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Microbiological quality and nutritional values of honey bee pollen

Mikrobiologische Qualität und Nährwerte von Honigbienenpollen

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Summary

The present study was aimed at examining the microbiological, physicochemical, and antioxidant properties of bee pollen samples collected from beekeepers in western Turkey. The results ranged between 2.24–6.87 log CFU/g, 1.98–3.77 log CFU/g, <1.00–2.51 log CFU/g, 4.19–5.30 log CFU/g, and 1.98–3.74 log MPN/g for total mesophilic aerobic bacteria, yeasts, molds, lactic acid bacteria, and total coliform, respectively. *S. aureus* was under detectable level. *Salmonella* spp. and *E. coli* O157:H7 were not detected in the pollen samples. The mean value of the dry matter, ash, titratable acid, pH, ascorbic acid, total carbohydrate, protein, fat, total phenolic content, FRAP, and TEAC of the bee pollen samples were 76.760%, 1.993%, 3.376%, 4.52, 32.047 mg/100g, 5.394 mg/g, 27.609 mg/g, 5.519%, 4.664 mg GAE/g, 29.644 µmol Trolox/g, and 1.736 µmol Trolox/g, respectively. The color values of the tested pollens were 56.923, 7.556, 26.456, 27.604, and 74.138 for *L**, a*, b*, C*, and H*, respectively. Although the samples were collected mainly from one province (Muğla) of the Aegean Region (a geographical region by the Mediterranean Sea), a significant variation in the pollen compositions was observed.

Keywords: Bee pollen, food safety, physicochemical, antioxidant, microbiological quality

Introduction

Bee pollen has been known as a rich food source for centuries. It is also recognized as a valuable apitherapeutic product in health and nutrition applications (Gonzalez et al., 2005; Kačániová et al., 2012; Estevinho et al., 2012; Matuszewska et al., 2021). Honey bees use pollen as a food source due to its high content of protein, carbohydrate, lipid, mineral, vitamin, carotenoid, phenolic compound, flavonoid, sterol, and terpene content (Feás et al., 2012 Thakur and Nanda, 2018; Thakur and Nanda, 2021; Matuszewska et al., 2021). It is commonly named ‚'the only perfectly complete food" because of its high content of nutrients (Asmae et al., 2021). Bee pollen has an important role in human nutrition with rich compositions of carbohydrates, proteins, enzymes, fatty acids, carotenoids, phenolic compounds, flavonoids, sterols, terpenes, minerals, and vitamins. Pollen also has a high content of free amino acids, especially essential amino acids and fatty acids such as Omega-3 and Omega-6 (Estevinho et al., 2012; Feás et al., 2012; Hani et al., 2012 Thakur and Nanda, 2018; Thakur and Nanda, 2020).

Due to the demand for natural and functional products in recent years, bee pollen has been consumed by children, the elderly, pregnant women, and patients easily and frequently. The microbiological and functional properties of bee pollen can be affected by production methods and storage conditions. Bee pollen can be easily contaminated by bees, air, plant parts, insects, animals, people, and other agricultural equipment (Hani et al., 2012). Considering the nutritional content, various microorganisms can grow in bee pollen. If the harvesting, storage, and marketing applications are not suitable, molds can grow and cause intoxication by producing mycotoxins such as aflatoxin and ochratoxin A (Gonzalez et al., 2005). Bee pollen is a popular product due to its positive effects on health, so it must be a safe food. Bee pollen is gaining commercial interest due to its high nutritional value and medicinal properties. Since bee pollen is a preferred food in terms of functional and nutritional components, it is very important to provide the bee pollen with nutritious, good organoleptic properties and high microbiological quality for consumers (Nogueira et al., 2012; Barbieri et al., 2020).

Researchers worldwide have been dedicating great attention to bee pollen. Bee pollen contains nutritive compounds. The content of bee pollen varies greatly according to its botanical and geographical origins. Studies have recently been undertaken on the physical and chemical composition, the microbiological quality, and the identification of the compounds present in bee pollen (Mauriello et al., 2017; Al-Kahtani et al., 2020; Isopescu et al., 2020; Keskin and Özkök, 2020; Soares de Arruda et al., 2021; Bayram, 2021). These samples were obtained from different regions and originated from various plants. These researches could be classified according to the analyses of the antifungal effect (Özcan et al., 2003), fatty acid amounts (Karagözoğlu et al., 2012; Kostić et al., 2017), phenolic composition (Avşar et al., 2016; Mayda et al., 2019; Karkar et al., 2020), antimicrobial properties (Avşar et al., 2016; Spulber et al., 2018), antioxidant activity (Avşar et al., 2016; Selamoglu et al., 2016; Karkar et al., 2018; Atmaca, 2019; Şahin and Kemal 2019; Saral et al., 2019; Dulger Altıner et al., 2020), mineral composition (Altunatmaz et al., 2017; Adaškevičiūtė et al., 2019), microbiological hazard (Sandıkçı Altunatmaz and Yılmaz Aksu 2016; Anjos et al., 2019), physicochemical characteristics and sugar compositions (Başdoğan et al., 2019; Al-Kahtani et al., 2020; Mayda et al., 2020), and aflatoxin contents (Arslan and Durmaz, 2019). Allover these studies few researchers investigated the microbiological quality of the bee pollen. Generally, the research has focused on physicochemical properties, especially antioxidant activity. This study investigated the microbiological and physicochemical properties and antioxidant capacity. Thus, it offers a broader perspective on the status of bee pollen.

This study aimed to determine the microbiological quality and some functional properties of bee pollen due to its promising effect on human health. In this context, the microbial, physicochemical, and antioxidant properties of bee pollen obtained by beekeepers from western Turkey were analyzed. The objectives of the present study are; i) evaluation of microbial flora occurring in bee pollens, ii) determination of physicochemical properties and (iii) detection of antioxidant properties.

Material and method

Chemicals, media, and reagents

In this study; saline peptone water (0.1%, SPW, Merck, 67362, USA), Plate Count Agar (PCA, Merck, 105463, USA), De Man Rogosa Sharpe Agar (MRSA, Oxoid, 1153R, USA), Dichloran Rose Bengal Chloramphenicol Agar (DRBCA, Oxoid, CM0728, USA), Saboraud Dekstroz agar (SDA, RTA Biological Products, 2044, Turkey), Lauryl Sulphate Tryptose Broth (LSTB, Himedia 0000307168, India), Brilliant Green Bile Broth (BGBB, BD, 8250526, USA), Baird Parker Agar Base (BPA, Merck, 1.05406, USA), Mannitol Salt Agar (MSA, 1.05404, Germany), EC medium (Acumedia, Neogen, NCM0271A, USA), GLISA-Rapid *E. coli* O157 Test Kit (Merck, 1.04141, Germany), *Salmonella*-Shigella Agar (SSA, 02045, RTA, 3007, Turkey), and Eosin Metilen Blue Agar (EMBA, 307051066, Besimik, Turkey) were used media for microbiological analyses.

The chemicals were used in this study as follows: phenolphthalein (Merck, K37895633 935, Germany), sodium hydroxide (NaOH) (Tekkim Kimya, TK.170510, Turkey), oxalic acid (Aldrich, 194131, USA), 2.6-dichlorophenoldophenol (Acros Organic, 450700050, USA), hydrochloric acid (HCl) (Merck, K45964117 438, Germany), glucose (Merck, K50301442 909, Germany), phenol (Tekkim, TK.201122, Turkey), sulfuric acid (H_2SO_4) (ISOLAB, 970.026, Germany), copper (II) sulfate (Merck, 1.02790, Germany), potassium sodium tartrate tetrahydrate (Merck, 108087, Germany), bovine serum albumin (BSA) (Sigma, 1002928241, USA), and petroleum ether (Tekkim, TK.150370.01000, Turkey).

The extraction solution was prepared by mixing the hydrochloric acid (HCI, Merck, K45964117 438, Germany) and methanol (Tekkim, TK.120320, Turkey) in a ratio of 1:80 for the antioxidant capacity detection.

Collection of samples

Ten bee pollen samples were purchased from beekeepers from Muğla province in Turkey (Figure 1). Fresh and dry pollen samples were collected from seven regions. Six fresh bee pollens (1, 2, 6, 8, 9, and 10) and four dry bee pollens (3, 4, 5, and 7) were analyzed (Figure 2). All the samples were transferred to the laboratory in a thermobox within original packages aseptically. The bee pollen samples were held at –18°C until analyses. The details of the samples are presented in Table 1.

Journal of Food Safety and Food Quality 73, Heft 5 (2022), Seiten 139–168

FIGURE 1: *Geographic locations of honey bee pollen samples.*

Microbiological properties

25 g of each sample was added into 225 mL saline peptone water and homogenized by a stomacher (Bioxia, Thane, India) for 1 minute. Afterward; serial dilutions were carried out for the enumeration of total mesophilic aerobic bacteria (TMAB), the total count of yeast, mold, lactic acid bacteria (LAB), total coliform, and *S. aureus* (ISO 6887-1, 2017). TMAB, lactic acid bacteria, and the total count of yeast and mold were counted according to the FDA-BAM online (2001a), ISO 15214:1998 (1998), and FDA-BAM online (2001b) for the detection of the general microbial profile of samples, respectively. Total coliform bacteria were tested according to FDA-BAM online (2013) as a hygiene indicator for evaluating the production and storage condition of the samples. The tests of *S. aureus*, *E. coli* O157:H7, and *Salmonella* spp. were performed with methods described by ISO 6888 (2004), ISO 16654:2001 (2010), and FDA-BAM online (2011) for food safety.

Physicochemical properties

Honey bee pollen samples were tested for total dry matter (%) (Zhichneg, ZFD-5090, China) (AOAC, 1997), total ash content (%) (Protherm, PLF 11016, Turkey) (AOAC, 1997), titratable acidity (gluconic acid, %) (AOAC, 1995), pH (Adwa, AD1000, Romania) (AOAC, 1995), color as CIE color scale (Konica Minolta Spectrophotometer CM-3600d, Japan), and total lipids content (%) using soxhlet extraction (Almeida-Muradian et al., 2005).

The following determinations were carried out following the methodologies described by Hışıl (2004), Taylor (1995), and Gornall et al. (1949): ascorbic acid (mg/100g) was determined spectrophotometrically (Shimadzu, UV-1800, Japan); total sugar content (mg/g) was measured by the phenol-sulfuric acid method; and total protein content (mg/g) was carried out using Biuret method.

Antioxidant properties

The extraction process was applied before determining the antioxidant capacity. For extraction, 10 g samples were mixed with 25 mL extraction solution. Afterward, the mixtures were kept in a shaking incubator (WiseBath, WSB 30, Korea) for 30 min at room temperature, and then they were placed at 4°C for 24 hours. The pellets were discarded after centrifugation (10000 × g, 5 min) (Sigma, 3-30K, Germany). The supernatants were collected and stored at 4°C.

The extracts were used for total phenolic content (gallic acid equivalent) and antioxidant activity of pollens. The total phenolic content was conducted by using the Folin-Ciocalteu assay (Franke et al., 2004). The antioxidant activity of pollen samples was evaluated using the TEAC (Trolox Equivalent Antioxidant Capacity by ABTS) (Re et al., 1999) and FRAP (ferric reducing ability of plasma) (Benzie and Strain 1996).

Statistical analyses

All experiments were carried out with two replicates and two parallels (n=4). All statistical analyses were performed with the SPSS statistical package program (IBM SPSS Statistics Version 22; USA) by ANOVA variance analysis. The significance levels of $p<0.05$ were used for statistical differences. The significant difference between the means was established by Duncan Tests. The relationship between variables was assessed by Pearson's correlation and presented in the form of the correlation matrix.

FIGURE 2: *The photograph of honey bee pollen samples.*

Result and discussion

Microbiological properties

The counts of TMAB, total yeast, total mold, LAB, total coliform, and *S. aureus* are presented in Table 2. Correlation analysis (Pearson) showed that the TMAB count is strongly (R=0.508) associated with the yeast count (Table 6). The mean count of TMAB, total yeast, LAB, and total coliform were 4.43 log CFU/g, 2.72 log CFU/g, 4.75 log CFU/g, and 2.93 log MPN/g, respectively. *S. aureus*, *Salmonella* spp., and *E. coli* O157:H7 were not detected in the bee pollen samples. Although there has been no legislation on pollen or beekeeping products in the Turkish Food Codex, the ‚Limits of Pathogenic Microorganisms' supplement has been used in the Turkish Food Codex Microbiological Criteria Regulation to evaluate the conformity of the product to legal limits (TGK, 2011). The bee pollen samples have met the pathogenic criteria of the legislation due to the absence of *E. coli* O157:H7, *Salmonella* spp., and *S. aureus*. National pollen standards only existed in a few countries (Switzerland, Argentina, Bulgaria, Brazil, and Poland) in the world. The total count of aerobic bacteria and yeast-molds should not be more than 150×10^3 CFU/g (5.17 log CFU/g) and 10^2 CFU/g (2 log CFU/g) according to Argentina's regulation. Also, pathogenic bacteria should not be present in pollen products (Krell, 1996; Campos et al., 2008). 70% and 10% of the tested pollen samples are considered suitable in terms of TMAB and yeast-mold criteria in this regulation, respectively. Bee pollen samples were collected in Istanbul and some microbiological properties were tested. The counts ranged <1.00–3.48 log CFU/g, <1.00–2.04 log CFU/g, and <1.00–2.70 log CFU/g for TMAB, molds, and yeast, respectively. Besides, *E. coli*, *S. aureus*, *Enterococcus* spp., *Enterobacteriaceae, E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* were not detected (Sandıkçı Altunatmaz and Yılmaz Aksu 2016). The count of TMAB and yeast is higher in this study. This can be expected to relate to the higher moisture content of the samples. Sandıkçı Altunatmaz and Yılmaz Aksu (2016) were indicated the value of the moisture to be between 1.17% and 5.80%. Despite this, the lowest moisture content was observed in sample 7 (15.033%) in this study. The results of both experiments for *S. aureus*, *E. coli* O157:H7, and *Salmonella* spp. were similar. The values found for LAB are similar to those reported by Mauriello et al. (2017).

Campos et al., (2008) proposed that the microbiological properties of the pollen as *Salmonella* spp., *E. coli*, and *S. aureus* should not exist; the maximum value of the total aerobic bacteria and yeast-mold should be 10^5 CFU/g

TABLE 1: *Details about bee pollen samples.*

Samples	Region	Type	Source*
1	Fethiye/Muğla	Fresh	Flower
2	Marmaris/Muğla	Fresh	Sığla tree
3	Seydikemer/Muğla	Dry	Flower
4	Seydikemer/Muğla	Dry	Flower
5	Göcek/Muğla	Dry	Flower
6	Dalaman/Muğla	Fresh	Opium poppy
7	Köyceğiz/Muğla	Dry	Flower
8	Köyceğiz/Muğla	Fresh	Willow tree
9	Muğla/Muğla	Fresh	Flower
10	Fethiye/Muğla	Fresh	Opium poppy

*As represented by the beekeepers

TABLE 2: *Microbiological properties of honey bee pollen samples.*

Samples	TMAB log CFU/g	Total yeast log CFU/g	Total mold log CFU/g	LAB log CFU/g	Total coliform log MPN/g	*S. aureus* log CFU/g
1	5.16±0.021^{d}*	2.63±0.014^{bc}	2.11±0.047^{b}	4.63±0.776^{a}	3.74±0.006^{h}	<1.00±0.000^{a}
2	3.65±0.069^{b}	1.98±0.717^{ab}	<1.00±0.000^{a}	4.47±0.125^{a}	2.31±0.015^{b}	<1.00±0.000^{a}
3	5.41±0.024^{d}	2.79±0.010^{c}	2.51±0.028^{d}	4.77±0.010^{a}	3.06±0.081^{ef}	<1.00±0.000^{a}
4	5.63±0.014^{d}	2.75±0.027^{c}	<1.00±0.000^{a}	5.30±0.717^{a}	2.98±0.032^{e}	<1.00±0.000^{a}
5	3.48±0.675^{b}	2.11±0.047^{a}	2.19±0.020^{c}	5.00±0.000^{a}	2.55±0.009^{c}	<1.00±0.000^{a}
6	3.70±0.056^{b}	3.48±0.010^{d}	<1.00±0.000^{a}	4.58±0.691^{a}	3.04±0.051^{fg}	<1.00±0.000^{a}
7	6.87±0.012^{e}	3.77±0.015^{d}	<1.00±0.000^{a}	4.34±0.694^{a}	3.69±0.013^{h}	<1.00±0.000^{a}
8	2.24±0.337^{a}	2.64±0.007^{bc}	<1.00±0.000^{a}	4.19±0.701^{a}	1.98±0.032^{a}	<1.00±0.000^{a}
9	4.42±0.012^{c}	2.50±0.029^{abc}	<1.00±0.000^{a}	5.27±0.691^{a}	2.77±0.010^{d}	<1.00±0.000^{a}
10	3.75±0.011^{b}	2.57±0.017^{bc}	<1.00±0.000^{a}	4.96±0.686^{a}	3.15±0.044^{g}	<1.00±0.000^{a}

* n=4 (± standard deviation). Different lowercase letters indicate differences between rows (p<0.05).

TABLE 3: *Physicochemical properties of honey bee pollen samples.*

Samples	Total dry matter %	Total moisture %	Total ash %	pH	Titratable acid (gluconic acid) %	Acidity Milli-equivalent acid/Kg	Ascorbic acid (C vitamin) mg/100 g	Total carbo-hydrate mg/g	Protein mg/g	Total lipid %
1	70.611±0.148^{a}*	29.389±0.148^{a}	1.755±0.043^{a}	4.77±0.06^{de}	1.969±0.138^{a}	100.371±7.026^{a}	25.132±0.787^{b}	5.351±0.520^{abc}	18.965±1.661^{ab}	2.660±0.268^{a}
2	70.739±0.085^{a}	29.261±0.085^{a}	1.425±0.041^{b}	4.14±0.32^{bc}	2.693±0.360^{c}	137.286±18.346^{c}	18.383±3.643^{ab}	5.012±0.501^{ab}	34.188±1.724^{c}	6.618±2.390^{de}
3	77.508±0.127^{b}	22.492±0.127^{b}	2.143±0.109^{c}	4.22±0.02^{bc}	4.404±0.084^{e}	224.519±4.274^{e}	12.717±3.580^{ab}	5.638±0.531^{bc}	32.703±1.611^{c}	7.181±2.322^{de}
4	81.204±0.101^{c}	18.796±0.101^{c}	2.126±0.052^{c}	4.91±0.01^{e}	2.931±0.103^{c}	149.413±5.250^{c}	19.154±1.317^{ab}	5.416±0.467^{abc}	31.416±3.454^{c}	6.083±2.246^{cd}
5	83.085±0.264^{d}	16.915±0.264^{d}	2.165±0.093^{c}	4.76±0.04^{de}	3.627±0.308^{d}	184.882±15.717^{d}	63.074±19.449^{d}	6.067±0.557^{c}	32.282±6.959^{c}	5.298±0.554^{bcd}
6	77.982±1.636^{b}	22.018±1.636^{b}	2.370±0.058^{d}	5.66±0.22^{f}	1.722±0.003^{a}	87.794±0.167^{a}	42.297±1.772^{c}	6.034±0.607^{c}	24.807±3.956^{bc}	4.013±1.933^{abc}
7	84.967±0.212^{e}	15.033±0.212^{e}	2.228±0.020^{c}	4.64±0.22^{d}	3.705±0.060^{d}	188.887±3.048^{d}	25.776±2.030^{b}	4.776±0.348^{a}	29.188±5.214^{c}	5.547±1.098^{bcd}
8	75.642±3.098^{f}	24.358±3.098^{f}	2.345±0.076^{d}	3.71±0.01^{a}	6.905±0.117^{f}	352.020±5.981^{f}	19.818±0.149^{ab}	4.581±0.669^{a}	28.866±2.281^{c}	5.994±1.841^{cd}
9	67.886±0.275^{g}	32.114±0.275^{g}	1.622±0.102^{e}	4.07±0.01^{b}	3.485±0.096^{d}	177.664±4.905^{d}	6.328±5.974^{a}	5.804±0.557^{bc}	31.193±14.042^{c}	2.949±1.776^{ab}
10	72.273±0.221^{a}	27.727±0.221^{a}	1.756±0.052^{a}	4.31±0.15^{c}	2.319±0.189^{b}	118.215±9.623^{b}	87.799±19.969^{e}	5.259±0.283^{abc}	12.480±3.010^{a}	8.845±0.152^{e}

* n=4 (± standard deviation). Different lowercase letters indicate differences between rows (p<0.05).

Journal of Food Safety and Food Quality 73, Heft 5 (2022), Seiten 139–168

(5 log CFU/g) and 5×10^4 CFU/g (4.69 log CFU/g), respectively. When it is compared with the result of tested pollen; samples 1, 3, 4, and 7 had a higher count of TMAB, and samples 1 and 3 had a higher count of yeast-mold. TMAB counts are often used as food safety and sanitation indicator. A high count of mesophilic bacteria in a product indicated the condition of processing and storage that may lead to the growth of human-borne or animal-borne pathogens. It is also clear that the product has a high probability of containing such pathogens. Nevertheless, the presence of a high count of mold in a product increased the possibility of mycotoxin, and therefore health risk is raised (Ünlütürk and Turantaş 2003). Molds were detected in three of the samples tested. It was found that the rest of the samples were under detectable levels. Pollen samples collected from three different Algerian regions were examined and their results were in agreement with our findings in the total aerobic count, mold, and yeast. But, the values of total coliform (3–5.47 log CFU/g) were higher than the results of this study. As in this study, *S. aureus* and *Salmonella* spp. were not detected (Hani et al., 2012). Nogueira et al. (2012) reported that *Salmonella* spp., fecal coliform, and *E. coli* were absent in eight bee pollen samples. On the other hand, yeast and mold, and TMAB were detected in 50% and 12.5% of the samples. Belhadj et al. (2014) analyzed 15 pollen samples obtained from bee-keepers (2 samples) and local markets (13 samples). *Salmonella* spp. was observed in 7 samples, and Listeria spp. was detected in 10 samples. *S. aureus* was reported in 14 samples and the highest value was 8.32 log CFU/g. Our findings are in agreement with those found by Anjos et al. (2019). While TMAB results were lower than the values in this study, yeast and mold results were high. When these results are compared with this study, bee pollen samples, collected from Muğla and its region, have better bacteriological quality.

Since coliform bacteria are common in both the intestine and nature (soil, plants, etc.), they are considered indicators of sanitation in the food industry. The presence of high levels of coliform microorganisms in bee pollen indicates that the required hygienic measures have not been taken during the collection, production, storage, and sale stages. Total coliform counts of the bee pollen samples were found between 1.98 log MPN/g and 3.74 log MPN/g. Correlation analysis indicated a positive correlation between the total coliform and total yeast (R=0.563) and the TMAB (R=0.784) (Table 6). Sandıkçı Altunatmaz and Yılmaz Aksu (2016) and Anjos et al. (2019) reported that coliform bacteria were absent in tested bee pollen, conversely, Serra Bonvehi and Escolà Jordà (1997) determined that coliform bacteria were obtained in 55% of the bee pollen samples.

Physicochemical properties

The result of total dry matter, moisture, total ash, pH, titratable acidity, ascorbic acid, protein, total carbohydrate, and total lipid are shown in Table 3. The color values of the samples are presented in Table 4.

Bee pollen is divided into two groups dry and fresh (frozen). According to the Turkish Standards Institute (TSI) moisture could not exceed 10% for dry and 25% for fresh bee pollens (TS 10255, 2006). In this research, 40% of tested samples were purchased as dry (samples 3, 4, 5, and 7) and the rest of them were supplied as fresh (samples 1, 2, 6, 8, 9, and 10). The average dry matter content of the samples was 76.190%. The moisture content of the samples was between 15.033% and 32.114%. 20% of the tested samples have complied with the standard (Table 3). The moisture content of 12 pollen samples collected in Istanbul varies between 1.17% and 5.80% which are lower moisture content (Sandıkçı Altunatmaz and Yılmaz Aksu, 2016). Belhadj et al. (2014) stated the moisture content of pollens was between 18.11–36.29% in their study. Unlike our study, the moisture content of bee pollen samples (6.23%–2062%) was found to be lower (Keskin and Özkök, 2020).

The moisture content has an important role in the shelf life and organoleptic properties of pollens. A high value of moisture may promote microbial contamination mainly by yeast and mold and limit the effect of the drying process. Moisture content is related to collecting time from the pollen trap. Pollen has a hygroscopic structure, if the beekeepers don't harvest the pollen daily it can be easily exposed to high humidity environmental conditions. Also, low moisture content (under 3%) may cause undesirable effects such as discoloration, browning, Maillard reactions, fructose dehydration, loss of volatile compounds, and lipid oxidation which can affect the quality of the pollen (Estevinho et al., 2012; Nogueira et al., 2012; Kieliszek et al., 2018).

The pH value of bee pollen is important in texture, stability, and shelf life during storage (Nogueira et al., 2012). In this study, the pH values of pollen samples varied between 3.71 and 5.66. These results demonstrate that the samples are acidic (Table 3). These results are consistent

TABLE 4: *Color values of honey bee pollen samples.*

Samples	L*	a*	b*	C*	h*
1	59.605±1.249^{dt}	6.448±0.097^{c}	32.208±0.460^{e}	32.848±0.431^{d}	78.675±0.312^{d}
2	58.258±1.201^{c}	4.155±0.278^{a}	27.150±1.987^{d}	27.470±1.923^{c}	81.240±1.201^{e}
3	58.445±0.365^{c}	5.068±0.287^{b}	19.080±0.859^{b}	19.743±0.896^{b}	75.135±0.350^{c}
4	55.530±1.167^{b}	7.060±0.656^{d}	27.063±3.132^{d}	27.973±3.195^{c}	75.353±0.427^{c}
5	55.980±0.245^{b}	10.385±0.386^{f}	25.525±1.237^{cd}	27.555±1.288^{c}	67.853±0.300^{a}
6	52.775±0.396^{a}	3.710±0.099^{a}	14.790±0.588^{a}	15.250±0.578^{a}	75.913±0.588^{c}
7	58.958±0.339^{cd}	9.120±0.631^{e}	26.270±1.495^{d}	27.813±1.620^{c}	70.865±0.231^{b}
8	52.095±0.747^{a}	9.413±0.176^{e}	23.723±2.033^{c}	25.535±1.862^{c}	68.260±1.882^{a}
9	59.783±0.323^{d}	13.605±0.479^{g}	37.065±1.099^{f}	39.483±1.162^{e}	69.845±0.409^{b}
10	57.803±0.394^{c}	6.595±0.048^{cd}	31.690±0.470^{e}	32.368±0.459^{d}	78.243±0.197^{d}

† n=4 (± standard deviation). Different lowercase letters indicate differences between rows (p<0.05).

TABLE 5: *Antioxidant capacity of honey bee pollen samples.*

Samples	Total phenolic content mg GAE/g	FRAP µmol Trolox/g	TEAC µmol Trolox/g
1	8.219±0.547^{e}*	60.389±5.124^{f}	3.131±0.621^{e}
2	6.649±0.313^{d}	51.496±1.006^{e}	2.404±0.192^{d}
3	3.023±0.790^{ab}	18.007±1.470^{b}	0.796±0.096^{a}
4	3.780±0.581^{b}	23.721±2.184^{cd}	2.238±0.288^{cd}
5	3.091±0.448^{ab}	16.757±1.820^{b}	0.958±0.185^{a}
6	3.095±0.255^{ab}	17.802±1.160^{b}	0.958±0.054^{a}
7	2.572±0.423^{a}	9.882±1.336^{a}	0.546±0.082^{a}
8	3.223±0.656^{ab}	22.641±2.714^{c}	1.622±0.299^{b}
9	5.173±0.369^{c}	27.552±1.665^{d}	1.828±0.235^{bc}
10	7.810±0.550^{e}	48.195±5.080^{e}	2.884±0.502^{e}

* n=4 (± standard deviation). Different lowercase letters indicate differences between rows (p<0.05).

with findings in samples collected in İstanbul (3.75–4.30), Portugal (4.3–6.33), and Portugal (4.3–5.2) (Estevinho et al., 2012; Feás et al., 2012; Sandıkçı Altunatmaz and Yılmaz Aksu 2016).

The mean value of ash, an indicator of inorganic components, was 1.993%. The ash values of dry samples numbered 3, 4, 5, and 7 were found statistically insignificant ($p>0.05$) (Table 3). According to the Pearson correlation analysis, it was a positive relation between ash and dry matter (R=0.738) and a negative relation with moisture (R=–0738) (Table 7). The ash content of bee pollens in Spain and Portugal was noted as 1.63–2.20 g/100 g and 2–4% which is similar to the values of this study (Serra Bonvehi and Escolà Jordà 1997; Feás et al., 2012). Similarly, the contents of ash were examined in pollen samples from Romanian and found between 1.34–2.81 g/100g (Isopescu et al., 2020).

According to the legislation of Argentina, pollens should have the following aspects; maximum value of moisture of 8% and ash at 4%, and pH between 4 and 6. When the result of pollen samples was compared to this legislation, the moisture content is inconsistent because the minimum value was in sample 7 with 15.033%. The highest ash content was noted in sample 6 as 2.370% and it was in line with legislation. The pH values of the samples (except sample 8) were following the legislation (Coronel et al., 2004).

The color of food is the most important aspect of customer preference because is often the first element noticed in the appearance of a food product (Fellows, 2000). Pollens are seen in many colors from cream to brown, yellow, orange, red, green, and black due to botanical taxa and chemical composition (Almeida-Muradian et al., 2005). The color values of all samples were conducted by using the L^*, a^*, and b^* scale. Where L^*, degree of lightness, ranges from 0 (black) to 100 (white); a^*, degree of redness, from green (–) to red (+); and b^*, degree of yellowness, from blue (–) to yellow (+) (Anonymous, 1996; Harold, 2001). The colors of pollens are varied depending on factors such as diversity of plant, source, drying temperature, drying time, etc (Anonymous, 1996; Kieliszek et al., 2018). The results of L^*, a^*, and b^* ranged from 52.095 to 59.783, from 3.71 to 13.605, and from 14.790 to 37.065, respectively. According to the results, the tested pollen samples were tending to be redness and yellowness. The chroma (C^*) and Hue (h^*) values were calculated and ranged from 15.250 to 39.483 and from 67.853 to 81.240 (Table 4). Yook et al. (1998) carried out color values by the Hunter method. The L^* value was 74.5, indicating that these samples were lighter than our results. In the same study, a^* and b^* values were reported as 4.2 and 30.8. In another research, L^*, a^*, and b^* values were noted in order 42.8–58.4, 8.4–11.3, and 43.7–56.1 (De-Melo et al., 2016). Dulger Altıner et al. (2020) analyzed the colors of 20 bee pollen samples collected in Istanbul and found L^*; 56.42, a^*; 4.22, and b^*; 23.94. The values found for L^* are similar to those reported in the research (De-Melo et al., 2016), and the samples with lower a^* and b^* values are obtained in this study.

The organic acid composition depends on the species, age of the plant, and plant tissue (Moita et al., 2014). The organic acid composition was examined in bee pollen samples collected from regions of Turkey and gluconic acid was stated as the main acid (Kalaycıoğlu et al., 2017). The mean value of the bee pollen samples was 3.376% as gluconic acid. In addition, the mean value of acidity was 172.105 milli-equivalent acid/Kg. The acid content of the bee pollen samples was found significantly different ($p<0.05$) (Table 3). Correlation analysis indicated a negative correlation between the pH and titratable acidity (R=–0.663). While the acidity is increased, pH is decreased (Table 7). In a different study, the acidity of bee pollen samples from Portugal was mentioned between 13.5 and 21.2 meq NaOH/Kg (Anjos et al., 2019).

Vitamin C is the most common vitamin in nature. The average ascorbic acid (vitamin C) value of bee pollen samples was 32.048 mg/100g. Vitamin C is the least stable of the vitamins and especially; in the presence of oxygen long-term heating and light are the most important factors of degradation (Cemeroğlu et al., 2004). In the pollen samples, the lowest vitamin C was detected in sample number 9 (6.328 mg/100g) and the highest in sample number 10 (87.799 mg/100g). And the mean was 32.048 mg/100g (Table 3). Bayram (2021) found in their study that, although it had similar values with our results for its contents C 16.240 mg/100g. Barajas et al. (2012) studied the vitamin C content of pollens from two different regions ranging from 40.22 and 40.37 mg/100mg. 10 pollen samples were analyzed for chemical components and vitamin one of the analyzes was not detected in the tested pollens (Almeida-Muradian et al., 2005). The vitamin C content is mentioned to be between 70–560 mg/kg for dry bee pollens (Campos et al., 2008). Comparing, these findings with our results 8 samples (except 5 and 10) were compatible.

Proteins and amino acids that form part of the bioactive compounds in bee pollen have several benefits such as antibacterial, antioxidant, immunostimulating, anti-

TABLE 6: *Correlation matrix for the microbiological properties of honey bee pollen samples.*

R-Pearson Values*	1	2	3	4	5
1 Total yeast	1				
2 Total mold	–0.238	1			
3 Total coliform	0.563*	0.218	1		
4 LAB	–0.280	0.107	0.016	1	
5 TMAB	0.508*	0.142	0.784*	0.124	1

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

TABLE 7: *Correlation matrix for the physicochemical properties of honey bee pollen samples.*

R-Pearson Values*	1	2	3	4	5	6	7	8	9	10
1 Total dry matter	1									
2 Total moisture	–1.000*	1								
3 Total ash	0.738*	–0.738*	1							
4 pH	0.404*	–0.404*	0.356*	1						
5 L*	–0.317*	0.317*	–0.661*	–0.205	1					
6 a*	–0.015	0.015	–0.021	–0.384*	0.172	1				
7 b*	–0.505*	0.505*	–0.682*	–0.401*	0.640*	0.586*	1			
8 C*	–0.470*	0.470*	–0.635*	–0.421*	0.610*	0.664*	0.995*	1		
9 h*	–0.398*	0.398*	–0.524*	0.195	0.297	–0.783*	0.028	–0.071	1	
10 Titratable acid	0.168	–0.168	0.390*	–0.663*	–0.342*	0.412*	–0.135	0.072	–0.657*	1

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

Journal of Food Safety and Food Quality 73, Heft 5 (2022), Seiten 139–168

TABLE 8: *Correlation matrix for the physicochemical properties and antioxidant capacity of honey bee pollen samples.*

R-Pearson Values*	1	2	3	4	5	6	7
1 Ascorbic acid	1						
2 Protein	–0.487*	1					
3 Total carbohydrate	0.098	0.110	1				
4 Total lipid	0.312	–0.173	–0.209	1			
5 Total phenolic content	0.205	–0.503*	–0.052	–0.028	1		
6 FRAP	0.143	–0.400*	–0.114	–0.110	0.954*	1	
7 TEAC	0.099	–0.433*	–0.123	–-0.007	0.896*	0.876*	1

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

thrombotic, and anti-inflammatory activities. Proteins, one of the main components of bee pollen, vary between 10–40% depending on the source of a plant (Ares et al., 2018). The protein content of bee pollen is affected by the drying process, storage conditions, environment, and flower types (Estevinho et al., 2012). If the pollen is exposed to high drying process conditions (temperature and time), its protein content is decreased below 2g/100g. If the pollen is dried under suitable conditions, its amino acid values (>2.5 g/100g) can be preserved (Serra Bonvehi and Escolà Jordà 1997). The protein content of samples 1 (18.965 mg/g) and 10 (12.480 mg/g) was found below the specified value (Table 3). These samples considering they are fresh, the low protein content is thought to be due to storage conditions and/or flower composition. The protein values of the pollen ranged from 12.480 to 34.188 mg/g. The protein contents of the samples (except 1 and 10) were statistically insignificant ($p>0.05$) (Table 3). Similar results were found by Mayda et al. (2020). The average values of 20 bee pollen samples in Spain and 8 pollens in Portugal and Spain were 31.6 mg/g and 12.50–25.15g/100g. These agree with the result of this study (Serra Bonvehi and Escolà Jordà 1997; Nogueira et al., 2012).

Carbohydrates are one of the main components of pollen (Estevinho et al., 2012). Bee pollens always contain reducing sugar due to honey and nectar contained in the pellets. Although the total carbohydrate content of bee pollen varies according to the type of flower and the harvesting conditions, it is found in bee pollen at a rate of 15–60%. Monosaccharides are a great energy source for metabolism, while polysaccharides are stored as energy and act as structural components. While there is a high amount of glucose and fructose in bee pollen, there are also disaccharides, polysaccharides, oligosaccharides, and dietary fiber (Ares et al., 2018). The highest total sugar content was found in pollen sample 5 (6.067 mg g) and the lowest value in pollen sample 8 (4.581 mg/g) (Table 3). Serra Bonvehi and Escolà Jordà (1997) reported the average amount of sugar in the pollen as 32.9 g/100g which was lower than the result of this study (5.94 mg/g). In the same study, total lipid content was investigated (5.91 g/100 g) and it was correlated with the findings (5.519%) of this study. Feas et al. (2012) and Nogueira et al. (2012) found the carbohydrate 67.7% and between 69.68–84.25 g/100g. The carbohydrate was detected in higher amounts when compared to the results of Spulber et al. (2018).

The lipid content of pollen constitutes a part of its physicochemical properties. The lipid substances are present in the surface layer of the pollen grain. This layer also sticks the pollen grain together (Kostic et al., 2017). The content of lipid components is different depending on the botanical species and is between 1% and 20% (Estevinho et al., 2012; Ares et al., 2018). The mean value of lipid was found at 5.519% (Table 3). Feas et al. (2012) reported the average lipid content of pollen samples as 5.2% in their study. Researchers who tested 8 pollen samples reported lipid values as 2.35–3.06g/100g (Nogueira et al., 2012). In a study conducted in Colombia, it was reported as 1.14% and 3.8% (Barajas et al., 2012). The lipid content of the pollen samples analyzed in Brazil was calculated as 6%, and this value was consistent with the mean value of this study (Almeida-Muradian et al., 2005).

Antioxidant properties

Total phenolic content, FRAP, and TEAC analyzes were performed to determine the antioxidant properties of the pollen samples and the results are represented in Table 5.

Pollen is rich in polyphenolic compounds. Polyphenolic compounds are an important indicator of functional properties (Gullon et al., 2016). There is an increasing interest in polyphenolic rich foods due to the preventive effect on chronic diseases (O'Byrne et al., 2002). Phytochemicals such as phenolic compounds are beneficial for human health by reducing the risk of degenerative disease by inhibiting macromolecule oxidation and diminishing oxidative stress. Phenolic compounds neutralize active oxygen species with conjugated double bonds and hydroxyl groups in their structures (Pascoal et al., 2014).

Free radicals cause damage to protein, lipid, and DNA produced by many metabolic pathways in the human body. On the other hand, food oxidation can occur by free radicals. Antioxidants from natural sources like bee pollen gain importance in terms of preventing and controlling the harm of the free radicals. Components with antioxidant activity have a very important role in protection from diseases such as cardiovascular diseases and cancer which are based on oxidative stress. Therefore; it has been great interest in determining the antioxidant capacity of food. So, many different analytical methods are used to determine the antioxidant capacity in vitro. These methods have their advantages and limitation for evaluating antioxidant activity. However, it is not possible to measure the antioxidant activity by using a single method (Huang et al., 2005; Su et al., 2021). Therefore, FRAP and TEAC assays were used in this study (Table 5).

The lowest and highest values were obtained in order samples 7 and sample 1 for the total phenolic content, FRAP, and TEAC. It was identified as a very strong positive relation between phenolic content and FRAP ($R=0.954$), and phenolic content and TEAC ($R=0.896$) (Table 8). The findings of the three analyses are accordance in terms of antioxidant properties. The highest total phenolic content was 8.219 mg GAE/g and the lowest one was 2.572 mg GAE/g. FRAP and TEAC values of pollen products were found between 9.882–60.389 µmol Trolox/g and 0.546–3.131 µmol Trolox/g, respectively. Correlation analysis indicated a positive correlation between FRAP and TEAC (Table 8). The phenolic content was 18.55–32.15 mg GAE/g in 8 commercial bee pollens in Portugal and Spain and this was higher than in this study (Pascoal et al., 2014). In the same study, antioxidant capacity was evaluated by DPPH method and noted as 2.98–6.69 mg/mg extract. Feas et al. (2012) reported the phenolic content between 12.9–19.8 mg GAE/g. The phenolic content of bee pollen (3.6–10.9 mg GAE/g) collected in Brazil was similar to this study (Carpes et al., 2007). The phenolic content of bee pollens in Venezuela

were 496.65, 755, and 1540 mg GAE/100 g for water, methanol, and ethanol extraction solutions, respectively. The phenolic content of four bee pollen in China ranged 13.71 and 29.13 mg/g (Su et al., 2021). 11 bee pollen were obtained from the Black Sea region in Turkey and the phenolic content of the samples (15.73–26.92 mg GA/extract g) were higher than the result of this study (Alimoglu et al., 2021). TEAC values were detected as 0.50, 1.72, and 1.84 µmol TE/100 g in the same extraction, respectively (Pérez-Pérez et al., 2012). Anzer pollens (13 samples) were conducted for FRAP, DPPH, and phenolic content. FRAP values were expressed as 11.77–105.06 µmol Trolox/g, DPPH; 0.65–8.20 mg/mL, phenolic 44.07–124.10 mg/g (Ulusoy and Kolaylı 2014). The antioxidant properties of chestnut bee pollen, obtained from Zonguldak (Turkey), were 13.78 mg GAE/g for phenolic content, 0.19 mmol TEAC/g for CUPRAC, and 0.49 mg/ml for DPPH (Saral, 2013). The phenolic content, ABTS, and FRAP of chestnut bee pollen collected from Bursa, Balıkesir, Zonguldak regions (Turkey) were reported 16.19–38.82 mg GAE/g, 13.85–42.58 mg TE/g ABTS, and 1.53–4.75 mM TE/g, respectively (Karkar, 2018). In another study, phenolic content (266.93–434.24 mg GAE/g), DPPH (3.08–3.85 mg TE/g), and ABTS (1.80–5.98 mg TE/g) values were investigated in four different regions in Turkey (Mayda, 2019). Antioxidant capacity of 9 pollen samples in Brazil were mentioned as: phenolic content; 33.73–75.60 mg GAE/g, ABTS; 0.91–5.73 mg/mL, DPPH; 1.94–7.99 mg/mL, and FRAP; 1.82–8.77 mg/mL (Araújo et al., 2017). Dulger Altıner et al. (2020) were obtained 20 bee pollen samples from Istanbul (Turkey) and determined the total phenolic content, DPPH, CUPRAC (Copper (II) Ion Reducing Antioxidant Capacity), and ABTS as 147.10 mg GAE/g, 35.69 µmol TE/g, 83.24 µmol TE/g, and 48.96 µmol TE/g, respectively.

Conclusion

Pollen is widely preferred due to its nutritional properties by the consumer. It is consumed directly by consumers without any processing step and that may increase the health risk. From the hygienic point of view, microbiological safety is the main quality criterion. This study has presented substantial data about the characteristics of the bee pollens collected from different beekeepers based on their physical and chemical compositions, antioxidant capacity, and microbiological properties. Our results on microbial contamination of bee pollen show that a more comprehensive microbiological risk assessment is required in bee pollen for human consumption. The study demonstrated that bee pollen samples could provide good nutritional values. The values of physicochemical analyses mentioned significant compositions in bee pollen samples collected from the western part of Turkey. The results of the current study showed that all samples exhibited a strong antioxidant potential in vitro. In other respect, further studies are needed to clarify the type of antioxidant and antimicrobial compounds present in honey bee pollen and possible mechanisms of action for those and other constituents of pollen. This is important because the bee pollen would be beneficial not only as a dietary supplement but also as a functional food. The result of this study may help to establish standardization parameters.

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Authors' contributions

M.Ç. and N.Ö carried out the experiment. N.Ö. wrote the manuscript. M.Ç. reviewed the manuscript. M.Ç. developed the theory and performed the computations. N.Ö. verified the analytical methods. M.Ç. contributed to sample preparation. N.Ö. conceived of the presented idea and supervised the project. All authors discussed the results and contributed to the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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