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ORIGINAL RESEARCH ARTICLE

Comprehensive analysis of botanical origin and amino acid composition of bee pollen samples from various regions of Turkey

Ceren Mutlu[a], Cihadiye Candal-Uslu[b], Aslı Özkök[c], Andaç Koç[d], Hazal Özhanlı[e], Gökhan Akdeniz[f], Sultan Arslan-Tontul[g] and Mustafa Erbaş[h]

[a]Food Engineering Department, Engineering Faculty, Balıkesir University, Balıkesir, Turkey; [b]Nutrition and Dietetics, Faculty of Health Sciences, Kutahya Health Sciences University, Kütahya, Turkey; [c]Bee and Bee Products Application and Research Center (HARUM), Hacettepe University, Ankara, Turkey; [d]Food Engineering Department, Engineering Faculty, Gaziantep University, Gaziantep, Turkey; [e]Food Engineering Department, İzmir Institute of Technology, İzmir, Turkey; [f]Apiculture Research Center, Aegean Agricultural Research Institute, İzmir, Turkey; [g]Food Engineering Department, Agricultural Faculty, Selçuk University, Konya, Turkey; [h]Food Engineering Department, Engineering Faculty, Akdeniz University, Antalya, Turkey

ABSTRACT

This study aimed to investigate the botanical diversity in bee pollen and amino acid composition of Turkish bee pollen samples collected from different regions. The monofloral bee pollen samples belonged to three different plant families: Asteraceae, Fabaceae, and Ranunculaceae. Additionally, Agean, Central Anatolian, Black Sea, and Marmara Regions have monofloral bee pollen samples with higher than 85% pollen frequency. The free amino acid (FAA) composition and content of the bee pollen samples were analyzed, with the essential amino acids comprising 13.13–18.28% of the total amino acid content. The Central Anatolian region displayed the highest ratio of essential amino acids, and the Aegean region exhibited a more diverse profile of FAA. Principal Component Analysis was performed to evaluate the variation in the data, with the eigenvalues of the two factors explaining 68.58% of the total variability. Moreover, cluster analysis revealed distinct dendrograms based on palynological characteristics and FAA composition.

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Turkish bee pollen; regional variation; essential amino acid; palynological analyses; cluster analysis; Principal Component Analysis

Introduction

Bee pollen is referred to as the pollen collected by bees and transferred to the hive in the form of pollen loads. It serves as the primary and sole protein source in for the colony (Kieliszek et al., 2018). Following enrichment with honey and salivary secretions of bees, it is converted into bee bread through lactic acid fermentation (Kieliszek et al., 2018). A bee colony can collect 50–250 g of flower pollen daily (Kieliszek et al., 2018). Since ancient times, the possibility of using bee pollen for nutritional or medical purposes has been widely acknowledged (Ares et al., 2022; Li et al., 2018). Its preventive effects on aging, ulcerative colitis, arteriosclerosis, gastroenteritis, respiratory diseases, prostatic disorders, and allergic sensitization as well as its ability to protect the digestive and nervous systems, have been reported in various *in vivo* and *in vitro* studies (Altiner et al., 2021; Ares et al., 2022; Cornara et al., 2017; Denisow & Denisow-Pietrzyk, 2016; Kocot et al., 2018; Mărgăoan et al., 2019). With its unique structure and bioactive composition, pollen is referred to as "the life-giving dust" and is recognized as one of the functional foods in the food industry (Altiner et al., 2021).

The extensive therapeutic effects of bee pollen can be attributed to its chemical composition, which is highly affected by botanical origin, environmental conditions, and ecological factors (Kieliszek et al., 2018). Pollen has a high nutritive value, including proteins (7–40%), carbohydrates (24–60%), and lipids (1–18%) (Kieliszek et al., 2018). It also contains a high amount of unsaturated fatty acids, including essential fatty acids such as linoleic (10.76%) and linolenic acid (15.76%) (Al-Kahtani et al., 2021). Al-Kahtani et al. (2021) reported that harvested time had a significant effect on the concentration of essential fatty acid of bee pollen and the maximum values of unsaturated/saturated fatty acid ratio were found in bee pollen harvested during summer. In bee pollens from various botanical sources, 60 different forms of polyphenols, including phenolic acids and glycosylated and non-glycosylated flavonoids, have been identified (Li et al., 2018). Gercek et al. (2022) identified 23 phenolic

CONTACT Sultan Arslan-Tontul sultan.arslan@selcuk.edu.tr; sltnarslan07@gmail.com

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CM: Methodology, Formal analysis, Investigation. CCU: Formal analysis, Investigation. AÖ: Methodology, Investigation, Review and Editing. AK: Formal analysis. HÖ: Formal analysis. GA: Resources. SAT: Writing—Original Draft, Review and Editing. ME: Data Curation, Review and Editing, Supervision, Project administration.

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compounds in pollen samples collected from the Black Sea Region of Turkey, with rutin being the most abundant. Kaškonienė et al. (2015) analyzed the phenolic profile of 14 pollen samples collected from the Baltic Region and determined that 2-hydroxycinnamic acid, rutin, and quercetin were common in all samples. Additionally, it is characterized by high antioxidant activity due to its rich phenolic and flavonoid content (Kieliszek et al., 2018). Altiner et al. (2021) determined the antioxidant activity of Turkish pollens to be in the range of 48.96–111.40 μmol TE/g, 83.24–257.27 μmol TE/g, and 35.69–83.84 μmol TE/g, according to ABTS, CUPRAC, and DPPH assay, respectively. Moreover, bee pollen is also called a "vitamin bomb" due to its high content of soluble and insoluble vitamins (about 0.02–0.7%). Furthermore, it includes over 25 distinct micro- and macro-elements (Kieliszek et al., 2018). K, P, Mg, Ca, and Si were reported as the elements with the highest concentrations in pollen (Mayda et al., 2020). Yang et al. (2013) determined high levels of beneficial elements such as K, Ca, Mg, Zn, Fe, Mn, and Cu, while the contents of detrimental trace elements (Cd, Pb, and Hg) were primarily lower or not detected in 20 common varieties of monofloral bee pollen collected from China. These elements are vital for human nutrition since the appropriate regulation of metabolic pathways and physiological functions depends on minerals (Sattler et al., 2016).

Turkey is an important country where beekeeping activities are intensively carried out, thanks to its ecological richness. It is the second-largest producer of honey worldwide (Kalaycıoğlu et al., 2017). Turkey has one of the richest floral diversity including over 12,000 plant species (Cenet et al., 2017). The ecological variations in seven Turkish regions produce nectarous plants with excellent diversity for the production of honey (Cenet et al., 2017). As a result, bee pollen exports and manufacturing have increased in Turkey recently (Kalaycıoğlu et al., 2017).

Considering the diversity in plant resources and chemical differences in beekeeping products due to climate and geographical characteristics, it is important to examine the characteristics of different bee products of Turkey. In this study, palynological analysis of 93 bee pollen samples collected over a 2-year period (2018–2020) from seven regions in Turkey was performed, and the amino acid composition was determined. The relationship between botanical origin and flower pollen source was examined by multivariate analysis. This study is comprehensive research in terms of the number of samples collected and the regional diversity, providing valuable insights into the botanical diversity of pollen in Turkey, since there is no study conducted with such a large number of samples from every region of Turkey in the literature.

Materials and methods

Materials

A total of 93 samples of bee pollen were obtained from seven different regions of Turkey (Mediterranean, East Anatolian, Aegean, Southeast Anatolian, Middle Anatolian, Black Sea, and Marmara Region). The sampling was done in 2 years (2018–2020), and 44 of the pollens were obtained in the first year and the remaining were obtained in the second year. Bee pollen samples were collected with the support of Altıparmak Gıda San. ve Tic. A.Ş. and Ordu Beekeeping Research Institute, Turkey. The region and collection numbers according to the years of the bee pollen samples are shown on the map in Figure 1.

Palynological analysis of bee pollen samples

For palynological bee pollen analysis, 2 g of the mixed bee pollen samples were weighed, and 13 mL of ethanol was added and mixed with a vortex mixer. The homogenized samples were centrifuged

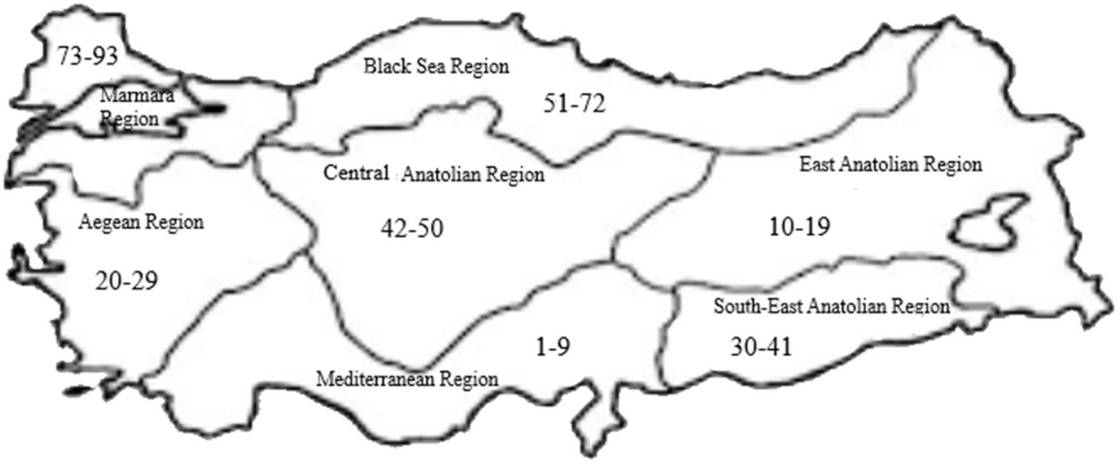

Figure 1. Map of Turkey showing the geographical distribution of bee pollen samples.

at 3500 rpm for 20 min and the supernatant was removed. A 13 mL of 50% glycerine solution was added over the precipitate and mixed with a vortex mixer. After the second homogenization, the sample was centrifuged at 3500 rpm for another 20 min. After centrifugation, the supernatant was removed, and the precipitate was allowed to dry at ambient temperature. Tubes were inverted on a blotting paper and the remaining precipitate in the tube was allowed to dry. The dried bee pollen sample was transferred onto a slide. The slide was heated on a heating tray at 30–40 °C to melt the basic-function glycerine-gelatine mixture. After melting, the pollen was spread onto an $18 \times 18\,mm^2$ coverslip and placed on two glass drumsticks to obtain a clear image during the examination (Barth et al., 2010). The prepared slides were monitored by an optical microscope (Nikon Eclipse E400, Tokyo, Japan). The identification of pollen spores was supported by literature data (d'Albore, 1997; Erdtman, 1969). The palynological results of bee pollen samples were classified as very frequent pollen (>85%), frequent pollen (45–85%), few frequent pollen (15–45%), rare frequent pollen (3–15%), and very rare frequent pollen (<3%) (Barth et al., 2010).

Color properties of bee pollen samples

The color measurement of pollen samples was performed by color meter (CR-400, Konica Minolta, Japan). A total of 5 g of pollen samples were weighed into the measurement vessel. The color properties were expressed with L^* [(0) black—(100) white], a^* [(+) red—(−) green] ve b^* [(+) yellow—(−) blue] values.

Moisture content (%) and water activity (a_w)

To determine moisture content, 2 g of bee pollen was weighted and dried at 70 °C to constant weight by drying chamber. Additionally, water activity of pollen samples was measured by water activity meter (Novasina LabSwift-aw, Switzerland).

Free amino acid composition

Free amino acid (FAA) composition of bee pollen samples was determined according to method of Li et al. (2016). To extract FAAs, 75% ethanol was added to the samples and ultrasonicated for 10 min at room temperature. The mixtures were centrifuged at 7100x*g* for 10 min and supernatant was obtained. A total of 360 µL of supernatant, 200 µL of 2,4-dinitrofluorobenzene (DNFB 10 mg/mL), 200 µL $NaHCO_3$ (0.5 M, pH 9.0), and 40 µL ultrapure water were mixed in a tube and it was kept in a water bath at 60 °C for 1 h. After cooling to room temperature, 800 µL KH_2PO_4 (0.01 M, pH 7.0) was added into the samples tubes and it was kept in the dark for 15 min. The mixture was filtered through a 0.45 µm filter into vials and 20 µL was injected into the HPLC-DAD system (Agilent 1260 Infinity II, Santa Clara, CA).

To perform chromatographic analysis, a C_{18} column (3.0 × 150 mm, 2.7 µm, InfinityLab Poroshell 120 EC-C18, Agilent, USA) was used as the analytical column, and sodium acetate (5 mM, pH 5.7)/tetrahydrofuran (95:5, mobile phase A) and methanol/water (80:20, mobile phase B) were used as mobile phases under gradient flow. The column temperature was set at 35 °C, mobile phase flow rate at 1 mL/min, and detector wavelength at 360 nm. The results were calculated using a standard curve prepared with different concentrations (2.84–113.64 mg/kg) of 18 amino acids, and expressed as dry bases.

Statistical analysis

The research was repeated for 2 years, and the analyses were conducted in parallel. ANOVA was used for data evaluation. All statistical calculations were performed using SAS Statistical Software (SAS Institute Inc., Cary, NC). Values are presented as mean ± standard error. Significance was evaluated using analysis of variance followed by Duncan's Multiple Range Test ($p < .05$). Principal component analysis (PCA) and hierarchal cluster analysis (HCA) were performed on the data of palynological taxa and amino acid composition.

Results

Palynological analysis

The palynological results indicate that bee pollen samples were classified as very frequent pollen (>85%), frequent pollen (45–85%), few frequent pollen (15–45%), rare frequent pollen (3–15%), and very rare frequent pollen (<3%) according to the Barth et al. (2010) and showed in Supplementary Table 1. In the monitored samples, 6 monofloral and 87 multifloral bee pollen types were detected. The monofloral bee pollen samples belonged to three different plant families. These are Asteraceae, Fabaceae, and Ranunculaceae. The monofloral bee pollen samples with higher than 85% pollen frequency originate from Aegean, Central Anatolian, Black Sea, and Marmara Regions.

Pollen from taxa belonging to *Paliurus spina-christi*, Brassicaceae, Ranunculaceae, Apiaceae, and Cistaceae were detected in the Mediterranean region, and a similar pattern was observed in the Aegean region. Additionally, in the multifloral samples of these two regions, flower pollen types from

Asteraceae, Cistaceae, Rosaceae, Berberidaceae, Fabaceae, Lamiaceae, and Brassicaceae were also detected. Due to the similar climatic characteristics specific to these two regions, similarities in botanical origin of pollen are also observed.

The pollen samples obtained from East Anatolian and Southeast Anatolian regions were predominantly multifloral. This result can be attributed to the region's climate and geographical location, which result in a scarcity of vegetation.

In the Central Anatolian and Marmara regions, pollen of *Helianthus annuus* were detected as a dominant taxon, ranging from 49.18% to 90.19%. This result is an expected result, since Central Anatolia and Marmara regions are the main regions where sunflowers are cultivated (Semerci, 2012). On the other hand, secondary pollen in the multifloral samples collected from these regions primarily originated from *Salix* sp., Cistaceae, *P, spina-christi,* Brassicaceae, *Castanea sativa*, Lamiaceae.

In the Black Sea region, the best-presented families for pollen collected were Fabaceae, *C. sativa, Salix* sp., Cistaceae, Papaveraceae, *Onobrychis* sp., *Trifolium* sp., and *H. annuus. C. sativa* pollen was detected both from Marmara and Black Sea regions since there are a lot of natural chestnut trees in these regions.

As a palynological results, it has been determined that the plant pollens detected in the palynological bee pollen analyses reflect the floral characteristics of the regions. For this reason, palynological analyses on bee pollen can be used in both geographical indication detection and vegetal origin determination of the regions.

Physical analysis

Statistical differences were determined in mean L^*, a^*, and b^* color values of 93 pollen samples collected from different regions of Turkey (Table 1). The L^* value ranged between 55.03 and 59.13, and it was higher in pollens obtained from the Central Anatolian, Mediterranean, and Black Sea regions. The a^* value varied between 6.78 and 9.64, and the positive a^* values indicated higher redness in all pollen samples. Lastly, the b^* value of pollens ranged from 27.54 to 36.05.

The moisture content (mc) and water activity (a_w) of bee pollen samples collected from seven different regions of Turkey showed statistically significant differences ($p > .05$) (Table 1). mc and a_w ranged between 6.71–16.67% and 0.39–0.64, respectively.

Chemical analysis

The composition of FAA in the bee pollen samples is presented in Table 2. In the present study, 18 amino acids were detected in the bee pollen samples, and their levels differed significantly according to the region ($p > .05$). The major amino acid found in the pollen samples was proline, with amounts ranging from 12130.3 to 17582.5 mg/kg. Moreover, the highest proline content was observed in pollen samples collected from the Aegean region. The bee pollen samples obtained from the Mediterranean region had a higher content of glutamine, alanine, and valine compared to those collected from other regions, with amounts of 535.24, 1223.84, and 289.50 mg/kg, respectively. The Aegean region exhibited a richer profile of FAA, with higher content of aspartic acid, hydroxyproline, glutamine, histidine, proline, methionine, cystine, tryptophan, leucine, lysine, and tyrosine compared to other regions. Furthermore, the highest phenylalanine content of 2428.35 mg/kg was determined in the pollen samples obtained from the Central Anatolia region. In addition, the pollen samples from the Black Sea region had a higher content of asparagine, serine, and isoleucine compared to samples from other regions. Notably, none of the samples contained detectable levels of threonine.

Moreover, the bee pollen samples obtained in this study were found to be a good source of essential amino acids in terms of human diet. Valine (137.58–289.50 mg/kg), methionine (28.83–137.01 mg/kg), tryptophan (51.95–97.54 mg/kg), isoleucine (424.75–665.03 mg/kg), phenylalanine (862.58–2428.35 mg/kg), leucine (256.27–493.03 mg/kg), and lysine (123.23–512.92 mg/kg) were the essential amino acids detected in the pollen samples collected from different regions of Turkey. The essential amino acids comprised a range of 13.13–18.28% of the total

Table 1. Moisture content (mc) and water activity (a_w) of pollen samples.

	Color parameters				
Region	L^*	a^*	b^*	mc (%)	a_w
Mediterranean	$58.99^{A} \pm 1.29$	$9.27^{A} \pm 0.43$	$34.39^{A} \pm 1.79$	$13.74^{B} \pm 1.14$	$0.58^{B} \pm 0.03$
East Anatolian	$55.39^{B} \pm 0.85$	$8.15^{BA} \pm 0.51$	$27.54^{C} \pm 1.03$	$6.71^{D} \pm 0.45$	$0.39^{D} \pm 0.02$
Aegean	$55.03^{B} \pm 2.03$	$9.64^{A} \pm 0.88$	$30.15^{BC} \pm 1.98$	$16.67^{A} \pm 1.41$	$0.64^{A} \pm 0.03$
Southeast Anatolian	$58.16^{BA} \pm 0.95$	$6.78^{B} \pm 0.59$	$30.25^{BC} \pm 1.03$	$9.46^{C} \pm 0.85$	$0.47^{C} \pm 0.03$
Central Anatolian	$59.13^{A} \pm 0.60$	$9.18^{A} \pm 0.58$	$36.05^{A} \pm 1.66$	$9.60^{C} \pm 0.68$	$0.48^{C} \pm 0.02$
Black Sea	$58.98^{A} \pm 0.64$	$9.47^{A} \pm 0.41$	$32.72^{BA} \pm 1.12$	$8.22^{DC} \pm 0.66$	$0.43^{DC} \pm 0.02$
Marmara	$57.68^{BA} \pm 1.02$	$8.48^{BA} \pm 0.57$	$32.26^{BA} \pm 1.14$	$10.24^{C} \pm 0.85$	$0.49^{C} \pm 0.02$

The superscript letters, in the same column, indicate that are significantly different by Duncan's multiple range test ($p < .05$).

Table 2. Free amino acid profile and amount of pollen samples (mg/kg in dry base).

	Regions						
Amino acid	Mediterranean	East Anatolian	Aegean	Southeast Anatolian	Central Anatolian	Black Sea	Marmara
Aspartic acid	$324.70^{C} \pm 47.05$	$172.31^{D} \pm 27.05$	$807.68^{A} \pm 38.84$	$633.30^{B} \pm 33.82$	$684.75^{B} \pm 39.76$	$333.23^{C} \pm 45.01$	$277.82^{C} \pm 43.18$
Glutamic acid	$74.05^{BA} \pm 9.81$	$49.47^{C} \pm 5.42$	$72.57^{BA} \pm 6.64$	$80.68^{A} \pm 8.01$	$77.43^{A} \pm 9.59$	$56.91^{BC} \pm 4.10$	$62.70^{CBA} \pm 6.72$
Hydroxy proline	$27.66^{BA} \pm 5.10$	$17.6^{2B} \pm 3.24$	$31.44^{A} \pm 3.78$	$29.02^{A} \pm 3.71$	$27.13^{BA} \pm 4.75$	$22.03^{BA} \pm 2.32$	$26.61^{BA} \pm 3.35$
Asparagine	$380.02^{BA} \pm 39.29$	$382.49^{BA} \pm 80.98$	$318.53^{BA} \pm 23.00$	$236.66^{B} \pm 34.16$	$376.65^{BA} \pm 34.22$	$419.99^{A} \pm 56.43$	$398.77^{A} \pm 46.22$
Serin	$1298.42^{B} \pm 136$	$1091.12^{B} \pm 195$	$1116.95^{B} \pm 126$	$873.35^{B} \pm 106$	$917.39^{B} \pm 227$	$1901.55^{A} \pm 227$	$900.35^{B} \pm 106$
Glutamine	$535.24^{A} \pm 40.70$	$291.44^{B} \pm 39.96$	$535.25^{A} \pm 50.80$	$356.01^{B} \pm 24.40$	$378.92^{B} \pm 36.65$	$532.00^{A} \pm 50.10$	$343.14^{B} \pm 32.09$
Histidine	$633.88^{A} \pm 60.31$	$332.74^{B} \pm 60.94$	$750.80^{A} \pm 84.29$	$375.71^{B} \pm 53.62$	$732.41^{A} \pm 70.55$	$412.025^{B} \pm 44.81$	$329.19^{B} \pm 42.38$
Proline	$13725.0^{CB} \pm 447$	$12130.3^{C} \pm 1085$	$17582.5^{A} \pm 1476$	$15059.9^{BA} \pm 1500$	$12008.0^{C} \pm 1383$	$11963.0^{C} \pm 744$	$11225.6^{C} \pm 691$
Alanine	$1223.84^{A} \pm 106$	$651.93^{B} \pm 71$	$1185.87^{A} \pm 150$	$1004.46^{A} \pm 64$	$909.79^{BA} \pm 47$	$1081.24^{A} \pm 107$	$1122.58^{A} \pm 77$
Valin	$289.50^{A} \pm 27.35$	$140.33^{C} \pm 14.16$	$209.33^{B} \pm 35.23$	$163.45C^{B} \pm 8.65$	$137.58^{C} \pm 14.35$	$213.98^{B} \pm 15.86$	$204.81^{B} \pm 18.34$
Methionine	$28.83^{C} \pm 6.97$	$31.50^{C} \pm 4.35$	$137.01^{A} \pm 41.34$	$37.68^{CB} \pm 6.69$	$50.56^{B} \pm 12.94$	$52.31^{B} \pm 3.56$	$45.41^{CB} \pm 3.27$
Tryptophan	$83.33^{BA} \pm 12.50$	$56.45^{B} \pm 16.16$	$97.54^{A} \pm 18.44$	$52.36^{B} \pm 8.11$	$51.95^{B} \pm 9.38$	$62.28^{B} \pm 4.56$	$60.09^{B} \pm 7.86$
Isolysin	$665.03^{A} \pm 34.07$	$660.16^{A} \pm 39.83$	$586.39^{B} \pm 95.23$	$495.22^{DC} \pm 29.54$	$424.75^{D} \pm 29.05$	$722.88^{A} \pm 29.24$	$551.72^{CB} \pm 16.2$
Phenylalanine	$1639.92^{B} \pm 135$	$1304.15^{CB} \pm 142$	$1512.73^{B} \pm 138$	$1537.93^{B} \pm 34$	$2428.35^{A} \pm 637$	$862.58^{C} \pm 62$	$1159.78^{CB} \pm 54$
Leucin	$258.24^{C} \pm 11.70$	$256.27^{C} \pm 19.22$	$493.03^{A} \pm 40.05$	$347.10^{B} \pm 8.01$	$324.96^{CB} \pm 9.87$	$305.88^{CB} \pm 24.62$	$316.19^{CB} \pm 28.5$
Cysteine	$32.72^{C} \pm 2.75$	$50.11^{B} \pm 4.47$	$45.12^{CB} \pm 4.96$	$87.66^{A} \pm 12.46$	$49.37^{B} \pm 4.52$	$31.13^{C} \pm 3.34$	$31.75^{C} \pm 3.17$
Lysin	$371.01^{B} \pm 67.01$	$123.23^{D} \pm 9.12$	$512.92^{A} \pm 77.71$	$216.79^{DC} \pm 16.05$	$211.09^{DC} \pm 13.41$	$327.47^{CB} \pm 35.90$	$325.75^{CB} \pm 41.0$
Tyrosine	$116.96^{BA} \pm 12.34$	$60.72^{C} \pm 3.86$	$150.15^{A} \pm 22.04$	$86.16^{CB} \pm 6.30$	$64.02^{C} \pm 8.73$	$97.39^{CB} \pm 9.24$	$105.18^{B} \pm 13.33$

The superscript letters, in the same line, indicate that are significantly different by Duncan's multiple range test ($p < .05$).

amino acid composition, with the highest ratio observed in the Central Anatolian region.

The relationship between very frequent, frequent, and few frequent pollen plant taxa and FAA of 93 bee pollen samples was investigated using PCA and HCA. The PCA results (Figure 2A) revealed that the eigenvalues of the two factors were 17.25 and 11.55, accounting for a total variability of 68.58%. A dendrogram of Turkish bee pollen samples based on palynological character and FAA composition was generated using cluster analysis, as shown in Figure 2B. According to HCA results, the samples could be divided into three main groups as Central Anatolian, Black Sea Region, and the others.

Discussion

Palynological analysis

This study is of particular importance as it identifies the original botanical sources of bee pollen by regions. As observed in this study, it has been reported that Mediterranean region honeys are predominantly produced with herbaceous plant pollen (Silici & Gökceoglu, 2007; Zerrouk et al., 2014). The palynological findings of bee pollen samples were consistent with a previous study of Zerrouk et al. (2014) who reported that Apiaceae was one of the most abundant families in pollen collected from central Algeria. Similarly, Silici and Gökceoglu (2007) found that Apiaceae pollen types were frequently observed in honey samples collected from the Mediterranean region. An interesting result was that the absence of pollen from common plants in the Mediterranean and Aegean regions, such as *Olea* sp. and *Citrus* sp., in this study. This result is supported by Silici and Gökceoglu (2007) who suggested that *Citrus* sp. may not be particularly attractive to foragers and may not serve as a significant source of pollen or nectar in this area, despite the honey sample being collected from the Mediterranean region. In a previous study by Cenet et al. (2017), honey samples were collected from the Southeast Anatolian region, and it was determined that the pollen composition consisted of 27 taxa belonging to 13 families. However, no dominant pollen was detected, indicating that all honey was noted as multifloral sources. Tosunoglu et al. (2023) also reported that the predominant pollen in honey samples collected from Gümüşhane, a province in the Black Sea region, belonged to *Trifolium* sp. taxa due to intensive livestock activities in this area. The Black Sea region yielded the highest number of unique pollen taxa compared to other regions. For instance, pollen originating from *C. sativa, Salix* sp., *H. annuus*, and Fabaceae taxa were exclusively determined as frequent pollen types in the Black Sea region. Asteraceae originated pollen was detected only in the Central Anatolia region, while *C. sativa, H. annuus,* and Lamiaceae-originated pollen was detected only in the Marmara region. The pollen of *Trifolium* sp., *Taraxacum* sp., Apiaceae, Berberidaceae, and Cistaceae taxa were exclusively determined in the Southeast Anatolian region.

In this study, bee pollen samples were collected from all regions over a period of 2 years, and palynological analyses were conducted. The results offer a broader perspective in terms of revealing the bee pollen species diversity of Turkey. As a result, Apiaceae, Asteraceae, Berberidaceae, Brassicaceae, Cistaceae, Fabaceae, Fagaceae, Lamiaceae, Papaveraceae, Ranunculaceae, Rhamnaceae, and Salicaceae plant families were found most frequent families in Turkey. Also, Mărgăoan et al. (2021) found dominantly Asteraceae, Brassicaceae, Fabaceae, Fagaceae, and Lamiaceae in Turkish bee pollen samples. Mayda et al. (2020) was determined similarly

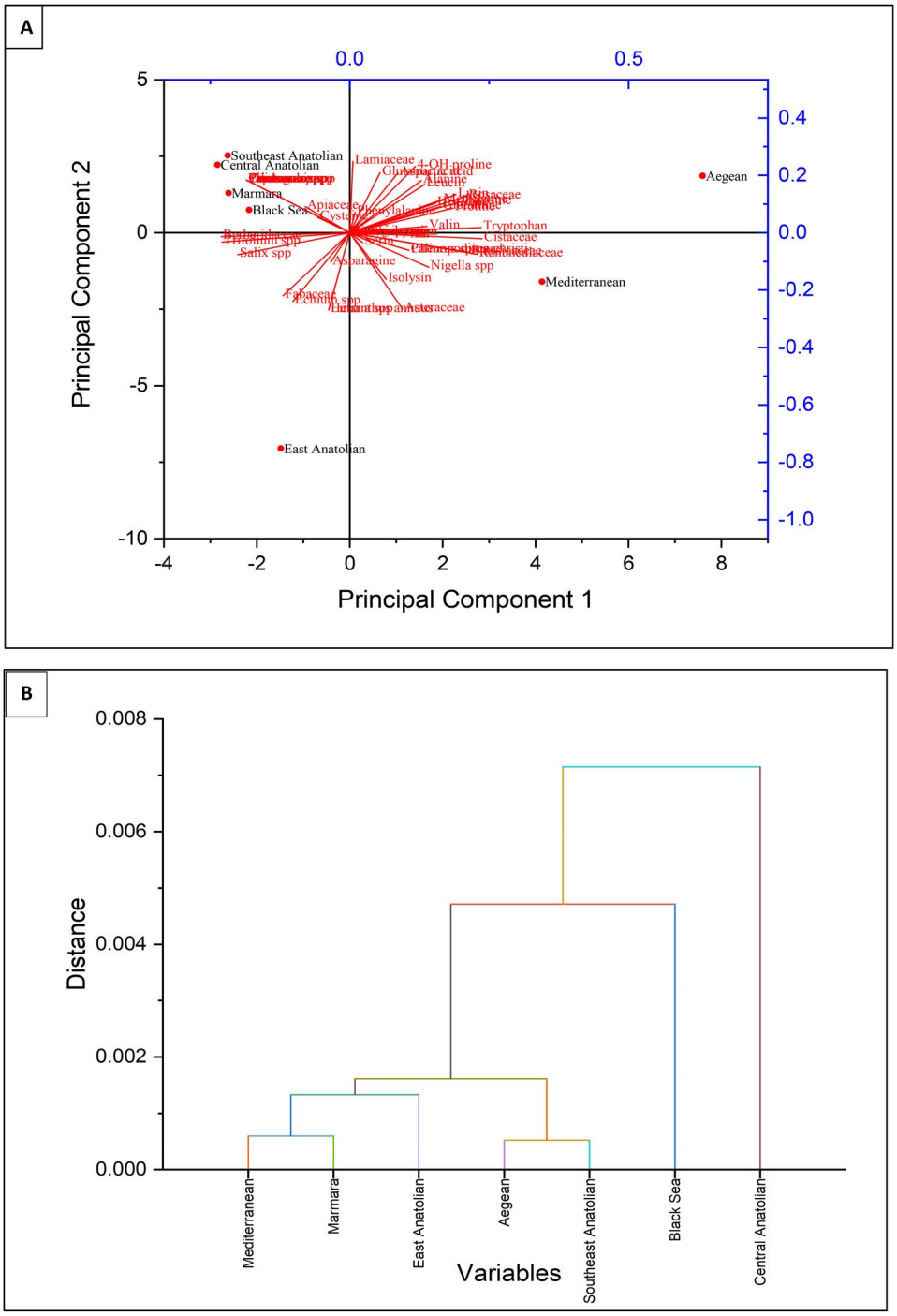

Figure 2. Principal component analysis (A) and hierarchical cluster analysis (B) of Turkish bee pollens.

Apiaceae, Brassicaceae, and Lamiaceae families were common in bee pollen samples from Turkey.

Physical analysis

The color of pollens is directly affected by their origin, and previous studies have yielded various results (Kieliszek et al., 2018). Typically, the color falls within a range of yellow, gray-white, orange, reddish, greenish, and blue tones with phenolic compounds, antocyanins, and carotenoids accounting for this variation (Kieliszek et al., 2018). The composition of these compounds is affected by several genetic and ecological factors. It was reported that high temperature, high ultraviolet radiation, low precipitation, and/or low latitudes were associated with increased floral anthocyanin production (Peach et al., 2020). Additionally, the color of pollens also depends on whether the thecae is already cracked open or if they are harvested from them (Kieliszek et al., 2018). Salazar-González et al. (2018) revealed that there was no trend in color throughout the pollen harvest year, however, there is a tendency every three and four months: between January–March and April–July, respectively, where hues change from yellow to orange. Altiner et al. (2021) reported min-max L^*, a^*, and b^* values for 20 Turkish bee pollens as 47.73–63.65, 1.54–7.19, and 19.92–30.39, respectively.

Castiglioni et al. (2019) researched the effect of botanical origin on morphological and physicochemical properties of 32 pollen samples collected from Marche, Italy, and reported a broad spectrum of color values. The authors found that L^*, a^*, and b^* color values of pollens changed between 28.6–67.6, (−1.9)–22.3, and 12.0–69.4, respectively.

In general, fresh bee pollen contains water ranging from 21% to 30% (Kieliszek et al., 2018). High water content poses a risk for pollen as elevated mc and a_w promote spoilage by molds and yeasts. Therefore, national standards recommend that the water content of bee pollen should not exceed 10 g/100 g (TSE, 2006). According to data of the present study, only pollen samples collected from East Anatolia and Black Sea Regions met the specifications. To meet this requirement, bee pollen is typically dried using appropriate techniques until its mc is below 5%, which is widely accepted as a safe level. Drying process helps preserve the chemical and microbial quality of pollen and extends its shelf life (Song et al., 2020). However, a mc of less than 3 g/100g is considered undesirable as it can lead to chemical reactions such as the Maillard reaction and lipid oxidation in pollen, resulting in discoloration and the formation of a hard texture (Kieliszek et al., 2018; Nogueira et al., 2012). Bee pollen samples obtained from East Anatolia had lower mc and a_w values compared to other regions, whereas the Aegean Region exhibited higher values for these parameters. This can be attributed to the predominantly wet pollen obtained from this area. It is hypothesized that these kinds of differences may be due to variations in the climatic conditions during the sampling periods in both years.

Chemical analysis

From the nutritional standpoint, amino acids are the main components of bee pollen (Ares et al., 2022). Paramás et al. (2006) reported that amino acids accumulate in the sporopollenin layer of pollens, and proline is one of the major amino acids, with an average content of 20.27 mg/g among 22 amino acids in Spanish pollen samples. Bayram et al. (2021) found that the proline content of pollens collected from five different locations in Turkey ranged between 8384.22 and 16670.79 mg/kg. These differences/similarities can be explained by the strong correlation between proline content and botanical origin (Serra Bonvehí & Escolà Jordà, 1997). It has been suggested that the reason for the generally high proline content is that honey bees prefer proline when choosing nectar, and proline is the main amino acid in most plants (Ares et al., 2022). On the other hand, ratio of proline and total FAA emerges as an effective indicator for evaluation of freshness with a recommended threshold of 0.65. However, the findings of the present investigation reveal a notable deviation from this recommended threshold, as the proline/FAA ratio fluctuated within the range of 0.03 to 0.69 across the analyzed pollen samples. The observed variation in the proline/FAA ratio suggests that a significant proportion of the examined pollen samples may have been subjected to suboptimal conditions during handling, storage, or transportation (Serra Bonvehí & Escolà Jordà, 1997). Ares et al. (2022) also reported that threonine was the least detected FAA among the 72 pollen samples. Bayram et al. (2021) determined that the FAA content of bee pollen collected from Central Anatolian, Marmara, and the Black Sea region ranged from 48.8 to 64.2 mg/g. The essential amino acid content of Turkish bee pollen samples fell within an acceptable range. Li et al. (2018) reported that dry bee pollen typically consisted of approximately 14–30% of the total amino acids, consisting of 20 essential amino acids. Additionally, bee pollen has been recognized as a good nutraceutical due to its high amino acid composition. Paramás et al. (2006) found that essential amino acids accounted for nearly %16 of total amino acids.

PC1 explained 41.08% of the variance and was associated with the content of tryptophan, as well as pollen taxa of Berberidaceae and *Trifolium* sp. Furthermore, PC2 explained 27.50% of the variance and was associated with hydroxyproline, as well as pollen taxa of Asteraceae, *H. annuus* and *Linaria* sp. According to PCA correlation results, a positive correlation ($p < .05$) was observed between glutamic acid and Ranunculaceae; serine and Asteraceae, Cistaceae, Fabaceae, Ranunculaceae, *Salix* sp.; glutamine and *Nigella* sp.; phenylalanine and Ranunculaceae; leucine and *Nigella* sp. It is worth noting that the pollen samples collected from the Mediterranean, Marmara, East Anatolian, Aegean, and Southeast Anatolian regions exhibited similarities in terms of pollen origin and FAA composition. Conversely, the bee pollen from the Black Sea Region and Central region formed separate clusters distinct from the other groups. The distinct separation of these clusters from other groups may be attributed to unique pollen taxa of the Black Sea region and dominant *H. annuus* pollen of Central region. The botanical composition of the Black Sea Region exhibits a distinct profile, likely influenced by the specific plant species prevalent in this geographic area. Furthermore, the dominance of *H. annuus* pollen in the Central Region appears to be a key factor contributing to the separation of this cluster. The prevalence of a single dominant pollen

source can significantly impact the overall composition of bee pollen in a given region.

Conclusions

This study provides comprehensive insights into the botanical origin, physical properties, and chemical composition of Turkish pollens collected from various regions across a 2-year period. The results reveal the presence of 6 monofloral and 87 multifloral pollen types, representing a wide range of plant species and families. Moreover, these findings contribute significantly to the understanding of the bee pollen species diversity in Turkey, highlighting the prevalence of certain plant families in different geographical areas. Additionally, essential amino acids, crucial for human diet, were present in varying amounts (13.13%–18.28%) across different regions, with the Central Anatolian region exhibiting the highest ratio. Cluster analysis based on palynological characteristics and FAA composition resulted in a dendrogram of Turkish pollens, providing insights into the relationships among different regions. Conversely, the pollen samples from the Black Sea Region and Central region formed separate clusters, indicating distinct characteristics.

This study enhances our understanding of the geographical variations in bee pollen characteristics in Turkey. The regional differences in palynological, physical, chemical, and biological aspects of bee pollen underscore the importance of considering the geographical origin when evaluating its quality and potential health benefits. The findings hold promise for potential applications in various fields such as medicine, food, and agriculture. Further research could explore the specific mechanisms underlying the observed variations in pollen characteristics and activity, leading to potential utilization of these resources in functional foods, supplements, or pharmaceutical products.

Disclosure statement

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ORCID

Sultan Arslan-Tontul http://orcid.org/0000-0003-1557-7948

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