged from 1.5 mEq/kg to 30 mEq/kg (Živkov-Baloš et al., 2018). Also, Boussaid et al. (2018) studied the physicochemical properties and bioactive characteristics of six honey samples from several floral origins in Tunisia. In this study, none of the analyzed samples were more than 50 mEq/kg as required by international regulations. Thus, all samples complied with the standards. It is assumed that different types of flowers with different origins, as well as changes in harvesting seasons, can be the reason for the difference in free acidity among honey samples (Boussaid et al., 2018). The free acidity suggested by CAC is below 50 mEq/kg. Ajlouni and Sujirapinyokul examined seven samples in their study; all samples were able to comply with the CAC range. The free acidity of honey samples in their study was from 10.25 ± 0.01 to 20.34±0.18 mEq/kg in Grey box and Banksia, respectively (Ajlouni & Sujirapinyokul, 2010). Several agents are recognized as probably responsible for the vast variation of the mentioned parameter (free acidity of honey). For instance, in some studies, the influences of different harvest periods as well as geographical and botanical origin on free acidity have been observed. It has also been reported that the fermentation process and elapsed time can affect and enhance the amount of free acidity (Mahmoodi-Khaledi et al., 2017).

Accordingly, the interpretation of physicochemical deviations in commercial honey should consider the complexity of production-to-market pathways, where multiple non-intentional factors may contribute to quality dispersion. Overall, the findings of this study are consistent with previous research demonstrating the significant influence of geographical origin, climate, and production practices on honey quality. These factors collectively contribute to the observed variability in physicochemical characteristics across different honey samples

(Mohammed, 2020). In a study in the Asir region, six samples of honey from three different altitudes were analyzed for assessment of the acidity parameter. The mean of all the studied samples complied with the international standards (38.63±17.17 mEq/kg). A comparison of the mean of the acidity parameter from three different altitudes indicated that the mean acidity increased with increasing altitude, which was similar to the current study (Ahamed, Abdallah, Abdalaziz, Serag, & Atallah, 2017). In opposition, Bouhala *et al*. (2020) observed a different range of free acidity, from 35.7 to 40.5 mEq/kg, and a range of HMF from 2.4 to 10.8 mg/kg. In this study, altitude affects the properties of honey. The study showed that HMF content was highly significantly negatively correlated with altitude (Bouhala, Ouchemoukh, Moussi, & Beldjoudi, 2020).

This study has some limitations; only physicochemical variables have been studied. These variables do help in quality assessment, but they cannot be regarded as completely conclusive as far as the identification of adulteration is concerned. For that matter, other methods like isotope ratio analysis, melissopalynology, or even chemometrics along with spectrometry could add some value to the study.

5. Conclusion

This study assessed the physicochemical quality of 76 commercial honey samples collected from 12 cities across different regions of Iran using key parameters including proline, diastase activity, HMF, reducing sugars, sucrose, F/G ratio, and free acidity. Overall, proline content ranged from 51.20 to 480.00 mg/kg (mean 215.60 mg/kg), diastase activity from 0.50 to 30.00 DN (mean 9.07 DN), HMF from 7.10 to 126.80 mg/kg (mean 36.05 mg/kg), reducing sugars from 30.80 to 83.70%, sucrose from 0.70 to 15.21%, F/G ratio from 0.10 to 2.50, and free acidity from 1.00 to 28.10 mEq/kg. Most samples complied with national (INSO) and international (Codex) quality limits. However, notable deviations were observed, particularly elevated HMF levels in some samples (especially from Isfahan) and reduced diastase activity or proline content in others. Clear regional variations were also evident: samples from Ahvaz and Shiraz generally showed higher average proline contents, while samples from Zahedan and Tehran tended to have lower proline values. These variations likely reflect differences in geographical origin, production practices, and storage conditions. The results underline the value of evaluating multiple physicochemical parameters together rather than relying on single indicators for assessing honey quality. The data provide useful baseline information for quality monitoring and regulatory control of commercial honey in Iran.

Acknowledgments

The authors would like to acknowledge the institutional support and technical assistance that facilitated access to the analytical data used in this study.

Authorship statement

Material preparation, data collection, and formal analysis were carried out by Sama Barzegar-Bafrouei, Solmaz Abedinzadeh, and Behrouz Tajdar-Oranj. The original draft was written by Sama Barzegar-Bafrouei and Solmaz Abedinzadeh. Parisa Sadighara and Amirhossein Abedini participated in reviewing, technical editing, and visualization. Methodology, Project administration, and Supervision were conducted by Anosheh Rahmani and Vahid Mofid. All authors read and approved the final manuscript before submission.

Use of Artificial Intelligence

Generative AI tools (ChatGPT, OpenAI, GPT-5.2) were used solely to improve the clarity, grammar, and academic style of the manuscript. The AI tools were not used for data analysis,

interpretation of results, or generation of scientific content. All scientific decisions, data analyses, and conclusions are the responsibility of the authors.

Conflict of Interest: The authors declare that they have no competing interests.

Data availability: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Funding Declaration: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics, Consent to Participate, and Consent to Publish declarations: Not applicable.

Clinical trial number: Not applicable

References

Abu-Farich, B., Masalha, M., Egbaria, E., Kmail, A., El Ghouizi, A., Weld Ali, D., . . . Saad, B. (2024). Physicochemical properties, chemical composition, antioxidant properties, and antibacterial effects of four palestinian honey varieties.

Ahamed, M. M. E., Abdallah, A., Abdalaziz, A., Serag, E., & Atallah, A.-b. E. H. (2017). Some physiochemical properties of Acacia honey from different altitudes of the Asir Region in Southern Saudi Arabia. *Czech Journal of Food Sciences, 35*(4), 321-327.

Ahmed, S. (2020). Effect of Bee Honey on Blood Glucose Level of Sudanese Patients with Type 2 Diabetes Mellitus. *Journal of Diabetes Research and Therapy, 6*(1), 1-5.

Ajlouni, S., & Sujirapinyokul, P. (2010). Hydroxymethylfurfuraldehyde and amylase contents in Australian honey. *Food chemistry, 119*(3), 1000-1005.

Al-Farsi, M., Al-Belushi, S., Al-Amri, A., Al-Hadhrami, A., Al-Rusheidi, M., & Al-Alawi, A. (2018). Quality evaluation of Omani honey. *Food chemistry, 262*, 162-167.

Awulachew, M. T. (2025). Re-evaluating Honey Quality: Key Factors Influencing Its Purity and Excellence. *Food and Drug Safety, 2*(1), 25-39.

Bako, T., Mamai, E. A., & Bature, B. J. (2019). Determination of Quality Parameters of Honey from Taraba State–Nigeria. *Chemical and Biomolecular Engineering, 4*(1), 1-9.

Belay, A., Solomon, W., Bultossa, G., Adgaba, N., & Melaku, S. (2013). Physicochemical properties of the Harenna forest honey, Bale, Ethiopia. *Food chemistry, 141*(4), 3386-3392.

Beykaya, M., Samancı, A. E. T., Samancı, T., & Önder, E. Y. (2026). Advanced characterization of raw honey authenticity using high-resolution sugar profiling and stable carbon isotope ratio analysis. *Journal of Food Measurement and Characterization*, 1-9.

Bhure, R. A., Alam, M., Nanda, V., Pawar, V. M., & Saxena, S. (2025). Exploring the impact of thermal processing on the quality attributes of honey: A comprehensive review. *Journal of Food Process Engineering, 48*(1), e70033.

Bogdanov, S. (2007). Authenticity of honey and other bee products: state of the art. *Bulletin USAMV-CN, 63*, 64.

Bogdanov, S. (2015). Book of honey: honey composition. Bee product science. 2009; 1–9. In.

Bouhala, A., Ouchemoukh, S., Moussi, A., & Beldjoudi, S. (2020). Altitude Effect on the Properties of Honeys from the Region of Jijel (Algeria). *POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES, 70*(2), 169-178.

Buba, F., Gidado, A., & Shugaba, A. (2013). Analysis of biochemical composition of honey samples from North-East Nigeria. *Biochem Anal Biochem, 2*(3), 139.

CABANero, A. I., Recio, J. L., & Ruperez, M. (2006). Liquid chromatography coupled to isotope ratio mass spectrometry: a new perspective on honey adulteration detection. *Journal of agricultural and food chemistry, 54*(26), 9719-9727.

CAC. (2001). Revised Codex standard for honey. Codex Standard 12-1982: rev. 1 (1987)., Rev 2, 2001. Standards and Standard Methods, Rome. In.

Can, Z., Yildiz, O., Sahin, H., Turumtay, E. A., Silici, S., & Kolayli, S. (2015). An investigation of Turkish honeys: their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food chemistry, 180*, 133-141.

Cilavdaroğlu, E. (2026). Proline in Honey: A Complementary Biochemical Indicator in Quality, Authenticity, and Adulteration Assessment. *Uludag Bee Journal, 26*, 1-14.

Czipa, N., Borbély, M., & Győri, Z. (2012). Proline content of different honey types. *Acta Alimentaria, 41*(1), 26-32.

da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). Honey: Chemical composition, stability and authenticity. *Food chemistry, 196*, 309-323.

Danieli, P. P., & Lazzari, F. (2022). Honey traceability and authenticity. Review of current methods most used to face this problem. *Journal of Apicultural Science, 66*(2), 101-119.

EC. (2002). European Council Directive 2001/110/EC of 20 December 2001 relating to honey. *Official Journal of the European Communities L, 10*, 47-52.

El Sohaimy, S., Masry, S., & Shehata, M. (2015). Physicochemical characteristics of honey from different origins. *Annals of Agricultural Sciences, 60*(2), 279-287.

Fairchild, G. F., Capps Jr, O., & Nichols, J. P. (2000). *Impacts of economic adulteration on the US honey Industry, Western agricultural Economics Association Annual meeting*. Retrieved from Vancouver:

Gebremariam, T., & Brhane, G. (2014). Determination of Quality and adulteration effects of honey from Adigrat and its surrounding areas. *Int J Technol Emerg Engin Res, 2*(10), 71-76.

Ghorbani, M., Saei-Dehkordi, S., Mohebbi, A., & Pak, A. (2017). Evaluation of some Chemical Quality Characteristics of honey produced in Iran. *Journal of food quality and hazards control, 4*(4), 113-118.

Gürbüz, S., Çakıcı, N., Mehmetoğlu, S., Atmaca, H., Demir, T., Arıgül Apan, M., . . . Güney, F. (2020). Physicochemical Quality Characteristics of Southeastern Anatolia Honey, Turkey. *International Journal of Analytical Chemistry, 2020*.

Iglesias, A., Feás, X., Rodrigues, S., Seijas, J. A., Vázquez-Tato, M. P., Dias, L. G., & Estevinho, L. M. (2012). Comprehensive study of honey with protected denomination of origin and contribution to the enhancement of legal specifications. *Molecules, 17*(7), 8561-8577.

Khalafi, R., Goli, S. A. H., & Behjatian, M. (2016). Characterization and classification of several monofloral Iranian honeys based on physicochemical properties and antioxidant activity. *International journal of food properties, 19*(5), 1065-1079.

Khandelwal, P., Paliwal, G., & Zade, V. (2020). The study and analysis of Apis dorsata honey collected from Wardha and Gadchiroli districts of Vidharbha in Ma harashtra. *Vi dyabharati I nternational Interdisciplinary Research Journal, 10*(1), 42-51.

Kirs, E., Pall, R., Martverk, K., & Laos, K. (2011). Physicochemical and melissopalynological characterization of Estonian summer honeys. *Procedia Food Science, 1*, 616-624.

Kukurova, K., Karovičová, J., Greif, G., Kohajdová, Z., & Lehkoživová, J. (2006). Determination of 5-hydroxymethylfurfural after Winkler and by the HPLC method for authentication of honey. *Chemical Papers, 60*(3), 186-191.

Kurt, A., Palabiyik, I., Gunes, R., Konar, N., & Toker, O. S. (2020). Determining Honey Adulteration by Seeding Method: an Initial Study with Sunflower Honey. *Food Analytical Methods*, 1-10.

Mahmoodi-Khaledi, E., Lozano-Sánchez, J., Bakhouche, A., Habibi-Rezaei, M., Sadeghian, I., & Segura-Carretero, A. (2017). Physicochemical properties and biological activities of honeys from different geographical and botanical origins in Iran. *European Food Research and Technology, 243*(6), 1019-1030.

Maieves, H. A., Züge, L. C. B., Teixeira, G. L., Cámara, M., Ribani, R. H., & Sánchez-Mata, M. C. (2020). Chemical Properties, Rheological Behavior, and Melissopalynological Analysis of Selected Brazilian Honeys from Hovenia dulcis Flowering. *Brazilian Archives of Biology and Technology, 63*.

Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food chemistry, 91*(3), 571-577.

Mehryar, L., & Esmaiili, M. (2011). *Honey and honey adulteration detection: A review.* Paper presented at the Proceedings of 11th International Congress on Engineering and Food.

Mehryar, L., Esmaiili, M., & Hassanzadeh, A. (2013). Evaluation of some physicochemical and rheological properties of Iranian honeys and the effect of temperature on its viscosity. *American-Eurasian J Agric Environ Sci, 13*(6), 807-819.

Mohammed, M. E. A. (2020). Factors Affecting the Physicochemical Properties and Chemical Composition of Bee's Honey. *Food Reviews International*, 1-12.

Naila, A., Flint, S. H., Sulaiman, A. Z., Ajit, A., & Weeds, Z. (2018). Classical and novel approaches to the analysis of honey and detection of adulterants. *Food Control, 90*, 152-165.

Nhlapho, S. (2023). *Investigation of sugars in honey for authentication and detection of adulterants using selected analytical techniques.* University of Johannesburg,

Nweze, J. A., Olovo, C. V., Nweze, I. E., John, O. O., & Paul, C. (2019). Therapeutic Properties of Honey. In *Honey Analysis*: IntechOpen.

Parviz, M., Karimi, F., Rezaei, M., Javanmard, M., Javadzadeh, M., & Allahdadi, G. (2015). Assessment of the physicochemical quality of Iranian honey. *Quality Assurance and Safety of Crops & Foods, 7*(5), 629-634.

Raweh, H. S., Badjah-Hadj-Ahmed, A. Y., Iqbal, J., & Alqarni, A. S. (2023). Physicochemical composition of local and imported honeys associated with quality standards. *Foods, 12*(11), 2181.

Saeed, M. A., & Jayashankar, M. (2020). Physico-chemical characteristics of some Indian and Yemeni Honey. *Journal of bioenergy and food science, 7*(2), 2832019.

Sak-Bosnar, M., & Sakač, N. (2012). Direct potentiometric determination of diastase activity in honey. *Food chemistry, 135*(2), 827-831.

Sant'ana, R. d. S., de Carvalho, C. A. L., OdaSouza, M., Souza, B. d. A., & Dias, F. d. S. (2020). Characterization of honey of stingless bees from the Brazilian semi-arid region. *Food chemistry*, 127041.

Santos-Buelga, C., & González-Paramás, A. M. (2025). Chemical composition of honey. In *Bee products–chemical and biological properties* (pp. 47-104): Springer.

Sapri, N., & Jamaludin, D. Determination of Pure and Adulterated Stingless Bee Honey Based on Dielectric Properties.

Se, K. W., Wahab, R. A., Yaacob, S. N. S., & Ghoshal, S. K. (2019). Detection techniques for adulterants in honey: Challenges and recent trends. *Journal of Food Composition and Analysis, 80*, 16-32.

Széles, É., Borbély, M., Hovánszki, D., Tisza, A., & Győri, Z. (2007). Studying of proline content and organic contaminants in Hungarian and Greek honey samples. *Cereal Research Communications, 35*(2), 1153-1156.

Tarapoulouzi, M., Mironescu, M., Drouza, C., Mironescu, I. D., & Agriopoulou, S. (2023). Insight into the recent application of Chemometrics in quality analysis and characterization of bee honey during processing and storage. *Foods, 12*(3), 473.

Tosun, M., Cengiz, M. M., Erdoğan, Y., & Turan, M. (2026). Physicochemical properties, phytohormone profiles and quality assessment of honey from the Çoruh Valley of Türkiye. *Journal of Food Measurement and Characterization*, 1-13.

Truzzi, C., Annibaldi, A., Illuminati, S., Finale, C., & Scarponi, G. (2014). Determination of proline in honey: comparison between official methods, optimization and validation of the analytical methodology. *Food chemistry, 150*, 477-481.

Yadata, D. (2014). Detection of the electrical conductivity and acidity of honey from different areas of Tepi. *Food Science and Technology, 2*(5), 59-63.

Zábrodská, B., & Vorlová, L. (2015). Adulteration of honey and available methods for detection–a review. *Acta Veterinaria Brno, 83*(10), 85-102.

Zhang, J., Sun, M., Elmaidomy, A. H., Youssif, K. A., Zaki, A. M., Kamal, H. H., . . . Abdelmohsen, U. R. (2023). Emerging trends and applications of metabolomics in food science and nutrition. *Food & function, 14*(20), 9050-9082.

Zielińska, S., Wesołowska, M., Bilek, M., Kaniuczak, J., & Dżugan, M. (2019). The saccharide profile of Polish honeys depending on their botanical origin. *Journal of Microbiology, Biotechnology and Food Sciences, 2019*, 387-390.

Živkov-Baloš, M., Popov, N., Vidaković, S., Ljubojević-Pelić, D., Pelić, M., Mihaljev, Ž., & Jakšić, S. (2018). Electrical conductivity and acidity of honey.

Table 1. Physicochemical quality parameters of honey samples from different Iranian cities
* Indicates that at least one sample exceeded the maximum or minimum limits established by

of	Longitude Latitude	Altitude above sea level	Number of samples	Proline (mg/Kg)		Sucrose (%)		Reducing sugar (%)		Diastase
				Mean± SD	Range	Mean± SD	Range	Mean± SD	Range	Mean± SD
d	59.5444490(36.3260620(	1027	**6**	206.14±21.90	180.00-232.70	5.62±2.94*	2.34-9.80	71.19±1.49	69.70-73.47	6.34±3.59*
	53.0587633 36.5661173	42	**6**	178.82±26.57*	152.94-211.76	7.18±3.03*	2.27-11.70	61.52±15.09*	30.80-68.79	11.81±5.01
	46.3037436 38.0784786	1402	**6**	290.03±138.08	95.30-449.30	4.18±2.07*	1.20-7.50	68.06±4.23*	61.20-72.80	9.87±4.92*
n	51.3807223 35.7007233	1214	**10**	161.36±51.89*	75.00-221.66	6.63±4.99*	0.80-15.21	65.29±4.60	54.66-72.08	6.68±2.48*
n	51.6715730(32.6721070(	1575	**6**	217.60±168.79	51.20-480.00	4.66±2.30	2.18-8.31	66.22±2.20*	62.75-68.95	10.62±3.59*
n	48.4920193 36.6820223	1678	**6**	176.91±19.07*	138.30-186.60	4.96±1.79	2.00-7.00	66.03±15.29*	38.09-83.60	13.36±4.74*
	50.9693923 35.8286443	1312	**6**	199.33±13.93	188.00-220.00	7.01±4.08*	4.42-14.85	72.07±4.33	66.98-79.26	12.12±9.44*
	48.7168943 31.3440633	20	**6**	314.00±92.09	198.00-421.00	2.88±1.05	1.60-4.30	78.23±4.48	71.60-83.70	9.31±2.39*
r	56.3079830(27.1946350(	17	**6**	234.04±52.93	182.03-298.59	2.58±1.92	0.70-5.00	65.54±0.29	65.01-65.80	9.58±2.99*
n	57.0726120(30.2845120(	1764	**6**	199.46±85.41*	111.40-330.00	6.77±2.83*	3.94-11.82	65.06±2.49*	62.00-68.46	5.93±2.67*
n	60.8558086 29.5021596	1386	**6**	143.89±35.33	74.04-173.18	5.79±4.97*	0.99-13.40	65.18±3.79*	60.00-69.20	8.85±5.05*
	52.5333800(29.5966570(	1519	**6**	301.83± 64.73	210.00-389.00	6.56±1.80*	4.40-9.1 0	74.01±2.33	70.30-77.10	6.03±1.98*
	Overall		**76**	**215.60±90.17**	**51.20-480.00**	**5.47±3.33**	**0.70-15.21**	**68.05±7.70**	**30.80-83.70**	**9.07±4.75**

relevant standards
-Values represent pooled descriptive statistics of all analyzed samples

Table 2. Hydroxymethylfurfural content, fructose-to-glucose ratio, and free acidity of honey samples from different Iranian cities

Name of the Cities	Number of samples	Hydroxymethylfurfural (mg/kg)		Fructose – Glucose Ratio		Free Acidity (mEq/kg)	
		Mean± SD	Range	Mean± SD	Range	Mean± SD	Range
Mashhad	**6**	35.73±25.46*	17.00-85.00	1.20±0.09	1.10-1.33	12.50±3.49	9.00-17.50
Sari	**6**	33.28±19.21*	14.22-59.30	0.91±0.01	0.90-0.94	7.55±1.43	5.42-9.31

Tabriz	**6**	32.66±20.53*	10.00-62.00	1.07±0.14	0.90-1.25	25.98±1.61	23.80-28.10
Tehran	**10**	26.17±14.00	7.10-47.00	1.11±0.26	0.71-1.60	12.12±8.52	1.00-23.50
Isfahan	**6**	65.04±36.53*	22.30-126.80	0.81±-0.11	0.62-0.94	16.33±6.62	5.00-23.00
Zanjan	**6**	45.50±34.03*	11.00-92.00	0.95±0.55	0.10-1.50	10.66±5.27	6.00-21.00
Karaj	**6**	26.33±8.55	14.80-36.00	1.11±0.11	0.98-1.24	10.51±0.97	9.22-11.71
Ahvaz	**6**	45.50±26.36*	15.70-85.00	1.20±0.21	0.90-1.50	13.25±7.41	7.00-25.00
Bandar Abbas	**6**	29.04±9.34	15.60-38.17	1.04±0.16	0.93-1.35	9.45±1.39	7.78-11.79
Kerman	**6**	39.23±14.95*	28.70-64.10	0.93±0.65*	0.30-2.20	14.77±4.24	7.40-19.94
Zahedan	**6**	21.46±5.92	15.20-31.10	1.33±0.67*	0.57-2.50	8.50±2.14	4.90-10.90
Shiraz	**6**	39.28±20.51	14.30-64.40	0.96±0.13*	0.70-1.09	15.55±1.26	13.80-17.00
Total	**76**	**36.05±22.67**	**7.10-126.80**	**1.05±0.34**	**0.10-2.50**	**13.04±6.42**	**1.00-28.10**

*Out of legal limits
Values outside the typical range reported for nectar honeys

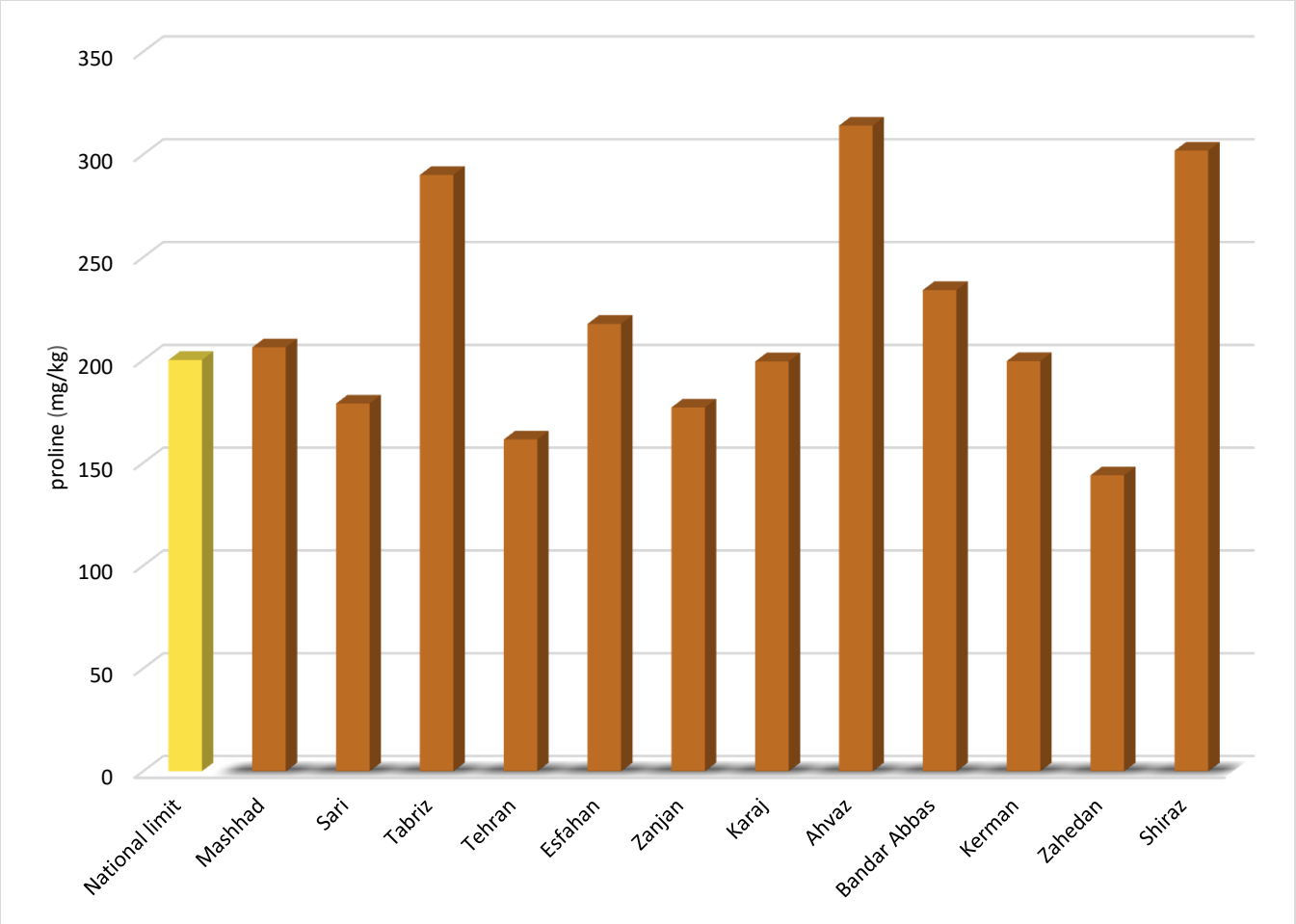


Figure 1. Comparison of proline content in honey samples from different Iranian cities

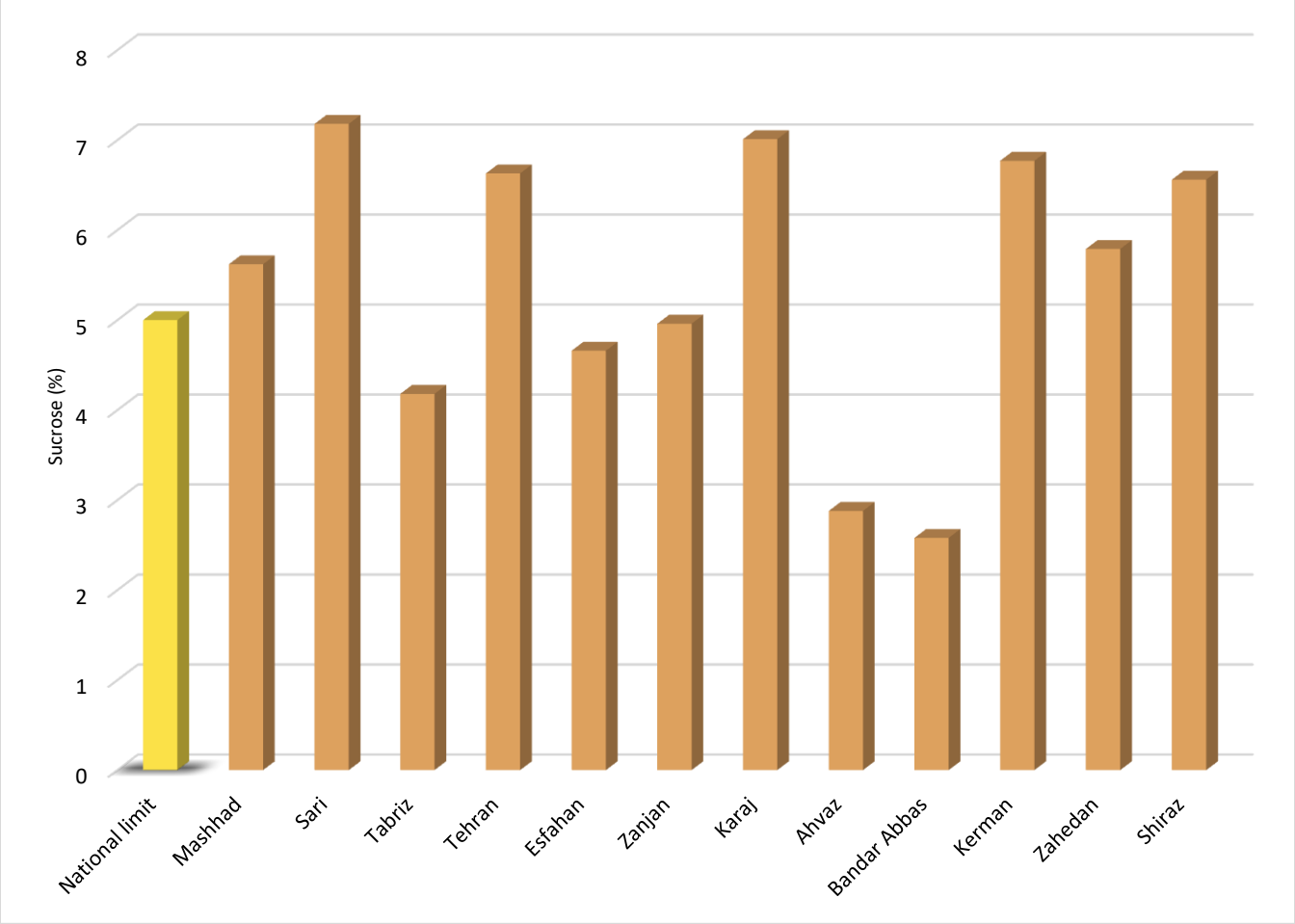


Figure 2. Sucrose content of honey samples collected from different Iranian cities

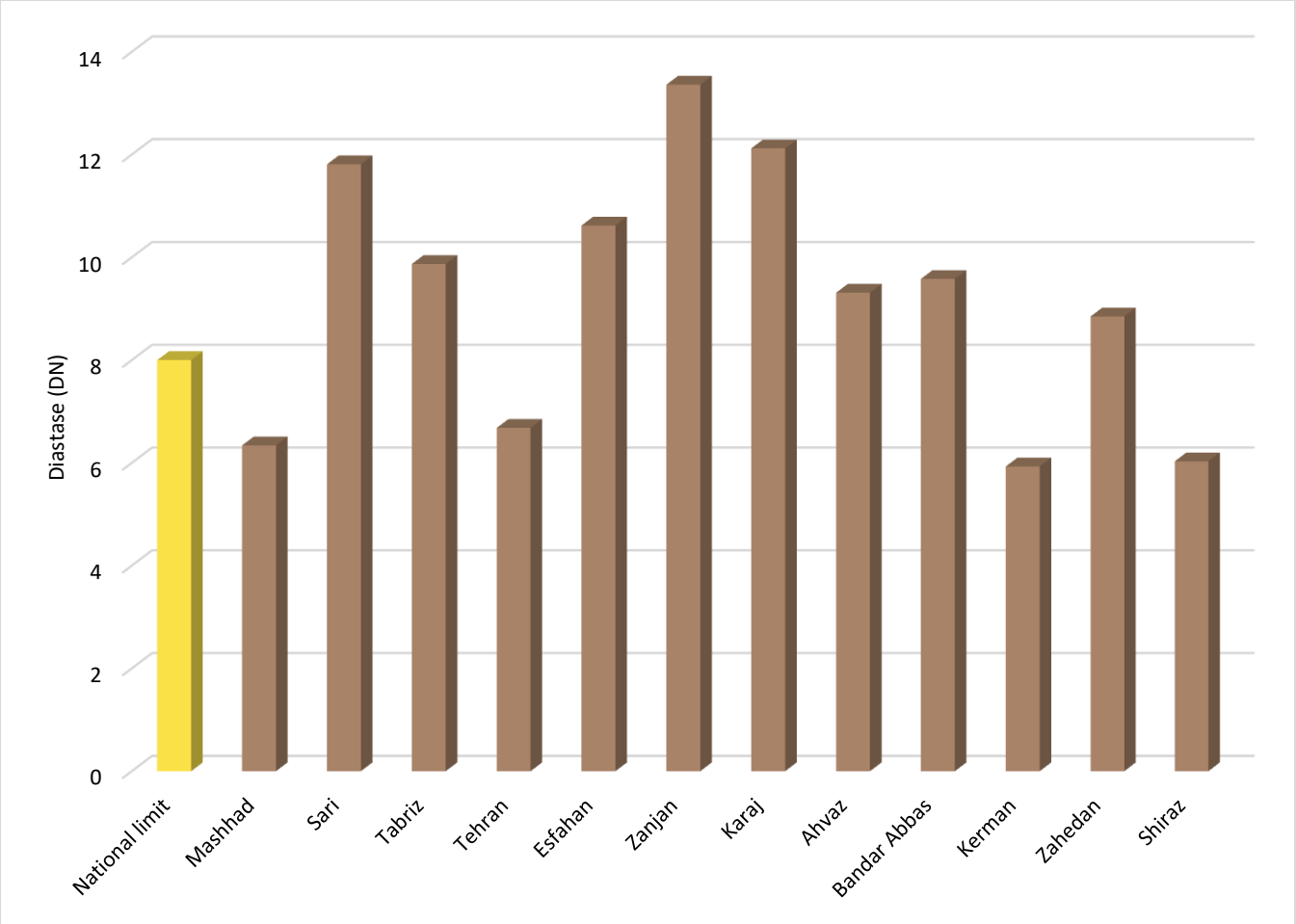


Figure 3. Diastase activity of honey samples from different regions of Iran

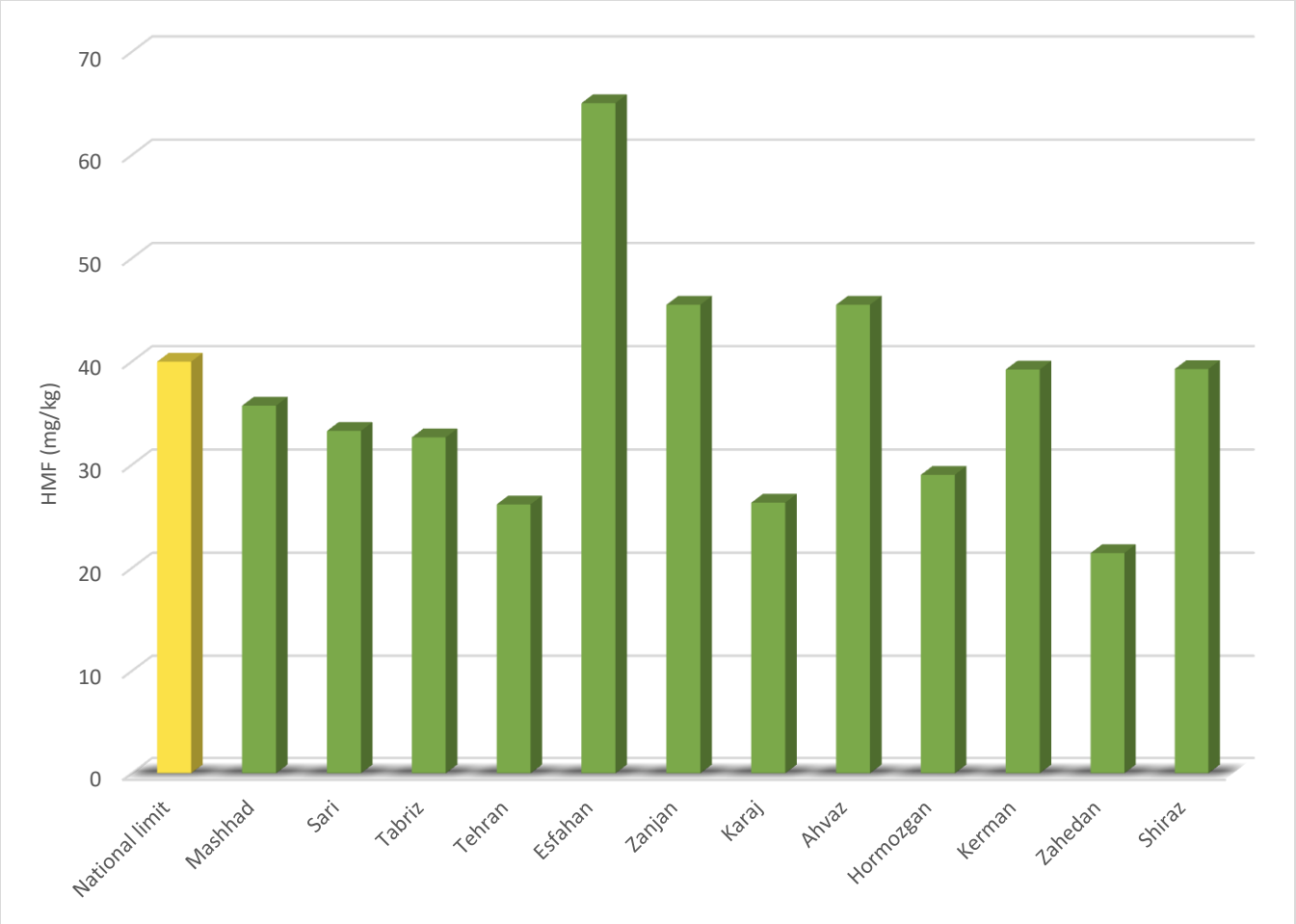


Figure 4. Hydroxymethylfurfural (HMF) levels in honey samples from various Iranian cities

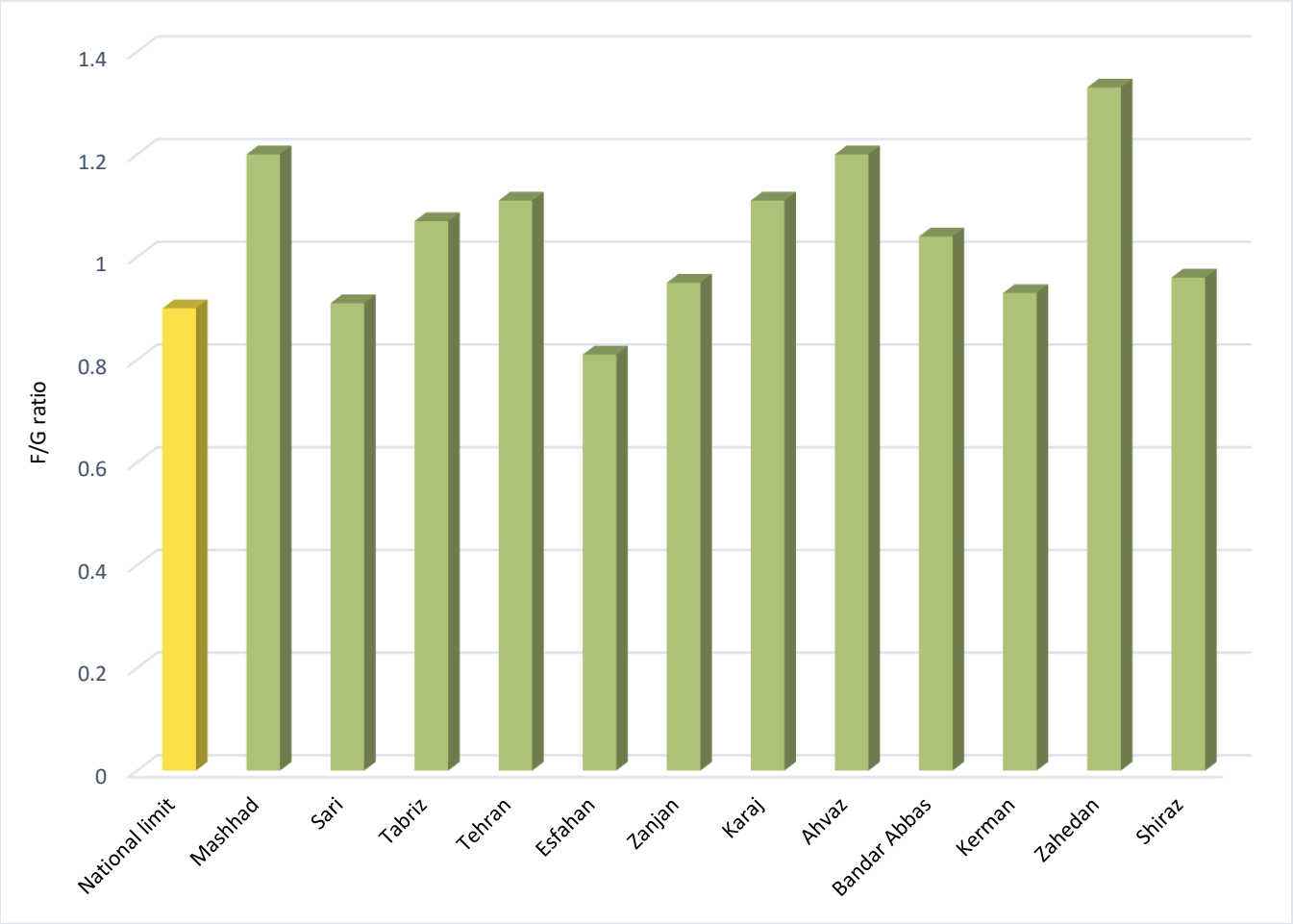


Figure 5. Fructose-to-glucose (F/G) ratio of honey samples from different Iranian regions

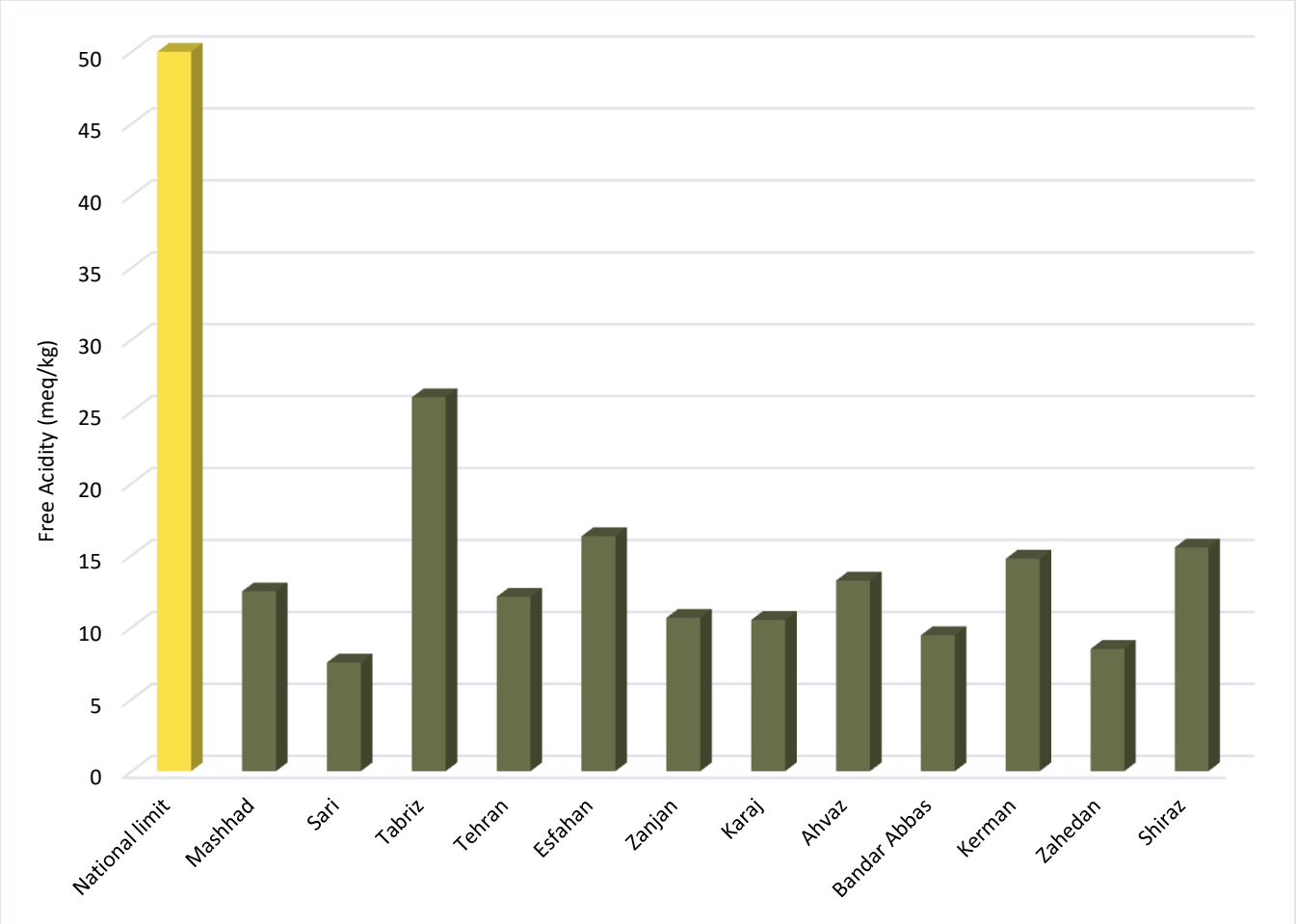


Figure 6. Free acidity levels of honey samples collected from different Iranian cities