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Quality and Regulatory Compliance of Commercial Blossom and Pine Honeydew Honeys in the Turkish Retail Market

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Abstract

In this study, 22 commercially available honeys produced in different regions of Türkiye, comprising 14 blossom and eight pine honeydew honeys, were evaluated. Honey quality was assessed through the combined evaluation of physicochemical and compositional characteristics. Physicochemical analyses were combined with comprehensive HPLC-RID profiling of major and minor sugars, including fructose, glucose, sucrose, maltose, xylose, arabinose, galactose, trehalose, melezitose, and raffinose. Moisture, free acidity, diastase number, electrical conductivity, proline, and HMF ranged from 15.20–17.80%, 9.33–24.50 mEq/kg, 8.33–41.66 DN, 210.00–1154.23 µS/cm, 273.17–855.09 mg/kg, and 7.47–83.52 mg/kg, respectively. Two blossom honey samples were classified as non-compliant with the established quality criteria, as both exceeded the regulatory HMF limit and one also failed to meet the minimum proline requirement, whereas all pine honeydew honey samples complied with the established criteria. Pine honeydew honeys showed significantly higher pH and electrical conductivity than blossom honeys ($p < 0.001$), whereas blossom honeys had significantly higher fructose ($p = 0.005$) and fructose + glucose contents ($p = 0.025$). Minor sugars showed considerable variation among individual samples. The combined evaluation indicated generally satisfactory quality and regulatory compliance among honeys sold in major chain supermarkets in Türkiye, with deviations in individual samples.

1. Introduction

Honey produced by *Apis mellifera* is classified according to its source as blossom honey, derived from plant nectar, and honeydew honey, originating mainly from plant secretions or the excretions of plant-sucking insects (Codex Alimentarius, 1981). Honey is composed predominantly of sugars, together with water and smaller amounts of organic acids, enzymes, amino acids, minerals, and other minor constituents (Lyoussi et al. 2022).

Global honey production is approximately 1.99 million tonnes annually. When considered in relation to a world population of approximately 8.32 billion, this corresponds to only about 0.24 kg of honey produced per capita (Worldometer, 2026; FAOSTAT, 2024). This relatively limited supply, together with the commercial value and demand for honey, may increase its vulnerability to economically motivated adulteration. At the same time, preserving its natural composition and quality requires careful control of processing and storage conditions. Türkiye ranked third among the world's leading honey-producing countries in 2024, after China and India, with an annual production of approximately 95.5 thousand tonnes (FAOSTAT, 2024). Moreover, Türkiye is the world's largest producer of pine honey, accounting for approximately 92% of global pine honey production (Varol & Yücel, 2026). Given Türkiye's prominent position in global honey production, reliable quality assessment is particularly important for verifying authenticity, monitoring changes during processing and storage, and ensuring compliance with established quality standards.

Honey quality is influenced by several factors, including botanical origin, processing, and storage conditions, and is therefore evaluated using a combination of physicochemical and compositional parameters (da Silva et al. 2016). Turkish Food Codex Communiqué on Honey (Communiqué No. 2020/7; hereafter, THC), which is aligned with the European Council Directive 2001/110/EC (Council, 2001) relating to honey, establishes regulatory limits for key physicochemical parameters and sugar composition criteria used in honey quality assessment. The principal criteria applicable to blossom and pine honey are summarized in Table 1.

Table 1
Quality Criteria for Blossom and Pine Honey According to the THC

Parameter	Blossom Honey	Pine Honey
Moisture (%)	≤ 20	≤ 20
Free Acidity (mEq/kg)	≤ 50	≤ 50
Diastase Activity (DN)	≥ 8	≥ 8
Electrical Conductivity (µS/cm)	≤ 800	≥ 800
Proline (mg/kg)	≥ 300	≥ 300
HMF (mg/kg)	≤ 40	≤ 40
Fructose + Glucose (F + G, %)	≥ 60	≥ 45
Fructose / Glucose (F/G)	0.9–1.4	1.0–1.4
Sucrose (%)	≤ 5	≤ 5
Maltose (%)	≤ 4	≤ 5

Physicochemical parameters provide information on different aspects of honey quality, including freshness, maturity, storage stability, and changes associated with processing and storage (Karadal et al. 2024). Moisture content is particularly important for storage stability, as excessive moisture increases the risk of fermentation and microbial deterioration (da Silva et al. 2022; Correa-Mosquera et al. 2022). pH and free acidity reflect the acidic characteristics of honey and may vary according to its organic acid composition and honey type (Muhammad & Sarbon, 2023; dos Santos et al. 2024). Diastase activity decreases with prolonged storage and heat exposure, whereas HMF formation increases under these conditions, making both parameters useful indicators of honey freshness and processing history (Makhloufi et al. 2020; Morales et al. 2009; Mouhoubi-Tafinine et al. 2018). Electrical conductivity is particularly useful for distinguishing blossom from honeydew honeys, while proline is commonly associated with honey maturity and authenticity (Mateo & Bosch-Reig, 1998; Kaškonienė et al. 2010; Meda et al. 2005).

Sugars constitute the major fraction of honey, with fructose and glucose being the predominant sugars. In addition to these major monosaccharides, honey contains disaccharides such as sucrose and maltose, which are included among the regulatory quality criteria for honey, as well as minor sugars such as

xylose, arabinose, galactose, trehalose, melezitose, and raffinose. The relative abundance and distribution of these sugars may vary among different honey types and provide additional information on their compositional characteristics (Islam et al. 2020; Uçurum et al. 2023).

The assessment of honey quality and authenticity should not rely on a single analytical parameter, as physicochemical and compositional characteristics provide information on different aspects of honey quality. Integrating physicochemical parameters with comprehensive sugar profiling may therefore provide a more complete assessment of commercially available honey (Lupu et al. 2025). In this study, moisture content, free acidity, pH, diastase activity, electrical conductivity, proline, and hydroxymethylfurfural (HMF) content were evaluated together with comprehensive HPLC-RID profiling of ten sugars, including both major and minor sugars, in retail blossom and pine honeydew honeys produced in Türkiye. This integrated approach was used to assess their quality, compositional differences, and compliance with established regulatory criteria.

2. Materials and Methods

2.1. Materials

2.1.1. Sample collection and preparation

A total of 22 commercial honey samples, comprising 14 blossom and eight pine honeydew honeys, were purchased from five major chain supermarkets in Türkiye. These retailers were selected because of their extensive nationwide retail networks and broad consumer accessibility, allowing the study to focus on honey products widely available through the mainstream retail market. According to the labelling information, the samples were produced and packaged in Türkiye by fifteen different manufacturers; however, this statement refers only to the place of production and packaging and does not indicate the botanical or geographical origin of the honey, since commercial honeys are typically obtained from beekeepers in different regions and marketed as blended products. Detailed labelling information is presented in Table 2. The blossom and pine honeydew honey samples were coded as B (B1–B14) and P (P1–P8), respectively.

Table 2
Labelling information of honey samples

Sample Code	Honey Type	Region of Manufacturer	Batch No
B1	Blossom	Mediterranean	227-01
B2	Blossom	Aegean	23–053
B3	Blossom	Marmara	202011007
B4	Blossom	Mediterranean	270-02
B5	Blossom	Mediterranean	232-04
B6	Blossom	Marmara	BCI2006
B7	Blossom	Central Anatolia	2020/09/35
B8	Blossom	Mediterranean	302-05
B9	Blossom	Marmara	BPC19.0129
B10	Blossom	Eastern Anatolia	UB-15-62
B11	Blossom	Eastern Anatolia	AB-25-05
B12	Blossom	Eastern Anatolia	31YP21
B13	Blossom	Mediterranean	286-03
B14	Blossom	Mediterranean	316-03
P1	Pine Honeydew	Mediterranean	56-01-2020
P2	Pine Honeydew	Marmara	202011007
P3	Pine Honeydew	Central Anatolia	56-01-2020
P4	Pine Honeydew	Mediterranean	317P21
P5	Pine Honeydew	Central Anatolia	232-04
P6	Pine Honeydew	Mediterranean	1127/04
P7	Pine Honeydew	Mediterranean	316-03
P8	Pine Honeydew	Mediterranean	212-03

2.1.2. Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade or high purity. Sodium hydroxide (NaOH, Sigma-Aldrich, Germany) and standard buffer solutions (pH 4.01, 7.00, and 10.01; Thermo Scientific, USA) were used for free acidity determination and pH meter calibration, respectively. Potassium iodide

(Fisher Bioreagents, USA), iodine (Merck, Germany), sodium chloride, starch, and citric acid monohydrate (Sigma-Aldrich, Germany), hydrochloric acid (IsoLab, Germany), and sodium phosphate (Carlo Erba, France) were used for diastase activity determination.

Potassium chloride (KCl) conductivity standard solutions (0.01, 0.1, and 1 M; Consort, Belgium) were used for electrical conductivity meter calibration. Ninhydrin and formic acid (Sigma-Aldrich, Germany), ethylene glycol monomethyl ether (Fisher Chemical, USA), 2-propanol (Carlo Erba Reagents, France), and L-proline (Merck, Germany) were used for proline determination. Methanol (CHROMASOLV, Sigma-Aldrich, Germany) and 5-hydroxymethylfurfural (HMF) standard (HPLC grade, J&K) were used for HMF determination.

The sugar standards used for HPLC-RID analysis included xylose, galactose, trehalose, and raffinose (Tokyo Chemical Industry Co., Ltd. [TCI], Japan), arabinose (Boston USA Chemistry LLC, USA), fructose (Sigma-Aldrich, Germany), glucose (Fisher Scientific, UK); sucrose and melezitose (BLDpharm, China), and maltose (Fisher BioReagents, Fisher Scientific). Acetonitrile (Sigma-Aldrich, Germany) was used as a chromatographic solvent.

2.2. Methods

All honey samples were kept in sealed glass containers and stored at room temperature in the dark prior to analysis. Before each analysis, samples were homogenized gently to ensure uniformity. All physicochemical analyses were performed in triplicate (n = 3), and results are expressed as mean ± standard deviation. All reagents were of analytical grade.

2.2.1. Moisture Content

The moisture content of the honey samples was determined from refractive index measurements using an Abbe refractometer, and the corresponding moisture values were obtained according to the refractive index–moisture relationship table (IHC, 2009).

2.2.2. Free Acidity and pH

The measurements of pH and free acidity were carried out following the analytical recommendations of the International Honey Commission (IHC, 2009). For free acidity determination, 10 g of honey was dissolved in 75 mL of distilled water. The pH of the prepared honey solution was measured at 25°C using a pH meter (Orion 3-Star, Thermo Fisher Scientific Inc.) prior to titration. The pH meter was calibrated with standard buffer solutions at pH 4.01, 7.00, and 10.01 before analysis. Subsequently, the same solution was titrated with 0.05 M NaOH until a pH of 8.30 was reached. Free acidity was calculated and expressed as milliequivalents per kilogram (mEq/kg) using Eq. (1).

Free acidity (mEq/kg) = 5 × V_{NaOH} consumed during titration (mL) (1)

Where V_{NaOH} is the volume of 0.05 M NaOH consumed during titration (mL).

2.2.3. Diastase Number

Diastase activity was determined according to the Turkish Standards Institution method (TS 13364) and expressed as the diastase number (DN) in Gothe units. Briefly, 10 g of honey was dissolved in distilled water and diluted to 100 mL. The honey solution was mixed with starch–buffer solution and incubated at 47°C for 1 h. After cooling, 0.1 N iodine solution was added, and the diastase number was determined based on the resulting colour change.

2.2.4. Electrical Conductivity

Electrical conductivity (EC) was determined according to the Turkish Standards Institution method (TS 13366). Honey corresponding to 20 g of dry matter was dissolved in 100 mL of ultrapure water. The conductivity meter (Consort C3010, Consort bvba, Turnhout, Belgium) was calibrated using 0.01, 0.1, and 1 M KCl standard solutions. Conductivity was measured at 20°C and expressed as µS/cm.

2.2.5. Proline Content

Proline content was determined spectrophotometrically according to the Turkish Standards Institution method (TS 13357). The method is based on the reaction of proline with ninhydrin under acidic conditions to form a coloured complex, the intensity of which is proportional to the proline concentration. Briefly, the honey solution was reacted with ninhydrin and formic acid and heated in a boiling water bath for 15 min. After cooling, 2-propanol was added, and the absorbance was measured at 510 nm against a blank using a double-beam UV–Vis spectrophotometer (Jasco V-650). Proline content was calculated using Eq. (2):

Proline (mg/kg) = (A_{sa}/A_{st}) × (A1/A2) × 80 (2)

Where A_{sa} is the absorbance of the sample solution, A_{st} is the absorbance of the proline standard solution, A_1 is the amount of proline used in the standard solution (mg), A_2 is the weight of the sample (g), and 80 represents the dilution factor.

2.2.6. HMF Content

HMF content was determined by HPLC-DAD according to the Turkish Standards Institution method (TS 13356). Briefly, 10 g of honey was diluted to 50 mL with ultrapure water (18.2 MΩ·cm resistivity; Sartorius H2O-I-1-UV-T Arium Comfort) and filtered through a 0.45 µm Minisart filter (Sartorius Stedim Biotech GmbH, Göttingen, Germany). An eight-point calibration curve was prepared using HMF standard solutions ranging from 0.1 to 20 ppm. The calibration curve showed good linearity, with the regression equation y = 143.32739x − 2.87996 and R^2 = 0.99996.

HPLC analysis was performed using a 1260 Infinity II system (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector (1260 Infinity II WR) and a C18 column (ACE 5, 250 mm × 4.6 mm, 5 µm). Isocratic elution was carried out with methanol: water (10:90, v/v) at a flow rate of 1.0 mL/min.

HMF was detected at 285 nm with a retention time of 8.036 min, while the column temperature was maintained at 30°C. Quantification was based on the corresponding peak areas using the calibration curve, and the results were expressed as mg/kg of honey.

2.2.7. Sugar Profile

Sugar profiling was performed by HPLC-RID according to the Turkish Standards Institution method (TS 13359). A 5 g honey sample was weighed into a beaker and dissolved in ultrapure water (18.2 MΩ·cm resistivity; Sartorius H2O-I-1-UV-T Arium Comfort). The solution was transferred to a 100 mL volumetric flask containing 25 mL of methanol and diluted to volume with ultrapure water. The resulting solution was filtered through a 0.45 µm membrane filter (Sartorius Stedim Biotech GmbH, Göttingen, Germany).

Sugar analysis was performed using an HPLC system (1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA) equipped with a refractive index detector (RID, 1260 Infinity II) and a Zorbax NH_2 column (4.6 × 250 mm, 5 µm; Agilent Technologies). Separation was performed under isocratic conditions using an acetonitrile: water mobile phase (80:20, v/v) at a flow rate of 1.3 mL/min, with both the column and detector temperatures maintained at 30°C. All samples were analysed in triplicate, and quantitative evaluation was based on mean peak areas.

Five-point calibration curves were prepared for sucrose, maltose, fructose, glucose, xylose, arabinose, galactose, trehalose, melezitose, and raffinose using analytical-grade standards. Sugar concentrations were quantified from the corresponding calibration curves based on peak areas and expressed as g/100 g of honey (%). The retention times, linearity ranges, regression equations, and coefficients of determination (R^2) for each sugar are presented in Table 3.

Table 3
Retention times, regression equations, coefficients of determination (R^2), and linearity ranges of sugars determined by HPLC–RID

No	Sugar Name	Retention time (min)	Linearity range (mg/L)	Regression equation	R^2
1.	Xylose	6.69	100–2500	y = 33.587x + 551.39	0.9989
2.	Arabinose	7.74	100–2500	y = 15.432x – 694.54	0.9983
3.	Fructose	8.68	5000–25000	y = 66.217x – 94.01	0.9995
4.	Glucose	10.60	5000–25000	y = 63.871x – 22.936	0.9993
5.	Galactose	11.56	100–2500	y = 34.045x – 3447.7	0.9950
6.	Sucrose	17.52	100–2500	y = 68.373x – 263.57	0.9995
7.	Maltose	22.24	100–2500	y = 47.419x – 214.16	0.9955
8.	Trehalose	25.07	100–2500	y = 64.651x – 2196.7	0.9979
9.	Melezitose	38.75	100–2500	y = 68.075x – 3636.3	0.9971
10.	Raffinose	49.51	100–2500	y = 58.219x – 2410.5	0.9979

2.3. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics, version 22 (IBM Corp., Armonk, NY, USA). The normality of the data was assessed prior to group comparisons. Differences between blossom and pine honeydew honeys were evaluated using an independent-samples t-test for normally distributed variables and the Mann–Whitney U test for variables that did not meet the normality assumption. Statistical significance was set at $p < 0.05$. The results of the statistical comparisons are presented in Table 6.

3. Results and Discussion

The quality of retail pine honeydew and blossom honeys was evaluated through physicochemical characterization and comprehensive sugar profiling. The results were assessed in relation to the quality criteria established by the THC and compared with findings reported for Turkish honeys and honeys from different geographical regions.

3.1. Physicochemical Characterization

3.1.1. Moisture Content

The moisture content of the honey samples ranged from 15.20% to 17.80%, with an overall mean of 16.18% (Table 4). Blossom honeys had a mean moisture content of 16.35%, while the mean value for pine honeydew honeys was 15.89%, as illustrated in Fig. 1. The difference between the two honey types was not statistically significant ($p = 0.113$). All samples remained below the maximum moisture limit of 20% established by the THC.

Previous studies have reported greater variability in the moisture content of Turkish honeys; Tornuk et al. (2013) reported values ranging from 10.92% to 17.99%, while Uçurum et al. (2023) reported ranges of 15.40–20.00% for pine honeydew and 15.70–18.50% for multifloral blossom honeys. In comparison, the retail honeys examined in the present study were confined to a relatively narrow moisture range, with none approaching the regulatory maximum. This limited variability indicates a relatively uniform moisture profile across the commercial samples despite differences in honey type. Studies from different geographical regions have reported considerable variation in honey moisture content. Terrab et al. (2002) reported mean moisture contents ranging from 16.8% to 20.3% among different Moroccan honey types, with individual values reaching 24.1%. Ayton et al. (2025) reported moisture contents ranging from

13.6% to 22.4% in Australian honey samples. Tigistu et al. (2021) reported a mean moisture content of 18.83%, with values ranging from 14.4% to 23.0% for honeys from Southern Ethiopia. In comparison, the commercial honeys analysed in the present study exhibited a lower overall mean (16.18%) and a narrower range (15.20–17.80%). The absence of high-moisture samples in the present dataset is favourable in terms of storage stability and reduced susceptibility to fermentation (da Silva et al. 2022).

3.1.2. Free acidity and pH

Free acidity and pH were evaluated together as complementary indicators of honey acidity. Free acidity values ranged from 9.33 to 24.50 mEq/kg, with an overall mean of 17.52 mEq/kg, while pH values ranged from 3.52 to 4.73. Pine honeydew honeys had higher mean free acidity (19.06 mEq/kg) and pH (4.45) than blossom honeys (16.64 mEq/kg and 3.89, respectively) (Fig. 2a,b). The difference in free acidity was not statistically significant ($p = 0.202$), whereas pH was significantly higher in pine honeydew honeys ($p < 0.001$). All samples remained below the maximum free acidity limit of 50 mEq/kg established by the THC (Fig. 2a).

Previous studies on Turkish honeys have shown variation in free acidity and pH among honey types. Relatively high pH values have been reported for pine honeys (4.48–4.71), whereas floral honeys exhibited considerable variation in free acidity, with reported means ranging from 14.94 to 38.18 mEq/kg (Akbulut et al. 2009; Gürbüz et al. 2020; Ucuncu et al. 2026). Yiğit et al. (2024), however, reported higher free acidity in flower than pine honeydew honey (22.5 vs. 18.6 mEq/kg). Manzanares et al. (2011) similarly found significantly higher pH and acidity in honeydew than blossom honeys, whereas studies on floral and commercial honeys from Tunisia and Ethiopia showed broader variation in these parameters (Boussaid et al. 2018; Damto et al. 2023). In the present study, pine honeydew honeys showed higher values for both pH and free acidity than blossom honeys, indicating a clear compositional difference between the two honey types.

3.1.3. Diastase Number (DN)

The diastase number (DN) of the honey samples ranged from 8.33 to 41.66, with an overall mean of 21.48. Pine honeydew honeys had a higher mean DN (24.73) than blossom honeys (19.63), although the difference was not statistically significant ($p = 0.279$). All samples met the minimum DN requirement (≥ 8) established by the THC (Fig. 3).

Previous studies on Turkish honeys have reported considerable variation in diastase activity among honey types. Küçük et al. (2007) reported DN values of 17.7, 17.9, and 23.0 for chestnut, heterofloral, and rhododendron honeys, respectively, while Çetintaş (2025) found a maximum diastase activity of 23.04 Schade units among 20 different honey samples. Sahinler (2007) further showed that diastase activity differed among honey types and decreased markedly during heating and storage.

Studies from different countries have likewise demonstrated wide variability in diastase activity. Venezuelan stingless bee honeys showed DN values of 2.6–3.5 in Meliponini and up to 35.6 in Trigonini honeys (Vit and Pulcini, 1996), while Babacan et al. (2002) reported DN values ranging from 4.3 to 36.8 in raw honeys of different floral origins from the United States, including clover, orange, pepper, and buckwheat honeys.

In this context, the DN values obtained in the present study (8.33–41.66) are generally consistent with the variability reported for Turkish and international honeys, although some samples reached values above the upper ranges reported in several of these studies. The higher mean DN observed in pine honeydew honeys compared with blossom honeys is also consistent with the general pattern reported for honeydew and darker honey types.

3.1.4. Electrical conductivity

The electrical conductivity (EC) values of blossom honeys ranged from 210.00 to 678.77 µS/cm, with a mean of 436.29 µS/cm, whereas pine honeydew honeys ranged from 872.10 to 1154.23 µS/cm, with a mean of 1028.63 µS/cm (Table 4). EC was significantly higher in pine honeydew honeys than in blossom honeys ($p < 0.001$). According to the THC, EC should be below 800 µS/cm for blossom honeys and above this threshold for pine honeydew honeys. All samples complied with these criteria, resulting in a clear differentiation between the two honey types (Fig. 4).

Previous studies on Turkish honeys have likewise reported lower EC values for blossom honeys and higher values for pine and chestnut honeys (Yücel and Sultanoğlu, 2013; Simsek et al. 2012; Cengiz et al. 2018; Kanbur et al. 2021). Similar patterns have also been reported internationally, with honeydew, forest, and darker honeys generally showing higher EC values than blossom honeys (Kaškonienė et al. 2010; Escriche et al. 2017; Ng et al. 2023).

The clear separation observed in the present study supports the usefulness of EC for distinguishing blossom and pine honeydew honeys.

3.1.5. Proline Content

The proline content of the honey samples ranged from 273.17 to 855.09 mg/kg, with an overall mean of 456.22 mg/kg (Table 4). Blossom and pine honeydew honeys exhibited comparable proline levels, with mean values of 452.32 and 463.05 mg/kg, respectively, with no statistically significant difference between the two honey types ($p = 0.868$). As illustrated in Fig. 5, 21 of the 22 samples met the minimum proline requirement (≥ 300 mg/kg) established by the THC. Only one blossom honey sample (B1) fell below this limit, with a proline content of 273.17 mg/kg.

Studies on Turkish honeys have demonstrated considerable variation in proline content according to botanical origin and honey type. In a large-scale analysis of 373 Turkish pine honeydew honeys, proline concentrations ranged from 158 to 960 mg/kg, with a mean of 408.57 mg/kg (Uçurum et al. 2023). Kıvrak et al. (2017) reported a broader range of approximately 280–1780 mg/kg in Turkish honeys, with relatively high levels in certain unifloral and honeydew types. Yiğit et al. (2024) reported a mean proline content of approximately 619 mg/kg across pine, blossom, and thyme honeys, with the highest mean value in pine

honey (≈ 703 mg/kg), followed by thyme (≈ 637 mg/kg) and blossom honey (≈ 516 mg/kg). Similarly, Can et al. (2015) reported values ranging from 282 to 845 mg/kg, with higher concentrations in darker honeys such as chestnut, heather, chaste tree, and astragalus and lower concentrations in lighter blossom honeys such as acacia.

Substantial variability has also been reported for Spanish, Algerian, Italian, and Malaysian honeys. Spanish honeydew honeys showed particularly high proline concentrations (mean ≈ 1069 mg/kg; range 664–1689 mg/kg) compared with blossom honeys (mean ≈ 602 mg/kg; range 310–1057 mg/kg) (Manzanares et al. 2011). Algerian honeys from different botanical origins contained approximately 202–680 mg/kg proline, with higher concentrations generally observed in honeydew honeys (Ouchemoukh et al. 2007). In Italian unifloral honeys, acacia showed relatively low proline levels (≈ 230–250 mg/kg), whereas sunflower, chestnut, and honeydew honeys exhibited higher concentrations (≈ 590–665 mg/kg) (Truzzi et al. 2014). Malaysian stingless bee honeys displayed an even broader range of 38.8–797.2 mg/kg, further demonstrating the variability associated with botanical, geographical, and entomological origin (Hassan et al. 2023).

Taken together, the proline concentrations observed in the present study fall within the broad ranges reported for Turkish and international honeys. However, unlike several previous studies reporting distinctly higher proline levels in honeydew or darker honeys, only a small difference was observed between blossom and pine honeydew honeys in the present sample set. This finding highlights the considerable natural variability of proline among honeys and suggests that proline content should be interpreted together with botanical origin and other physicochemical parameters rather than as a stand-alone indicator of honey type.

3.1.6. Hydroxymethylfurfural (HMF) Analysis by HPLC-DAD

The hydroxymethylfurfural (HMF) content of the honey samples ranged from 7.47 to 83.52 mg/kg, with an overall mean of 25.77 mg/kg (Table 4). Blossom honeys had a higher mean HMF content (29.14 mg/kg) than pine honeydew honeys (19.88 mg/kg), although the difference between the two honey types was not statistically significant ($p = 0.188$). Among the 22 samples analysed, only two blossom honey samples, B1 and B12, exceeded the maximum permissible HMF limit of 40 mg/kg established by the THC, with values of 83.52 and 52.04 mg/kg, respectively. All pine honeydew samples remained within the specified limit (Fig. 6).

Studies on Turkish honeys have generally reported relatively low HMF levels, although considerable variation has been observed depending on honey type, processing, and storage conditions. Pine honeys from Western Anatolia showed HMF values ranging from 1.54 to 23.42 mg/kg, all remaining below the international limit (Akbulut et al. 2009). Özcan and Ölmez (2014) reported values of 0.38–42.0 mg/kg for Turkish honeys of different botanical origins, with most samples remaining within acceptable limits. Similarly, floral honeys from city of Gümüşhane exhibited relatively low HMF concentrations of 0.37–18.89 mg/kg (Ucuncu et al. 2026). In contrast, honeys from Southeastern Anatolia displayed substantially greater variability, with HMF concentrations ranging from 1.10 to 166.25 mg/kg, and a small proportion of samples exceeding the legal limit (Gürbüz et al. 2020).

Considerable variability in HMF content has also been reported for Greek, Moroccan, Australian, and Chinese honeys. Greek honeys generally exhibited low HMF levels, with lower concentrations reported for honeydew than for blossom honeys (Pasias et al. 2017). Moroccan honeys showed HMF concentrations ranging from 3.2 to 52.6 mg/kg, with some samples exceeding the European limit of 40 mg/kg and substantial variation among botanical origins (Terrab et al. 2002). Australian honeys displayed an even broader range of 0.36–74.9 mg/kg, with low HMF levels in fresh, unprocessed honeys and higher concentrations in some processed samples (Ajlouni and Sujirapinyokul 2010). In contrast, Chinese monofloral Dendropanax dentiger honey exhibited consistently low HMF concentrations of 0.71–2.91 mg/kg (Wang et al. 2021).

Overall, the HMF concentrations observed in the present study fall within the broad variability reported for Turkish and international honeys, although B1 and B12 showed values above the regulatory limit. The higher mean HMF content observed in blossom honeys compared with pine honeydew honeys is also compatible with the variation reported among different honey types in previous studies. Since HMF formation is influenced by processing and storage conditions as well as honey composition, elevated HMF values should be interpreted together with other quality indicators, particularly diastase activity, rather than being attributed to a single factor (Pasias et al. 2017).

3.2. Comprehensive Sugar Profiling by HPLC-RID

The sugar profiles of the honey samples were comprehensively characterized by HPLC-RID, covering the major sugars considered as regulatory parameters (sucrose, maltose, fructose, and glucose), along with the minor sugars (xylose, arabinose, galactose, trehalose, melezitose, and raffinose).

3.2.1. Major Sugars and Regulatory Quality Parameters

The sugar profiles of the honey samples obtained by HPLC-RID are presented in Table 5. As expected, fructose and glucose were the predominant sugars in both blossom and pine honeydew honeys. Blossom honeys had higher mean fructose (39.16%) and glucose (31.10%) contents than pine honeydew honeys (36.47% and 29.58%, respectively). Fructose was significantly higher in blossom honeys ($p = 0.005$), whereas the difference in glucose content was not statistically significant ($p = 0.122$). The mean F + G content was also significantly higher in blossom honeys (70.27%) than in pine honeydew honeys (66.36%) ($p = 0.025$). In contrast, the mean F/G ratios were similar at 1.26 and 1.25 for blossom and pine honeydew honeys, respectively ($p = 0.676$). The F + G contents and F/G ratios of the individual honey samples are presented in Fig. 7. According to the THC, F + G should be at least 60% for blossom honeys and 45% for pine honeydew honeys, while the F/G ratio should range from 0.9–1.4 and 1.0–1.4, respectively (Table 5). Sucrose and maltose contents ranged from 0.06 to 0.93% and from 0.34 to 1.33%, respectively (Table 5). As illustrated in Fig. 8, sucrose (a) and maltose (b) contents in all samples remained below their respective maximum limits established by the THC. Overall, all honey samples met the sugar composition criteria established by the THC.

Previous studies on Turkish honeys have similarly identified fructose and glucose as the predominant sugars and generally reported low sucrose levels (Silici and Yılmaztürk, 2025; Çetintaş, 2025). Higher F + G contents in blossom honeys than in pine honeys have also been reported, consistent with the pattern observed in the present study (Aslan & Kibar, 2024). Regarding the F/G ratio, Uçurum et al. (2023) reported mean values of 1.51 for pine and 1.20 for multifloral blossom honey, while Çobanoğlu et al. (2023) reported values of 1.40 and 1.36, respectively. In contrast to these reports, the mean F/G ratios observed in the present study were highly comparable between blossom (1.26) and pine honeydew (1.25) honeys.

Studies conducted on honeys from other countries have also reported similar sugar composition patterns. Ouchemoukh et al. (2010) reported fructose and glucose contents of 35.99–42.57% and 24.63–35.06%, respectively, in Algerian honeys, with F/G ratios ranging from 1.11 to 1.36, closely corresponding to the values observed in the present study. Studies on Spanish honeys have likewise demonstrated variations in F + G and F/G ratios according to honey type and botanical origin (Manzanares et al. 2011; Escuredo et al. 2014). In Serbian honeys, Baloš et al. (2020) reported higher F + G contents in multifloral and meadow honeys (74.52 and 72.73%, respectively) than in honeydew honey (69.15%), consistent with the pattern observed in the present study.

3.2.2. Minor Sugar Profile

Although fructose and glucose constitute the major fraction of honey sugars, the analysis of minor sugars provides a more comprehensive characterization of its carbohydrate composition. In the present study, minor sugars occurred at relatively low concentrations and showed considerable variation among the analysed samples (Table 5). Trehalose was detected in all samples (0.23–1.22%), whereas xylose and arabinose occurred sporadically and galactose was not detected. Melezitose was found in both blossom and pine honeydew honeys, reaching 1.00 and 0.93%, respectively. Raffinose showed a more distinct distribution, being detected mainly in pine honeydew honeys (up to 0.93%) and in only one blossom sample (0.43%).

Studies on Turkish honeys have likewise revealed considerable variability in minor sugar composition. Can et al. (2015) reported that arabinose and galactose were not detected, while trehalose occurred only in some honey types; melezitose was generally detected at low levels (approximately 0.5%) but reached higher concentrations in pine and oak honeys. More recently, Beykaya et al. (2026), using HPLC-RID to characterize 150 Turkish raw honeys, reported low melezitose concentrations (ND–0.45%) together with other minor sugars such as turanose and erlose.

Studies on honeys from other countries have likewise demonstrated considerable variability in minor sugar composition. In Spanish monofloral honeys, Manzanares et al. (2014) reported trehalose concentrations of 0.62–2.66% and melezitose levels of 0.08–3.69%, illustrating the wide variation of these sugars among honey types. Pascual-Maté et al. (2018) similarly found trehalose at low concentrations (< 0.35%), while melezitose and raffinose occurred across different honey types, with the highest mean melezitose concentration observed in honeydew honeys (0.50%). In Moroccan honeys, Bouddine et al. (2022) reported an even more heterogeneous pattern: trehalose was detected in only a limited number of samples, whereas melezitose occurred in 19 of 23 honeys (0.02–0.46%) and raffinose was detected in all samples at concentrations not exceeding 0.32%. These findings demonstrate that minor sugars can vary considerably in both occurrence and concentration among honeys and, although some sugars such as melezitose are frequently associated with honeydew honey, their presence is not restricted to a single honey type.

The minor sugar profile observed in the present study is therefore consistent with the broad variability reported in the literature, particularly the occurrence of melezitose in both blossom and pine honeydew honeys and the variable distribution of trehalose and raffinose.

Table 4
Physicochemical characteristics and quality assessment of blossom and pine honeydew honey samples (mean ± SD)

Sample ID	Moisture (%)	pH	Free Acidity (mEq/kg)	Diastase Number (DN)	Electrical conductivity (µS/cm)	Proline (mg/kg)	HMF (mg/kg)
B1	16.60 ± 0.20	3.80 ± 0.02	10.50 ± 0.50	20.83 ± 0.00	353.00 ± 2.61	273.17 ± 9.59	83.52 ± 0.34
B2	16.53 ± 0.12	4.24 ± 0.01	17.50 ± 0.50	35.71 ± 0.00	528.47 ± 0.68	443.84 ± 20.60	26.38 ± 0.86
B3	16.13 ± 0.12	4.49 ± 0.01	18.67 ± 0.29	41.66 ± 0.00	628.00 ± 1.18	442.28 ± 15.71	7.47 ± 0.33
B4	17.80 ± 0.00	4.06 ± 0.01	18.17 ± 0.29	16.66 ± 0.00	628.57 ± 1.96	358.60 ± 15.95	31.11 ± 0.16
B5	17.00 ± 0.00	3.65 ± 0.01	20.00 ± 1.00	16.66 ± 0.00	429.53 ± 1.37	404.29 ± 16.30	25.67 ± 0.54
B6	15.60 ± 0.00	3.52 ± 0.00	12.50 ± 0.00	8.33 ± 0.00	210.00 ± 0.89	442.76 ± 7.55	14.76 ± 0.07
B7	16.60 ± 0.00	3.53 ± 0.01	18.50 ± 0.50	16.66 ± 0.00	262.43 ± 0.23	466.08 ± 10.54	12.10 ± 0.18
B8	16.33 ± 0.12	3.84 ± 0.01	16.17 ± 0.29	8.89 ± 0.79	353.57 ± 1.03	461.15 ± 10.66	24.27 ± 0.10
B9	15.87 ± 0.12	4.02 ± 0.01	17.00 ± 0.50	14.70 ± 0.00	424.90 ± 10.10	334.90 ± 13.92	27.18 ± 0.22
B10	15.60 ± 0.00	3.72 ± 0.01	22.50 ± 1.00	25.00 ± 0.00	360.67 ± 2.41	855.09 ± 28.11	15.86 ± 0.26
B11	15.33 ± 0.12	3.65 ± 0.00	18.17 ± 0.29	16.66 ± 0.00	269.77 ± 0.50	724.75 ± 5.36	35.44 ± 0.84
B12	15.67 ± 0.12	3.95 ± 0.03	10.50 ± 0.50	32.74 ± 2.10	619.43 ± 2.42	398.03 ± 12.64	52.04 ± 0.32
B13	16.87 ± 0.12	3.78 ± 0.01	16.83 ± 0.58	10.83 ± 1.18	361.00 ± 0.30	400.36 ± 13.39	28.54 ± 0.27
B14	17.00 ± 0.00	4.21 ± 0.01	16.00 ± 0.50	9.44 ± 0.79	678.77 ± 0.50	327.31 ± 9.66	23.63 ± 0.65
P1	16.20 ± 0.00	4.46 ± 0.01	22.33 ± 0.29	35.71 ± 0.00	999.53 ± 3.01	694.35 ± 18.47	21.54 ± 0.87
P2	15.20 ± 0.00	4.46 ± 0.00	20.00 ± 0.00	41.66 ± 0.00	872.10 ± 0.72	434.47 ± 16.69	7.61 ± 0.16
P3	15.60 ± 0.00	4.10 ± 0.01	9.33 ± 0.29	31.25 ± 0.00	1123.10 ± 2.82	377.24 ± 5.02	29.28 ± 0.20
P4	15.60 ± 0.00	4.39 ± 0.01	21.83 ± 0.29	20.83 ± 0.00	928.77 ± 3.93	544.78 ± 3.12	12.41 ± 0.09
P5	16.60 ± 0.00	4.62 ± 0.02	23.50 ± 1.00	18.51 ± 0.00	1154.23 ± 1.37	501.52 ± 14.24	23.72 ± 0.20
P6	15.93 ± 0.12	4.73 ± 0.01	16.17 ± 0.29	16.66 ± 0.00	1102.67 ± 4.34	396.27 ± 12.52	21.77 ± 0.14
P7	16.40 ± 0.00	4.38 ± 0.01	24.50 ± 0.50	14.70 ± 0.00	970.10 ± 5.01	387.51 ± 10.67	25.06 ± 0.32
P8	15.60 ± 0.20	4.45 ± 0.01	14.83 ± 0.29	18.51 ± 0.00	1078.57 ± 1.03	368.22 ± 9.76	17.64 ± 0.52

Table 5
Sugar profiles and quality assessment of blossom and pine honeydew honey samples (mean ± SD; ND: not detected)

Sample ID	Xylose (%)	Arabinose (%)	Galactose (%)	Trehalose (%)	Melezitose (%)	Raffinose (%)	Sucrose (%)	Maltose (%)	Fructose (%)	Glucose (%)	F + G (%)	F/G	To (%
B1	0.18 ± 0.01	ND	ND	0.35 ± 0.01	ND	ND	0.06 ± 0.01	1.33 ± 0.00	39.67 ± 0.19	32.96 ± 0.43	72.64	1.20	74
B2	0.53 ± 0.04	ND	ND	0.66 ± 0.06	0.18 ± 0.01	ND	0.37 ± 0.04	1.08 ± 0.04	38.12 ± 0.17	31.25 ± 0.40	69.37	1.22	72
B3	ND	0.79 ± 0.02	ND	0.23 ± 0.01	ND	ND	0.29 ± 0.03	ND	43.23 ± 0.71	32.59 ± 2.01	75.81	1.33	77
B4	ND	ND	ND	0.88 ± 0.04	0.41 ± 0.02	0.43 ± 0.03	0.06 ± 0.01	0.78 ± 0.01	37.25 ± 0.40	29.22 ± 0.39	66.47	1.27	69
B5	ND	ND	ND	0.63 ± 0.04	ND	ND	0.16 ± 0.01	1.24 ± 0.01	39.62 ± 0.16	33.12 ± 0.63	72.74	1.20	74
B6	ND	ND	ND	1.05 ± 0.04	ND	ND	0.14 ± 0.02	0.47 ± 0.04	39.41 ± 0.14	29.70 ± 0.38	69.11	1.33	70
B7	ND	ND	ND	0.71 ± 0.02	0.30 ± 0.01	ND	0.41 ± 0.02	1.25 ± 0.05	38.99 ± 0.23	29.51 ± 0.23	68.50	1.32	71
B8	ND	ND	ND	0.68 ± 0.03	0.21 ± 0.01	ND	0.27 ± 0.04	1.30 ± 0.02	38.70 ± 0.51	31.09 ± 1.00	69.78	1.24	72
B9	ND	ND	ND	0.71 ± 0.01	0.82 ± 0.02	ND	0.14 ± 0.01	1.05 ± 0.08	38.26 ± 0.27	31.07 ± 0.10	69.33	1.23	72
B10	ND	ND	ND	0.97 ± 0.02	0.46 ± 0.03	ND	0.11 ± 0.02	0.71 ± 0.01	40.20 ± 0.22	29.64 ± 0.29	69.84	1.36	72
B11	ND	ND	ND	0.89 ± 0.04	0.57 ± 0.01	ND	0.13 ± 0.01	1.00 ± 0.04	39.31 ± 0.40	29.71 ± 0.76	69.02	1.32	71
B12	0.12 ± 0.00	ND	ND	0.41 ± 0.03	0.20 ± 0.01	ND	0.13 ± 0.03	0.94 ± 0.02	39.28 ± 0.27	33.95 ± 0.81	73.23	1.16	75
B13	ND	ND	ND	0.65 ± 0.01	0.67 ± 0.04	ND	0.16 ± 0.01	1.32 ± 0.04	39.14 ± 0.22	31.78 ± 1.52	70.92	1.23	73
B14	ND	ND	ND	0.49 ± 0.03	1.00 ± 0.05	ND	0.16 ± 0.01	1.23 ± 0.09	37.09 ± 0.17	29.86 ± 0.17	66.95	1.24	69
P1	0.80 ± 0.06	ND	ND	0.73 ± 0.01	ND	0.43 ± 0.02	0.20 ± 0.01	0.47 ± 0.02	36.88 ± 0.10	27.45 ± 1.42	64.33	1.34	66
P2	ND	0.89 ± 0.04	ND	0.25 ± 0.01	ND	ND	0.49 ± 0.02	ND	41.71 ± 0.19	33.19 ± 0.67	74.90	1.26	76
P3	0.13 ± 0.01	ND	ND	0.37 ± 0.03	0.40 ± 0.03	0.49 ± 0.01	0.24 ± 0.01	0.71 ± 0.06	38.17 ± 0.33	34.77 ± 0.79	72.94	1.10	75
P4	0.44 ± 0.01	ND	ND	0.87 ± 0.03	ND	0.71 ± 0.06	0.18 ± 0.01	0.74 ± 0.01	36.60 ± 0.24	28.63 ± 0.93	65.23	1.28	68
P5	ND	ND	ND	1.22 ± 0.10	0.30 ± 0.03	0.75 ± 0.03	0.06 ± 0.02	0.34 ± 0.01	33.58 ± 0.71	26.80 ± 0.63	60.37	1.25	63
P6	0.10 ± 0.01	ND	ND	0.83 ± 0.06	0.38 ± 0.01	ND	0.17 ± 0.01	0.82 ± 0.03	35.01 ± 0.49	27.60 ± 0.27	62.61	1.27	64
P7	ND	ND	ND	0.64 ± 0.01	0.93 ± 0.06	0.93 ± 0.08	0.43 ± 0.04	1.06 ± 0.02	35.56 ± 0.10	28.19 ± 0.56	63.75	1.26	68
P8	ND	ND	ND	0.70 ± 0.02	0.35 ± 0.01	0.52 ± 0.01	0.12 ± 0.01	1.22 ± 0.02	36.80 ± 0.14	29.97 ± 0.44	66.77	1.23	69

Table 6
Statistical comparison of physicochemical and major sugar parameters between blossom and pine honeydew honeys. (Values are expressed as mean ± standard deviation; Bold p-values indicate statistically significant differences ($p < 0.05$))

Parameter	Blossom honey (n = 14)	Pine honeydew honey (n = 8)	*p*-value
Moisture (%)	16.35 ± 0.70	15.89 ± 0.48	0.113
Free acidity (mEq/kg)	16.64 ± 3.42	19.06 ± 5.20	0.202
pH	3.89 ± 0.29	4.45 ± 0.18	**< 0.001**
Diastase number (DN)	19.63 ± 10.49	24.73 ± 10.06	0.279
Electrical conductivity (µS/cm)	436.29 ± 154.19	1028.63 ± 101.01	**< 0.001**
Proline (mg/kg)	452.32 ± 155.72	463.05 ± 112.76	0.868
HMF (mg/kg)	29.14 ± 19.11	19.88 ± 7.05	0.188
Fructose (%)	39.16 ± 1.47	36.47 ± 2.87	**0.005**
Glucose (%)	31.10 ± 1.57	29.58 ± 2.91	0.122
Fructose + glucose (F + G, %)	70.27 ± 2.56	66.36 ± 5.05	**0.025**
Fructose/glucose (F/G) ratio	1.26 ± 0.06	1.25 ± 0.07	0.676

4. Conclusion

This study provides an integrated assessment of the physicochemical quality and sugar composition of commercially available blossom and pine honeydew honeys produced in Türkiye. Overall, the samples showed good compliance with the quality criteria established by the THC. All samples met the requirements for moisture, free acidity, diastase activity, electrical conductivity and major sugar composition, while 21 of the 22 samples complied with the minimum proline requirement. HMF was the main parameter showing non-compliance, with two blossom honey samples exceeding the maximum permissible limit. These findings indicate generally satisfactory quality among the commercial honeys examined, while also revealing sample-level variation that may reflect differences in processing or storage conditions.

The comparative analysis further demonstrated compositional differences between blossom and pine honeydew honeys. Pine honeydew honeys had significantly higher pH and electrical conductivity, with electrical conductivity providing particularly clear separation between the two honey types. In contrast, blossom honeys showed significantly higher fructose and F + G contents, whereas moisture, free acidity, diastase activity, proline, HMF, glucose and F/G ratio did not differ significantly between the groups. Minor sugars showed considerable variation among individual samples, providing additional information on carbohydrate composition beyond the major sugars.

The combined evaluation of conventional physicochemical parameters and comprehensive HPLC-RID sugar profiling provides a useful approach for assessing the quality and compositional characteristics of retail honeys. The findings also highlight the value of evaluating multiple quality indicators together rather than relying on a single parameter when characterizing commercially available blossom and pine honeydew honeys produced in Türkiye.

Declarations

AI Declaration: AI was not used in generating the manuscript, except for linguistic, stylistic, and grammatical corrections.

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Figures

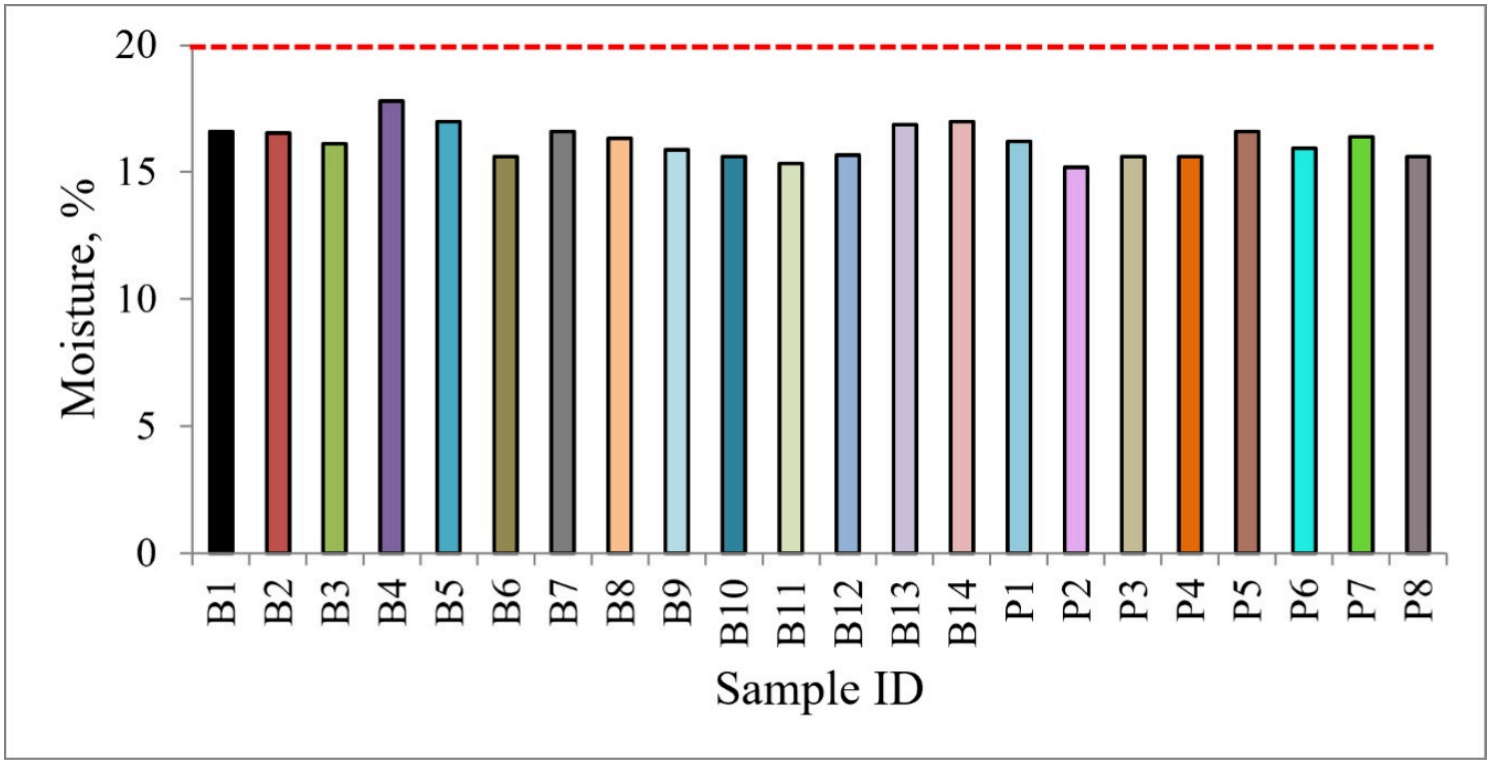


Figure 1

Moisture content (%) of the honey samples. The red dashed line indicates the maximum moisture limit (20%) established by the THC.

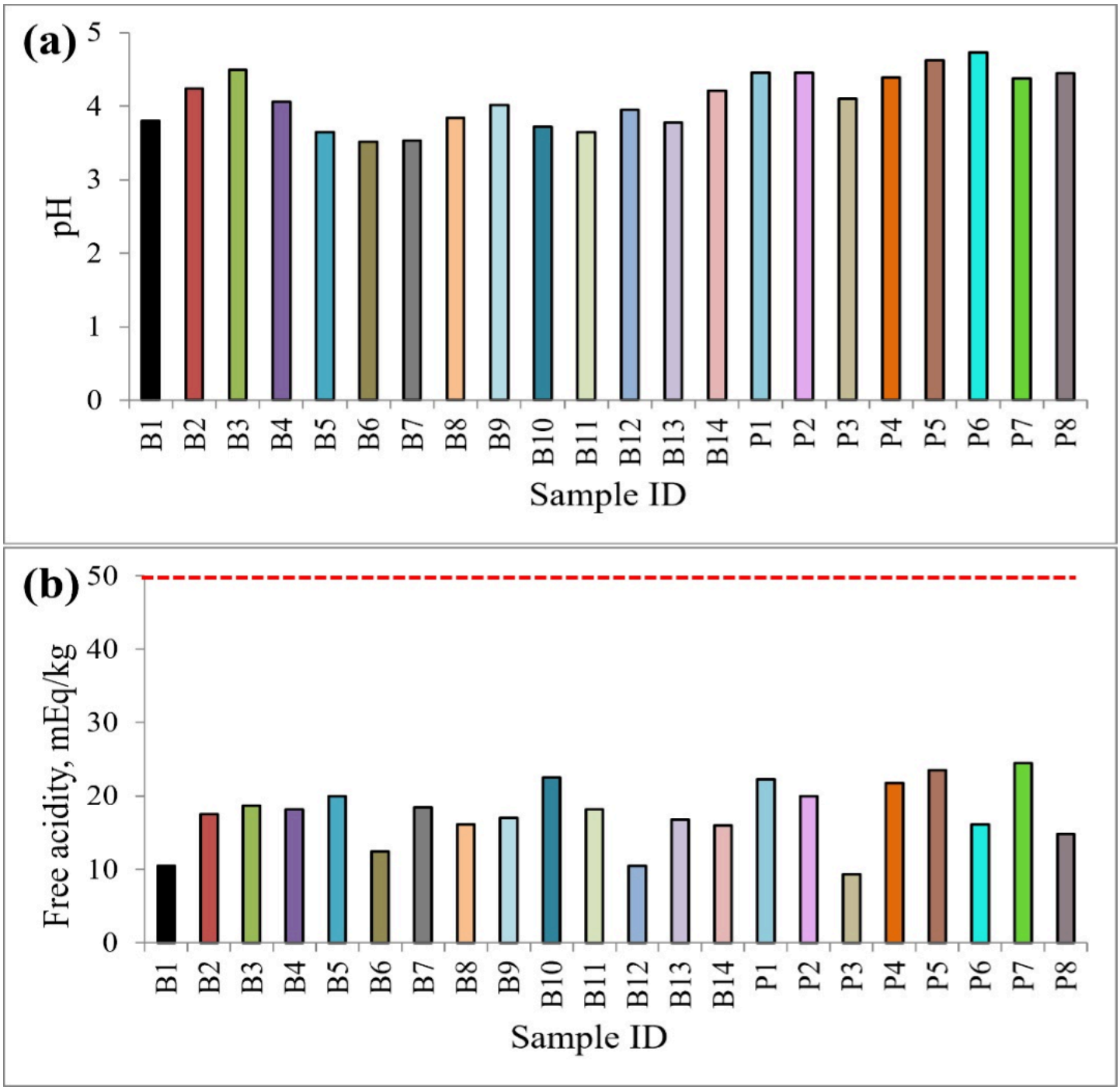


Figure 2

Free acidity and pH values of the honey samples; (a) free acidity values and (b) pH values of the samples. The dashed line indicates the maximum free acidity limit established by the THC.

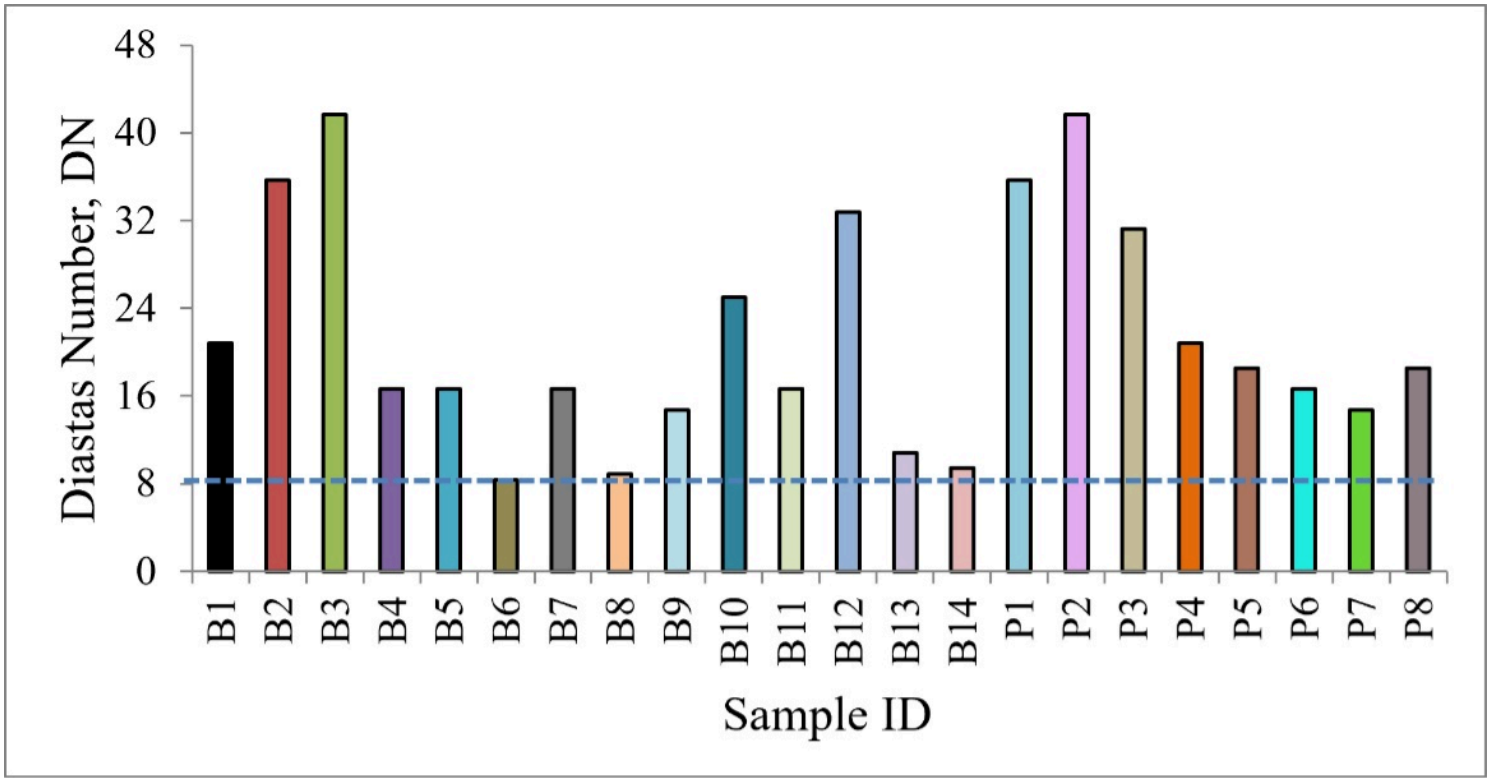


Figure 3

Diastase number (DN) of the honey samples. The blue dashed line indicates the minimum diastase activity level established by the THC.

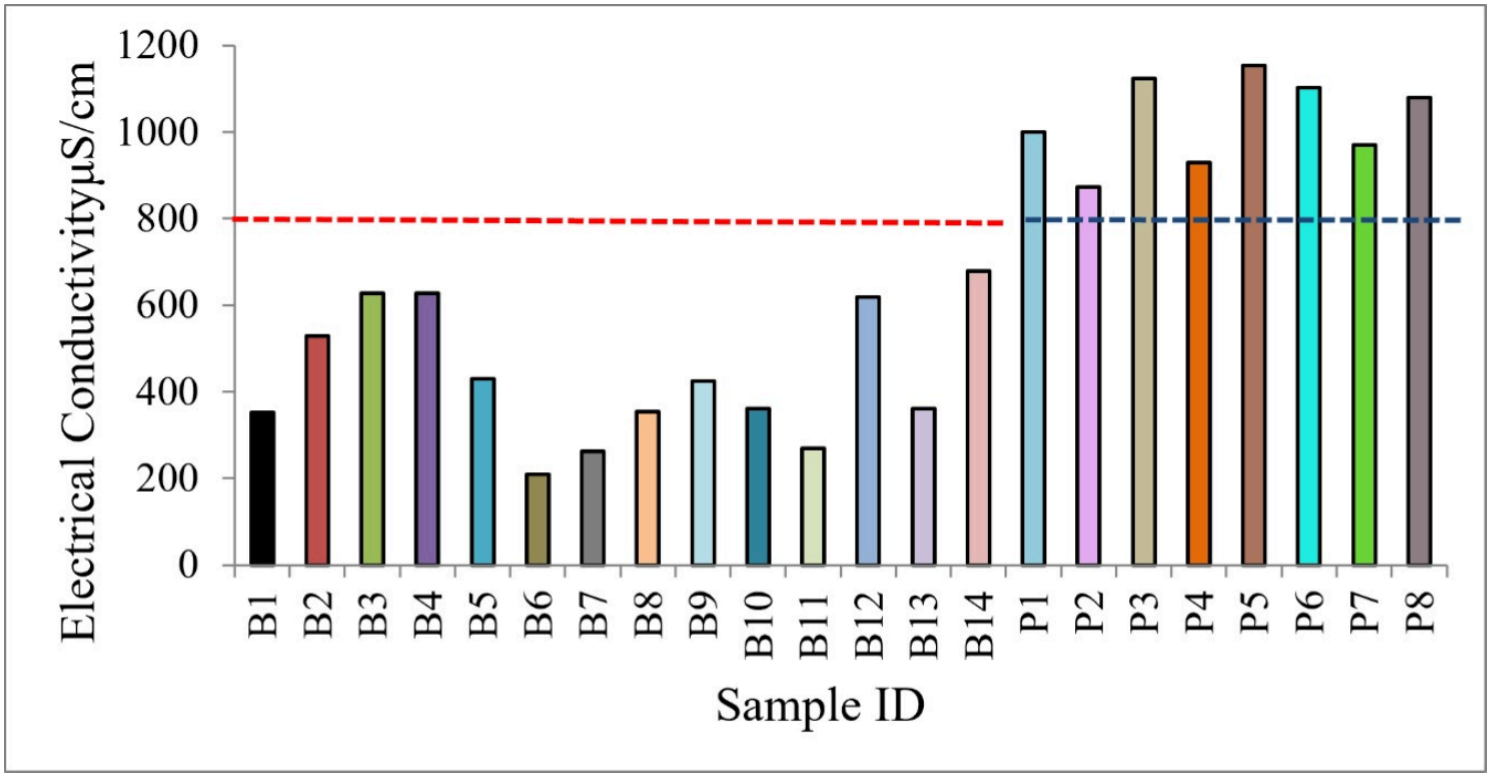


Figure 4

Electrical conductivity (μS/cm) of the honey samples. The red dashed line indicates the maximum limit for blossom honeys, while the blue dashed line represents the minimum limit for pine honeydew honeys, as established by the THC.

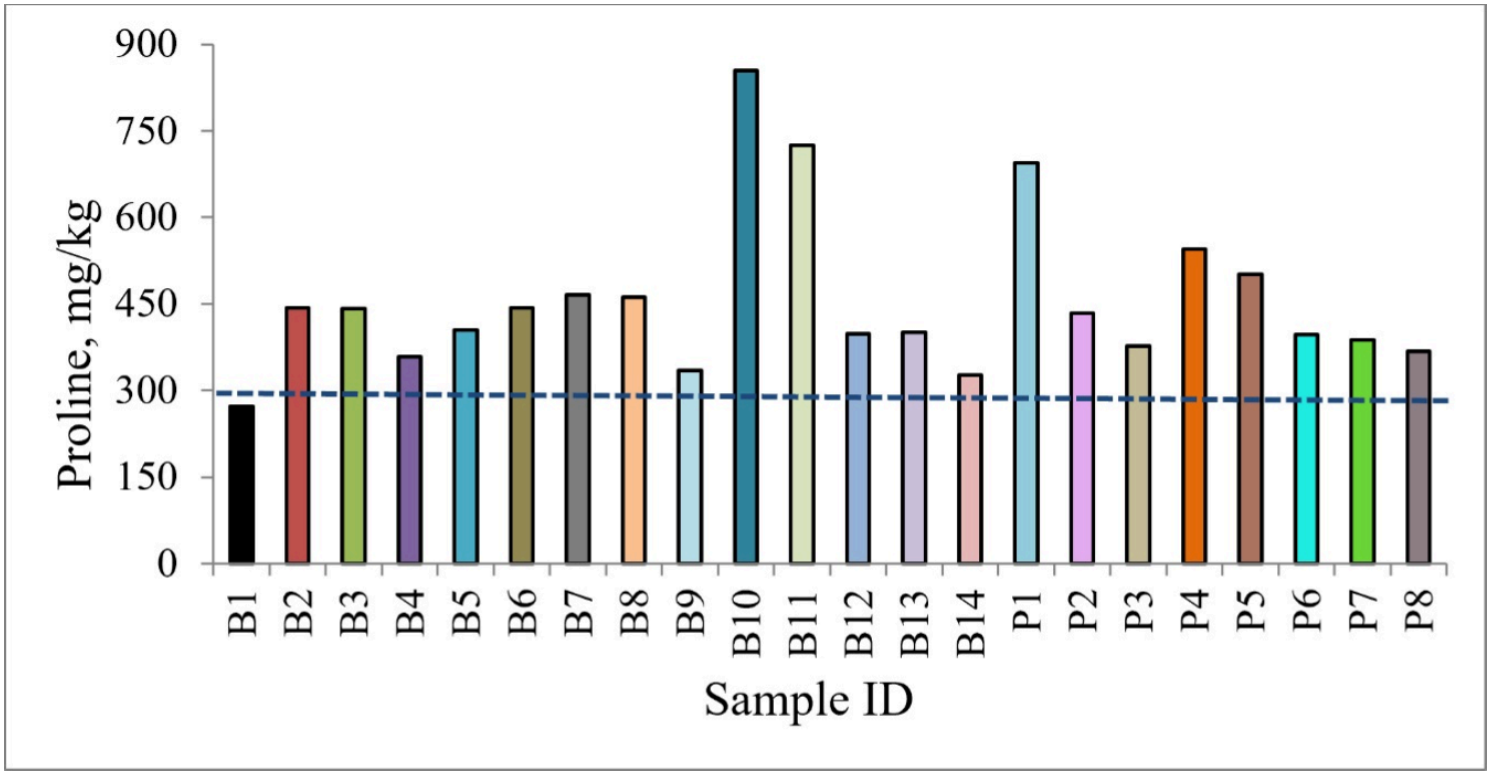


Figure 5

Proline content of the honey samples. The blue dashed line indicates the minimum proline level established by the THC.

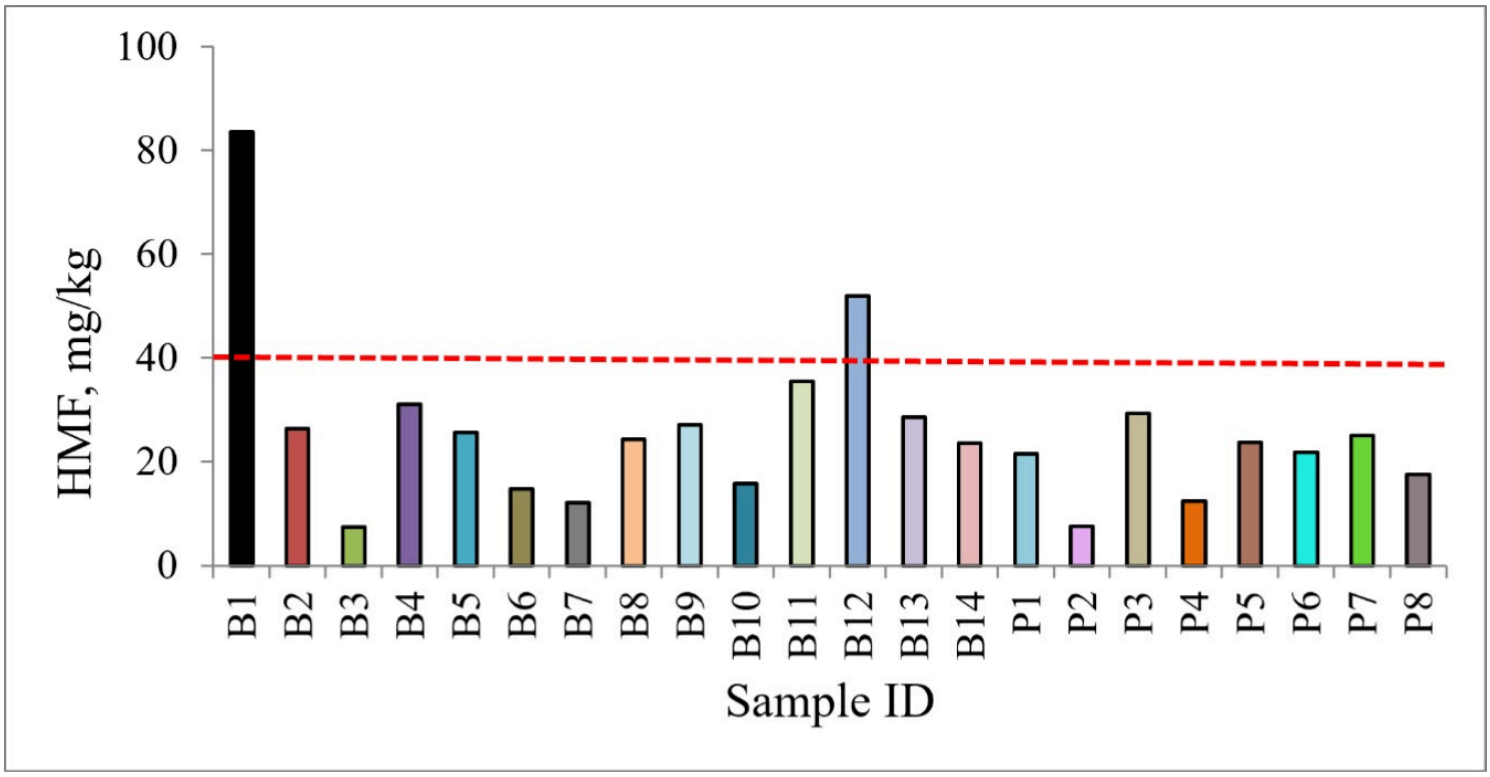


Figure 6

HMF content of the honey samples. The red dashed line indicates the maximum HMF level established by the THC.

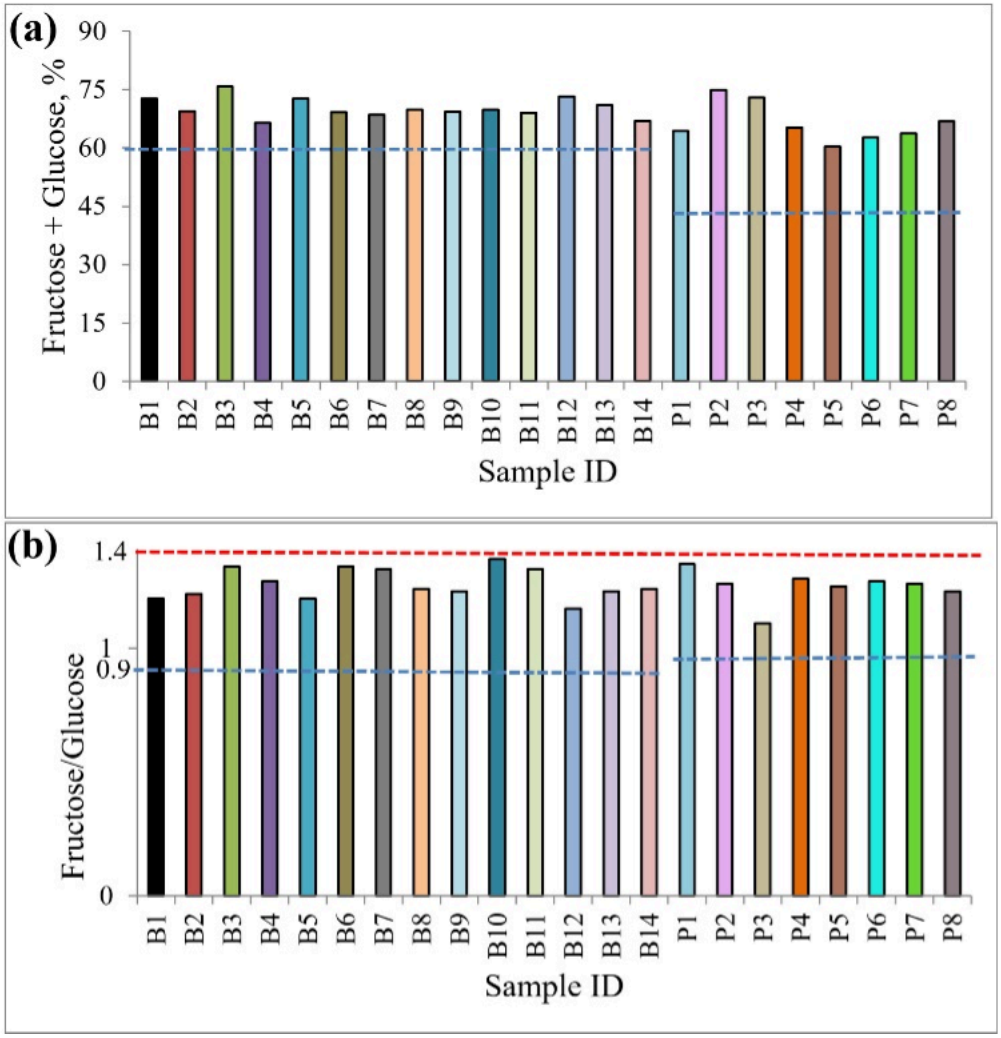


Figure 7

Fructose + glucose (F+G) content (a) and fructose/glucose (F/G) ratio (b) of the honey samples. The blue dashed lines indicate the minimum limits, while the red dashed line indicates the maximum limit established by the THC.

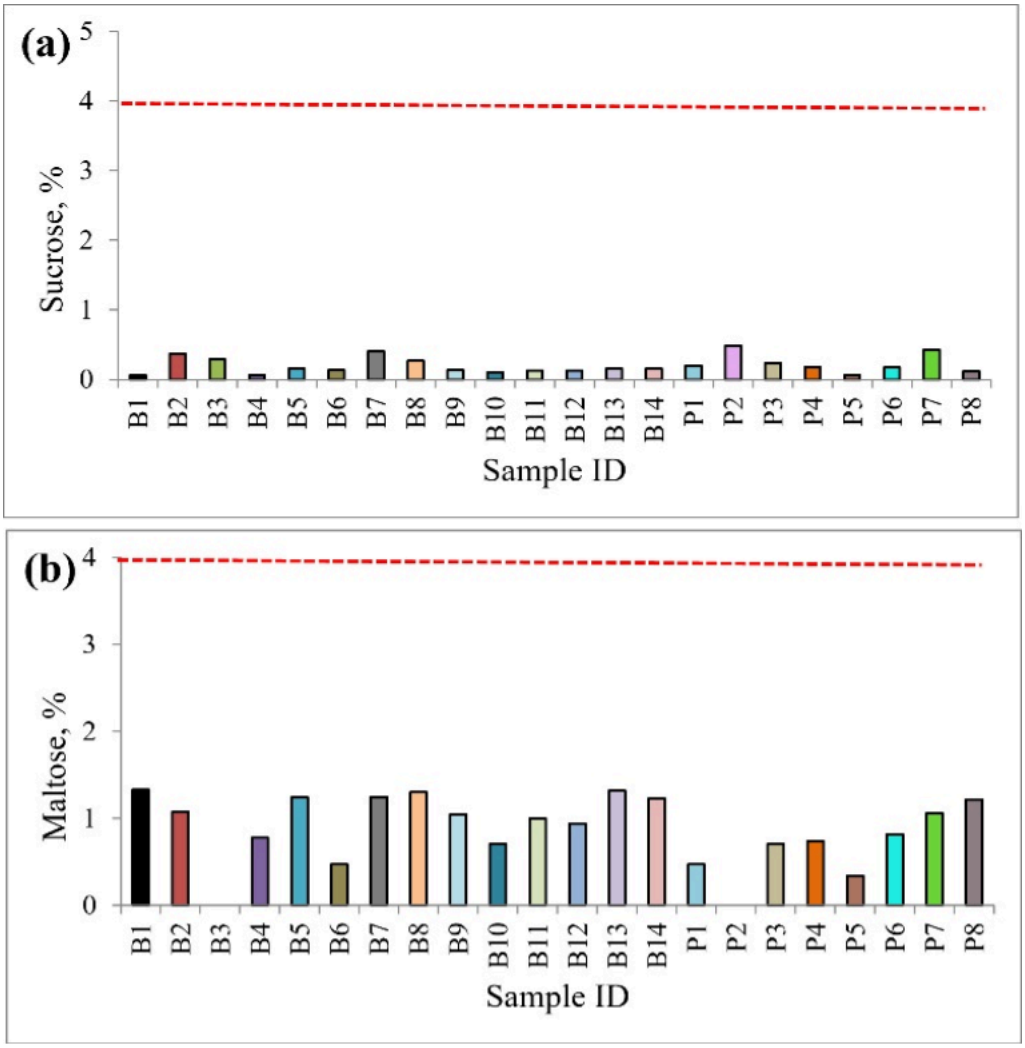


Figure 8

Sucrose (a) and maltose (b) contents of the honey samples. The red dashed lines indicate the maximum limits established by the THC.

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