



Comparison of The Bioactive Content and Antioxidant Capacity of Bee Bread Samples from Different Beehives

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ABSTRACT

Bee bread has recently gained a lot of attention as a nutritional supplement among bee products due to its functional properties. However, the bioactive composition of bee bread varies depending on many different variables. The aim of this study was therefore to characterize the bioactive ingredients of 12 different bee bread samples originating from the same apiary but from different hives and to evaluate the differences between them. For this purpose, the samples of bee bread were analyzed for total phenolic content, total flavonoid content and antioxidant activity. The analysis revealed that the bee bread samples contained total phenolics in the range of 8.4 ± 0.61 to 26.38 ± 1.83 mg GAE/g, whereas their total flavonoid levels ranged between 0.53 ± 0.04 and 6.90 ± 0.67 mg QE/g. In addition, antioxidant activity values were found to vary from 1.41 ± 0.07 to 7.01 ± 0.14 mg TE/g. According to the results, the bioactive potential of the bee bread samples supports the possible use of this product both as a functional food and in various industrial fields.

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Introduction

Bee bread is a nutrient-rich and healthy bee product (Kieliszek et al., 2018). Bees produce this product by combining pollen with digestive enzymes and storing it in honeycomb cells (Vásquez and Olofsson, 2009). When bee bread is produced in the hive, it combines with honey, pollen, and various bee secretions, and subsequently undergoes lactic acid fermentation. The mixture turns into bee bread within about two weeks, so that the fermented bee bread can be stored in the hive for a longer period of time (Bogdanov, 2011). This product contains honey, pollen and the secretions of the bees' salivary glands. Bee bread plays an important role in nutrient and development as a source of protein, vitamins and lipids for worker bees after three days of feeding with royal jelly. It should be bee bread is similar to pollen in composition, but it is richer in amino acids, protein, lipids, *carbohydrates*, *vitamins*, minerals, polyphenols and phenolic acids. Leucine aminopeptidase, acid phosphatase and alpha-glucosidase are the most common digestive enzymes found in bee workers (Gilliam et al., 1989). In addition, the removal of the multi-layered wall surrounding the pollen grain by fermentation makes it easier for humans to digest, which has a positive effect on nutrient absorption (Şimşek et al., 2023; Aylanc et al, 2023; Aylanc et al, 2021a; Silici, 2015). Bee bread is not only a source of energy and protein for bee

colonies, but also an important building block for a healthy bee colony. Bee bread plays a crucial role in fundamental processes in the bee colony, including larvae growth, the resilience of worker bees and the queen's ability to reproduce. The essential nutrients present in bee bread support the rapid growth and developmental processes of young bees, while simultaneously contributing to the maintenance of vital metabolic functions in older bees through proteins, vitamins, minerals, and other bioactive compounds. In particular, the healthy development of larvae is made possible by the rich protein and amino acid content of bee bread (Gümüş and Karslı, 2024; Kieliszek et al., 2018).

Bee bread, phenolic compounds found in have been reported to have anti-inflammatory, antioxidant, anticancer, and antidiabetic activities, as well as effects on enzyme activation, hormone regulation, and gene expression (Aylanc et al., 2021a). Bee bread the composition varies depending on its seasonal conditions and botanical origin (Baltrušaitytė et al., 2007). Bee bread serves as the primary source of nourishment for larvae and young worker bees that are responsible for producing royal jelly within the hive, and due to the fermentation process it undergoes, bee bread possesses a higher nutritional value and enhanced bioavailability of nutrients when compared

to bee pollen. Due to its high free amino acid content and easily digestible sugars, it is a more easily digestible food (Bakour et al., 2019). Bee bread contains protein, fat, and lactic acid and a high amount of carbohydrates. Bee bread has a higher bioavailability than pollen and can be partially digested through fermentation (Khalifa et al., 2020). Bee bread is considered an important source of bioactive compounds with antioxidant, antibacterial, anti-inflammatory, and anticarcinogenic activities (Kolaylı et al. 2024). Today, bee products are among the most valuable natural products (Kabakcı, 2024). In a study conducted by Karadağ et al. (2020), it was reported that there are 294 species comprising 304 plants from 57 families in the Muş Alparslan University campus area, including herbaceous, shrubby, and tree forms. Species belonging to the Apiaceae, Asteraceae, and Boraginaceae families were found to be densely populated in the campus area.

The aim of this study was to determine the total phenolic, total flavonoid, and antioxidant activity values of bee bread obtained from different honeybee colonies located on the Muş Alparslan University campus.

Materials and Methods

Materials

To obtain the bee bread samples used in the study, 12 randomly selected 10-frame hives containing bees and an equal number of brood frames were chosen from 60 honeybee colonies located on the Muş Alparslan University campus. The queens used in the study were one-year-old Caucasian hybrids. Twelve empty frames were then placed in these hives. Each empty frame was placed as the 9th frame in the hive, following the hive's frame arrangement.

The colonies were inspected every 3 days to check the bee bread stocks in the frames. After approximately 1.5 months, it was determined that a large portion of the bee bread had been stored, and the frames were harvested using a perga spoon. The collected bee bread (perga) was stored at -20°C for analysis.

Methods

Preparation of the Samples for Extraction

Extraction of the bee bread samples was performed using a modified version of the method recommended by Mayda et al. (2020).

Determination of the Total Phenol Content

The total phenol content of the extracts was analyzed according to the Folin-Ciocalteu method recommended by Singleton et al. (1999).

Determination of the Total Flavonoid Content

The flavonoid concentration of the extracts was determined through an adapted version of the method described by Zhishen et al. (1999).

Determination of Antioxidant Activity

The antioxidant activity of the extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay method as recommended by Brand-Williams et al. (1999)

Statistical Analysis

All analytical results are presented as mean values $\pm$ standard deviation (SD) for each extract. The statistical significance of the findings was evaluated through one-way ANOVA, and differences among groups were further distinguished using Tukey's post hoc test. Statistical analyzes were performed using the MINITAB 21 software package.

Research Results and Discussion

Natural compounds from plants, classified as primary or secondary metabolites, have attracted the attention of researchers since ancient times. Although secondary metabolites do not take part directly in metabolic pathways, they contribute significantly to plant defense mechanisms and adaptation against environmental stress conditions. Phenolic compounds belong to the secondary metabolites and are of great importance due to their functional properties and have been extensively studied by researchers. Since phenolics are found in all plants, research has mainly focused on the discovery of plant sources rich in phenolic compounds (Saini et al., 2024).

From this perspective, the number of studies on bee bread, which has been reported in various studies to be a significant source of phenolic compounds, has increased significantly in recent years (Gerçek et al., 2024; Bayram et al., 2024; Hashemirad et al., 2024; Çolak et al., 2025). Therefore, in this research, assays for total phenolics, flavonoids, and antioxidant activity were carried out on freshly collected bee bread samples obtained from 12 distinct beehives. According to the results, the samples exhibited total phenolic levels ranging from 8.4 to 26.38 mg GAE/g.

Among all the analyzed bee bread samples, the maximum concentration of total phenolic compounds was quantified in sample BB1, indicating that this particular sample possessed the richest phenolic profile within the studied samples. In contrast, the lowest level of total phenolic content was detected in sample BB10, suggesting that this sample contained comparatively fewer phenolic constituents than the others (Figure 1).

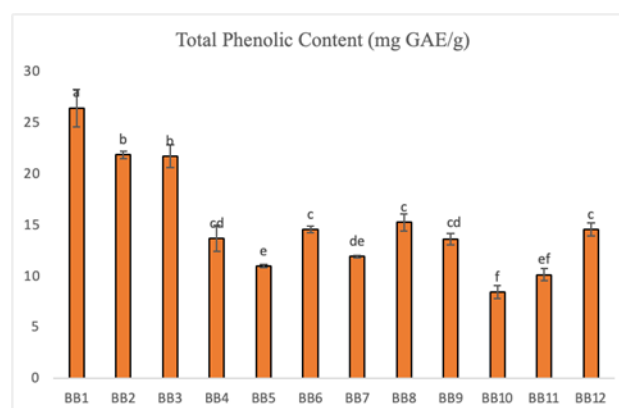


Figure 1: Total content of phenolic substances in the bee bread samples (distinct superscript letters a–f show significant differences, $p < 0.05$)

However, consistent with the trend observed in the total phenolic content, the maximum total flavonoid concentration was identified in sample BB1, with a measured value of 6.9 mg QE/g, whereas the minimum total flavonoid level was quantified in sample BB10, where the concentration was determined as 0.53 mg QE/g (Figure 2).

Similarly, numerous scientific studies have investigated the total phenolic content of bee bread samples. These studies often focused on samples obtained from various botanical sources and different geographical regions. The findings have provided valuable comparative information, revealing how environmental conditions, floral diversity, and regional characteristics can significantly influence the phenolic composition of bee bread. In the comprehensive study conducted by Mayda et al. (2020), bee bread samples collected from different regions of Türkiye (from the provinces of Ankara, Bursa, Kırklareli, and Rize) were analyzed to determine their phenolic and flavonoid contents. The results indicated that the total phenolic content of the samples ranged between 8.26 ± 0.299 and 12.71 ± 0.170 mg GAE/g. In terms of flavonoids, the concentrations were found to vary from 1.81 ± 0.040 to 3.74 ± 0.055 mg QE/g. These findings demonstrate that the phytochemical composition of bee bread may differ significantly depending on its geographical origin. The researchers determined from the palynological analysis that not only the geographical origin but also the botanical origin of these samples differed, and they related the differences in content obtained to this variability.

Similarly, in a study conducted by Sawicki et al. (2022), the total phenolic content in extracts of bee bread samples from Poland prepared with 80% methanol was determined to be $8,23 \pm 0,24$ mg GAE/g, which is in a similar range to the values obtained in our study. The total flavonoid content was reported to be about 5 mg QE/g. In another study conducted with 12 different bee bread samples of *Apis mellifera carpatica* bees from regions of Romanian geography and different botanical origin, the characterization of the bioactive compounds of the extracts prepared with 70% ethanol and ultrasonic water bath method was similar to our study.

According to the data obtained in this study, the total phenolic and flavonoid contents of the bee bread extracts varied between 7,10–18,30 mg GAE/g and 0,45–1,86 mg QE/g, respectively. Based on the results of the palynological analysis, the researchers found that they could not detect any significant differences between the dominant plant families observed in the samples.

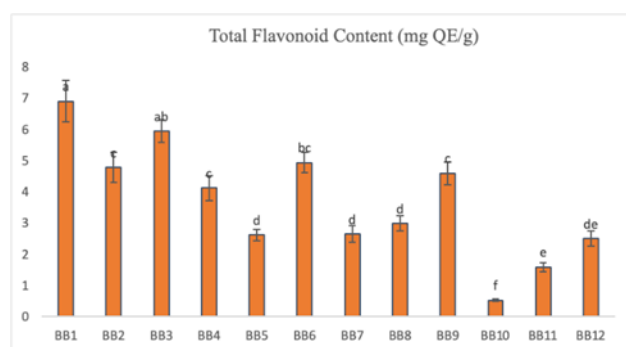


Figure 2: Total flavonoid content of the bee bread samples (different letters (a-f) indicate statistically significant differences ($p < 0.05$))

Bee products are functional foods with bioactive properties such as antioxidant, antimicrobial, anti-inflammatory, anti-cancer, immunomodulatory and anti-allergic (Hashemirad et al., 2024). One of the key characteristics of these natural products lies in their strong antioxidant potential. This property enables them to neutralize reactive oxygen species and free radicals, which are known to cause oxidative damage at the cellular level. By reducing oxidative stress, they help to protect cells and tissues from harmful effects, thereby playing a supportive role in the prevention of various diseases. Therefore, interest in the antioxidant potential of bee bread, one of these natural resources, has increased significantly in recent years (Martinello and Mutinelli, 2021). The current investigation evaluated the antioxidant capacity of bee bread sourced from the same apiary but originating from different hives by employing the DPPH method. The antioxidant activity values of fresh bee bread samples ranged from 1.41 to 7.62 mg TE/g (Figure 3). Similarly, the content of bioactive substances in extracts prepared by the ultrasound-assisted methanol method from 37 different fresh bee bread samples from different regions of Ardahan province in Türkiye was characterized in detail (Aksoy et al., 2024). In this study, the radical scavenging activities of the samples were determined through the DPPH assay. The results showed that the antioxidant activity values ranged from 3.13 to 3.90 mg TEAC/g. Furthermore, the researchers showed that the antioxidant activity was positively related to the total phenolic content, demonstrating that as the number of phenolic compounds increased, the antioxidant potential of the samples also rose proportionally. In addition, the antioxidant activity of ethanolic extracts of uniflorous (*Lotus*, *Trifolium*, *Xeranthemum* spp.) and multiflorous bee bread samples, which were shown to be of different botanical origin, was found to range from 1.2 to 5.5 mg TEAC/g. The highest value for antioxidant activity was found in the unifloral sample of *Trifolium* spp. and the lowest in the multifloral bee pollen sample (Çobanoğlu et al., 2023). This clearly shows that the botanical differences of the samples are an effective factor for antioxidant activity. In another study in which the antioxidant activity of bee bread samples from bee species (*Apis cerana*, *Apis mellifera*, *Trigona* spp.) was investigated, significant differences were found between the samples; the highest antioxidant activity values were found in bee bread samples from *A. cerana*, *Trigona* spp. and *A. mellifera*, respectively. The researchers reported that bee bread samples from *Apis cerana* had higher values in terms of antioxidant activity, total phenolic and flavonoid content and microbiological quality than bee bread samples from other species (Jaya et al., 2020). These results clearly show that different bee species have a decisive influence on the bioactive content of bee bread. These results showed that the total phenolic and flavonoid content and antioxidant activity of the bee bread samples used in our study were similar to the profiles reported in the literature. However, some differences were also found. These differences support many studies in the literature and are probably due to the effects of geographical origin, botanical source, collection time, harvesting methods and processing conditions on the content of bioactive compounds in the bee bread samples. Although the samples used in our study were collected under the same

conditions (geographical origin, processing conditions and harvest time), the data obtained show that the fermentation processes may vary between bee colonies and that this may affect the content. Furthermore, it is hypothesized that in addition to the predominant plant sources, minor plant sources can also significantly influence the profile of the bee bread. Although no direct analysis of the botanical origin of the bee bread samples was conducted in this study, it can be assumed that the distribution of minor plants contributing to the botanical composition of the samples is not identical and therefore there may be some differences in plant diversity that affect the content. In fact, the observed differences in phenolic compounds extracted from plants can be explained in this way. This clearly shows that the diversity of plant species contributing to the composition of bee bread and the proportions of these species can have a significant impact on the total phenolic and flavonoid content.

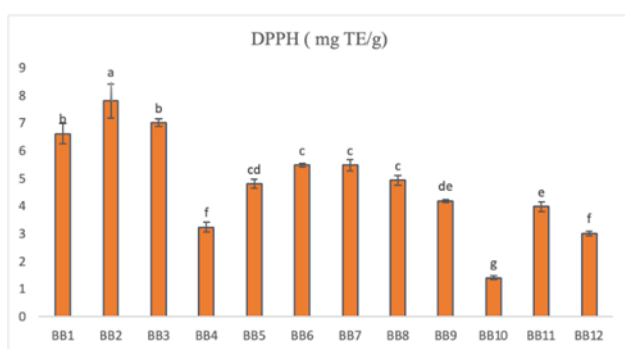


Figure 3: Results on the antioxidant activity of bee bread samples (a–f lettering reflects statistically significant differences, $p < 0.05$)

Conclusion

Thanks to its bioactive characteristics, bee bread has become a remarkable naturally occurring product in recent years, both as a dietary supplement and in apitherapy and therapeutic uses. In this study, the phytochemical profiles of bee bread samples obtained from the same apiary but from different hives were comparatively evaluated. The values obtained for total flavonoids, total phenols, and antioxidant activity showed the remarkable nutritional potential and therapeutic properties of the product. The results underline the importance of vegetation and environmental conditions for the phytochemical profile of this bee product. In this context, more extensive research linking bee bread to its botanical and geographical origin will contribute to its more effective and controlled use as a functional food.

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