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Characterization of Anatolian Bee Breads by Principal Component Analysis Based on Their Physicochemical and Chemical Characteristics

Anadolu Arı Ekmeklerinin Fizikokimyasal ve Kimyasal Özelliklerine Dayalı Temel Bileşen Analizi ile Karakterizasyonu

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Abstract

Bee bread is a fermented bee product which is the mixture of pollen and honey. This substance is actually the main source of food for honey-bee workers. Due to its rich therapeutic properties, bee bread has gained an increasing interest for human consumption in recent years. The purpose of this study was to compare the physicochemical characteristics and chemical composition of bee bread samples collected from seven different regions in Anatolia. Various measurements such as pH, electrical conductivity, colour, colour intensity, and optical density were performed. Together with the mentioned, the content of moisture, ash, total protein, and free acidity were analyzed. Principal component analysis (PCA) was applied to analyze results in order to classify the bee breads from different regions. This study contributes to the chemical and physicochemical knowledge of this scarcely explored natural bee-product.

Keywords: Bee bread, Perga, Total protein content, Colour analysis, Free acidity

Özet

Arı ekmeği, polen ve bal karışımı olan fermente bir arı ürünüdür. Bu madde aslında bal arısı işçilerinin ana besin kaynağıdır. Arı ekmeği, zengin tedavi edici özelliklerinden dolayı son yıllarda insan tüketiminde artan bir ilgi görmektedir. Bu çalışmanın amacı, Anadolu'nun yedi farklı bölgesinden toplanan arı ekmeği örneklerinin fizikokimyasal özelliklerini ve kimyasal bileşimini karşılaştırmaktır. pH, elektriksel iletkenlik, renk, renk yoğunluğu ve optik yoğunluk gibi çeşitli ölçümler yapılmıştır. Bunlarla birlikte nem, kül, toplam protein ve serbest asit içeriği analiz edildi. Farklı bölgelerden arı ekmeklerini sınıflandırmak için sonuçları analiz etmek için temel bileşen analizi (PCA) uygulanmıştır. Bu çalışma, nadiren keşfedilen bu doğal arı ürününün kimyasal ve fizikokimyasal bilgisine katkıda bulunmaktadır.

Anahtar Kelimeler: Arı ekmeği, Perga, Total protein miktarı, Renk analizi, Serbest asitlik
Abbreviations: PCA, Principal component analysis; OD, Optical density, ANOVA, Analysis of variance

1. INTRODUCTION

Honey, bee-pollen, propolis, royal jelly, and bee venom are among the most popular bee products which have been used in traditional medicine. These products have been known to increase body resistance due to their bioactive ingredients (Kolaylı & Keskin, 2020). In the recent years, scientific researches on the therapeutic effects, different bioactivities, physicochemical characteristics, and chemical compositions of bee products have been increased on a large scale (Alvarez-Suarez, 2017; Bogdanov, 2011; Kaygusuz et al., 2016; Nainu et al., 2021; Tezcan et al., 2011).

Bee bread (perga) is also a unique bee product which is poorly known until a few years ago due to the difficulties in collecting (Bakour et al., 2022). However, the appropriate collecting methods that beekeepers could perform without destroying the hive were improved. Thus, this product have been provided to human consumption. Bee bread is produced by the older female adult bees mixing pollen grains with nectar, honey, and other bee secretions such as digestive enzymes in their saliva. The mixture is subjected to lactic acid fermentation and becomes bee bread in about two weeks. As a fermented product, it is kept with a thin layer of honey and bee wax (Bakour et al., 2022).

The therapeutic properties of bee bread samples such as antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic activities were reported in the literature (Bakour et al., 2022; Mohammad et al., 2020; Nagai et al., 2004; Urcan et al., 2017; Pełka et al., 2021). The chemical compositions of bee bread from worldwide were also studied by many authors (Adaškevičiūtė et al., 2019; Bakour et al., 2019; Dranca et al., 2020; Ivanišová et al. 2015; Mohammad et al., 2020; Sobral et al., 2017; Urcan et al., 2018; Zuluaga et al., 2015). According to these studies, bee bread mainly composed of water, protein, carbohydrates, fatty acids, organic acids, vitamins, minerals, and several bioactive compounds.

Anatolia is pretty rich with regard to endemic vegetation due to its geographical location. Different bee products such as honey, bee-pollen, and bee venom from Anatolia have been evaluated by our group before (Aftab et al., 2021; Akay et al., 2021; Kalaycıoğlu et al., 2017; Kalaycıoğlu et al., 2021). High antioxidant activities and nutritional contents such as minerals, vitamins, sugar, and organic contents were reported in these studies. Anatolian bee-

bread was evaluated by several groups (Bayram et al., 2021; Beykaya et al., 2021; Kaplan et al., 2016). However, studies on bee bread seem to be limited when compared with other bee products. It is shown that both the composition of bee bread and the amounts of the compounds varies depending on the region, melliferous plants and climatic conditions of that region, and collecting season (Andjelkovic et al., 2012; Baltrušaitytė et al., 2007). Bayram et al. (2021) and Beykaya et al. (2021) used chemometric techniques in order to show the discrimination between bee breads.

The objective of the present work was to determine some physicochemical properties such as pH, electrical conductivity, colour analysis, colour intensity, and optical density of bee bread samples collected from seven different regions in Anatolia. The chemical analysis such as moisture, ash, total protein contents and free acidity were also determined. Moreover, the data were evaluated by principal component analysis to discriminate the bee bread samples according to their origins.

2. MATERIALS and METHODS

2.1. Bee Bread Samples and the Sample Preparation for the Analysis

Bee bread samples were collected from different locations such as Bingöl, Kırşehir, Ankara, Trabzon, Zonguldak, Erzurum and Niğde during 2021 (Figure 1). All samples were stored at 4°C until further analysis.

Figure 1. Location map of sample sites

Bee bread samples were finely grounded in a laboratory mortar. Fifty hundred mg of powdered samples were accurately weighed. Each sample was extracted with 10 mL of

deionized water by vortexing for 5 min at 3000 rpm and sonicating for 15 min. The mixture was centrifuged at 3500 rpm for 15 min. The supernatant was filtered through 0.45 µm pore-sized micro filters before analysis. These extracts were used for the measurement of pH, colour intensity, colour analysis, optical density, electrical conductivity and the determination of total protein content and free acidity.

2.2. Chemicals

Sodium hydroxide (NaOH), sodium chloride (NaCl), potassium chloride (KCl), Coomassie Brilliant Blue G-250, ethanol, phosphoric acid (H_3PO_4), bovine serum albumin (BSA) were purchased from Merck (Darmstadt, Germany).

2.3. Physicochemical Analysis of Bee Bread Samples

2.3.1. Determination of pH and Electrical Conductivity

A pH meter (Thermo Scientific, USA) was used to measure the pH values of the bee bread samples.

Electrical conductivity of the samples was measured using a conductivity meter (Wissenschaftl Techn, Weilheim, Germany). The instrument was calibrated using 0.01 M KCl. The results were reported as mS/cm. The electrical conductivity of the deionized water was determined below 5 mS/cm.

2.3.2. Colour Analysis, Colour Intensity, and Optical Density

The colour analysis of the bee bread samples was measured according to Pfund colour scale which the widely used in the colour measurement of honey. Briefly, the absorbance value of the samples was meaured at 635 nm using a Shimadzu 1800 UV-VIS spectrophotometer (Japan). Pfund values of the samples were calculated according to the following equation (Sant'ana et al., 2013) (Equation 1).

$$mm\ Pfund = -38.70 + (371.39xA) \text{ (Equation 1)}$$

mm Pfund: Colour intensity in Pfund scale

A: Absorbance value of bee bread sample

The colour intensity was measured using the method of Beretta et al. (2005). The absorbance of bee bread extracts was measured at 450 nm and 720 nm using a spectrophotometer. The difference between the absorbance at 450 nm and 720 nm was expressed as mAU.

For the determination of optical density (OD), the absorbance of each bee bread sample extract was measured at 530 nm against deionized water using a spectrophotometer (Wakhle, 1997).

2.4. Chemical Analysis of Bee Bread Samples

2.4.1. Moisture Content

The moisture contents of the bee bread samples were measured by the loss on drying technique using a moisture analyzer (Shimadzu, Japan). The samples were weighed on moisture balance and superheated until the end of the drying period by infrared. The temperature was set at 105 °C. All the samples reached equilibrium at 10 min. The results were recorded as g/100g moisture.

2.4.2. Ash Content

Ash content of the bee bread samples was determined according to the methods of AOAC (1999). Each sample was accurately weighed as 0.2000 g and was placed in crucibles. Then, the samples were incinerated at 550 °C in a burning muffle for 3 h. After cooling at room temperature, the obtained ash was weighed. Ash percentage (g ash/100 g bee bread) of the samples were calculated according to the Equation 2:

$$\%Ash = \frac{m_{ash} x 100}{m_{sample}} \text{ (Equation 2)}$$

2.4.3. Total Protein Content

The total protein content of the bee bread samples was analyzed by the Bradford method with a little modification (Bradford, 1976). Twenty mg of Coomassie Brilliant Blue G-250 was dissolved in 10 mL 95% ethanol solution (95% ethanol-5% water, v/v). Then, 20 mL 85% H_3PO_4 solution (85% H_3PO_4 – 15% water, v/v) were added and the resulting solution was diluted to a final volume of 100 mL. Fifty hundred µL of each bee bread sample extract was added to 5 mL of this solution. The Coomassie Brilliant Blue forms a protein-dye complex. After 5 minutes of incubation, absorbance was measured at 595 nm against an albumin standard solution of bovine serum (10-100 µg/0.1 mL) in 0.15 M NaCl.

2.4.4. Determination of Free Acidity

Free acidity was determined by potentiometric titration. The solution was titrated with 0.05 mol/L NaOH to pH 8.30. The results were expressed in miliequivalent of acid per kg of bee bread.

2.5. Statistical Analysis

All the analysis was done three replicates. The results were given as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied to evaluate the significance of differences in the obtained mean values of the bee bread samples at $p \leq 0.05$. Principal component analysis (PCA) was also used to visualize the differences and similarities among the bee bread samples. All the statistical calculations were performed using Minitab 16 statistical software program for Windows.

3. RESULTS and DISCUSSION

3.1. Physicochemical Characterization of Bee Bread

The physicochemical parameters of the bee bread samples were presented in Table 1. As it is seen from the table, all the samples were acidic (pH: 3.66-4.16) that insures their freshness. Among the samples, the bee bread collected from Trabzon was the most acidic (pH: 3.66) followed by Zonguldak (pH: 3.84). The lowest acidity was detected in bee bread collected from Bingöl (pH: 4.16). Analysis of variances revealed that the values of pH of the tested bee breads were not identified as statistically different ($p > 0.05$). The pH values found in this study are comparatively in line with past reports for bee bread pH value: 4.04 in a Romanian bee bread (Dranca et. Al., 2020) and between 4.11- 4.44 in Lithuanian bee breads (2.93–4.08) (Adaškevičiūtė et al., 2019).

The electrical conductivity (EC) of all studied bee bread samples ranged from 4.63 to 5.64 mS/cm. The highest EC value was determined in bee bread samples from Trabzon (5.64 mS/cm) whereas the lowest EC value was recorded for Ankara (4.63 mS/cm). Performed ANOVA revealed that these differences were statistically significant at $p \leq 0.05$.

The colour of bee bread samples were evaluated using Pfund scale, colour intensity, and optical density. The highest Pfund value belonged to Zonguldak bee bread with 109.8 mm whereas the lowest Pfund value was registered with Trabzon bee bread (61.58 mm). The other samples were changed between 65.28 mm and 95.00 mm. USDA-approved colour standards (1985) reported that the samples having Pfund scale between 50 mm and 85 mm are light amber in colour whereas between 85 mm-114 mm value shows amber (USDA, 1985). According to this standard, bee breads from Zonguldak and Erzurum were amber in colour whereas all the other samples were light amber. There is a significant difference in colour between all studied types of bee bread ($p \leq 0.05$).

Colour intensities of the bee bread samples were represented by the AB450. AB450 values ranged from 94.03 to 194.6 mAU (Table 1). The results showed that there is a significant difference between studied types of bee bread in colour intensity ($p \leq 0.05$). Zonguldak bee bread, which showed the highest Pfund value, also presented the highest colour intensity (194.6 mAU) followed by Erzurum (129.6) and Bingöl (107.6 mAU) bee breads while Trabzon showed the lowest colour intensity (92.66 mAU). The correlation between colour and colour intensity was found to be as 0.89.

The optical density which is a means of colour classification were changed between the 0.27 (Trabzon bee bread) and 0.40 (Zonguldak bee bread) mAU. A statistically significant difference was obtained for the optical density of the samples ($p \leq 0.05$). The correlation between colour and colour intensity was found to be as 0.86.

Table 1. Physicochemical characteristics of bee bread samples

Bee bread	pH	EC (mS/cm)	Colour (Pfund scale, mm)	Colour intensity (mAU)	Optical density (mAU)
Ankara	3.91 ± 0.03	4.63 ± 0.02	65.28 ± 1.12	94.03 ± 4.16	0.32 ± 0.03
Bingöl	4.16 ± 0.05	5.06 ± 0.03	80.15 ± 2.63	107.6 ± 4.16	0.36 ± 0.01
Erzurum	4.03 ± 0.04	5.02 ± 0.04	95.00 ± 3.09	129.6 ± 4.6	0.38 ± 0.02
Kırşehir	3.84 ± 0.01	5.01 ± 0.04	69.00 ± 1.65	97.6 ± 2.3	0.29 ± 0.01
Niğde	3.92 ± 0.02	5.40 ± 0.05	69.00 ± 2.10	98.2 ± 3.4	0.29 ± 0.01
Trabzon	3.66 ± 0.02	5.64 ± 0.03	61.58 ± 2.18	92.66 ± 4.1	0.27 ± 0.01
Zonguldak	3.80 ± 0.01	5.10 ± 0.04	109.8 ± 3.9	194.6 ± 2.4	0.40 ± 0.04

Each of the physicochemical characteristics mentioned in this study were reported for Anatolian bee breads for the first time.

3.2. Chemical Characterization of Bee Bread

Table 2 displays the moisture, ash, total protein content and free acidity in bee bread samples. Moisture content is closely related with quality and stability of bee products. Bee bread is known as a hygroscopic material which is highly reactive to fungal or microbial molecules. In order to avoid this, bee bread is generally stored in dried or frozen form. The moisture of bee bread samples at 105°C was found to be between 11.54-23.07 g/100g. Bee bread collected from

Trabzon showed the highest moisture content (23.0 g/100g) whereas Kırşehir bee bread presented the lowest moisture content (11.54 g/100g). Performed ANOVA revealed that these differences were statistically significant at $p \leq 0.05$. The results obtained in this study were in consistent with the other bee bread samples from Anatolia. Beykaya et al. (2021) reported the moisture content of 10 bee bread sample between 10.13-18.10 g/100g. In the study of Kaplan et al. (2016), 8 Anatolian bee bread samples showed different moisture contents changing between 11.41 and 15.89 g/100g.

Table 2. Chemical characteristics of bee bread samples

Bee bread	Moisture (g/100g)	Ash (g/100g)	Total protein (g/100g)	Free acidity (mEq/kg)
Ankara	19.60 ± 0.05	1.97 ± 0.01	20.16 ± 1.12	531 ± 17
Bingöl	17.54 ± 0.02	2.93 ± 0.02	22.18 ± 2.63	493 ± 12
Erzurum	20.00 ± 0.09	2.78 ± 0.04	23.85 ± 3.09	518 ± 16
Kırşehir	11.54 ± 0.01	2.82 ± 0.03	23.09 ± 1.65	548 ± 18
Niğde	12.96 ± 0.03	2.85 ± 0.05	24.02 ± 2.10	527 ± 15
Trabzon	23.07 ± 0.10	2.44 ± 0.01	21.67 ± 2.18	569 ± 20
Zonguldak	19.04 ± 0.04	2.39 ± 0.02	22.45 ± 3.9	558 ± 18

The content of ash which attributes to any inorganic materials such as mineral, was between 1.97 (Ankara bee bread)-2.93 (Bingöl bee bread) g/100g. There is no significant difference remarked between samples in ash conttent ($p > 0.05$). The result was in the similar range for bee bread samples from different regions in Anatolia and the world. Kaplan et al. (2016) reported the ash content of Anatolian bee bread samples as 1.93-2.62 g/100g. The mean ash content for Columbian bee bread was reported as 2.45 (Zuluaga et al., 2015) and 3.42% in Romanian bee bread (Dranca et. al., 2020).

Standard protein level in bee pollen is reported as 15 g/100g. The total protein of all bee bread samples was found to be above this standard as seen in Table 2. The highest total protein content belonged to Niğde bee bread (24.02 g/100g) whereas Ankara bee bread had the lowest total protein content (20.16 g/100g). The results were found to be significantly different ($p \leq 0.05$). In the study of Kaplan et al. (2016) total protein contents of Anatolian bee breads

changed between 14.82 and 24.26 g/100g. A Romanian bee bread was reported as including 18.60 g/100g (Dranca et. al., 2020). The mean total protein content of bee bread reported in the literature changed as follows: 19 g/100g in Anatolian bee bread (Beykaya et al., 2021), 19.96 g/100g in Moroccan bee bread (Bakour et al., 2019), 22.26 g/100g in Malaysian bee bread (Mohammad et al., 2020), and 23.1 g/100g in Columbian bee bread (Zuluaga et al., 2015).

Free acidity ranged from 493 meq/kg in bee bread sample from Bingöl and 569 mEq/kg in Trabzon bee bread. These results confirmed the acidic character of the bee bread. It was revealed that the differences in free acidity of bee bread samples were statistically significant at $p \leq 0.05$. Free acidity values found in this study were in consistent with the other studies from literature. The free acidity of a Romanian bee bread was found to be as 543.2 mEq/kg (Dranca et. al., 2020). The mean free acidity of Moroccan bee bread samples was reported as 400 mEq/kg (Bakour et al., 2019).

3.3. PCA Analysis

In order to identify similarities and specificities of bee bread samples analyzed, principal component analysis (PCA) was conducted. Correlated variations with each other was detected whereas the contribution of some variations have lower. As can be seen from Figure 2, except colour intensity, none of the variables are correlated with any principal components (PCs) directly. The negative part of first component (PC1) is related with free acidity, EC, and moisture; the rest of the variables are related with the positive part of PC1.

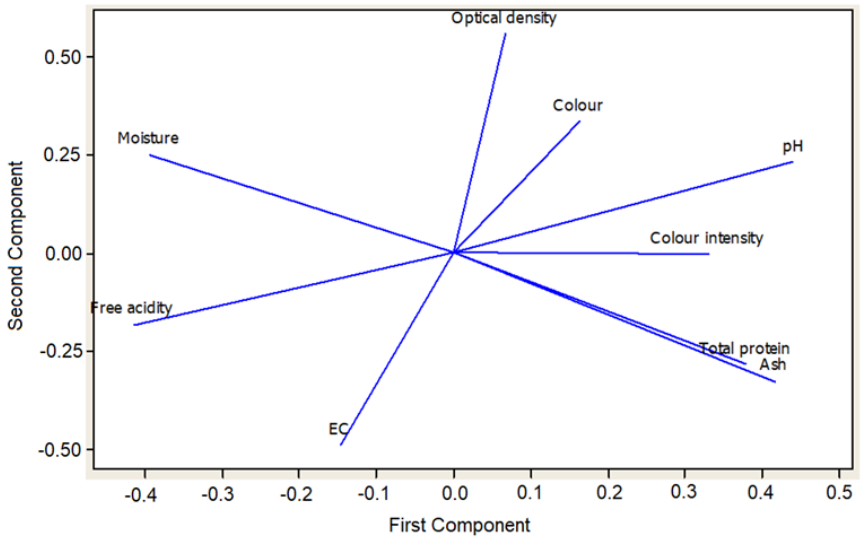

Figure 2. Distribution of the loadings plot for PC1- PC2, generated from a correlation-matrix PCA

The score plot (Figure 3) shows that bee breads separated into four groups. Erzurum and Bingöl bee breads are grouped in the positive part of both PC1 and PC2. Kırşehir and Niğde

bee breads are also close to each other in the positive part of PC1 and negative part of PC2. The samples collected from Ankara and Zonguldak are in negative part of PC1 and positive part of PC2. Only the sample collected from Trabzon is an outlier on the bottom left. The reason for that is significantly lower pH and higher EC values than those of other samples. Therefore, the given variables can be used to distinguish these bee bread samples.

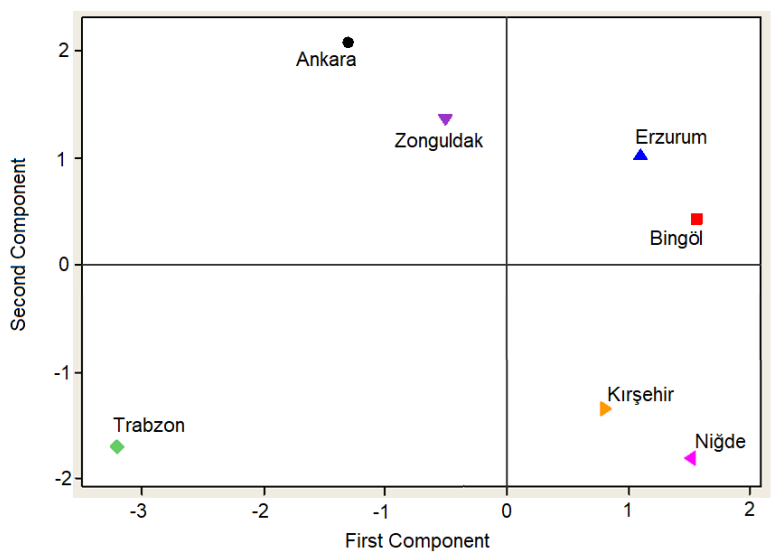

Figure 3. Score plot of bee bread samples on PC1-PC2.

4. CONCLUSION

In recent years, the interest in finding biologically active natural products have been increased with the human population growth. Bee products, which include many components nececssary for basic life functions, are one the important natural products. It is necessary to investigate the chemical properties of these marketed products to contribute to the standardization. However, the scientific studies on bee bread are still scarce. In this study, some physicochemical characteristics and chemical properties of 7 Anatolian bee bread collected from different regions were presented. The results revealed statistically significant differences among the samples in terms of electrical conductivity, colour analysis, moisture, ash, protein content and free acidity. According to the results, bee bread can be preferred as a good source of protein. Moreover, this study shows that PCA appear to be a potential tool for discrimination of bee bread using their physicochemical and chemical profiles tested.

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DECLARATIONS

The author declare that has no conflicts of interest.

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