European Food Research and Technology (2024) 250:2961–2971
https://doi.org/10.1007/s00217-024-04597-7

ORIGINAL PAPER

Antioxidant activity and phenolic content of bee breads from different regions of Türkiye by chemometric analysis (PCA and HCA)

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Received: 4 April 2024 / Revised: 20 May 2024 / Accepted: 2 June 2024 / Published online: 18 July 2024
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Abstract

The objective of this study was to assess the antioxidant activity and phenolic compounds of bee bread samples obtained from various regions in Türkiye The goal was to characterize and classify 15 of bee bread samples based on their geographical origins. This investigation employed chemometric techniques, specifically principal component analysis (PCA) and a hierarchical clustering algorithm (HCA), for the inaugural comprehensive analysis of all data encompassing antioxidant activity and phenolic compounds in bee bread. The primary objective was to unveil potential clustering patterns among of bee bread samples based on their geographical origins. According to the results, the Total Phenolic Content (TPC) of bee bread samples ranged from 4.393 to 14.917 mg GAE/g dw, while the Total Flavonoid Content (TFC) exhibited variation within the range of 0.681 to 3.504 mg QE/g dw. *p*-OH benzoic and *p*-coumaric acids were detected in all samples. Other phenolic compounds were identified in different proportions among the bee bread samples. This study demonstrated the successful application of the PCA chemometric method to assess the antioxidant activity and phenolic compounds of bee bread samples from various regions of Türkiye. The results revealed effective clustering of the bee bread samples based on their geographical origins.

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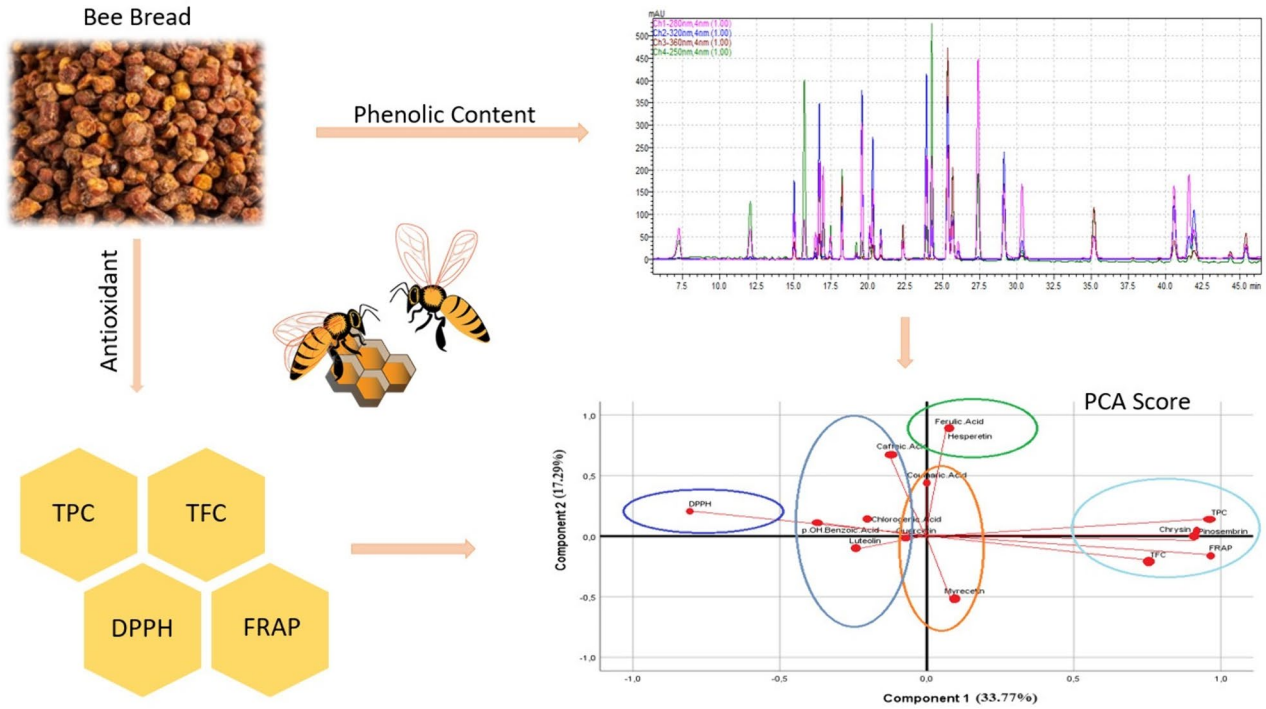
Graphical Abstract



Keywords Bee Bread · Chemometric analysis · Antioxidant activity · Phenolic Content

Introduction

In recent years, rising concerns regarding synthetic foods and food additives have fueled a growing interest in natural products for achieving balanced and healthy nutrition. This shift in consumer preferences is reflective of an increasing awareness of the potential health implications associated with synthetic food and additives. In this regard, there is a growing interest in research focused on natural functional products, particularly bee products (such as honey, pollen, bee bread, propolis etc.), which are high in nutritional value and have positive effects on health [1, 2]. Bee pollen is created when the pollen gathered by honey bees from flowers combines with their self-produced nectar secretions [3]. After being deposited into the hive, this pollen is stored within the cells of the comb. Through a blend of organic acids, honey, and digestive enzymes released by the bees, bee bread (perga) is formed through lactic acid fermentation [1, 2]. Bee bread, typically formed during fermentation, exhibits differences from bee pollen in many ways, despite their similarities. Bee bread contains more sugar and less starch compared to bee pollen. Moreover, bee bread is easily digestible because it has a higher content of B vitamins [4] and amino acids than bee pollen [5]. The chemical composition of bee bread can vary depending on factors such as the plant's origin, the nutritional state of the plant, geographical conditions, soil and harvesting, as well as the degree of storage and processing [3]. These variables encompass phenolic compounds, which play a pivotal role in biological activities, exerting influence over the diversity and quantity of phytochemicals. As a result, this leads to diverse therapeutic properties [2].

The primary objective of this study is to assess the antioxidant activity and phenolic profiles of bee bread samples collected from various regions on different dates in Türkiye. The aim is to characterize and classify 15 bee bread samples based on their geographical origin. Additionally, employing multivariate analysis, specifically principal component analysis (PCA) and a hierarchical clustering algorithm (HCA), this study provides a comprehensive examination of total phenolic and flavonoid content, antioxidant capacities (DPPH· and FRAP), and phenolic constituents. Notably, this study represents the first investigation into the potential clustering of Türkiye bee bread samples from various regions based on their geographical origin.

Methods

Chemicals

HPLC- grade methanol,acetonitrile solutions, all phenolic compounds standards and all chemicals used for antioxidant testing were purchased from Sigma-Aldrich (Munich, Germany).

Table 1 Code, Sample Region and Year

Code	Sample Region	Year
BB1	Kırşehir	2020
BB2	Afyon-1	2022
BB3	Bingöl-1	2022
BB4	Siirt	2022
BB5	Muğla-1	2022
BB6	Çanakkale	2022
BB7	İzmir	2022
BB8	Amasya-1	2022
BB9	Manisa	2022
BB10	Zonguldak	2022
BB11	Kütahya	2022
BB12	Afyon-2	2021
BB13	Muğla-2	2022
BB14	Bingöl-2	2022
BB15	Amasya-2	2022

Sample collection and preparation

Fifteen samples of bee bread were collected between the years 2020–2022 by experienced beekeepers owing different beehives from various regions of Türkiye. The codes for the bee bread samples are provided as between BB1to BB15 in Table 1. The map illustrating the collection locations of bee bread from different regions of Türkiye is presented in Fig. 1.

Each sample of bee bread was weighed at 3 g, and 30 mL of methanol was added. The extraction process was carried out in a shaker at room conditions for 24 h. At the end of the period, to remove any potential particles, the mixture was first filtered through regular filter paper, followed by blue tape-filtered paper. After the filtration process was completed, the concentration was calculated as mg/mL. The samples were divided into two parts for the determination of antioxidant activity and phenolic content. The samples were stored at + 4°C until the analyses were conducted.

Total phenolic contents (TPC) and total flavonoid content (TFC)

The determination of total phenolic content was conducted according to the method proposed by Slinkard and Singleton [6]. The fundamental principle of the method relies on the formation of a colored complex using Folin-Ciocalteu reagent. In brief, both samples and standards were mixed with 680 µL of distilled water and 400 µL of 0.2 N Folin reagent into tubes. Subsequently, 25 µL from each sample was added into the tubes. 25 µL of gallic acid standard at

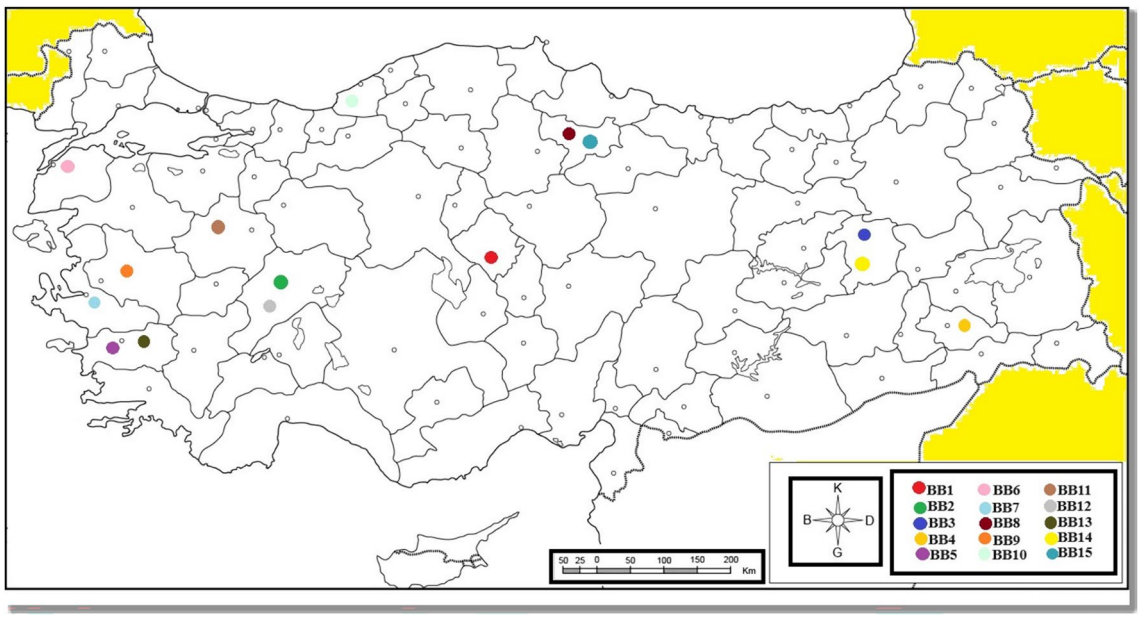


Fig. 1 Geographical location of the different region in Türkiye

different concentrations was added into the standard tubes. Finally, 400 µL 10% Na_2CO_3 was added to create an alkaline environment, and the tubes were incubated in a dark environment at room temperature for 2 h. At the end of the incubation period, the spectrophotometric reading at 760 nm was performed using a (Thermo Scientific Evolution Spectrophotometer (TM 201 UV–VIS) located in Madison, USA). Gallic acid ranging from 0.032 to 1 mg/mL was utilized as the standard. The results were expressed in terms of mg GAE/g dw sample.

The Fukumoto and Mazza [7] method was adapted for the aluminum chloride colorimetric technique, employing quercetin as the reference standard. Quercetin (5 mg) was dissolved in 100% methanol and diluted to achieve a concentration range of 0.03125–1 mg/mL. Subsequently, 0.5 mL of the test samples were combined with 0.1 mL of 10% $Al(NO_3)_3$ and 0.1 mL of 1 M NH_4CH_3COO in a test tube. The mixture was allowed to incubate at room temperature for 40 min. The reaction mixture was then analyzed using a UV–Vis spectrophotometer (Thermo Scientific Evolution TM 201 UV–VIS Spectrophotometer located in Madison, USA), with measurements taken at 415 nm.

Determination of antioxidant activity

The FRAP and DPPH· radical tests were chosen as the most commonly utilized methods in the literature to determine the antioxidant activities of bee bread samples.

Ferric reducing antioxidant power (FRAP) test

The total antioxidant potential of each bee bread sample solution was determined using the ferric (III) reduction/antioxidant capacity (FRAP) assay, following the methodology described by Benzie and Strain [8]. This assay as a quantification of antioxidant power. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6) with 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM $FeCl_3$. To each 100 µL of the sample, 3 mL of the reagent was added and thoroughly mixed. Absorbance was measured at 593 nm after a 4-min incubation period. A standard curve was generated using various concentrations of $FeSO_4$ and the results were expressed as mmol $FeSO_4$/g dw.

1,1-Diphenyl-2-picrylhydrazyl (DPPH· Radical) test

The scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was determined following the method by Molyneux [9]. The analysis mixture comprised 750 µL of bee bread sample extracts and 750 µL of a 40 mM methanolic DPPH· solution. Following a 50-min incubation in darkness at room temperature, absorbance measurements were taken at 517 nm. The results were expressed as the SC_{50} value in mg/mL, representing the concentration required to scavenge 50% of the radicals. The results were given relative to the trolox standard (Trolox dilutions ranged from 0.032 to 1.0 mg/mL).

The phenolic content

For the phenolic component analysis using RP-HPLC–PDA, enrichment was accomplished through liquid–liquid extraction. Initially, 10 mL of the bee bread sample extracts, the solvent was evaporated using a rotary evaporator at 40 °C. Subsequently, 10 mL of purified water was introduced, and the pH was adjusted to 2 using concentrated HCl. The organic phases resulting from three extractions with diethyl ether and ethyl acetate were combined. After complete solvent removal, the residue in the flasks was dissolved in 2 mL of methanol, filtered through a 0.45 µm filter (RC membrane, 0.45 µm), and inject to the device for phenolic analysis [10].

The phenolic compositions of bee bread samples were determined using a Shimadzu Liquid Corporation LC 20AT HPLC system equipped with a PDA detector and a C18 column (250 mm × 4.6 mm, 5 µm; GL Sciences). The chromatographic method employed for this analysis utilized a calibration and validation process with a set of 25 phenolic standards [10]. The mobile phase consisted of (A) 2% acetic acid in water and (B) acetonitrile:water (70:30), employing a gradient program. Both sample and standard injection volumes were set at 20 µL, with a column temperature of 30 °C and a flow rate of 1.0 mL/min.

Statistical analysis

Statistical analysis of this study was performed using the SPSS 26.0 software program. Descriptive statistics of total phenolic content, total flavonoid content, ferric reducing antioxidant power and DPPH· (1,1-diphenyl-2-picrylhydrazyl) radical of bee breads samples were determined, and calculating the standard deviations of the data. In this study, principal component analysis (PCA) was applied to determine the main components of TPC, TFC, antioxidant parameters (FRAP, DPPH·) and phenolic content. In addition, hierarchical cluster analysis (HCA) was applied to determine the relationship between bee breads samples.

Results and discussion

Total phenolic and total flavoid content of bee bread

Bioactive compounds are secondary metabolites that have attracted considerable attention due to their beneficial effects

on health, encompassing attributes such as antioxidant, antimicrobial, and anti-inflammatory properties. Found in trace amounts in foods, these compounds demonstrate a diverse range of chemical structures and functionalities, which vary depending on the plant species [11, 12]. The total phenolic and flavonoid content of the 15 bee bread samples belonging to different geographical regions of Türkiye, collected in different periods, was determined, and the results of samples are given in Table 2. The total phenolic content of the bee bread samples varies between 4.393 and 14.917 mg GAE/g dw. The highest TPC result was founded in sample BB6, while the lowest TPC result was founded for sample BB15. The total flavonoid content of the bee bread samples varies between 0.681 and 3.504 mg QE/g dw. The highest TFC result was founded in sample BB9, while the lowest TFC result was founded for sample BB15. Mayda et al. [13] reported that the TPC contents of bee bread samples collected from different provinces of Türkiye ranged from 8.26 to 12.71 mg GAE/g, while the total flavonoid contents (TFC) ranged between 1.81 and 4.44 mg QE/g. The TPC results of this study are consistent with some of the bee bread samples in this present study, while it is observed that the TFC results are higher than those in this present study. Beykaya et al. [14] reported that TPC result of bee bread ranged from 11.90 to 14.77 mg GAE/g from different provinces of Türkiye. The average TPC results of our samples were found to be slightly lower than those reported in this present study in the literature. This could be attributed to the fact that the samples were sourced from different locations. Aksoy et al. [11] reported that the total phenolic values of the samples of bee bread collected from the Ardahan province center and districts in the northeast of Türkiye ranged between 11.70 and 18.35 mg GAE/g, while the highest total flavonoid content value in the samples was 2.82 mg QE/g in a sample. The flavonoid contents of some provinces in present study show similarities in the results. In another study, it was reported that the total phenolic content (TPC) of bee bread from Romania and India ranged between 5.67 and 12.83 mg GAE/g [15]. It is possible to say that the TPC results of the samples in present study are in line with those of this present study in the literature. In another study, green synthesis of silver nanoparticles (AgNPs) was achieved using aqueous extracts of bee bread, and the antioxidant potential of the obtained AgNPs was preliminarily evaluated. The total phenolic content (TPC) in the aqueous extracts ranged from 7.95 to 21.46 mgGAE/g sample, while the total flavonoid content (TFC) ranged from 3.43 to 14.32 mgQE/g sample [16]. In an another study, Ilie et al. [17] reported that the TPC of bee bread obtained from Romania ranged from 7.10 to 18.30 mg GAE/g, while the TFC ranged from 0.45 to 1.86 mg QE/g of sample. It is seemed consistent with the outcomes of our study, despite minor variations.

Antioxidant activity

Bee bread, a fermented mixture of pollen and honey consumed by honeybees, has garnered attention for its potential health benefits. Research suggests that bee bread is abundant in phenolic compounds, known for their antioxidant activity. Consumption of bee bread may reduce the risk of degenerative diseases, decrease oxidative stress, and inhibit macromolecule oxidation [18, 19]. The FRAP assay, one of the most widely used methods for assessing antioxidant activity in the literature, is based on the spectrophotometric detection of a colored complex formed during the reduction of Fe^{+3} to Fe^{+2}. In this present study, the Ferric Reducing

Table 2 Antioxidant Actvities of Bee Bread Samples

Samples	TPC mg GAE/g	TFC mg QE/g	FRAP (μmol$FeSO_4$/g)	DPPH SC_{50} mg/mL
BB1	7.550 ± 0.179	1.563 ± 0.095	38.051 ± 0.325	1.703 ± 0.025
BB2	6.959 ± 0.204	2.071 ± 0.109	47.972 ± 0.077	1.365 ± 0.011
BB3	6.157 ± 0.488	1.351 ± 0.257	52.305 ± 0.558	1.446 ± 0.269
BB4	4.633 ± 0.035	1.242 ± 0.086	34.176 ± 0.239	2.214 ± 0.085
BB5	5.271 ± 0.074	1.826 ± 0.196	50.897 ± 0.674	1.462 ± 0.005
BB6	14.917 ± 0.216	2.970 ± 0.271	175.143 ± 0.205	0.242 ± 0.000
BB7	5.708 ± 0.144	1.551 ± 0.227	58.570 ± 0.328	0.871 ± 0.008
BB8	5.196 ± 0.161	0.904 ± 0.116	32.579 ± 0.245	2.195 ± 0.027
BB9	5.836 ± 0.052	3.504 ± 0.104	75.982 ± 0.078	0.752 ± 0.026
BB10	4.396 ± 0.355	0.962 ± 0.167	38.952 ± 0.323	2.126 ± 0.016
BB11	7.038 ± 0.155	1.394 ± 0.121	45.743 ± 0.391	1.095 ± 0.003
BB12	6.597 ± 0.125	1.811 ± 0.001	65.279 ± 0.923	1.175 ± 0.005
BB13	5.543 ± 0.047	1.430 ± 0.006	30.613 ± 0.541	1.757 ± 0.011
BB14	4.393 ± 0.063	0.987 ± 0.072	24.151 ± 0.356	2.065 ± 0.019
BB15	5.151 ± 0.036	0.681 ± 0.039	21.997 ± 0.082	2.890 ± 0.049
Trolox				0.0004 ± 0.0001

Antioxidant Power (FRAP) was chosen to determine the antioxidant activitiy of the bee bread samples. The bee bread samples exhibited a range of Fe (III) reducing power, varying between 21.997 to 175.143 µmol$FeSO_4.7H_2O$/g dw. The highest FRAP value was observed in sample BB6, while the lowest FRAP value was recorded for sample BB15. Kalaycıoğlu et al. [20] found that of bee bread samples FRAP values ranged from 0.28 to 2.46 mmol Fe(II)/g. The FRAP values results of this study were found to be slightly higher than the results of the present study. In a study conducted by Suleiman et al. [21], it was founded that 106.7 µmol (Fe^{+2}/L).

The SC_{50} values of the samples for DPPH· radical scavenging activity ranged from 0.242 to 2.890 mg/mL, with sample BB6 exhibiting the highest scavenging effect, while sample BB15 showed the lowest scavenging activity. Sönmez et al. [22] was reported that the FRAP value of Anatolian bee bread was 19.887 µmol Trolox/g, while the DPPH· radical SC_{50} value was determined to be 0.878 mg/mL. Although some differences were observed in certain samples compared to study in the literature, our antioxidant results generally align with similar outcomes.

Correlation is a statistical method to determine the relationship between two or more variables and the degree of significance of this relationship. This method is frequently used in the evaluation of scientific study results. One of the most widely used correlation coefficients today is the Pearson correlation coefficient. When determining the correlation between two variables, one of them is the dependent variable and the other is the independent variable. The correlation coefficient is called a numerical value that calculates the level of relationship between dependent and independent variables and shows the direction of this level of variability. The correlation coefficient is denoted by r and its value varies between -1.0 and + 1.0. In this study, Pearson correlation analysis was performed for TPC, TFC, FRAP and DPPH· data of bee bread samples obtained from different locations (Table 3).

Pearson correlation coefficients for this study showed that there was a positive correlation between TPC and TFC and FRAP, and the coefficients were $r_p = 0.578$ ($p = 0.024$) ($p < 0.05$ significance level) and $r_p = 0.909$ ($p = 0.000$) ($p < 0.01$ significance level), respectively. There was also a negative correlation between TPC and DPPH with a correlation coefficient of r_p = -0.670 ($p = 0.006$) ($p < 0.01$ significance level). There was a positive correlation between TFC and FRAP with a coefficient of $r_p = 0.738$ ($p = 0.002$) ($p < 0.01$ significance level), while there was a negative correlation between TFC and DPPH with a coefficient of r_p = -0.819 ($p = 0.000$) ($p < 0.01$ significance level). In addition, a negative correlation was observed between FRAP and DPPH· with a coefficient of r_p = -0.789 ($p = 0.000$) ($p < 0.01$ significance level). A negative correlation between DPPH· radical and other tests is logical. In this case, lower concentration actually implies a greater antioxidant capacity.

Table 3 Pearson correlation coefficients for TPC, TFC, FRAP and DPPH data of bee bread samples obtained from different locations

		TPC	TFC	FRAP	DPPH
TPC	r_p	1	**0.578***	**0.909****	**− 0.670****
	p		**0.024**	**0.000**	**0.006**
TFC	r_p	0.578*	1	**0.738****	**− 0.819****
	p	0.024		**0.002**	**0.000**
FRAP	r_p	0.909**	0.738**	1	**− 0.789****
	p	0.000	0.002		**0.000**
DPPH	r_p	− 0.670**	− 0.819**	− 0.789**	1
	p	0.006	0.000	0.000	

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Results showed that TPC, TFC, DPPH· and FRAP values have differentiation according to the location of bee bread (Fig. 2).

Distribution of TPC, TFC, FRAP, and DPPH·, averages have been seen at the lowest averages respectively bee bread samples number of BB14, BB15, BB15, and BB6.

Phenolic compounds profile of bee bread

Quercetin was detected only in samples BB5, BB9, and BB13 in the present study, while it was not detected in the other samples. The presence of flavonol glycoside derivatives, particularly quercetin, kaempferol, isorhamnetin, and methylherbacetin derivatives, has been detected in Moroccan's bee bread samples [23]. Urcan et al. [16] found that bee bread samples were rich in flavonoids such as myricetin, quercetin, and luteolin, and high levels of phenolic acids such as *trans-p*-coumaric acid and ellagic acid were also detected. Comparing our study with the literature, in the present research, *p*-coumaric acid, *p*-hydroxy benzoic acid, and *t*-cinnamic acid were detected in all samples, while ellagic acid was not detected in any of the samples (Table 4). The composition of bee bread, which is influenced by the plants visited by bees, can vary significantly based on factors such as geographical location, seasonality, and local flora diversity. Therefore, the absence of ellagic acid in our samples could be attributed to the specific floral sources prevalent in the region. Isidorov et al. [24] utilized GC–MS analysis to identify naringenin, kaempferol, apigenin, isorhamnetin, and quercetin in bee bread sourced from Latvia, Russia, and Poland. In our samples, quercetin was identified solely in samples labeled BB5, BB9, and BB13, whereas apigenin was not determined in any of the samples. Aksoy et al. [11] reported in their study that among the phenolics, high proportions of *p*-hydroxybenzoic acid and ferulic acid were

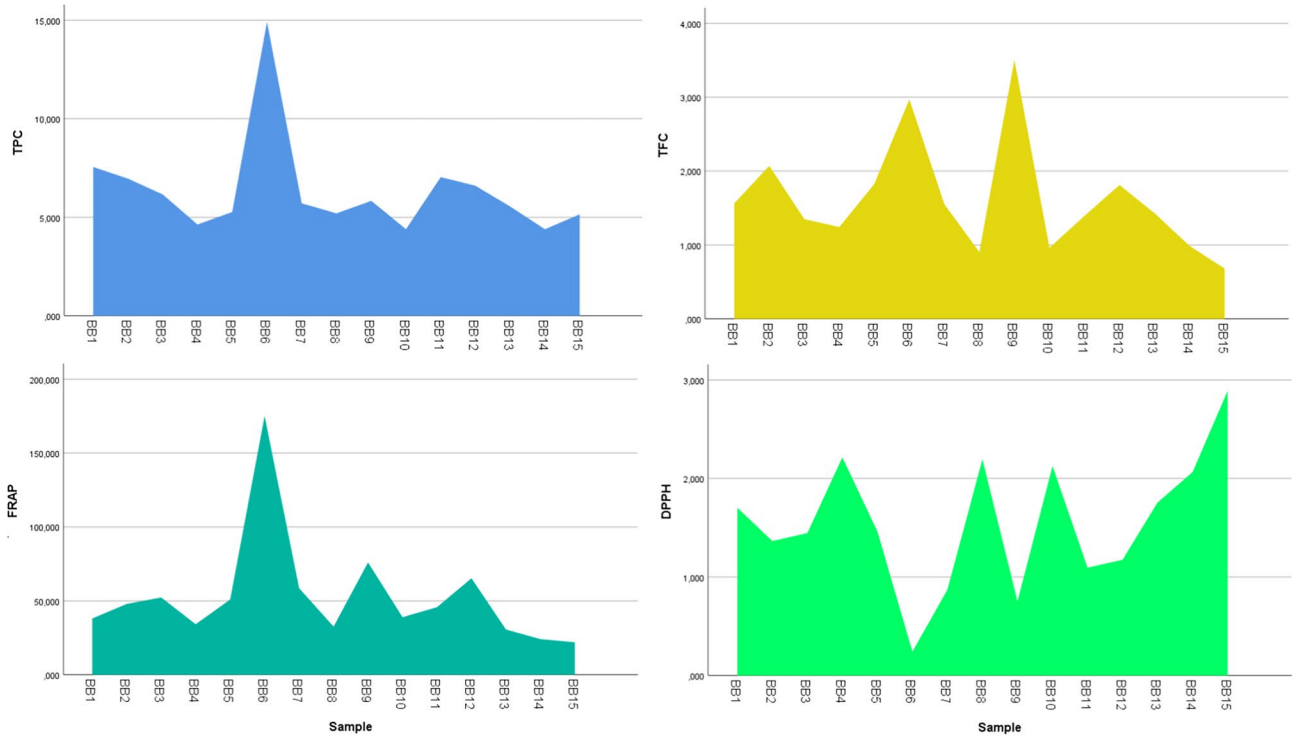


Fig. 2 Comparison of TPC, TFC, DPPH and FRAP values have differentiation according to the location of bee bread

observed. When comparing our results with the results of this study, we determined that *p*-hydroxybenzoic acid was detected in all samples except BB6, while ferulic acid was only identified in our BB1 sample. It is hypothesized that these differences stem from variations in vegetation, climate conditions, geographical origin, storage conditions, and the collection process of bee bread [11].

Principal component analysis (PCA)

The chemometric analysis of the tested bee bread samples' TPC, TFC, FRAP, DPPH·, and phenolic content commenced with the establishment of a data matrix. Principal component analysis (PCA) is a technique which with fewer new variables that are the linear components of the variables, that may be used to uncover the variance structure of the original *p* variable. Principal component was determined for TPC, TFC, DPPH· and FRAP, by PCA. Eigenvalues and variance percentages of PCA analysis is provided in Table 5. and graph is provided in Fig. 3. It is indicated that variance explanation ratios over 70% were sufficient in the PCA analysis [25]. For PCA, PC1, PC2, PC3, and PC4 explained 83.58% of the total variation, while PC1 explained 33.77%, PC2 explained 17.29%, PC3 explained 12.58%, PC4 explained 11.17% and PC5 explained 8.47% of the total variation. According to Kaiser rules, eigenvalues of greater than 1.0 are accepted as the principal component and descriptor of the variance [26]. In PCA, eigen values of PC1 (5.066), PC2 (2.594), PC3 (1.932), PC4 (1.676), and PC5 (1.271) were greater than 1.0.

Hierarchical cluster analysis (HCA)

Various techniques are used to classify the analyzed samples into groups. Hierarchical Cluster Analysis (HCA) is the most commonly used method. Hierarchical Cluster Analysis (HCA) was used for clustering the analyzed samples. Number of clusters graphic of bee bread samples is shown at Fig. 4. Additionally, by displaying a hierarchy, it makes it possible to identify similarities and differences both within and between groups [27].

The results of HCA are generally presented in a dendrogram a plot that shows the organization of bee bread samples and their relationships in tree form. The grouping problem in HCA can be solved using one of the two popular methods: agglomerative or divisive. The HCA of samples of bee bread identified two major categories (Fig. 5). According to the HCA results, the most striking issue was that the BB5 bee bread sample was separated from all other samples and formed a main branch alone. This result is supported by the phenolic content and TPC, TFC, DPPH·, and FRAP data.

Table 4 Phenolic Content of Bee Bread Sample

µg phenolic /g sample															
Standards	**BB1**	**BB2**	**BB3**	**BB4**	**BB5**	**BB6**	**BB7**	**BB8**	**BB9**	**BB10**	**BB11**	**BB12**	**BB13**	**BB14**	**BB15**
Gallic Acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Protocatechuic Acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chlorogenic acid	–	–	–	27.068	–	–	–	–	–	–	–	–	–	–	–
p-OH Benzoic acid	17.323	19.542	5.845	11.893	12.967	-	7.257	11.258	5.554	84.512	27.545	12.325	12.166	16.057	11.653
Epicatechin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Caffeic acid	11.523	7.901	4.649	10.719	–	–	–	–	–	–	13.691	3.406	–	2.877	2.347
Syringic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
m-OH Benzoic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Rutin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ellagic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
p-Coumaric acid	45.816	34.785	23.173	23.636	95.407	16.639	6.983	8.193	6.070	24.700	15.096	19.562	8.397	8.342	10.511
Ferulic acid	10.985	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Myrecetin	–	80.723	–	–	–	–	251.933	-	310.478	–	–	–	133.213	–	–
Resveratrol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Daidzein	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Luteolin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	64.074
Quercetin	–	–	–	–	367.720	–	–	–	5.701	–	–	–	102.909	–	–
t-Cinnamic acid	199.616	19.010	60.219	-	48.965	24.094	-	110.978	9.792	-	42.381	-	72.839	61.112	96.170
Apigenin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hesperetin	22.729	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnetin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chrysin	7.441	6.590	11.563	-	-	101.006	-	-	-	8.389	6.300	17.135	2.906	-	-
Pinosembrin	12.456	9.152	13.630	-	-	105.831	-	-	-	-	4.382	2.340	5.013	-	-
CAPE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Curcumin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*:-:not detected

Table 5 Eigenvalues and percentage of variance for investigated parameters of PCA analysis

	Eigenvalue	Variability (%)	Cumulative (%)
PC1	5.066	33.772	33.772
PC2	2.594	17.292	51.064
PC3	1.932	12.878	63.941
PC4	1.676	11.174	75.116
PC5	1.271	8.473	83.588

Conclusions

Bee bread is acknowledged as a functional food owing to its wealthy chemical composition and bioactive properties. The demand for natural products is steadily increasing day by day. Consequently, there is considerable interest, particularly in bee products. This represents a comprehensive investigation utilizing chemometric methods to explore the antioxidant activities and phenolic contents of bee bread collected from various regions of Anatolia. It is evident from the analysis of all bee bread samples collected from different regions of Türkiye that PCA effectively distinguishes between the samples.

According to our findings, numerous studies are required for the standardization of Anatolian bee bread. We believe this study will contribute to future research efforts.

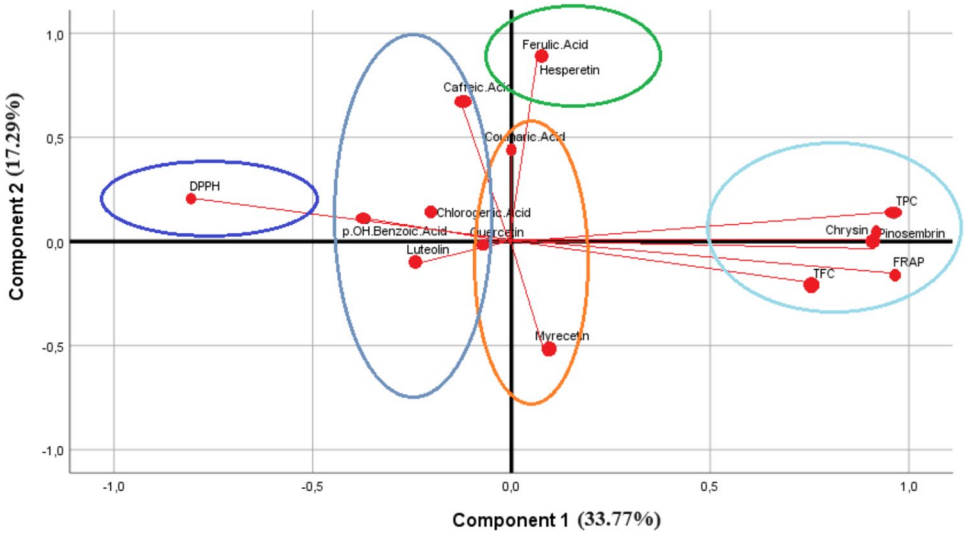


Fig. 3 PCA score plot bases on the TPC, TFC, DPPH, FRAP and phenolic acids parameters

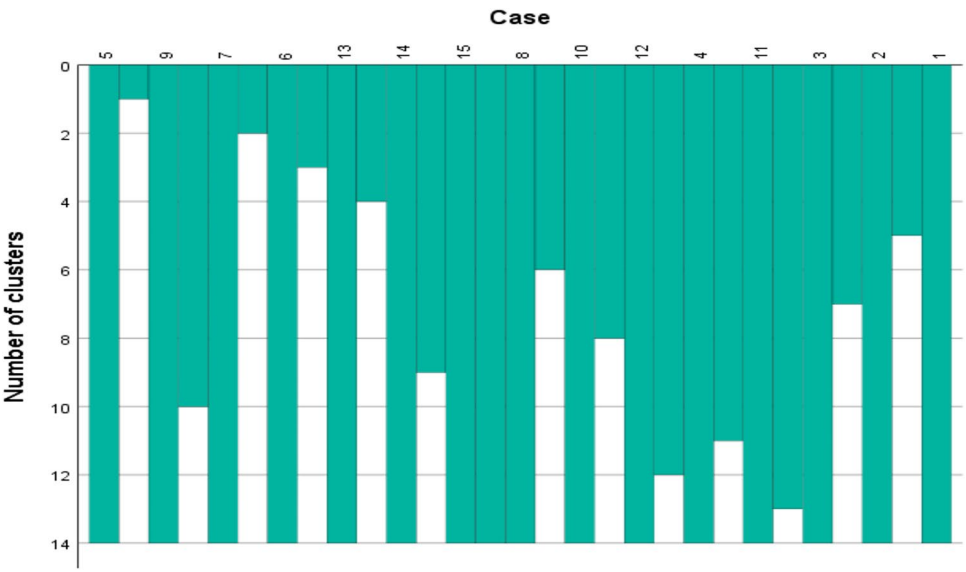


Fig. 4 Number of clusters graphic of bee bread samples

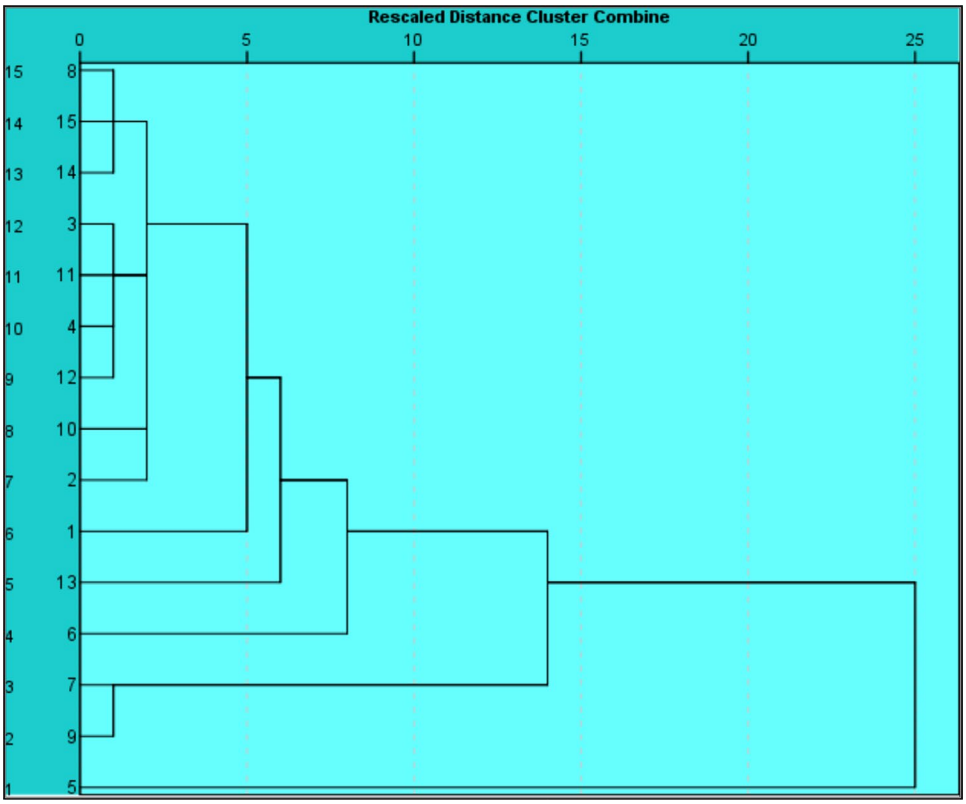


Fig. 5 The HCA dendrogram according to TPC, TFC, DPPH, FRAP and phenolic acids of bee bread samples

Acknowledgements None

Author contributions ZC Investigation, Conceptualization, Methodology, Data curation, Formal analysis, Writing-Original draft preparation, and Editing. BG statistical analysis. YK Phenolic analysis. SK Writing-Original draft preparation. All authors have read and approved the final manuscript.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK).

Data availability Data will be made available on request.

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.

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