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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

PHYSICOCHEMICAL, BIOCHEMICAL AND SENSORY CHARACTERIZATION OF BEE BREAD FROM BURSA (TÜRKİYE) REGION

Türkiye'de Bursa bölgesinde Arı Ekmeğinin Fizikokimyasal, Biyokimyasal ve Duyusal Karakterizasyonu

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ABSTRACT

Bee bread, also known as perga, is a product created through anaerobic lactic fermentation, meticulously crafted by bees. Worker bees mix collected pollen with nectar and their specialized enzymes, then pack and store this nutrient-dense substance in honeycomb cells. Bee bread is highly esteemed as a valuable food source due to its rich protein content, antioxidants, phenolic compounds, vitamins, and minerals. Its health benefits have been increasingly recognized in recent years. This study aims to investigate the physical and chemical properties, as well as the aroma constituents, of bee bread samples sourced from Bursa and its surrounding areas. The analysis includes measurements of moisture content (17.89%), ash (2.53%), crude fat (9.16%), and crude protein (19.06%). Additionally, total phenolic content was determined 9.91 mg gallic acid equivalent per gram (mg GA/g), total flavonoid content at 0.32 mg quercetin equivalent per gram (mg QE/g), CUPRAC activity at 12.97 mg Trolox equivalent per gram (mg Trolox/g), and TEAC activity at 0.55 mM Trolox per milliliter (mg Trolox/mL). Aromatic compounds were identified and their percentage ratios determined using Solid Phase Microextraction (SPME) combined with Gas Chromatography-Mass Spectrometry (GC-MS). These findings align with previous research in the field, although significant variations among parameters are noted due to factors such as geographic location, climate, vegetation, collection time, and sample collection methodology.

Keywords: **Bee Bread, Physicochemical Characterization, Bioactivity, Volatile Compounds, Sensory Properties**

ÖZ

Arılar tarafından toplanan polen, çeşitli enzimler ve bal ile karıştırılarak petek gözlerinde depolanır ve burada fermantasyona uğrar. Bu süreç sonunda anaerobik laktik fermantasyon ürünü olan arı ekmeği oluşur. Sonuçta arı ekmeği; işçi arıların topladıkları poleni, salgıladıkları nektar ve özel enzimlerle karıştırıp, petek hücrelerinde paketleyip depoladıkları değerli bir besin maddesidir. Arı ekmeği, yüksek

protein içeriği, antioksidanlar, fenolik bileşikler, vitamin ve mineraller ile sağlığa birçok faydalı etkisi olan ve son yıllarda değeri keşfedilmeye başlanan önemli bir arı ürünüdür. Bu çalışmanın amacı, Bursa ve çevresinden temin edilen arı ekmeği örneğinin fiziksel, kimyasal özellikleri ve aroma maddelerinin incelenmesidir. Bu çalışmada kül, nem, yağ, protein, antioksidan aktivite, toplam fenolik madde, toplam flavonoid madde ve aroma analizleri gerçekleştirilmiştir. Aroma bileşenlerinin tanımlanması ve yüzde oranlarının belirlenmesi, SPME tekniği kullanılarak GC-MS cihazında yapılmıştır. Arı ekmeğinin nem içeriği %17.89, kül içeriği %2.53, ham yağ içeriği %9.16, ham protein içeriği ise %19.06 olarak tespit edilmiştir. Toplam fenolik madde miktarı 9.91 mg GAE/g, toplam flavonoid madde miktarı 0.32 mg QE/g, CUPRAC aktivitesi 12.97 mg Trolox/g ve TEAC aktivitesi 0.55 mM Trolox/mL olarak belirlenmiştir. Sonuçlar literatürdeki diğer çalışmaları desteklemektedir. Parametreler arasındaki anlamlı farklılığın, arı ekmeğinin elde edildiği yer, iklim, bitki örtüsü, toplama zamanı ve toplama yöntemi gibi faktörlerden kaynaklandığı düşünülmektedir.

Anahtar Kelimeler: **Arı Ekmeği, Fizikokimyasal Karakterizasyon, Biyoaktivite, Uçucu Bileşikler, Tanımlayıcı Duyusal Analiz**

GENİŞLETİLMİŞ ÖZET

Amaç: Arı ekmeği, polenin arı vücut salgıları ile karıştırılıp petek gözlerinde depolanması ve burada fermantasyona uğraması sonucu oluşan doğal bir üründür. Arı ekmeği polenin fermente halidir ve karbohidrat, protein, yağ, vitamin ve mineral içeriği bakımından zengin bir üründür. Çalışmanın amacı, Bursa yöresinden elde edilen arı ekmeği örneğinin biyokimyasal karakterizasyonunun, antioksidan özelliklerinin, uçucu bileşenlerinin, tanımlayıcı duyusal özelliklerinin belirlenmesidir.

Gereç ve Yöntem: Çalışmada kullanılan arı ekmeği örneği 2021 yılında Bursa ilinden temin edilmiş olup analize kadar -18°C' de depolanmıştır. AOAC metodu kullanılarak, arı ekmeğinin nem içeriği, konveksiyonlu bir fırın içerisinde 105°C'de kurutularak sabit ağırlıkta gravimetrik olarak belirlenmiştir (AOAC, 2005). Ham protein değeri, Kjeldahl metodu kullanılarak tayin edilmiştir. Azot yüzdesini ham protein yüzdesine dönüştürmek için 6.25'lik bir dönüşüm faktörü kullanılmıştır. Ham yağ, AOAC yöntemine (AOAC, 1984) göre bir soxhlet cihazı ve *n*-hekzan kullanılarak ekstrakte edilmiştir. Numunelerin kül içeriği gravimetrik olarak belirlenmiştir (AOAC, 2005). Numunenin toplam fenolik içeriği Singleton ve Rossi (1965) tarafından tanımlanan Folin-Ciocalteau yöntemine göre belirlenmiştir. Toplam fenolik içerik, gallik asit ile hazırlanan standart eğrinin denkleminden hesaplanmıştır. Numunedeki toplam fenolik bileşik miktarı "mg GAE/ g numune" olarak ifade edilir. Numunenin toplam flavonoid içeriği Chang ve arkadaşları (2002) tarafından geliştirilen yöntemle belirlenmiştir. Standart olarak Kuersetin kullanılmıştır. Toplam flavonoid içeriği standart eğri denklemi yardımıyla hesaplanmıştır. Sonuçlar "mg QE (kuersetin eşdeğeri)/g" cinsinden ifade edilir. ABTS. analizi Re ve arkadaşlarının (1999) geliştirdiği yöntemle belirlenmiştir. Sonuçlar "mg TEAC troloks eşdeğer antioksidan kapasitesi)/g" cinsinden ifade edilir. CUPRIC-ION Arı ekmeğinin Antioksidan Kapasitesini Düşürücü etkisi Apak ve arkadaşları (2004) tarafından geliştirilen yöntemle bulunmuştur. Neocuproin (Nc) ve Cu(II) tarafından oluşturulan Cu(II)-Nc kompleks materyalinin spektrofotometrede 450 nm'de absorbe olan Cu(I)-Nc şelatına indirgenmesine dayanmaktadır (Thermo Scientific, Multiskan™ GO Microplate Spectrophotometer, USA). Uçucu Bileşik Analizleri Uçucu bileşiklerin tanımlanması ve miktarının belirlenmesi için Katı faz mikro ekstraksiyon tekniği (SPME) Gaz Kromatografisi-Kütle Spektrometresi (GC-MS) (GC 6890, MS 6890N, Agilent Technologies, Wilmington, DE, ABD) kullanılmıştır. Arı ekmeği örneğinin tanımlayıcı duyusal analizi Spectrum™ yöntemi kullanarak yapılmıştır. Yaşları 25-55 arasında değişen 6 eğitimli panelist (2 erkek ve 4 kadın) tarafından yürütülmüştür (Meilgaard ve diğerleri, 1999). Elde edilen sonuçlar örümcek ağı diyagramı ile gösterilmiştir.

Bulgular: Bursa Bölgesinden temin edilen arı ekmeğinin nem içeriği %17.89, kül içeriği %2.53, ham yağ oranı %9.16 ve ham protein oranı %19.06 olarak tespit edilmiştir. Toplam fenolik madde miktarı 9.91 mg GA/g, toplam flavonoid madde miktarı 0.32 mg QE/g, CUPRAC aktivitesi 12.97 mg Trolox/g ve TEAC aktivitesi 0.55 mM Trolox/ml olarak belirlenmiştir. GC MS analizi sonucunda numunede aldehitler, ketonlar, yağ asitleri, hidrokarbonlar ve karboksilik asitlerin yaygın olduğu tespit edilmiştir. Tanımlayıcı duyusal analiz sonucunda, arı ekmeği

için sekiz parametre belirlenmiştir. Bunlar; meyveli/kiraz, çam/reçine, ıhlamur çiçeği, kuru kayısı/erik, oksitlenmiş/balık yağı, ransit, odunsu, buruk. Panelistler tarafından arı ekmeği parametrelerine verilen puanlar sırasıyla; 2.83 meyveli/kiraz, 2.83 çam/reçine, 2.33 ıhlamur çiçeği, 2 kuru kayısı/erik, 1.5 oksitlenmiş/balık yağı, 4.25 ransit, 2 odunsu, 3.33 keskin olarak belirlenmiştir.

Sonuç: Bursa Bölgesine ait arı ekmeği örneğinin nem içeriği, kül içeriği, ham yağ içeriği, ham protein içeriği, antioksidan aktivite, toplam fenolik madde, toplam flavonoid madde ve aroma analizleri gerçekleştirilmiştir. Yapılan analizler doğrultusunda elde edilen sonuçlar literatürdeki diğer çalışmaları desteklemektedir. Arı ekmeğinin bileşimi iklim, coğrafi koşullar, bitki örtüsü, botanik orjin, arının türü, toplama zamanı, toplama yöntemi, depolama koşulları ve ekstrakte ediliş şekli gibi birçok faktörden etkilenmekte buna bağlı olarak parametreler arasında anlamlı farklılığa sebep olduğu düşünülmektedir.

INTRODUCTION

Bee products have gained significant traction as functional foods among consumers in recent years, owing to their rich nutritional content and bioactive properties. These products have emerged as compelling candidates for safeguarding and promoting human health. Within the realm of traditional and complementary medicine, apitherapy has garnered attention for its utilization of various bee-derived substances including honey, beeswax, pollen, propolis, apilarnil, royal jelly, bee bread and bee venom (Ekici and Gölgeli, 2021). Bee bread also known as perga, represents a natural nutrient used by bees to nourish offspring. Honey bees collect pollen from plants, mixing it with their digestive enzymes, honey and beeswax before storing it in honeycomb cells, where it undergoes lactic acid fermentation, culminating in the formation of bee bread within approximately two weeks (Gilliam, 1979).

Bee bread is renowned for its comprehensive nutritional profile, comprising essential amino acids, vitamins C, B1, B2, E, H, carotenoids and anthocyanins, saccharase, amylase, phosphatase enzymes and a myriad of minerals (Mutsaers et al, 2005). This nutrient-rich composition renders bee bread a vital protein source rich in essential amino acids, fats minerals, vitamins and flavonoids, serving as the primary sustenance for bees (Karaman et al., 2017). With approximately 20% protein, 3% lipid, 24-35% carbohydrates, 3% vitamins and minerals, bee bread emerges as a functional food owing to its potent ingredients, endowing it with antioxidant, antimicrobial, antiviral, antiarrhythmic, antibiotic, anti-inflammatory and anticancer properties (Khalifa et al.,2020; Ekici and Gölgeli, 2021). Bee bread is similar to bee pollen in terms of its compositional properties, but it has a richer content and contains proteins, amino acids, carbohydrates, lipids, vitamins, minerals, phenolic acids and polyphenols (Aylanc et al. 2021a). Moreover, bee bread, in its fermented state, surpasses pollen in nutritional value and digestibility, making it more readily assimilated by organisms (Habryka et al. 2016).

Bee bread is a natural product like other beekeeping products and has many nutritional, functional and biological properties due to the compounds in its structure. The biochemical characterization and biological activity properties of bee bread (perga) exhibit significant variability, influenced by factors such as geographical region, climate, bee species and plant types (Karataş and Şerbetçi, 2008). This study aims to elucidate the biochemical characterization, biological activity properties and antioxidant capacity of bee bread produced in the Bursa (Türkiye) region. Additionally, we seek to conduct analyses of volatile aroma compounds and descriptive sensory evaluations of bee bread, thereby contributing to a comprehensive understanding of its multi faceted attributes.

MATERIALS AND METHODS

Sample

Bee bread was obtained from honey producer in Bursa region during spring of 2021 and stored at -18°C until further analysis.

Chemicals

All chemicals used in the experiments were of analytical grade. Ethanol, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), sulfuric acid, gallic acid, trolox (6-hydroxy-2,5,7,8-tetramethylchroman carboxylic acid), quercetin, sodium chloride, 2-methyl valeric acid, 2-methyl-3-heptanone, Folin-ciocalteau solution, sodium carbonate, potassium acetate, aluminum nitrate from Sigma, Kjeldahl tablet, hydrochloric acid, hexane, were obtained from Merck.

Preparation of Extract from Bee Bread

Extraction procedure outlined by Zhou et al. (2015) was followed with some modifications. Briefly 1.5 g of bee bread sample was weighed and coarsely ground using a laboratory mortar. The ground bee bread was transferred a centrifuge tube and 10 ml (95%) ethanol was added. The sample was then subjected to ultrasonic bath treatment for 60 minutes at 40 °C to ensure homogenous disintegration. Subsequently, the tubes were centrifuged at 5000 rpm and 40 °C for 30 minutes. This procedure was repeated twice. The resulting supernatants were collected in a 25 ml beaker. This mixture was completed to 25 ml with 95% ethanol. It was filtered through 45 µm pore-sized micro filters before analysis (Mayda, 2019). The resulting extract was utilized for the determination of total phenolic substance amount, total flavonoid substance amount and ABTS. activity, CUPRAC activity.

Chemical Analysis of Bee Bread Samples

Moisture Content

Using the AOAC method, the moisture content of bee bread was determined by drying it gravimetrically in a convection oven at 105 °C to constant weight (AOAC, 2005). Moisture contents of the samples were measured using the loss on drying technique with the help of a moisture analyzer. (Shimadzu, Japan). Firstly, bee bread sample was ground to achieve homogeneity. Subsequently, 3 g of the sample was weighed and dried in an oven at 105 °C until it reached a constant weigh. After allowing it to cool down, the sample was weighed on a precision balance, and the percentage moisture content was calculated. The results were recorded as g/100g moisture.

Ash Content

The ash content of the bee bread samples was determined following the methods outlined in AOAC (1999). To determine the total ash, a 5 g portion of bee bread sample was weighed into a crucible of known weight and burned in a muffle furnace at 550 °C until a carbon-free white ash was obtained. Subsequently, the crucible was placed in a desiccator to cool and then reweighed. The procedure was repeated twice. The percentage ash content was calculated.

Total Protein Content

The protein content of the bee bread was determined using the Kjeldahl method (AOAC, 1990). A homogenized 1 g sample was weighed into the combustion tube and 1 Kjeldahl tablet (Merck) was added. Then 15 mL of H_2SO_4 (98%) was added, and the combustion process continued gradually until the blue clear color was obtained. Subsequently, the distillation process was applied to the solution in balloons. Upon completion of the, distillation process, the distillate was titrated with 0.1 N HCl (Merck) until a gray-lilac color was formed. The amount of HCl consumed in the titration was substituted into formula to determine the percentage nitrogen. The protein content was then calculated by multiplying this value by factor of 6.25.

Total Crude Fat Content

Fat content was determined using the Velp SER 148 solvent extraction system. Initially, 5 grams of sample was weighed on filter paper and placed in the extraction cartridge. The cartridge containing the sample was then inserted into the extraction system. Hexane was utilized as the solvent for extraction, with the process comprising 90 minutes immersion, 90 minutes washing and 90 minutes recovery processes at 130°C, respectively. Following the extraction procedure, the sample container containing the extracted oil was placed in an oven at 105°C overnight to ensure complete drying. Subsequently the oil content of the sample was calculated by weighing.

Analysis of Total Phenolic Content

The total phenolic content of the sample was determined according to the Folin-Ciocalteau method as recommended by Singleton and Rossi (1965). Gallic acid was used as standard. Total phenolic content was calculated using the equation derived from the standard curve prepared with gallic acid. The total amount of phenolic compounds in the sample is expressed as “mg GAE/ g sample”.

Total Flavonoid Content Analysis

The total flavonoid content of the sample was determined using the method developed by Chang et al. (2002), with quercetin (QE) as the standard. The total flavonoid content was calculated using the standard curve equation and expressed as "mg QE (quercetin equivalent)/g".

ABTS·+ Antioxidant Capacity Determination

The ABTS·+ antioxidant capacity of the bee bread was determined following the method developed by Re et al. (1999). The results were expressed as "mg TEAC (trolox equivalent antioxidant capacity)/g".

CUPRIC-ION Reducing Antioxidant Capacity Determination

The CUPRIC-ION Reducing Antioxidant Capacity of bee bread was assessed using the method developed by Apak et al. (2004). This method is based on the reduction of the Cu(II)-Nc complex to form the Cu(I)-neocuproine chelate, which exhibits absorbance at 450 nm. Spectrophotometric measurements were conducted using a Thermo Scientific Multiskan™ GO Microplate Spectrophotometer (USA).

Determination of Volatile Compounds

Volatile compound analysis was performed using Solid-Phase Microextraction (SPME) combined with Gas Chromatography-Mass Spectrometry (GC-MS) (GC 6890, MS 6890N, Agilent Technologies, Wilmington, DE, USA) for identification and quantification of volatile compounds. A HP-INNOWax column (60 m x 0.250 mm id x 0.25 µm film thickness) was used (J&W Scientific, Folsom, CA, USA). For the analysis, 5 g of bee bread was placed into a 40 mL SPME vial (Supelco, Bellafonte, USA) along with 5 ml of saturated sodium chloride solution and 2.5 µL of internal standard (consisting of 0.1 µL of 2-methyl valeric acid and 0.6 µL of 2-methyl valeric acid in 1 mL of methyl-3-heptanone). The mixture was incubated in a 50°C water bath (GFL, Model 1103, Burgwedel, Germany) for 30 minutes. Subsequently, the SPME fiber (2 cm-50/30 µm DVB/Carboxen/PDMS stable flex, Supelco, Bellafonte, USA) was exposed to the vial in the 50°C water bath for another 30 minutes, then injected into the GC-MS system. The carrier gas flow rate was set at 1.2 mL/min, with a furnace program initiating at 40°C for 5 minutes, followed by a ramp of 10°C/min to 230°C, maintaining the final temperature for 20 minutes. The National Institute of Standards and Technology (NIST, 2008) and Wiley Registry of Mass Spectral Data (Wiley, 2005) libraries were used for identification of volatile components. The amount of volatile components was determined based on their proportional abundance (Avsar et al. 2004).

Descriptive Sensory Assessment

The bee bread sample was stored under refrigerator conditions (4^0 C) until analysis. Before evaluation, the sample was allowed to equilibrate to room temperature for 30 minutes. Descriptive sensory analysis of the products was conducted using the Spectrum™ method; with six trained panelists (2 males and 4 females) aged between 25 and 55 (Meilgaard et al. 1999). The results obtained were presented using a spider web diagram.

RESULTS

Chemical Composition

The results of the nutritional composition, as shown in Table 1, indicated that proteins (19.06 ± 0.45 g/100 g Bee Bread) and fat (9.16 ± 0.06 g/100 g Bee Bread) were the primary macronutrients in bee bread. Minor components included ash (2.53 ± 0.21 g/100 g Bee Bread) and moisture (17.89 ± 0.13 g/100 g Bee Bread).

Table 1. The chemical content of bee bread.

Composition	Amount (g/100g Bee Bread)
Moisture	17.89 ± 0.13
Ash	2.53 ± 0.21
Protein	19.06 ± 0.45
Fat	9.16 ± 0.06

Antioxidant activity

Biochemical activity of bee bread was evaluated as a result of the study. In Table 2 the results of the biochemical activity were shown. Total phenolic content of bee bread was determined as 9.91 ± 0.87 mg GAE/g. The total amount of flavonoid substance was determined as 0.32 ± 0.07 mg QE/g for bee bread. Cu(II) ion reducing antioxidant capacity was 12.97 ± 1.8 mg Trolox/g for bee bread. ABTS radical scavenging activity of bee bread was calculated as 0.55 ± 0.001 mM Trolox/mL.

Table 2. Antioxidant activity, total phenolic and total flavonoid contents of bee bread.

Total amount of phenolic substance (mg GAE/g)	9.91 ± 0.87
Total amount of flavonoid substance (mg QE/g)	0.32 ± 0.07
ABTS (mM Trolox/mL)	0.55 ± 0.001
CUPRAC (mg Trolox/g)	12.97 ± 1.8

Descriptive Sensory Profile

The results of descriptive sensory evaluation were presented Figure 1. The sensory panel identified eight flavor descriptors for bee bread; fruity/cherry, pine/resin, linden flower, dried apricot/prune, oxidized/fish oil, rancid, woody, acrid. According to the scores provided by the sensory panel for the flavor of bee bread; fruity/cherry with value of 2.83, pine/resin with value of 2.83, linden flower with value of 2.33, dried apricot/prune with value of 2, oxidized/fish oil with value of 1.5, rancid with value of 4.25, woody with value of 2, acrid with value of 3.33.

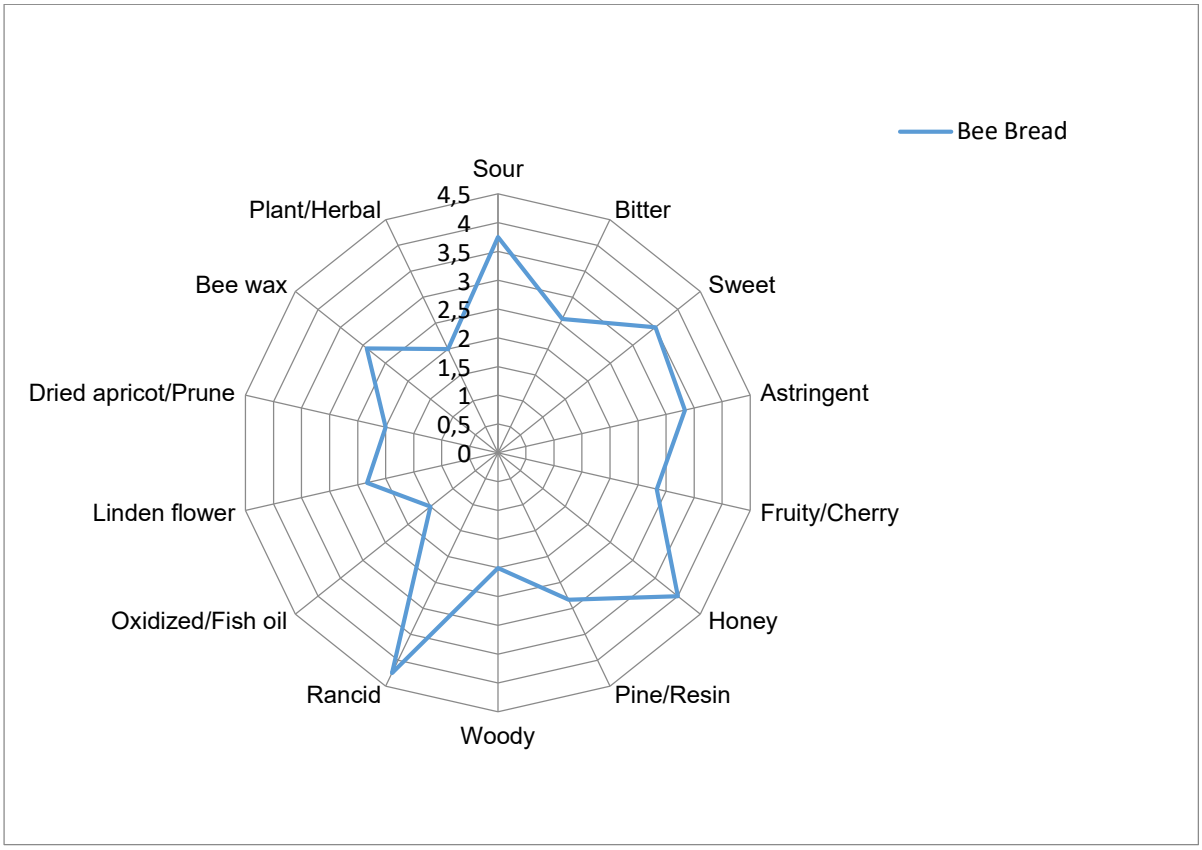

Figure 1. Sensory profile of bee bread.

Volatile Compounds

Volatile compounds determined in bee bread sample were presented in Table 3. As a result of the GC MS analysis, aldehydes, ketones, fatty acids, hydrocarbons and carboxylic acids were common in the sample.

Table 3. Aroma compounds detected in bee bread.

Compound	Odor	**Mean** (µg/100g)
Acetic acid	Sour, pungent, vinegar	16.92±5.73
Acetic acid, hexyl ester	Fruit, herb	0.08±0.11
Benzyl Alcohol	Sweet, floral	0.10±0.01
Borneol	Camphor	0.09±0.03
Butanoic acid	Rancid	7.29±8.24
Decanoic acid, methyl ester	Wine	0.51±0.15
Dodecane	Alkane	0.41±0.12
Dodecanoic acid, ethyl ester	-	0.17±0.04
Dodecanoic acid, methyl ester	-	0.84±0.47
Phenylethyl alcohol	Floral	0.20±0.13
Phenol	Phenolic	0.05±0.04
Furfural	Bready	1.17±0.73
Heptanoic acid	Cheesy	0.35±0.5
Hexanal	Green, fruity, oil	0.04±0.05
Hexanoic acid	Fatty, cheesy, soft	8.02±2.78
Hexanoic acid, ethyl ester	Apple peel, fruit	0.11±0.07
Hexanoic acid, methyl ester	Fruit, fresh, sweet	1.25±0.81
Camphene	Rosemary, volatile oil	0.13±0.05
Camphor	Camphor	0.62±0.48
Limonene	Lemon, citrus	1.47±0.48
Linalool	Floral, sweet, lavender	2.12±1.73
Methyleugenol	Clove, spice	0.34±0.17
Oxime-, methoxy- phenyl-	Honey	0.70±0.33
Nonanal	Waxy, Citrus	1.40±0.55
Nonanoic acid	Waxy, grass, oil	2.96±0.04
Nonanoic acid, ethyl ester	-	0.23±0.04
Octanoic acid	Cheesy	9.77±2.97
Octanoic acid, methyl ester	Orange	1.37±0.65
Octanoic acid, ethyl ester	Fruit, fat	0.50±0.01
Eugenol	Clove, honey	0.40±0.19
Pentanoic acid	Cheesy	0.36±0.05
Tetradecane	Alkane	0.33±0.1
1,8-Cineole	Mint	1.95±2.13
2-Phenylindolizine	-	0.38±0.54
2-Undecanol	Waxy	0.05±0.07
Pyrazine, 2, 6-dimethyl-	Chocolate	0.16±0.23
2-Butanoic acid 3-methyl-	-	3.85±3.03
Pentanoic acid, 3-methyl-	-	3.85±5.45
3-metil-2(5H)-furanone	-	0.07±0.04
2-Cyclohexen-1-one, 3,5,5 trimethyl-	-	0.55±0.47
4-Ketoisophorone	Moldy	0.14±0.09
6-Methyl-5-Hepten-2-One	Green, citrus,	0.78±0.12
ρ-Cymene	Solvent, gasoline, citrus	0.79±0.31
ρ-Cymen-2-ol	Citrus, moldy	0.02±0.03
ρ-Cymen-3-ol	Citrus, moldy	0.05±0.07
Alpha pinene	Pine, turpentine	0.09±0.07
Alpha Terpinolene	Terpenic	0.36±0.52
Beta Myrcene	Spice	0.35±0.41
Beta Thujone	-	0.38±0.36

Odor description source: flavornet.org

DISCUSSION

Current literature suggests that bee pollen and bee bread are excellent sources of PUFAs, which are essential for human nutrition and cannot be synthesized by the body. However, scientific research on bee bread is limited, highlighting the need for further studies (Silici, 2015). This study explores the physical and chemical properties, as well as the aroma constituents, of bee bread sample collected from Bursa (Türkiye) region. The analyses covered moisture, ash, protein, fat, antioxidant activity, total phenolic content, total flavonoid content, and aroma profile. It is well-established that the composition and nutritional value of bee products are influenced by plant species and environmental conditions (Küçük et al., 2024). Studies on bee bread from various regions remain scarce.

Regarding chemical composition, Karlıdağ et al. (2021) found that the protein content of fermented pollen was 18.70%. Mayda et al. (2020) reported that the average protein content of bee bread samples from different regions of Türkiye was 22.2%. Dranca et al. (2020) observed a protein ratio of 18.60% for bee bread sourced from the Iasi region of Romania. A study conducted in Colombia determined that the protein content of bee bread ranged from 19.10% to 27.30 % (Zuluga et al., 2015). In our study, the protein content of bee bread was found to be 19.6%, aligning with previous findings. Bee bread is known for its high protein content and it has been reported that the protein content may vary depending on the botanical origin (Waykar, 2016; Kaplan et al., 2016; Mohammad et al., 2021).

Bakour et al. (2019) reported a lipid content of 1.90 g/100 g in bee bread, while Kaplan et al. (2016) found lipid content ranging from 5.93 g/100 g to 11.55 g/100 g in samples from various regions. Another study reported that the lipid contents of 15 different bee bread samples collected in Colombia ranged between 1.65% and 5.50%. The study suggested that this variability is related to the fatty acid, carotene and vitamin contents of the pollen in the bee bread (Zuluaga et al., 2015). Andjelkovic et al. (2012) reported a range of 4.51% to 4.92% in Serbian bee bread. Our study revealed the fat content of bee bread to be 9.16%. The lipid content of bee bread varies greatly depending on the plant origin of the pollen. Lipids are represented by fatty acids, which contribute to the biological value of this bee product (Urcan et al., 2017).

Previous studies reported moisture content in bee bread as 15.6% (Zuluaga et al., 2015), 11.4% to 15.9% (Kaplan et al., 2016), and 9.85% (Bakour et al., 2017). Mayda et al. (2020) found moisture content in bee bread from various regions in Türkiye to range from 17.7% to 22.3%. In a different study conducted in Türkiye, it was determined that the moisture content of bee bread samples was in the range of 11.54-23.07 g/100g (Kalaycıoğlu, 2022). In our study, the moisture content was 17.89%. Bee bread has a high water content due to the hygroscopic properties of the pollen in its structure. This characteristic, along with the absorption of environmental moisture and the presence of bee secretions and honey, gives bee bread its sticky and moist texture (Mohammad et al., 2021).

The ash content in our study was 2.53%. Literature values for ash content include 2.45% (Zuluaga et al., 2015) and 3.32% (Bakour et al., 2019). A study in Malaysia found the ash content of bee bread produced by stingless bees to be 1.7 g/100 g (Ismail et al., 2018). Hazır and Özer (2019) reported an ash content of 8.14 g/100 g in their bee bread sample from Kayseri. Differences in ash content may be attributed to regional and bee species variations.

Biochemical characterization of bee bread is crucial for understanding its health effects and biological activities. In our study, the total phenolic content was 9.91 mg GAE/g. Karlıdağ et al. (2021) found the phenolic content of fermented bee pollen to be 6.12 mg GAE/g. Bayram et al. (2021) reported an average total phenolic content of 8.26 mg GAE/g in bee bread from various locations. Zuluaga et al. (2015) determined the phenolic content of Colombian bee bread to be 8.9 mg GAE/g. Literature shows significant variation in total phenolic content, influenced by factors such as the collection region, climate, bee breed, and plant species (Bayram et al., 2021).

The total flavonoid content in our study was 0.32 mg QE/g. Karlıdağ et al. (2021) reported 2.73 mg QE/g in fermented pollen sample. Mayda et al. (2020) observed an average content of flavones/flavonols 1.81 mg QE/g in bee bread from different regions in Türkiye. Urcan et al. (2018) noted that flavonoid content in bee bread is typically lower in flavones/flavonols compared to flavanones/dihydroflavonols. Another study reported total flavonoid content of bee bread 13.56 to 18.24 µg QE/g (Ivanišová et al., 2015). Zuluaga et al. (2015)

found flavonoid content to range from 1.9 and 4.5 mg QE/g.

The ABTS radical scavenging activity of bee bread was measured as 0.55 mM Trolox/mL. Mayda (2020) reported ABTS capacity values of bee bread between 0.375 mg TEAC/g and 1.55 ± 0.12 mg TEAC/g. Another study showed ABTS capacity ranging from 4.86 mg TEAC/g to 5.70 mg TEAC/g (Čeksterytė et al., 2016). Zuluaga et al. (2015) reported ABTS capacity between 46.1 µmol TEAC/g and 76.3 µmol TEAC/g, with an average of 61.5 µmol TEAC/g.

The Cu(II) ion reducing antioxidant capacity was 12.97 ± 1.8 mg Trolox/g in our study. Kutlu et al. (2023) reported CUPRAC values ranging from 9.18 ± 0.06 mg Trolox/g to 11.93 ± 0.76 mg Trolox/g. While specific studies on bee bread's CUPRAC antioxidant capacity are limited, Özparlak et al. (2017) reported CUPRAC reducing power activities of pollen extract equivalent to 77.12 mg TE/g. Ulusoy and Kolayli (2014) noted CUPRAC reducing power values between 33.1 µmol TE/g and 91.8 µmol TE/g in their study on Anzer bee pollen. Zuluaga et al. (2015) and Kocot et al. (2018) highlighted that the protein, minerals, and phenolic compounds contribute to the natural antioxidant properties of bee bread.

Information on volatile compound profiles in bee products is limited, with most studies focusing on honey (Starowicz et al., 2021). Kaškonienė et al. (2007) examined volatile compounds in honey and bee bread, noting that while some volatile components were common to both, bee bread had a slightly different profile. Compounds such as dimethyl sulfide, pentannitrile, furfural, benzaldehyde, nonanal, benzylnitrile, and decanal were found in both bee bread and honey, while others like 2-methylbutylnitrile, 3-methyl pentanoic acid, benzeneacetaldehyde, linalool, and octanoic acid were unique to honey.

According to HS-SPME/GC-MS results, Starowicz et al., (2021) identified 20 volatiles in beeswax and honey, 32 in bee bread, and 33 in pollen. Bee bread contained 32 volatile compounds, including 15 alkanes, 4 aldehydes, 5 acids, 2 benzenes, 2 ketones, and other compounds like disulfides, furans, pyrroles, and lactones (Starowicz et al., 2021). In our study, we identified 49 volatile aromatic compounds in bee bread. Kolaylı et al., (2024) reported 119 volatile aromatic compounds in the bee breads from Anatolia by SPME-GC-MS.

Volatile components are key to determining the taste and aroma of bee products. Bee bread's volatile profile differs from that of honey (Mayda, 2019). Common aroma components in bee bread include aldehydes, ketones, acids, alcohols, hydrocarbons, benzene, furan derivatives, and terpenes. Notable compounds in bee bread from monofloral and polyfloral sources include 1-heptadecen and acetic acid (Mărgăoan et al., 2020).

Bee bread is a mixture of honey and bee pollen, and the volatile aromatic compounds in this mixture play an important role in shaping its aroma profile (Kolaylı et al., 2024).

The unique sensory properties of bee products result from the combined and synergistic effects of their volatile compounds. This study identified six odor descriptors for bee bread: honey-like, sweet, acidic, pungent, waxy, and plant-based (Starowicz et al., 2021).

A recent study conducted a sensory analysis of 10 bee bread samples from various Turkish companies with 13 panelists (4 women and 9 men, aged 20-65). The sensory profile included descriptors such as fermented taste, bitterness, saltiness, sourness, caramelized taste, floral aroma, fruity aroma, sour smell, animal feed smell, distinctive smell, floral smell, and fruity smell (Soykan-Çiftçi, 2022). The taste, aroma, and color of bee bread (perga) vary depending on the pollen source plant (National Nutrition Council Bee and Bee Products Scientific Commission Report, 2022).

The composition of bee bread is influenced by factors such as climate, geographical conditions, plant variety, bee species, collection methods, storage conditions, and extraction techniques. The results of this study align with existing literature and suggest that significant differences in parameters are largely due to the location, climate, vegetation, collection time, and storage conditions of bee bread.

Conclusion: Based on the literature review, studies on the composition, phenolic profile and bioactive properties of bee bread produced in Türkiye are limited. Therefore, we believe that this study makes a significant contribution to the existing body of research. In this study, the biochemical characterization, antioxidant properties, volatile components, and descriptive sensory properties of bee bread were examined. The findings indicate that bee bread has a high antioxidant capacity and significant nutritional value from a nutritional

physiology perspective. The results suggest that bee bread could be a valuable source of nutrients and bioactive compounds for food supplements and functional foods. Consequently, it was concluded that bee bread can be consumed as a functional food. However, further studies are needed to develop new functional foods related to bee bread.

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