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ORIGINAL PAPER

Unveiling the geographical signatures of Turkish honey through multivariate analysis of its physicochemical, chemical, and biological properties

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Abstract

Verification of geographical origin and identification of region-specific properties of honey have gained increasing attention due to their influence on consumer preferences and market value. In this study, it was aimed to classify a total of 121 unifloral, multifloral, and honeydew honeys collected from different geographical regions of Türkiye using a linear discriminant analysis (LDA) model. The stepwise function was employed to identify the key variables based on physicochemical, chemical, and biological properties contributing most significantly to geographical differentiation. Except for titratable acidity and proline content, the examined honey properties, including basic physicochemical properties, amino acid profiles, and biological activities, showed significant differences among the seven geographical regions. The honey samples exhibited wide botanical diversity, and their quality parameters were largely compliant with legal standards. Honey samples collected from Black Sea region showed higher total phenolic and flavonoid contents and antioxidant activity. Aegean honeys exhibited stronger anti-hyaluronidase effects, and Eastern Anatolia honeys demonstrated greater inhibitory effect against *Staphylococcus aureus* ATCC 25923. The LDA model achieved classification accuracies of 91.7% for original cases and 85.5% after cross-validation. Function 1 was mainly associated with higher aspartic acid content and lower water activity, while Function 2 was characterized by stronger *S. aureus* ATCC 25923 inhibition, higher cysteine, and lower aspartic acid levels. The honey samples collected from Marmara (94.1%) and Central Anatolia (95.2%) were clearly distinguishable, while those from the Mediterranean (73.5%) and Black Sea (80.0%) regions showed lower cross-validated discrimination, particularly in relation to the Central Anatolia samples, due to overlapping properties.

Keywords Geographical origin · Geographical markers · Linear discriminant analysis · Aspartic acid · Honey bioactivity

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Introduction

The geographical origin of honey is of great importance in terms of its chemical composition and biological effects. Furthermore, the natural properties, quality, and reputation of geographical origin influence consumer perception and preferences and determine the market price of honey. According to the European Economic Community's labeling regulations, leading honey-producing countries are required to accurately indicate the geographical and botanical origin of their honey on labels [1, 2].

Considering the importance of geographical origin in verifying the origin of honey, from basic analytical techniques to various spectrometric techniques (Fourier transform infrared, near infrared, inductively coupled plasma, and nuclear magnetic resonance), chromatographic methods (gas, liquid, and ion chromatography), and biological techniques such as polymerase chain reaction have been assessed to accurately identify origin of honey [1, 2]. The moisture content, conductivity, acidity, hydroxymethylfurfural (HMF) content, diastase activity, sugar profile, amino acids, phenolic compounds, organic acids, volatile compounds, and elemental composition of honey, along with its antioxidant activity, are reported parameters used to distinguish and determine the geographical origin of honey [2–6].

The discriminative power of these parameters and the classification of honey samples based on their geographical origin are interpreted using multivariate data analysis techniques [7–9]. Linear discriminant analysis (LDA) is a supervised multivariate statistical method used for data classification. LDA aims to determine the optimal discriminant functions that assign individuals or groups to one of several predefined populations based on multiple measured variables [8, 10]. Furthermore, the stepwise linear discriminant method (SLDA) can also be applied to obtain the discriminant variable(s) that stand out in the classification [10]. LDA is widely applied a discriminant analysis model in previous studies for the evaluation of botanical and geographical origins of honey [6, 8, 11–13]. For example, it has been successfully applied to characterize acacia honey from Romania [14]; clover and citrus honeys from Egypt [15]; assa-peixe, capixingui, eucalyptus, and citrus honeys, as well as various wildflower honeys from Brazil [16]; and different honey types from Russia, including polyfloral, clover, fireweed, linden, raspberry, buckthorn, white acacia, and lavender honeys [17]. In these studies, LDA was applied to analyze the physicochemical properties, fluorescent fingerprints, and spectral reflectance characteristics of honey samples, yielding satisfactory classification accuracies.

Türkiye is a significant honey producer country, ranking second in the world with an annual production of approximately 115,000 tons according to data provided by FAOStat for 2023 [18]. This prominence is attributed to its rich floral diversity, genetic resources of honeybees, and favorable climatic conditions [19]. Although various studies have been conducted on the classification of honey produced in Türkiye, these studies have generally been limited in terms of geographical scope and botanical diversity [5, 20–23]. Therefore, this study aimed to classify a total of 121 unifloral, multifloral, and honeydew honey samples collected from 54 provinces of the country using LDA model based on their physicochemical, chemical, and biological properties. Besides, SLDA was applied to identify the key variables contributing to classification, thereby highlighting the parameters that play the most significant role in the geographical discrimination of honey.

Materials and methods

Honey samples collection

Honey samples were collected directly from beekeepers under authentic production conditions, ensuring representation of the predominant botanical varieties of each region, with the support of Altıparmak Food Industry and Trade Inc. (İstanbul, Türkiye) and the Ordu Apiculture Research Institute (Ordu, Türkiye). A total of 121 honey samples were collected from various regions during two consecutive harvest seasons (2018–2019 and 2019–2020). The geographical information and regional distribution of all honey samples obtained are provided in Fig. 1, and their botanical origin information is provided in Supplementary Material 1. The honey samples were stored in glass jars at 25 °C in a dark place without any pre-treatment until analysis.

Materials

Chemicals used in spectrophotometric and chromatographic analyses were purchased at analytical and chromatographic purity by Honeywell (Michigan, USA), Isolab Laborgeräte GmbH (Germany), and Merck (Darmstadt, Germany). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 strains were supplied in lyophilized form from American Type Culture Collection (ATCC, Rockville, USA), and media, chemicals, and other consumables used in microbiological analyses were supplied at high purity by Merck (Darmstadt, Germany).

Methods for melissopalynological and physicochemical analyses

Melissopalynological analysis was carried out via reported method by Louveaux et al. [24], and the pollen identification

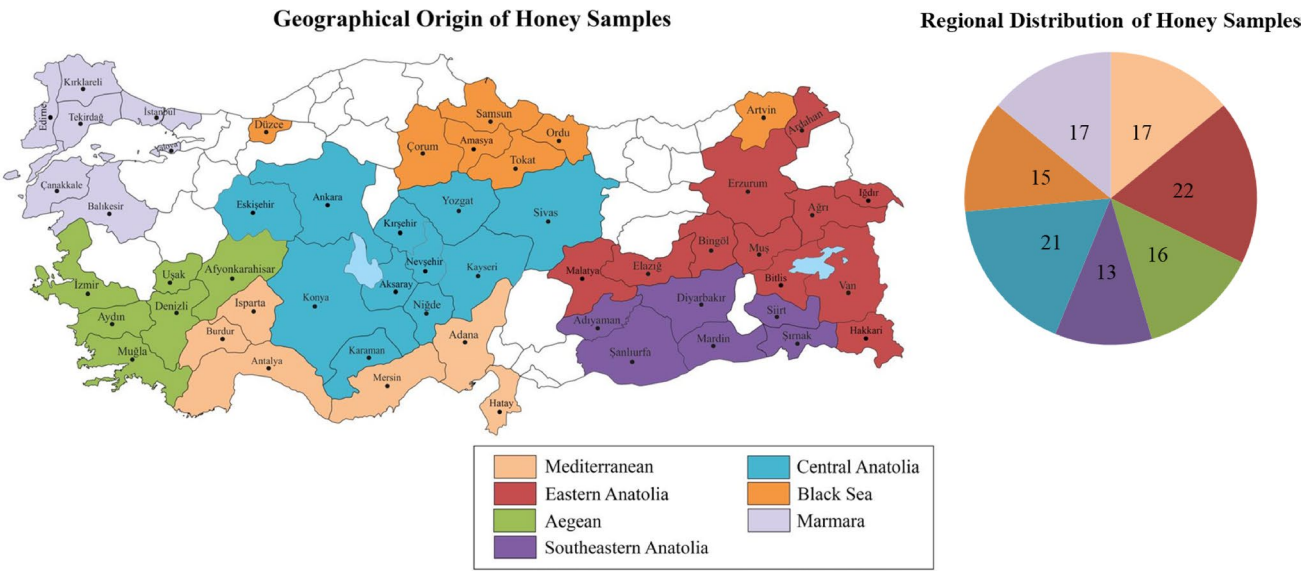


Fig. 1 Map showing the geographical distribution of honey samples collected from different cities and regions of Türkiye

was performed based on reference preparations and literature data. Pollen content of honey samples was also specified as follows: predominant > 45%; secondary 16–45%; important minor 3–15%; and minor < 3% [24, 25].

Unifloral honey is generally defined when the pollen of a single plant species constitutes more than 45% of the total pollen content, and the honey is named after that botanical source. Nevertheless, certain exceptions exist because some plants naturally produce relatively low amounts of pollen. Species such as lavender, thyme, and citrus are considered "under-represented" plants; therefore, a pollen proportion of around 10% is considered sufficient for these plants to characterize the honey. In contrast, chestnut is classified as an "over-represented" plant due to its abundant pollen production, and chestnut honey typically requires approximately 70% chestnut pollen to be considered unifloral [24, 25]. When no single pollen type predominates, the honey is described as mixed floral or multifloral honey. Honeydew honey, however, is distinguished by the presence of honeydew elements, including fungal spores and hyphae. A honey sample is classified as honeydew (secretory) honey when the ratio of honeydew elements (HDE) to total pollen number (TPN) exceeds 3. Furthermore, samples with an HDE/TPN ratio greater than 4.5 are regarded as high-quality honeydew honey [24, 25].

Color analyses (L^*, a^*, and b^*) of honey samples were determined using a colorimeter (CR-400, Konica Minolta, Japan). Before color measurement, the device was calibrated using a white reference plate. Approximately 40 g of each honey sample was then transferred from the device's transparent container into a 30 mm diameter for color determination. Besides, color intensity was calculated using the absorbance difference measured at 450 and 720 nm wavelengths, according to the method reported by Habib et al. [26] via following equation.

$$Color\ intensity\ (mAU) = (Abs_{450} - Abs_{720})\ x\ 1000$$

Electrical conductivity [27], pH, and titration acidity analyses were performed according to the methods detailed by Akbulut et al. [28]. Moisture content was determined by drying 2 g of the honey sample in an oven at 70 °C until a constant weight was achieved. The water activity values (a_w) of the samples were measured using a Novasina LabSwift-a_w (Switzerland).

Methods for chemical analyses

The diastase number of honey samples was determined according to the method reported by Bogdanov [29] and International Honey Commission [27]. Buffered honey solutions (1.59 M acetate buffer, pH 5.3) (20%, w/v) prepared for analysis were reacted with starch solution (2%, w/v) and then incubated in a water bath at 40 °C. Color reactions were formed with iodine solution (0.0007 N) in samples taken at specific intervals, and absorbance values were measured at a wavelength of 660 nm. Measurements continued with 5–10 min intervals until the absorbance value fell below 0.235, and an absorbance-time graph was plotted for each sample to determine specific time (min) for absorbance of 0.235 value. The diastase number was calculated by following equation [29], and results are expressed as diastase number (DN, Schade units).

$$Diastase\ number = \frac{300}{Specific\ time\ for\ Absorbance_{0.235}}$$

The HMF content of honey samples was determined using an HPLC-DAD system (Agilent 1260 Infinity II, USA). The aqueous honey solutions were prepared at a 1:10 (w/v) ratio, containing 0.5 mL of Carrez I and Carrez II solutions for clarification [30]. The C18 column (3.0 × 150 mm, 2.7 µm; InfinityLab Poroshell 120 EC-C18, Agilent, USA) was used in the analyses, the detector wavelength was set to 280 nm, and the mobile phase was acetonitrile: water (5:95) at a flow rate of 1 mL/min. The column temperature and injection volume were adjusted to 32 °C and 20 µL, respectively. HMF amounts of samples were expressed in mg/kg using a calibration curve (0.05-10 mg/kg) [31].

The fructose, glucose, sucrose, and maltose contents of honey samples were determined using the method reported by Yuan et al. [32] with some modifications. Aqueous honey solutions (10%, w/v) were filtered through a 0.45 µm membrane filter, transferred to vials, and injected into the HPLC-RID system at 10 µL volume. During the analysis, an NH_2 column (4.6 × 250 mm, 5 µm; Kromasil 100-5-NH_2, Sweden) was used, and a mobile phase of acetonitrile: water (75:25, v/v) was applied at a flow rate of 1.0 mL/min. The column and detector temperatures were 30 °C, and the results were calculated as g/100 g sample using calibration curves (0.005–0.75 g/100 g) [32].

The amino acid composition of honey samples was determined via the method reported by Li et al. [33] with some modifications. Honey samples were extracted with 75% ethanol at a 1:4 (w/v) ratio, stirred with a vortex for 1 min, and then subjected to ultrasonication in a water bath system at room temperature for 10 min, with the temperature monitored manually. After centrifugation (7100 x g, 10 min), 360 µL of supernatant was mixed with 200 µL of 2,4-dinitrofluorobenzene (DNFB, 10 mg/mL), 200 µL of $NaHCO_3$ (0.5 M, pH 9.0), and 40 µL of ultrapure water. The mixture was incubated at 60 °C in the dark for 60 min and then cooled to room temperature. After that, 800 µL of KH_2PO_4 (0.01 M, pH 7.0) was added to the mixture, and they were left in the dark for 15 min. Finally, the samples were filtered through a 0.45 µm filter and injected into the HPLC-DAD system in a volume of 20 µL. During analysis, a C18 column (3.0 × 150 mm, 2.7 µm; InfinityLab Poroshell 120 EC-C18, Agilent, USA) was used, and gradient flow was applied with sodium acetate (5 mM, pH 5.7)/tetrahydrofuran (95:5, mobile phase A) and methanol/water (80:20, mobile phase B). Gradient flow program was 0.01 min 10% B, 0.01–20 min 10–20% B, 20–25 min 20–50% B, 25–34 min 50–55% B, 34–50 min 55–100% B, 50–55 min 100 − 10% B, and 55–60 min 10% B. The column temperature was set at 35 °C, the mobile phase flow rate at 1 mL/min, and the detector wavelength at 360 nm [33]. The results were calculated as mg/kg of sample using a calibration curve prepared with different concentrations (0.03-113.64 mg/kg) of a mixture of amino acids, including aspartic acid, glutamic acid, 4-hydroxyproline, asparagine, serine, glutamine, histidine, proline, alanine, valine, methionine, cystine, tryptophan, isoleucine, phenylalanine, leucine, cysteine, lysine, and tyrosine, and were expressed on a dry weight basis.

The total phenolic content was determined using the Folin-Ciocalteu method. Absorbance measurements were performed at 760 nm (Cary 60 UV-Vis, Agilent, USA), and the results were evaluated using a gallic acid curve (0.1–10 mg/100 g) as mg GAE/100 g sample [34].

The total flavonoid content was determined based on the method reported by Bueno-Costa et al. [35] according to the principle of complex formation with $AlCl_3$. Absorbances measured at 415 nm were evaluated using a quercetin standard curve (0.1–10 mg/100 g), and the results were calculated as mg QE/100 g sample.

The total carotenoid content was determined by extraction of compounds by n-hexane/acetone (60:40 v/v, including 0.1% BHT) solution according to the reported method of Boussaid et al. [34]. Absorbances measured at 450 nm were evaluated with the β-carotene standard curve (0.001-0.5 mg/100 g) and the results expressed as mg β-carotene/100 g sample.

Methods for biological activity analyses

Biological activity of honey samples was assessed for antioxidant activity using the Trolox Equivalent Antioxidant Capacity (TEAC) and Cupric Reducing Antioxidant Capacity (CUPRAC) methods, antibacterial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, and anti-hyaluronidase activity by hyaluronidase inhibition level. The methods are detailed in a previous study reported by Mutlu et al. [36].

In the TEAC antioxidant activity analysis, honey extracts prepared with phosphate buffer (pH 7.4) were centrifuged, and samples taken from the upper phase (5, 10, 15, and 20 µL) reacted with 1 mL of $ABTS^+$ solution adjusted to an absorbance of 0.700 ± 0.02. After 6 min, the absorbance was measured at 734 nm. In the CUPRAC antioxidant activity analysis, 1 mL each of $CuCl_2$, neocuproine, and CH_3COONH_4 buffer (pH 7.0) was mixed with the sample extract (x mL) and water (1.1-x mL) to achieve absorbance values in the range of 0.20–0.40 at 450 nm wavelength. The mixtures were left in the dark for 30 min, then absorbances were measured at 450 nm. The results were calculated as µmol TE/g sample using a calibration curve created with Trolox standard solutions in the range of 0–25 µmol/g [36].

The antibacterial effectiveness of honey samples against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 strains was measured by measuring zone diameters according to the disk diffusion test protocols [37]. In the analysis, *E. coli*

ATCC 25922 and *S. aureus* ATCC 25923 strains were incubated in sterile nutrient broth medium at 37 °C for 24 h. Then the growing cells were diluted with sterile Ringer's solution to a cell density of 10^7-10^8 CFU (colony forming unit)/mL (0.5 McFarland unit) and incubated in about 20 mL of nutrient agar medium. After 2 h of diffusion of diluted microorganism cells into the culture medium, sterile discs prepared by diffusing 100 µL of honey solution prepared with 70% ethanol (50:50, w/v) were placed into 9-cm petri dishes with two discs in each petri and incubated again at 37 °C for 24 h. At the end of incubation, the width and length diameters (mm) of the inhibition zones formed around the discs were measured, and the results are given as the averages of these measurements. Besides 100 µL of 70% ethanol diffused sterile disk and a commercial ampicillin disc (10 µg/mL; BD BBL™ Sensi-Disc™, Becton Dickinson, USA) were used as controls.

In the anti-hyaluronidase activity assay, aqueous honey extracts (0.40 g/mL) were prepared by mixing the samples at 25 °C for 24 h under horizontal shaking. The extract was then centrifuged (7100 x *g* for 10 min), and 100 µL of the resulting supernatant was incubated with 200 µL of hyaluronidase enzyme (310 U/mL) and 200 µL of phosphate buffer solution (0.2 M, pH 7.0, including 77 mM sodium chloride-0.01% bovine serum albumin) at 37 °C for 10 min. After a period, 200 µL of trisodium phosphate solution (0.3 M, pH 5.35, containing 0.03% hyaluronic acid sodium salt) was added to the mixture and incubated at 37 °C for 45 min. Subsequently, 2 mL of 24 mM sodium acetate-79 mM acetic acid-0.1% bovine serum albumin solution (pH 3.75) was added to the mixtures and kept at room temperature for 10 min. Hyaluronidase enzyme activity was inhibited at a water bath (100 °C) for 1 min, followed by placing an ice bath for 2 min. The absorbance of the samples was measured at 600 nm, and the results were expressed as the inhibition rate (%) compared to the control sample, containing 100 µL of distilled water instead of honey extract.

Statistical analysis

Sample collection was conducted over two consecutive years, with physicochemical and chemical analyses performed in duplicate and biological analyses conducted in quadruplicate. SAS statistical software (v.9.00, SAS Institute Inc., USA) and IBM SPSS Statistics version 23.0 (IBM Corp., USA) were used for statistical analyses. Numerical variables were presented as "mean ± standard error." One-way analysis of variance (ANOVA) with Duncan's Multiple Range Test for post hoc comparisons was used to compare physicochemical, chemical, and biological measurements among geographical origins. Stepwise LDA was performed to determine whether the geographical origin of honey could be classified based on its physicochemical, chemical, and biological properties. In this analysis, the geographical region was used as the grouping (dependent) variable, while the honey properties, showing significant regional differences according to ANOVA served as predictor (independent) variables. In stepwise LDA, the first two discriminant functions were evaluated because they explained the largest proportion of variance in group separation [38], and allow straightforward visualization and interpretation of group separation. The leave-one-out classification was used for model validation, where each observation was classified based on discriminant functions generated from all other observations. For all statistical analyses, *p*-value below 0.05 was considered statistically significant.

Results and discussions

Regional differences in botanical origin and physicochemical properties

According to the botanical origin results obtained, a total of 57 unifloral, 53 multifloral, and 11 pine honey samples were included in the study (Supplementary Material 1). The unifloral samples were derived from 13 different plant species (*Astragalus* spp., *Castanea sativa*, *Centaurea* spp., *Chenopodium* spp., *Cistus* spp., *Hedysarum* spp., *Helianthus annuus*, *Onobrychis* spp., *Paliurus spina-christi*, *Plantago* spp., *Salix* spp., *Teucrium* spp., and *Trifolium* spp.) and 11 families (Apiaceae, Asteraceae, Brassicaceae, Citreae, Ericaceae, Fabaceae, Geraniaceae, Lamiaceae, Malvaceae, Poaceae, and Rosaceae), demonstrating the wide botanical diversity contributing to honey production in Türkiye. The plant taxa that serve as primary sources of flower honey in Türkiye have been reported to originate from several botanical families, including Asteraceae, Fabaceae, Fagaceae, Myrtaceae, Malvaceae, Brassicaceae, Scrophulariaceae, Lamiaceae, and Oleaceae [39]. Similarly, Çobanoğlu et al. [40] indicated that pollen grains from Fabaceae, Asteraceae, and Lamiaceae were most frequently identified in honey samples, underscoring the importance of these families as key nectar sources in Turkish apiculture.

When the regions were evaluated individually, honey samples from the Mediterranean region were found to be predominantly multifloral, although unifloral honeys from *Astragalus* spp., *Teucrium* spp., *Trifolium* spp., Brassicaceae, Citreae, as well as pine honey were found. The Aegean region was characterized largely by pine honey, with additional unifloral honeys from *Chenopodium* spp., *Onobrychis* spp., *Paliurus spina-christi*, Apiaceae, and Fabaceae, as well as multifloral types.

Central Anatolia contributed predominantly multifloral honeys (14 of 21 samples), but also unifloral honeys from *Astragalus* spp., *Chenopodium* spp., *Centaurea* spp., *Salix* spp., Rosaceae, and Poaceae. Honey samples from the Southeastern Anatolia region consisted mostly of Poaceae-origin honeys, along with unifloral samples from *Astragalus* spp., *Plantago* spp., *Trifolium* spp., Geraniaceae, and Malvaceae, in addition to multifloral honeys. The Eastern Anatolia region was mainly represented by *Astragalus* spp. (7 of 22 samples), along with unifloral honeys from *Cistus* spp., *Centaurea* spp., *Hedysarum* spp., Asteraceae, Fabaceae, and several multifloral samples.

Honey originating from the Black Sea region typically contained predominant or secondary amounts of *Castanea sativa* pollen, along with unifloral honeys from *Helianthus annuus*, *Astragalus* spp., *Cistus* spp., *Trifolium* spp., Ericaceae, and several multifloral samples. Since the Marmara region shares a similar flora with the Black Sea region, the honey samples from this region were primarily associated with *Castanea sativa* and *Helianthus annuus*, as well as *Paliurus spina-christi*, Lamiaceae, and some multifloral honeys. Similarly, Özkök et al. [41] reported that *Castanea sativa* Mill. honeys were from provinces in the Marmara and Black Sea regions, while *Helianthus annuus* botanical origin honeys were from the Marmara region.

Table 1 shows that some physicochemical properties of honey samples, such as L^*, a^*, and b^* color parameters, color intensity, electrical conductivity, moisture content, water activity, and pH, varied significantly ($p<0.01$) except for titratable acidity ($p>0.05$) across different regions of Türkiye.

Color analysis revealed that the L^*, a^*, and b^* values of honey samples ranged between 17.43-55.21, − 2.33-4.44, and 4.19-24.49, respectively. Honey samples from the Central Anatolia region were found to have higher L^* values than other regions, meaning a lighter color. In terms of color intensity, honey samples from the Eastern Anatolia region were found to be less intense, that was more intense in samples collected from the Black Sea region, with color intensity ranging from 36.80 to 1688.50 mAU. A study reported that the color intensity of honey samples ranged from 273.90 to 2874.80 mAU [26]. Honey color differences were influenced by factors such as nectar source, pollen color, non-enzymatic browning reactions, and mineral content [42]. The regional differences observed in this study are likely attributable to variations in the botanical origin of nectar sources, which are influenced by the climate and soil properties of each region, ultimately resulting in differences in the color of the honey samples. According to the findings of Karabagias et al. [43] honey color intensity ranged between 164 and 405 mAU depending on botanical source, with orange blossom honey characterized by lighter coloration and pine honey presenting darker color properties. Color intensity of pine honeys included in the presented study also ranged from 185 to 710 mAU.

The electrical conductivity values of honey samples ranged from 139.75 to 1645.50 µS/cm; honey from the Aegean region had the highest values, while honey from the Eastern, Southeastern, and Central Anatolia regions had the lowest values. Changes in electrical conductivity are associated with variations in the mineral, organic acid, free amino acids, and protein contents of honey [26, 44, 45].

The moisture content of honey samples ranged from 10.27 to 16.64 g/100 g, and a_w ranged from 0.49 to 0.64, remaining within the limits specified in the honey regulation [46], indicating appropriate preservation and storage conditions [45]. Honey collected from the Black Sea and Marmara regions showed higher moisture and a_w values, while honey from the Eastern and Southeastern Anatolia regions showed lower values. It was assessed that climatic differences between regions have a significant effect on the moisture content and a_w values of honey samples, with lower moisture content and a_w values in drier regions and higher values in more humid regions.

Regional differences in chemical properties

Chemical properties of honey samples were assessed with diastase number, HMF, fructose, glucose, saccharose, maltose, total phenolic, flavonoid, and carotenoid contents, and amino acid composition, and the results obtained are presented in Tables 1 and 2. It has been determined that Eastern Anatolia region honey samples had the highest diastase values and lower HMF contents than those of samples from other regions (Table 1). At the same time, as expected, this inverse relationship was also seen in the results from other regions, and it was determined that Marmara region honeys had lower diastase and higher HMF content. Low diastase activity and high HMF content mainly reflect thermal processing applied to honey or prolonged storage conditions. In honey that has been stored for a long time and/or under inappropriate conditions and subjected to heat treatment, the HMF content increases while diastase activity decreases [47]. Furthermore, the diastase number and HMF content of the honey samples examined ranged from 0.79 to 72.41 and 0.72 to 83.59 mg/kg, respectively. It was determined that some honey samples did not meet the limit values reported for diastase number (> 8) and HMF (< 40 mg/kg) [46]. It is likely that heat treatments exceeding 45 °C, possibly applied to facilitate honey processing, may lead to a decrease in diastase activity and an increase in HMF content in these honeys, with the extent of the changes depending on the intensity and duration of the heat exposure, resulting in noncompliance with legal limits.

Table 1 Physicochemical, chemical, and bioactive properties of honey samples collected from different regions

	Mediterranean	Eastern Anatolia	Aegean	Southeastern Anatolia	Central Anatolia	Black Sea	Marmara	Sign
Color								
*L**	$24.23^{CB}\pm0.53$	$26.16^{B}\pm0.50$	$23.66^{C}\pm0.33$	$23.30^{C}\pm0.57$	$28.27^{A}\pm1.19$	$24.83^{CB}\pm1.37$	$25.27^{CB}\pm0.65$	**
*a**	$0.88^{BA}\pm0.19$	$0.00^{C}\pm0.13$	$1.24^{A}\pm0.30$	$0.47^{BC}\pm0.23$	$0.21^{C}\pm0.18$	$1.38^{A}\pm0.34$	$0.55^{BC}\pm0.19$	**
*b**	$10.59^{BAC}\pm0.33$	$8.04^{D}\pm0.26$	$9.60^{C}\pm0.28$	$7.60^{D}\pm0.34$	$11.53^{BA}\pm0.56$	$10.19^{BC}\pm0.99$	$11.79^{A}\pm0.59$	**
Color intensity (mAU)	$360.24^{B}\pm36.86$	$195.87^{D}\pm15.96$	$377.80^{B}\pm27.89$	$225.42^{CD}\pm34.96$	$235.84^{CD}\pm25.42$	$676.47^{A}\pm92.14$	$324.45^{CB}\pm39.99$	**
Conductivity (μS/cm)	$497.32^{C}\pm54.53$	$219.14^{D}\pm5.91$	$816.44^{A}\pm67.44$	$306.87^{D}\pm32.16$	$330.89^{D}\pm17.29$	$697.27^{BA}\pm76.55$	$618.65^{BC}\pm56.77$	**
Moisture content (g/100 g)	$13.36^{B}\pm0.25$	$12.18^{C}\pm0.17$	$12.98^{B}\pm0.21$	$12.02^{C}\pm0.17$	$12.99^{B}\pm0.14$	$14.24^{A}\pm0.23$	$14.16^{A}\pm0.22$	**
Water activity	$0.55^{C}\pm0.00$	$0.54^{D}\pm0.00$	$0.57^{B}\pm0.00$	$0.53^{D}\pm0.00$	$0.56^{B}\pm0.00$	$0.59^{A}\pm0.00$	$0.58^{A}\pm0.00$	**
pH	$4.20^{B}\pm0.07$	$3.91^{C}\pm0.03$	$4.50^{A}\pm0.08$	$4.03^{C}\pm0.04$	$3.99^{C}\pm0.04$	$4.23^{B}\pm0.05$	$4.22^{B}\pm0.05$	**
Titratable acidity (meq/kg)	$28.48^{A}\pm1.58$	$31.10^{A}\pm1.31$	$30.59^{A}\pm1.54$	$29.08^{A}\pm1.61$	$30.12^{A}\pm1.23$	$36.23^{A}\pm2.81$	$32.81^{A}\pm2.36$	-
Diastase number (DN, Schade units)	$17.96^{B}\pm1.47$	$23.16^{A}\pm1.96$	$16.88^{B}\pm0.89$	$16.84^{B}\pm1.18$	$21.05^{BA}\pm1.17$	$21.68^{BA}\pm2.46$	$18.10^{B}\pm1.45$	**
HMF (mg/kg)	$25.99^{BA}\pm3.71$	$19.50^{B}\pm2.63$	$25.25^{BA}\pm3.54$	$23.04^{BA}\pm3.07$	$24.30^{BA}\pm2.59$	$20.53^{BA}\pm4.39$	$30.41^{A}\pm3.55$	*
Sugar composition (g/ 100 g)								
Fructose	$41.19^{CB}\pm0.64$	$41.73^{B}\pm0.39$	$38.17^{D}\pm0.72$	$43.70^{A}\pm0.56$	$41.36^{CB}\pm0.35$	$39.96^{C}\pm1.04$	$40.92^{CB}\pm0.40$	**
Glucose	$33.63^{CD}\pm0.96$	$35.48^{CB}\pm0.49$	$31.69^{D}\pm0.79$	$36.44^{B}\pm0.39$	$37.10^{B}\pm0.62$	$33.97^{C}\pm1.02$	$39.44^{A}\pm1.01$	**
Saccharose	$1.65^{A}\pm0.11$	$1.82^{A}\pm0.09$	$1.79^{A}\pm0.08$	$1.82^{A}\pm0.11$	$1.30^{B}\pm0.12$	$1.30^{B}\pm0.10$	$1.02^{B}\pm0.14$	**
Maltose	$1.55^{A}\pm0.43$	$0.26^{B}\pm0.02$	$0.23^{B}\pm0.02$	$0.20^{B}\pm0.01$	$0.25^{B}\pm0.02$	$0.44^{B}\pm0.07$	$0.64^{B}\pm0.12$	**
Total phenolic (mg GAE/100 g)	$48.31^{BA}\pm2.67$	$42.85^{BC}\pm2.69$	$53.15^{A}\pm2.26$	$33.32^{C}\pm2.88$	$42.72^{BC}\pm3.88$	$53.13^{A}\pm5.56$	$43.01^{BC}\pm2.24$	**
Total flavonoid (mg QE/100 g)	$2.83^{BAC}\pm0.24$	$2.58^{BC}\pm0.15$	$2.57^{BC}\pm0.15$	$2.24^{C}\pm0.13$	$3.08^{BA}\pm0.21$	$3.33^{A}\pm0.34$	$2.71^{BAC}\pm0.23$	*
Total carotenoid (mg β-carotene/100 g)	$0.07^{CD}\pm0.01$	$0.05^{D}\pm0.00$	$0.07^{CD}\pm0.00$	$0.05^{D}\pm0.00$	$0.09^{B}\pm0.01$	$0.09^{CB}\pm0.01$	$0.15^{A}\pm0.01$	**

** and *significance levels of $p \leq 0.01$ and $0.01 < p \leq 0.05$, respectively

Table 2 Amino acid composition of honey samples (mg/kg) from different regions

Compounds	Mediterranean	Eastern Anatolia	Aegean	Southeastern Anatolia	Central Anatolia	Black Sea	Marmara	Sign
Aspartic acid	$16.94^{B}\pm1.40$	$10.53^{C}\pm0.73$	$9.91^{C}\pm1.84$	$48.63^{A}\pm3.60$	$14.37^{B}\pm0.69$	$3.37^{D}\pm0.62$	$4.06^{D}\pm0.70$	**
Glutamic acid	$10.16^{A}\pm0.60$	$9.56^{A}\pm0.38$	$7.59^{B}\pm0.33$	$9.20^{A}\pm0.27$	$7.88^{B}\pm0.17$	$6.40^{C}\pm0.85$	$4.36^{D}\pm0.08$	**
4-OH proline	$4.62^{B}\pm0.99$	$1.50^{D}\pm0.15$	$3.86^{CB}\pm0.37$	$0.97^{D}\pm0.17$	$2.35^{DC}\pm0.15$	$5.10^{B}\pm0.81$	$10.11^{A}\pm1.48$	**
Asparagine	$4.83^{C}\pm0.45$	$6.57^{A}\pm0.51$	$6.38^{A}\pm0.33$	$4.75^{C}\pm0.58$	$6.32^{A}\pm0.43$	$5.30^{CB}\pm0.48$	$5.92^{BA}\pm0.35$	*
Serine	$11.26^{C}\pm1.20$	$20.87^{A}\pm1.44$	$6.67^{D}\pm0.50$	$10.31^{DC}\pm0.80$	$16.99^{B}\pm1.79$	$11.04^{C}\pm1.57$	$12.79^{C}\pm1.37$	**
Glutamine	$11.65^{A}\pm1.52$	$12.76^{A}\pm0.79$	$12.61^{A}\pm1.28$	$8.04^{B}\pm0.33$	$13.45^{A}\pm0.87$	$7.46^{B}\pm0.78$	$14.27^{A}\pm1.24$	**
Histidine	$9.92^{DCB}\pm1.52$	$7.28^{D}\pm0.46$	$10.55^{CBA}\pm0.87$	$7.77^{DC}\pm0.48$	$13.47^{A}\pm0.70$	$11.63^{BA}\pm1.56$	$10.84^{BA}\pm0.96$	**
Proline	$760.25^{A}\pm52.33$	$664.23^{A}\pm28.20$	$689.29^{A}\pm42.21$	$627.29^{A}\pm38.50$	$680.74^{A}\pm33.17$	$603.18^{A}\pm51.72$	$603.18^{A}\pm51.72$	-
Alanine	$27.03^{BA}\pm1.66$	$25.03^{BA}\pm1.03$	$23.35^{CB}\pm1.51$	$15.68^{E}\pm0.77$	$20.43^{DC}\pm0.95$	$18.58^{ED}\pm1.33$	$27.71^{A}\pm2.40$	**
Valine	$7.26^{CB}\pm1.18$	$8.76^{CB}\pm0.45$	$7.07^{C}\pm0.90$	$7.21^{CB}\pm0.75$	$9.26^{B}\pm0.49$	$6.61^{C}\pm0.54$	$14.30^{A}\pm1.12$	**
Methionine	$2.78^{B}\pm0.57$	$2.74^{B}\pm0.13$	$3.19^{B}\pm0.29$	$6.20^{A}\pm0.72$	$2.89^{B}\pm0.23$	$2.78^{B}\pm0.24$	$3.45^{B}\pm0.25$	**
Cystine	$2.26^{A}\pm0.39$	$1.56^{B}\pm0.13$	$0.39^{C}\pm0.13$	$1.22^{B}\pm0.06$	$1.78^{BA}\pm0.08$	$1.76^{BA}\pm0.15$	$1.67^{B}\pm0.13$	**
Tryptophan	$1.02^{A}\pm0.27$	$0.46^{B}\pm0.05$	$0.57^{B}\pm0.11$	$0.40^{B}\pm0.14$	$0.62^{B}\pm0.06$	$0.31^{B}\pm0.07$	$0.51^{B}\pm0.08$	*
Isoleucine	$118.67^{B}\pm12.06$	$156.83^{A}\pm4.13$	$150.14^{A}\pm4.76$	$154.48^{A}\pm4.69$	$145.47^{A}\pm1.25$	$157.12^{A}\pm2.05$	$152.75^{A}\pm2.23$	*
Phenylalanine	$30.48^{B}\pm4.03$	$25.21^{B}\pm3.06$	$61.54^{B}\pm21.01$	$162.81^{A}\pm40.28$	$40.93^{B}\pm7.47$	$39.97^{B}\pm3.66$	$52.70^{B}\pm8.53$	**
Leucine	$28.07^{DC}\pm4.85$	$44.14^{CB}\pm4.33$	$64.97^{BA}\pm19.88$	$44.77^{CB}\pm10.29$	$74.21^{A}\pm8.08$	$15.29^{D}\pm1.84$	$28.26^{DC}\pm5.94$	**
Cysteine	$0.81^{B}\pm0.10$	$1.66^{A}\pm0.13$	$0.61^{CB}\pm0.09$	$0.60^{CB}\pm0.15$	$0.31^{D}\pm0.03$	$0.67^{B}\pm0.08$	$0.35^{DC}\pm0.05$	**
Lysine	$16.07^{B}\pm2.15$	$16.52^{B}\pm0.75$	$8.37^{D}\pm1.36$	$21.24^{A}\pm1.91$	$17.03^{B}\pm1.25$	$13.84^{CB}\pm1.27$	$11.26^{DC}\pm0.95$	**
Tyrosine	$46.44^{A}\pm9.90$	$24.92^{CB}\pm2.34$	$14.21^{DC}\pm2.50$	$21.08^{DCB}\pm3.93$	$31.96^{B}\pm3.03$	$11.68^{D}\pm1.31$	$13.33^{DC}\pm1.98$	**

** and *significance levels of $p \leq 0.01$ and $0.01 < p \leq 0.05$, respectively

In terms of sugar composition, the fructose content of honeys ranged from 30.60 to 51.65 g/100 g, and the glucose content ranged from 24.12 to 48.33 g/100 g. Fructose was found to be higher in honeys from the Southeastern Anatolia region, while glucose was found to be higher in honeys from the Marmara region (Table 1), especially in samples originating from *Helianthus annuus* honeys. Sucrose and maltose amounts ranged from 0.00 to 3.52 g/100 g and 0.11–9.45 g/100 g, respectively, and it was determined that the maltose amount in three samples originating from the Mediterranean region exceeded the 4 g/100 g specified limit for honey [46].

In the evaluation of amino acid composition (Table 2), it was determined that differences between regions had statistically significant ($p<0.05$; $p<0.01$) effects on the amino acid content except for proline ($p>0.05$). Mediterranean region honeys were found to have higher levels of glutamic acid, cysteine, and tryptophan. Marmara region honeys had higher levels of 4-OH proline, glutamine, alanine, and valine, and Eastern Anatolia region honeys had higher levels of serine compared to other regions. Besides, Southeastern Anatolian honeys were considerably rich in essential amino acids of methionine, phenylalanine, and lysine compared to other regions.

The proline amino acid, which is found in the highest amounts in honey samples, is considered an indicator of honey's naturalness and ripeness [48]. The proline content was found to range between 261.10 and 1408.12 mg/kg. Honey from the Mediterranean region contained relatively higher levels of proline, whereas Black Sea and Marmara honeys had lower proline contents. It has also been observed that all honey samples except for four samples (CA17, BS7, BS10, and MA16 in Supplementary Material 1) meet the legal limit (> 300 mg/kg) for proline content applied in Türkiye [46].

It was found that the total phenolic, flavonoid, and carotenoid contents of the honey samples were significantly ($p < 0.05$; $p < 0.01$) different depending on region. Honey samples from the Black Sea region had higher total phenolic and flavonoid content than those from other regions, while Southeastern Anatolia region honeys had lower values, Table 1. Similarly, honey samples from the Southeastern Anatolia region had low total carotenoid content. It has been determined that honey samples obtained from the Marmara region, especially those originating from *Helianthus annuus*, had higher carotenoid content than honey obtained from other regions. The total phenolic content of the honey was determined to be in the range of 13.00-132.07 mg GAE/100 g, the flavonoid content was 0.75–7.30 mg QE/100 g, and the total carotenoid content was 0.30–1.01 mg β-carotene/100 g. It has been reported that the bioactive compound amount and profile vary depending on the plant flora, the geographical origin, and the production season of the year [7]. The results obtained also showed that regions with greater plant diversity, such as the Aegean, Mediterranean, and Black Sea regions, had higher phenolic compound compared to relatively arid regions such as Central, Eastern, and Southeastern Anatolia.

Regional differences in biological properties

The biological activities of honey samples were evaluated in terms of antioxidant, antibacterial, and anti-hyaluronidase properties, presented in Fig. 2. The results showed statistically significant differences among regions in all parameters ($p < 0.01$). These differences are particularly considered to be due to changes in phenolic compounds, which are closely related to plant source diversity. Türkiye encompasses a rich and diverse botanical landscape across multiple climatic zones. The findings suggest that the botanical diversity (Supplementary Material 1), shaped by the distinct properties of each region, contributes to variations in the biological properties of the honey samples.

It has been noted that antioxidant, antibacterial, and anti-hyaluronidase activities have positive correlation with total phenolic and flavonoid contents [49, 50]. Besides low pH value, high osmotic stress, organic acids, hydrogen peroxide (H_2O_2) content, and bioactive peptides have substantial effect on antimicrobial activity of honey [49, 51].

In terms of antioxidant activity, the results obtained by the TEAC and CUPRAC methods revealed that honeys from the Black Sea region had the highest values, while those from the Southeastern Anatolia region had the lowest values, and these findings were consistent with the total phenolic and flavonoid contents. The TEAC and CUPRAC values of honey samples ranged from 0.88 to 7.99 and 0.34–10.63 µmol TE/g, respectively. These values were within the ranges reported in the literature 0.34–7.46 µmol TE/g for TEAC [4, 52] and 0.27–23.80 µmol TE/g for CUPRAC [3, 53].

In antibacterial activity test, honey samples were found to exhibit varying levels of inhibitory effect on *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 strains. The inhibition zone diameters ranged from 6.00 to 9.13 mm for *E. coli* ATCC 25922 and from 6.00 to 14.50 mm for *S. aureus* ATCC 25923. Honey from the Black Sea region had higher antibacterial activity against *E. coli* ATCC 25922, while honey from the Eastern Anatolia region showed higher antibacterial activity against *S. aureus* ATCC 25923. Moreover, various studies have reported that honey samples have a higher antibacterial effect on the *S. aureus* ATCC 25923 strain as found in this study and that $Gram^+$ bacteria are generally more sensitive to the antibacterial effect of honey than $Gram^-$ bacteria [54–56]. It has been notified that $Gram^-$ bacteria are more resistant to the antibacterial effect of bee products due to their double cell wall composed of lipopolysaccharides and proteins [57].

In terms of anti-hyaluronidase activity, honey from the Aegean region had the highest values, while honey from the Marmara region had the lowest values. The anti-hyaluronidase activity values of the samples ranged from 21.35%

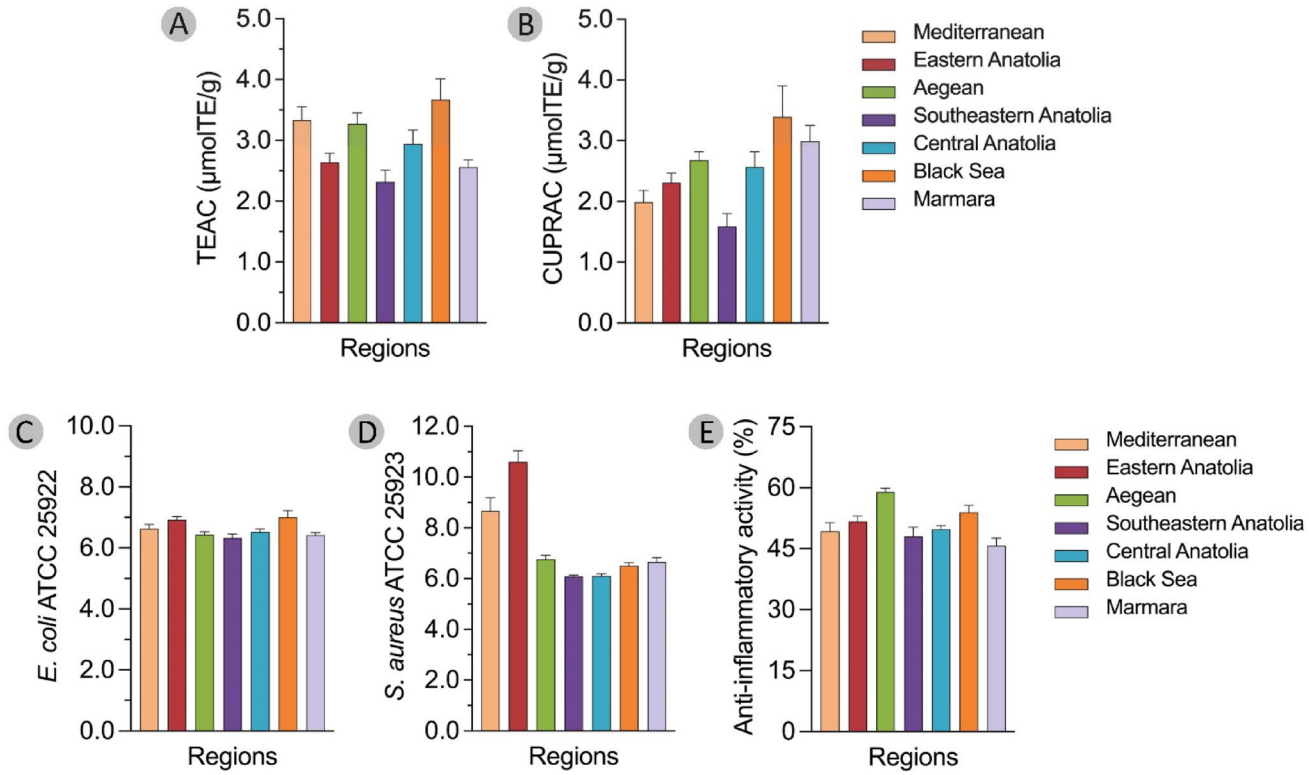


Fig. 2 Regional differences in TEAC (**A**) and CUPRAC (**B**) antioxidant activities, antibacterial activity against *E. coli* ATCC 25922 (**C**) and *S. aureus* ATCC 25923 (**D**), and anti-hyaluronidase activity (**E**) of honey samples ($p < 0.01$)

to 79.78%. These results are similar to the hyaluronidase inhibition values reported for different types of honey (12.0%-85.0%) [58]. In another study, the anti-hyaluronidase activities of three different types of heather honey at a concentration of 0.75 g/mL were reported to be 46.60%, 53.00%, and 54.20%, respectively [59]. Within the scope of this study, the anti-hyaluronidase activity value of the solution of the sample identified as heather honey (BS11, Supplementary Material 1) at a concentration of 0.4 g/mL was determined to be 49.31%, and this result was consistent with the literature data despite the concentration difference. Furthermore, in a study conducted by Silva et al. [60] using blackberry, citrus, and lavender honeys, it was reported that the anti-hyaluronidase activity value of the samples did not significantly change with increasing concentration after a concentration level of 0.02 g/mL. The strong anti-hyaluronidase activity observed in Aegean honeys, particularly those with pine botanical origin, may be associated with their relatively high phenolic content. Previous studies have demonstrated a strong correlation between high phenolic content and anti-inflammatory effects [61, 62]. In addition to phenolic compounds, previous studies have also reported that certain proteins present in pine honey may contribute to its anti-hyaluronidase activity [63].

Multivariate approach to the geographical characterization of honey by LDA

LDA was performed to evaluate whether the variables could distinguish honey samples based on their geographical origin, and the results are presented in Table 3; Fig. 3. Regional differences among honey samples were evaluated using their physicochemical, chemical, and biological parameters, which exhibited statistically significant variation across regions.

A stepwise procedure based on Wilks' Lambda identified 22 variables that significantly contributed to group separation. While all functions were significant, we interpreted the first two functions because they explain the most variation in group separation [38]. The two functions explained 63.9% of the variance (Function 1 explained 39.1% variance and Function 2 explained 24.7% variance). Function 1 was mainly associated with higher aspartic acid content (structure coefficient = 0.461) and lower water activity (-0.340). Function 2 was characterized by higher *S. aureus* ATCC 25923 inhibition (0.427), cysteine content (0.320), and lower aspartic acid content (-0.417). When samples were classified using the discriminant functions, 91.7% of the original cases were correctly assigned to their respective region. Besides, generated model has been validated by leave-one-out cross-validation method for assessing its predictive capacity and the cross-validated classification accuracy of the model was determined to be 85.5%. These results demonstrated the significant effect of geographical origin on the composition of honey.

Accordingly, when all results are evaluated collectively, honey samples from the Marmara (94.1%) and Central Anatolia (95.2%) regions can be clearly distinguished from those of other regions. In contrast, samples from the Mediterranean (73.5%) and Black Sea (80.0%) regions showed lower cross-validated distinguishability due to similarities in their physicochemical, chemical, and biological properties, and they overlap particularly with samples from the Central Anatolia region, which is located between those two regions (Fig. 1).

Table 3 Linear discriminant analysis classification results for honey samples from different regions, including predicted group membership, original and cross-validated accuracy

Regions	Predicted group membership							Total	%
	Mediterranean	Eastern Anatolia	Aegean	Southeastern Anatolia	Central Anatolia	Black Sea	Marmara		
Original (91.7%)									
Mediterranean	32	2	0	0	0	0	0	34	94.1
Eastern Anatolia	2	41	0	0	1	0	0	44	93.2
Aegean	0	0	27	1	2	0	2	32	84.4
Southeastern Anatolia	0	0	0	24	2	0	0	26	92.3
Central Anatolia	0	0	1	0	40	1	0	42	95.2
Black Sea	0	0	2	0	1	26	1	30	86.7
Marmara	0	0	0	0	2	0	32	34	94.1
Cross-validated (85.5%)									
Mediterranean	25	2	0	0	6	1	0	34	73.5
Eastern Anatolia	1	38	0	0	5	0	0	44	86.4
Aegean	0	1	26	1	2	0	2	32	81.3
Southeastern Anatolia	0	0	2	22	2	0	0	26	84.6
Central Anatolia	0	0	1	0	40	1	0	42	95.2
Black Sea	0	0	2	0	3	24	1	30	80.0
Marmara	0	0	0	0	2	0	32	34	94.1

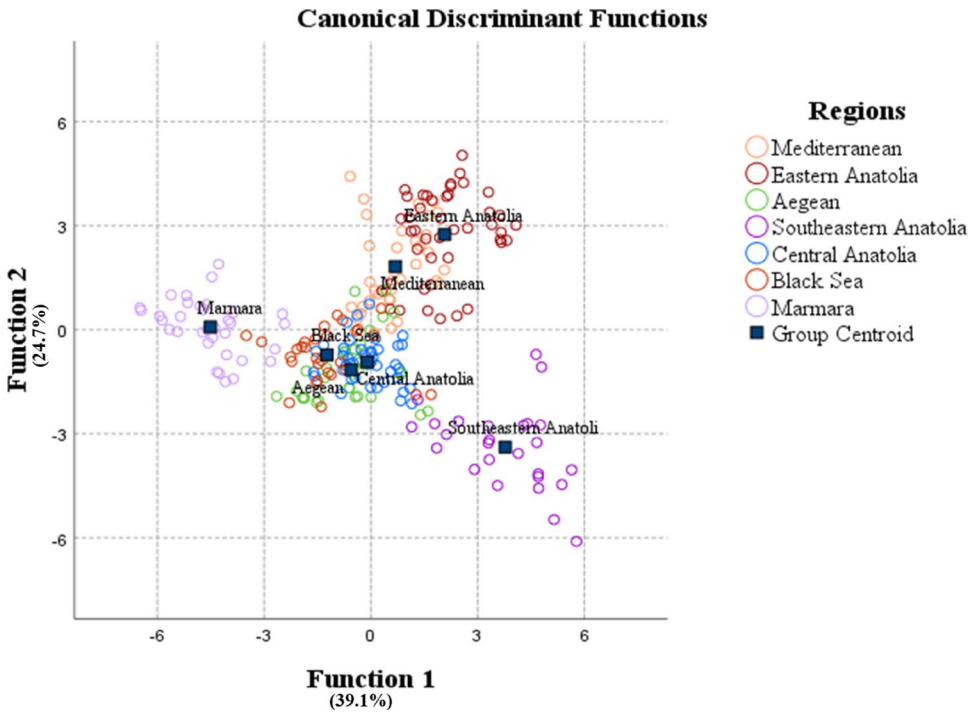


Fig. 3 Linear discriminant analysis scatter plot illustrating the separation of honey samples from different regions according to canonical discriminant functions

Aspartic acid and cysteine amino acids have emerged as two important chemical parameters in regional origin distinctions. Similarly, it has been stated in some previous studies that amino acid composition can be a parameter for the discrimination of honey samples according to their botanical and geographical origins [5, 64]. Sun et al. [64] reported that tryptophan, proline, aspartic acid, glutamic acid, lysine, and serine amino acids were important variables in the discrimination of jujube, rape, chaste, acacia, and lungan honeys' botanical origin and achieved 100% of overall classification rate. Gürbüz and Kıvrak [5] used support vector machine, partial least squares discriminant analysis (PLS-DA), and random forest (RF) methods to assess amino acid composition for discrimination 51 multifloral honey samples coming from Siirt and Şırnak cities located in the Southeastern region. It was emphasized that the PLS-DA method provided the best overall classification, with tyrosine, glutamic acid, methionine, glutamine, histidine, arginine, and glycine being the prominent parameters [5]. This discrepancy suggested that the parameters contributing most to sample differentiation may vary when transitioning from samples collected from a limited geographical area (Southeastern Anatolia region) to those produced across a broader region (seven regions). Findings from previous studies by Fechner et al. [6, 12] also support this observation. Fechner et al. [6] investigated the geographical origin authentication of 50 honey samples from Formosa, Chaco, Corrientes, and Misiones provinces of Argentina using moisture, color, electrical conductivity, pH, HMF, and elemental composition parameters. LDA achieved an overall classification accuracy of 76.0% when only mineral composition data were used, which increased to 94.0% when all variables were included. Discriminant function analysis identified color and electrical conductivity as the most influential physicochemical parameters, whereas pH and HMF content contributed less to the discrimination [6]. In another study, 141 honey samples from only Corrientes province of Argentina were classified according to their psychochemical properties, and LDA results indicated that the most important distinguishing variables were moisture, conductivity, pH, acidity, and HMF than color, reducing sugar, diastase and proline variables with an accurate classification ratio of 65.8% [12].

Karabagias et al. [65] conducted a study on Greek honeydew honey samples derived from *Pinus* spp. and *Abies cephalonica* collected from eight different locations (Halkidiki, Evia, Samos, Thassos, Messinia, Karditsa, Lakonia, and Arkadia), the LDA model achieved cross-validation accuracies of 76.9% for pine and 80.6% for fir honeys. Pine honeys from Samos and Thassos, as well as fir honeys from Karditsa, Messinia, and Lakonia, were successfully discriminated from samples originating from other regions. The main variables contributing to this discrimination were identified as maltose, fructose, raffinose, turanose, glucose, sucrose, proline, phenylalanine, moisture, formic acid, and fructose+glucose contents, and the fructose+glucose/moisture, fructose/moisture, and glucose/moisture ratios. de Oliveira et al. [16] have also developed a rapid discrimination method based on spectral reflectance techniques combined with discriminant analysis models, including

principal component analysis (PCA), PLS-DA, and LDA, for the classification of unifloral and multifloral honey samples collected from 23 different locations in Paraná, Brazil. Their results showed that, during the training stage, the LDA model achieved superior classification accuracy (84.1%) for geographical origin discrimination compared with PLS-DA, indicating strong initial discriminatory capability. However, during the testing stage, the performance of LDA declined to 79.4%, whereas the PLS-DA model demonstrated greater robustness, reaching an accuracy of 82.4%. Nevertheless, LDA showed satisfactory performance and contributed to the differentiation of both floral origin and geographical provenance of the honey samples. In another study, forty-seven fresh honey samples collected from seven administrative zones within the three major districts of Gojjam, Gondar, and Wollo in the Amhara region of Ethiopia were evaluated using LDA for geographical discrimination. The classification accuracies obtained for honey samples from Gojjam, Gondar, and Wollo were 78.3%, 33.3%, and 73.3%, respectively, with an overall cross-validation accuracy of 70.21% [66]. These findings suggest that the evaluated parameters (total phenolics, flavonoids, and antioxidant contents) were insufficient for reliable regional discrimination using the LDA approach. Similar observations were noted in the present study, where these parameters did not emerge as the most significant discriminant variables.

Despite the comprehensive analytical approach applied in this study, several limitations should be acknowledged. First, a potential confounding effect exists between geographical and botanical origin, since honey samples collected from different regions may also differ in their predominant floral sources. Although melissopalynological analysis was conducted to characterize the botanical composition of the honey samples, the extent to which botanical variability influenced the analyzed physicochemical and biological parameters was not quantitatively evaluated. In addition, the inclusion of different honey types (nectar and honeydew honeys) may introduce additional compositional variability. While the LDA model demonstrated good discriminatory potential with a cross-validated classification accuracy of 85.5%, the relatively high number of variables and limited sample size may increase the risk of overfitting and limit the generalizability of the model. Therefore, further validation studies with larger sample sizes are needed to confirm the classification model. Future studies incorporating larger sample sizes and more controlled evaluation of botanical origin would help to clarify in detail the relative contributions of floral and geographical factors to honey composition and bioactivity.

Conclusions

In this study, the melissopalynological, physicochemical, chemical, and biological activity properties of nectar and honeydew honeys representing all regions of Türkiye were collected and examined to investigate regional differences, and the parameters affecting regional distinctions were evaluated using multivariate statistical approaches. The results indicated that differences in regional conditions may lead to variations in the botanical properties and chemical composition of honey, as well as in its antioxidant, antibacterial, and anti-hyaluronidase activities. The evaluation conducted using LDA has confirmed to be an effective approach for the geographical classification of honey with high original (91.7%) and cross-validated (85.5%) classification accuracy. While the Marmara and Central Anatolia regions were clearly distinguished from other regions with more than 90% accuracy rate, samples from the Mediterranean (73.5%) and Black Sea (80.0%) regions showed similarities in physicochemical, chemical, and biological properties, particularly with those from Central Anatolia. Moreover, a stepwise discriminant approach has revealed key parameters that stand out in regional differentiation, such as aspartic acid and cysteine, water activity, and inhibition of *S. aureus* ATCC 25923. Based on these parameters, distinct compositional patterns were observed among regions; notably, aspartic acid levels were higher in Southeastern Anatolia, *S. aureus* ATCC 25923 inhibition activity was more pronounced in Eastern Anatolia, and cysteine levels were lower in the Aegean region. Overall, this comprehensive analytical and chemometric assessment applied to verify the origin of honey provides a scientific basis for enhancing the value of regionally specific honeys and promoting them in the international market. It may also provide a framework for future studies on honey traceability and origin verification in other honey production regions worldwide.

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tion, Methodology, Data curation, Formal analysis. Meltem Öztürk: Investigation, Methodology, Data curation, Formal analysis. Fatma Ersöz: Methodology, Data curation, Formal analysis. Aslı Özkök: Methodology, Data curation, Formal analysis, Writing-Reviewing and Editing. Mustafa Erbaş: Supervision, Funding acquisition, Project administration, Conceptualization, Writing-Reviewing and Editing.

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Data availability The data are available on request, but they are not publicly shared.

Declarations

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

Ethical approval This research does not contain any studies on human or animal objects.

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