ISPEC Tarım Bilimleri Dergisi, 8(2): 461-473, 2024
ISPEC Journal of Agricultural Sciences, 8(2): 461-473, 2024
DOI: https://doi.org/10.5281/zenodo.11305923

Palynological, Protein, and Phenolic Profiling of Bee Pollen from Mersin: An Investigation

Duygu Nur ÇOBANOĞLU [1*]
[1] Bingöl University, Department of Crop and Animal Production, Vocational School of Food, Agriculture and Livestock, Bingöl
[*]Corresponding author: dncobanoglu@bingol.edu.tr

Abstract
Due to bee pollen's exceptional nutritional profile and unique phenolic composition, it has received significant attention recently. The aim of this study was to investigate the botanical origin, protein content, and phenolic composition of the eight bee pollen samples (BP01–BP08) from different districts of Mersin city, Türkiye. According to palynological analysis, pollen grains of 51 taxa belonging to 25 families were determined. BP01 and BP07 were bifloral, and the other samples were multifloral. Pollen belonging to the genera *Ceratonia* sp., *Helianthemum* sp. and *Olea* sp. were predominant in the samples. The protein content values ranged from 23.1±1.23–28.1±1.49 g/100g. The presence of 23 phenolic compounds was investigated in bee pollen samples by liquid chromatography-tandem mass spectrometry (LC-MS/MS), and 12 of them were detected. Taxifolin, caffeic acid, quercetin, oleuropein, and kaempferol were determined to be the most abundant phenolic components, respectively. The bee pollen sample with the low protein content (BP03) had the highest phenolic composition. Furthermore, protein content and phenolic composition showed significant variability based on geographical origin.

Research Article

Article History
Received :02.03.2024
Accepted :19.04.2024

Keywords
Bee pollen
LC-MS/MS
protein
phenolic
palynological
Mersin

1.Introduction

Plant pollen originates from the anther of stamen. During collection, honey bees blend the pollen grains with saliva and nectar, forming distinctive loads, store them in pollen baskets on their hind legs, and transport them to the hive (Komosinska-Vassev et al., 2015). These loads are collected by beekepers with pollen traps that are place at the entrance of hives and are called bee pollen (Aylanc et al., 2021).

Bee pollen is a highly nutritious substance that plays a crucial role in the development and health of bee colonies. Since ancient times, flower pollen has been utilized by humans for its nutritional benefits, although bee-collected pollen only began to be used for human nutrition after the Second World War (Kostić et al., 2015). Bee pollen contains essential nutrients such as trace elements (potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), sodium (Na), etc.), proteins (2.90–33.51 % w/w), carbohydrates (13–55 % w/w), lipids (1–13 % w/w), and vitamins (0.02–0.7 %) that are vital for the growth and well-being of bees and supports human health (Yang et al., 2013; Hsu et al., 2021). Following carbohydrates, proteins constitute primary components in bee pollen, thereby serving as the principal protein source vital for the survival of honey bees (Almeida-Muradian et al., 2005). The protein content of pollen appears to be crucial for the lifespan of honey bees. When pollen is lacking or has insufficient protein, it leads to decreased levels of total protein and vitellogenin in the hemolymph, impacting the longevity of honey bees (Liolios et al., 2015). Also, proteins and amino acids are critical for human growth, nitrogen balance, protein synthesis, and cell signaling (Baky et al., 2023). The protein concentration found in bee pollen serves as an indicator of its nutritional quality (Hsu et al., 2021).

Bee pollen contains phenolic compounds ranging from 3.0 % to 5.0 %, with the quantity and composition of these phenolics subject to significant variation based on the plant source (Mutlu and Erbas, 2023). Phenolic compounds, prevalent in plants as secondary metabolites, are integral to the biological activities observed in bee pollen, including antioxidant, anti-carcinogenic, antimicrobial, and anti-inflammatory properties (Li et al., 2018). These compounds are credited with diverse protective effects against ailments such as cancer, cardiovascular diseases, inflammatory disorders, and neurological degeneration. Bee pollen is a good source of polyphenols which have the ability to scavenge free radicals (Alvarez-Suarez, 2017; Rocchetti et al., 2019).

Researchs have shown that the bee pollen's chemical composition varies depending on the botanical diversity of the plants from which it is collected (Al-Kahtani, 2017). The botanical origin of bee pollen is determined by palynological analysis (Alimoglu et al., 2021; de Souza et al., 2019). Palynological studies determine the plant source of the bee pollen by evaluating the physical properties of the pollen grains such as shape, size, and ornemantation on the exine layer (Barth et al., 2010). Bee pollen is classified as monofloral, bifloral, trifloral, and multifloral according to the pollen analysis (Çobanoğlu, 2024).

Türkiye's geographical location has positioned it as one of the leading countries in apiculture, producing various bee products, including bee pollen (Şahin and Kemal, 2020). Studies have been conducted to determine the elemental content, and antioxidant properties of Turkish bee pollens. Furthermore, the botanical origins and total bioactive compounds of bee pollen from different regions of Türkiye have been determined (Altunatmaz et al., 2017; Dulger Altiner et al., 2020; Temizer, 2023).

Mersin, a province in southern Türkiye, is known for its rich floral diversity, with a significant number of endemic plant species. Beekeeping has been a traditional practice in Mersin province for generations. However, until today, studies on the identification of bee plants and investigation of composition of bee products from Mersin province, Türkiye have been limited (Mărgăoan et al., 2021; Görhan, 2021). Therefore, the main scope of the present study was to determine botanical origin,

protein content, and phenolic composition of bee pollen samples from Mersin province of Türkiye. This study aimed to provide a comprehensive understanding of the effect of the botanical composition of bee pollen produced in Mersin on the protein and phenolic content of bee pollen.

2. Materials and Methods

2.1. Sampling

In 2021, a total of eight fresh bee pollen samples were collected from various beehives in Mersin, Türkiye.

After collection, the bee pollen samples were promptly placed in foam boxes containing ice to maintain their freshness. They were transported to the laboratory. Upon arrival, the samples were kept in a dry place and immediately frozen at -20 °C to preserve their composition until further analyses could be conducted.

2.2. Palynological analysis

Palynological analysis has been used to identify the floral sources of bee pollen. Bee pollen samples evaluated the procedure described by Çobanoğlu, (2024). 2 g bee pollen was mixed with 13 mL 70:30 ethanol:distilled water. The mixture was kept in ultrasound bath for 5 minutes. Then, the homogeneous mixture was centrifuged at 3500 rpm using a centrifuge (Eppendorf SE, Hamburg, Germany) for 15 minutes. Following that, a 1:1 mixture of glycerin and water was used to dilute the residue. Next, a 10 µL suspension was applied to a microscope slide and fixed using glycerin gelatin containing basic fuchsin. Subsequently, the prepared slides were examined using an (Light Emitting Diode) LED optical microscope (Leica DM 2500, Leica Microsystems, Germany) at magnifications of 600× and 1000×. To assess the relative abundance of pollen types, at least 500 pollen grains were counted on each slide. All analyses were performed in triplicate for each sample to ensure the accuracy and reliability of the results. The pollen composition of the examined samples was categorized as follows:

Monofloral: Consisting of a single pollen type at a percentage of >80 % or 46–80 % if there is no pollen at 15–45 %.
Bifloral: Containing two kinds of pollen types, one at a percentage of 46–80 % and the other at a percentage of 15–45 %, or two types each at a percentage of 46–80 %.
Multifloral: Comprises pollen grains from multiple plant species.

2.3. Protein content

The total protein content was calculated by using the Micro-Kjeldahl method and by multiplying the nitrogen content determined, Gerhardt's digestion unit, and the Vapodest distillation system by a factor of 6.38 (Kıvanç and Yapıcı, 2015).

2.4. Pollen extraction for phenolic analysis

50 grams of sample were extracted in 500 mL of 70% ethanol (>96 %, Merck, Germany). The solutions were kept in ultrasound bath at 40 °C for an hour. The mixture was centrifuged at 3500 rpm for 15 minutes. Following extraction, the samples were filtered, and the collected filtrates underwent evaporation using a rotary evaporator at 40 °C to remove the solvent entirely (Çobanoğlu et al., 2023a). The resulting extracts were stored in tubes in the dark at -20 °C for further analyses.

2.5. Phenolic compound detection

Bee pollen extracs's phenolic compounds were determined by liquid chromatography-tandem mass spectrometry (LC/MS/MS, Thermo Scientific/TSQ Quantum Access Max). A C18 column (ODS Hypersil, 4.6 ID x 250 mm 5 m) was used to separate phenolic compounds at 30 °C with a flow rate of 0.7 mL min^{-1}. The injection volume was 20 µL, and the analysis was performed over the course of 20 minutes. As mobile phases, A (water containing 0.1 % formic acid) and B (methanol) were employed. Starting with 100 % A, the mobile phase mixture was run through the column for one minute. The gradient elution was then changed to be 5 % A-95 % B between minutes 1 and 22, and after minute 22, the 5 % A-95 % B phase was continued for an additional three minutes.

The system was then set up to run from 0% A to 100 % B for 25 to 30 minutes, at which point it was turned off. The capillary temperature was set at 300 °C, the vaporizer temperature at 350 °C, and the positive and negative spray voltages were set at 4000 V and 2500 V, respectively (Çobanoğlu et al., 2023b). Table1 contains the parameters for quantifying phenolic compounds.

Table 1. Phenolic compound standard's parameters

Phenolic compounds	Rt (min.)	MS [m/z]	MS /MS [m/z]	LOD (mg.L^{-1})	LOQ (mg.L^{-1})	Polarity
Gallic acid	8.92	169.7	80.50 126.20	0.061	0.203	-
Caffeic acid	15.27	179.7	135.20 136.20	0.047	0.157	-
Taxifolin	16.68	303.0	126.20 285.50	0.058	0.194	- -
Protocatechuic acid	12.13	153.8	110.40 92.50	0.049	0.162	-
Protocatechuic aldehyde	13.16	136.9	92.25 108.20	0.026	0.087	-
Sesamol	12.82	137.18	109.291 108.173	0.048	0.161	-
p-coumaric acid	17.00	163.9	94.30 120.20	0.116	0.387	-
Catechin	10.92	289.2	203.90 245.70	0.068	0.227	-
Epicatechin	11.26	291.5	123.30 139.30	0.045	0.151	+
Rosmarinic acid	17.82	359.18	134.30 162.20	0.029	0.095	-
Vanillin	15.87	150.91	92.30 136.10	0.023	0.076	-
Ferulic acid	17.19	193.35	134.10 178.00	0.061	0.204	-
4-OH-benzoic acid	18.12	137.90	66.60 94.60	0.031	0.104	-
Salicylic acid	18.13	137.14	65.51 93.26	0.030	0.099	-
Syringic Acid	15.45	183.07	123.2 77.3	0.192	0.643	-
Ellagic acid	19.47	300.90	284.797 174.151	0.087	0.289	-
Rosmarinic acid	17.82	359.18	134.30 162.20	0.029	0.095	-
Quercetin	20.58	301.00	152.1 179.9	0.038	0.123	-
Oleuropein	18.00	539.10	275.80 377.50	0.050	0.167	-
Rutin	18.26	609.37	300.60 301.70	0.007	0.024	-
Rezveratrol	18.45	228.98	107.20 135.10	0.030	0.099	+
Flavone	23.90	222.90	77.275 121.154	0.027	0.090	+
Kaempferol	21.68	286.97 165.00	153.00	0.055	0.184	+

2.6. Statistical Analysis

The experiments were carried out in triplicate, and the results were presented as the mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Variations were considered significant at a threshold of $p < 0.05$.

3. Results and Discussion

3.1. Botanical Origin

In this study, Table 2 shows the pollen spectra of eight bee pollen samples from Mersin, Türkiye.

Table 2. Palynological analysis' results, and geographical origin of bee pollen samples

Sample	Geographical Origin	Botanical Origin	Pollen Type%
BP01	Mersin	Bifloral (*Ceratonia* dominant)	***** **** *Ceratonia* sp. (58.8) *** *Helianthemum* sp. (16.2) ** *Achillea* sp. (14.7) * *Astragalus* sp.(0.7), Brassicaceae (0.7), *Cistus* sp. (0.7), *Thymus* sp. (0.7), sp. (0.7), *Ranunculus* sp. (0.7), *Robinia* sp. (0.7), Rosaceae (2.2), *Rumex* sp. (0.7), *Salix* sp. (0.7), *Sanguisorba* sp. (0.7), *Thalictrum* sp. (0.7)
BP02	Mersin	Multifloral	***** **** *** *Helianthemum* sp. (32.4), *Quercus* sp. (21.3), *Salix* sp. (26.9) ** *Paliurus* sp. (4.6) * *Astragalus* sp. (1.85), Brassicaceae (0.9), *Cistus* sp. (0.9), *Crateagus* sp (1.9), *Juglans* sp. (0.9), *Laurus* sp (0.9), Lotus sp. (2.8), *Morus* sp. (0.9), Myrtaceae (0.9), *Thalictrum* sp. (2.8)
BP03	Mersin	Multifloral	***** **** ****Astragalus* sp. (18.4), *Ceratonia* sp. (35.6), *Helianthemum* sp. (29.9) ** *Brassica* sp. (6.9) * *Citrus* sp. (1.1), *Erica* sp. (1.2), *Juglans* sp. (1.1), *Melilotus* sp. (1.1), Pinaceae (1.1), *Thalictrum* sp. (3.5)
BP04	Mersin	Multifloral	***** **** ****Ceratonia* sp. (27.1), *Olea* sp. (37.4) ** *Cistus* sp. (3.7), *Juglans* sp. (4.7), *Eucalyptus* sp. (10.3) * *Carex* sp. (0.9), Caryophllaceae (0.9), *Citrus* sp. (0.9), *Chenepodium* sp. (0.9), *Crepis* sp. (0.9), *Medicago* sp. (2.8), *Mentha* sp. (0.9), *Paliurus* sp. (2.8), *Rumex* sp. (2.8), *Salix* sp. (1.9), *Salvia* (0.9), *Thalictrum* sp. (0.9)
BP05	Mersin	Multifloral	***** **** *** *Astragalus* sp. (16.9), *Citrus* sp. (41.5) ** *Fragaria* sp. (5.6), *Lotus* sp. (8.9), *Olea* sp. (8.9), * Brassicaceae (3.4), *Carex* sp. (1.1), *Cistus* sp. (1.2), *Quercus* sp. (1.1), *Plantago* sp. (3.4), *Rumex* sp. (1.2), *Salix* sp. (3.4), *Taraxacum* sp. (3.4)
BP06	Mersin	Multifloral	***** **** *** *Cistus* sp. (38.4) ** *Brassica* sp. (11.6), *Cyanus* sp. (12.7), *Hedysarum* sp. (9.3), * *Ceratonia* sp. (1.2), *Chenepodium* sp. (1.2), *Crateagus* sp. (2.3), *Crepis* sp. (2.3), *Ferula* sp. (1.2), *Helianthus* sp. (2.3), *Helianthemum* sp. (1.2), *Helicrysum* sp. (2.3), *Juglans* sp. (2.3), *Onobrychis* sp. (2.3), *Pedicularis* sp. (1.2), *Plantago* sp. (2.3) Rosaceae (1.2), *Turgenia* sp. (1.2),
BP07	Mersin		*****

		Bifloral (*Pimpinella* dominant)	**** *Pimpinella* sp. (49.4)
			*** *Astragalus* sp. (16.5),
			** *Centaurea* sp. (3.9), *Cistus* sp. (5.2), *Cyanus* sp. (3.8), *Helianthus* (3.9), *Ranunculus* sp. (3.9), *Salix* sp. (3.5),
			* Amaranthaceae (1.3), *Crepis* sp. (1.3), *Fragaria* sp. (1.3), *Melilotus* (1.3), Morus sp. (1.6), *Quercus* sp. (1.6), *Rumex* sp. (1.3), *Verbascum* sp. (0.3)
BP08	Mersin	Multifloral	*****
			****
			*** *Cirsium* sp. (20.5), *Olea* sp. (43.8)
			** *Cistus* sp. (10.9), *Eucalyptus* sp. (4.1), *Helianthemum* sp. (13.7),
			* *Astragalus* sp. (2.7), Caprifoliaceae (1.4), *Erica* sp. (1.4), *Thalictrum* sp. (1.4),

*****: > 80 %; **** 46-80 %; *** 15-45 %; ** 3-15 %; * < 3

Palynological analysis of bee pollen samples revealed the presence of pollen grains from a total of 51 taxa belonging to 25 families of plants. The most commonly encountered pollen grains in the samples were from the families (insect-pollinated types) Fabaceae, Asteraceae, Cistaceae, and Apiaceae. The bee pollen samples presented different numbers of pollen taxa ranging from 9 (BP08) to 19 (BP06) types. Pollen belonging to *Ceratonia* sp., *Helianthemum* sp., and *Olea* sp. are most frequently genera represented in the samples. There were no common botanical species present in all the analysed samples. This observation suggests that the variability in pollen types presence may be linked to the geographical origin, reflecting the diverse flora within the beekeeping area.

In the study, two bee pollen samples were bifloral, the others were multifloral. The identified taxa from the bee pollen samples in our study were also observed in the pollen spectra of previous palynological studies carried out in Mediterrian regions of Türkiye (Silici and Gökceoglu, 2007; Temizer, 2023). *Ceratonia* sp. serves as an excellent resource for honey bee nourishment due to its advantageous characteristics like blooming in autumn, a season with limited plant flowering, production of nectar and pollen, significant nectar yield, and frequently visits by honeybees (Maha and Haddad, 2012). The pollen grain of *Ceratonia* sp. was dominant in BP01 (58.8 %) sample. A study was conducted that honey bees are actively utilizing olive flowers as a pollen source (Giovanetti, 2018), *Olea* sp. pollen grains were detected in bee pollen samples in this study. Particularly in the summer, honey bees choose the Cistaceae family as a source of pollen, finding it to be an exceptionally appealing source (Dimou et al., 2014). In this study, *Helianthemum* pollen was found as BP01 (16.2 %), BP02 (32.4 %), BP03 (29.9 %), BP06 (1.2 %), and BP08 (13.7 %). Some taxa (*Astragalus* sp., *Trifolium* sp., *Melilotus* sp. etc.) from the Fabaceae family are considered important resources used by bees (Küçükaydın et al., 2023; Sultana et al., 2022). Pollen grains belonging to the Apiaceae family were frequently found in the samples, like in other studies conducted in the Mediterrian region of Türkiye (Silici and Gökceoglu, 2007). *Cirsium* sp. pollen grain has already been reported in Eastern Anatolia bee pollen (Çobanoğlu et al., 2021). *Eucalyptus* sp. trees are grown along the coastlines of the Aegean and Mediterranean regions of Anatolia, with their flowering period is between from May to September. Pollen grains of *Eucalyptus* plant in the bee flora of Mersin province were determined in two (BP04, BP08) samples (Görhan, 2021).

3.2. Protein Content

The protein content showed statistical differences ($P<0.05$) and ranged from 22.8%±1.21 (BP07) to 28.1%±1.49 (BP01) (Table 3), (Figure 1).

Table 3. Protein content of bee pollen samples

	BP01	BP02	BP03	BP04	BP05	BP06	BP07	BP08
Protein	28.11±1.49 a	23.655±1.25b c	23.056±1.22b c	26.643±1.4a b	23.695±1.25b c	27.606±1.46 a	22.811±1.2 c	23.3±1.23b c

*Results are given as g/100 g. Mean values (n=3) with the different letters in a line indicate statistical differences among the results that determined by Tukey test ($p<0.05$)

Protein content results were similar to the results reported by Kostić et al. (2015), from Serbia (14.81–27.25 %), Estevinho et al. (2012), from Portugal (24.23–34.18%) and Mayda et al. (2020), for pollen samples from Türkiye (17.6–22.2 %). Nevertheless, Taha et al. (2019), found lower values (15.19–20.23 %) in bee pollen from Saudi Arabia. The significant variation in protein levels could potentially be impacted by factors such as floral sources, geographical origin, and storage conditions (Oroian et al., 2022).

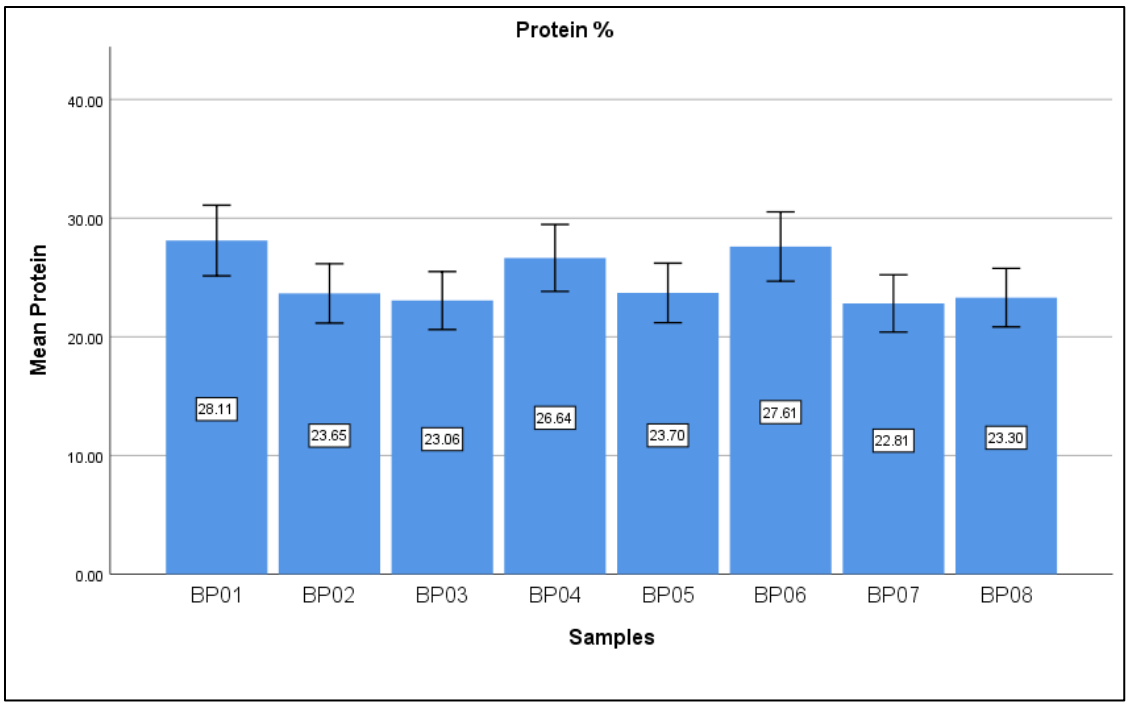

Figure 1. Protein content of bee pollen samples

Bee pollen's protein content stands out as its key defining feature, often serving as the primary criterion for assessing quality. It's typically categorized into three grades based on protein levels: excellent (above 25 %), average (20–25 %), and poor (below 20 %) (Somerville, 2001). In the current study, BP01, BP04, and BP06 were categorized excellent, other samples were classified as average according to this classification.

3.3. Phenolic compounds

For their wide range of functions, such as, anti-diabetic, antimicrobial, anti-inflammatory, antioxidant, and anti-hyperlipidemia effects, phenolic compounds have recently increased in attention (Ares et al., 2018). The results obtained by LC-MS/MS for the eight bee pollen samples from Mersin are presented in Table 4.

Table 4. Phenolic compounds of bee pollen samples

	BP01	BP02	BP03	BP04	BP05	BP06	BP07	BP08
Gallic acid	12.49±0.78 cd	4.94±0.31e	37.9±2.37 a	nd	16.58±1.04b	9.34±0.6	nd	nd
Caffeic acid	nd	nd	2.24±0.14 b	nd	0.71±0.04 c	4.1±0.26a	nd	0.2±0.012 d
Taxifolin	nd	0.37±0.02c	104.4±6.5 a	9.46±0.6b	5.55±0.34 bc	nd	nd	10.79±0.68b
Protocatechuic acid	nd	nd	nd	nd	nd	nd	nd	nd
Protocatechuic aldehyde	nd	nd	nd	nd	nd	nd	nd	nd
Sesamol	nd	nd	nd	nd	nd	nd	nd	nd
p-coumaric acid	nd	nd	nd	nd	nd	5.48±0.34 a	nd	nd
Catechin	nd	nd	nd	nd	nd	nd	nd	nd
Epicatechin	nd	nd	nd	nd	nd	nd	nd	nd
Rosmarinic acid	nd	nd	nd	nd	nd	nd	nd	nd
Vanillin	nd	0.96±0.06b	1.06±0.07 b	1.63±0.1a	1.1±0.07b	1.76±0.1a	nd	nd
Ferulic acid	nd	nd	nd	nd	nd	nd	nd	nd
4-OH-benzoic acid	nd	nd	nd	0.05±0.003a	nd	nd	nd	nd
Salicylic acid	nd	nd	nd	nd	nd	nd	nd	nd
Syringic Acid	4.05±0.25b	nd	nd	nd	nd	nd	4.69±0.3 a	nd
Ellagic acid	nd	nd	nd	1.05±0.06 a	nd	nd	nd	nd
Rosmarinic acid	nd	nd	nd	nd	nd	nd	nd	nd
Quercetin	nd	nd	31.44±1.96a	22.7±1.4b	nd	15.65±0.98c	nd	6.45±0.4d
Oleuropein	nd	nd	0.05±0.003d	2.31±0.14 c	3.95±0.25 b	nd	nd	9.8±0.61a
Rutin	nd	nd	nd	nd	nd	nd	nd	nd
Rezveratrol	0.8±0.05a	nd	nd	nd	nd	nd	0.55±0.03b	nd
Flavone	nd	nd	nd	nd	nd	nd	nd	nd
Kaempferol	nd	nd	7.7±0.48a	nd	nd	6.32±0.39 b	nd	nd

*Results are given a mean value of three replicates±standart deviation. Mean values (n=3) with the different letters in a column indicate statistical differences among the results that conducted by Tukey test ($p<0.05$). The results are given as µg/g. Nd: not detected.

The optimized chromatographic conditions enabled the measurement of 23 phenolic compounds in bee pollen samples. Of the 23 phenolic compounds analysed, 12 were determined in samples. Gallic, caffeic, syringic, and ellagic acids, taxifolin, vanillin, quercetin, oleuropein, rezveratrol, and kaempferol were determined in the samples. Taxifolin, gallic acid, and quercetin were the most abundant phenolic compounds of the investigated bee pollen samples, respectively. However, the 4OH benzoic acid, ellagic acid, and rezveratrol levels were at remarkably low amounts. The mean levels of taxifolin, gallic acid, quercetin, and kaempferol were high in the BP03 multifloral sample (Figure 2). Additionally, the BP06 bee pollen had high vanillin content, the BP04 bee pollen had high ellagic acid content, and the BP05 sample had high oleuropein content.

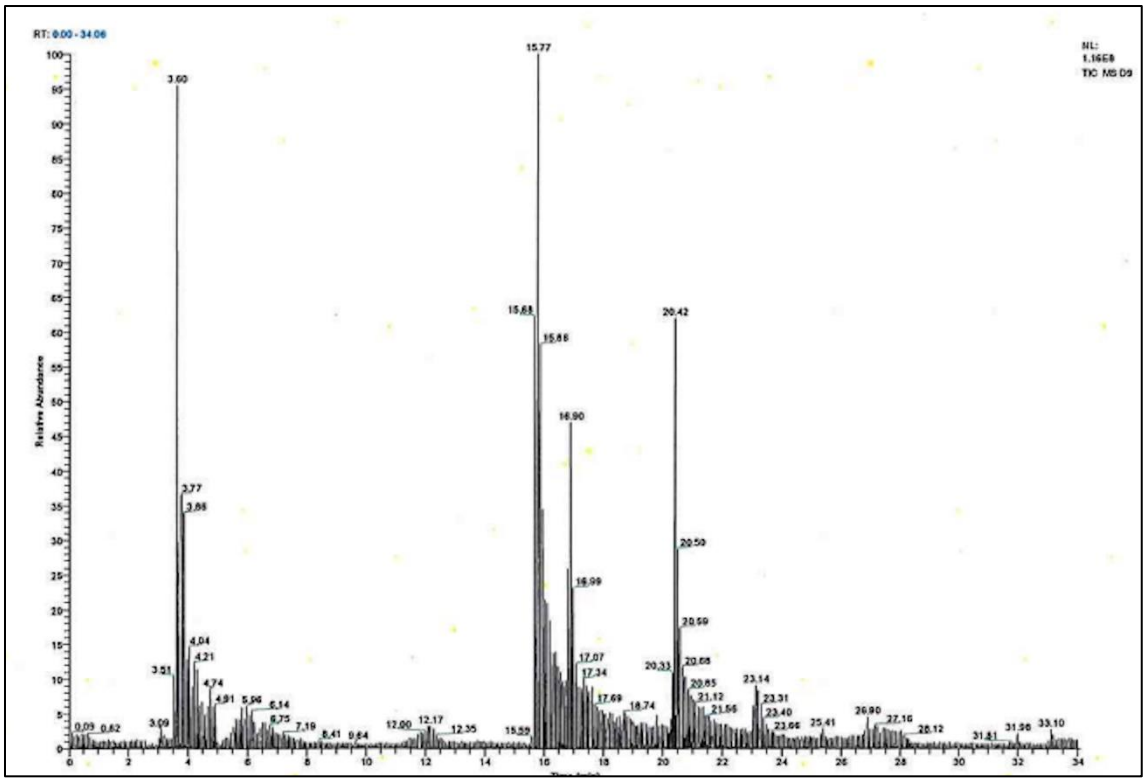

Figure 2. Chromatogram of BP03

In a study conducted by Mutlu and Erbas (2023), phenolic compounds were investigated in monofloral/multifloral bee pollen samples from different regions in Türkiye. It was reported that the amounts of rosmarinic acid, naringenin, rutin, and apigenin were 155.38, 51.91, 34.31, and 29.16 mg/100 g, respectively. In another study conducted a bee pollen sample collected from Bayburt, Türkiye, the major phenolic compounds such as rutin, kaempferol, quercetin, myricetin, and *p*-coumaric acid were determined 115442.25 ± 7774.28, 9870.72 ± 790.14, 7849.8 ± 528.63, 2220.70 ± 177.76, 1508.98 ± 91.89 µg/kg, respectively (Gercek et al., 2021). Rutin, which was detected at high levels in bee pollen in previous studies (Adaškevičiūtė et al., 2022; Çobanoğlu, 2024), was not detected in Mersin bee pollen. In this study, taxifolin was found 0.37±0.02-104.4±6.5 µg/g, while in another study with bee bread collected from Türkiye, was found 3.1±0.3-5.3±0.3 µg/g (Çobanoğlu et al., 2023b). The gallic acid wasn't found in the ethanolic extract of chestnut bee pollen by Karkar et al., (2018), but it was determined in five samples with this study. Quercetin, the third most abundant phenolic component in bee pollen, was also found in bee pollen in previous studies (Bridi et al., 2022; Gercek et al., 2021). With this study, the phenolic compound oleuropein detected in bee pollen samples, but it was not found in a previous study from Türkiye (Çelik et al., 2022). Kaempferol was detected 7.7±0.48 and 6.32±0.39 as µg/g in BP03, BP06, respectively and it was found 6.37-100.18 µg/g in another study (Çobanoğlu, 2024). BP03 had the most abundant phenolic compounds among the samples. Both the amount and type of phenolics in bee pollen may vary depending on the geographical location and conditions during production. Even if the samples originate from the same botanical source, these factors can lead to divergent outcomes (De-Melo et al., 2016).

4. Conclusions

Bee pollen is a bee product that provides the development of bees in the hive and is also recommended to be consumed by humans due to its content. The chemical content of bee

pollen varies depending on the plant diversity and environmental conditions in the environment where it is produced. Therefore, content determination studies of bee pollen are important for both beekeeping and human consumption. In this study, the botanical origin, protein and phenolic content of bee pollen produced in Mersin province of Türkiye were evaluated. The samples were divided into bifloral and multifloral as botanical source. It was found that the protein content of bee pollen samples was higher in bifloral samples. No such distinction was found in phenolic content. The findings underscore the importance of understanding the diverse factors influencing bee pollen composition, offering significant implications for its utilization in various fields, including nutrition, health, and apiculture.

References

Adaškevičiūtė, V., Kaškonienė, V., Barčauskaitė, K., Kaškonas, P., Maruška, A., 2022. The Impact of fermentation on bee pollen polyphenolic compounds composition. *Antioxidants*, 11(4): 645.

Alimoglu, G., Guzelmeric, E., Yuksel, P.I., Celik, C., Deniz, I., Yesilada, E., 2021. Monofloral and polyfloral bee pollens: Comparative evaluation of their phenolics and bioactivity profiles. *Lwt*, 142(5): 110973.

Al-Kahtani, S.N., 2017. Fatty acids and B vitamins contents in honey bee collected pollen in relation to botanical origin. *Sicientific Journal of King Fasal University*, 18(2):41–48.

Almeida-Muradian, L.B., Pamplona, L.C., Coimbra, S., Barth, O.M., 2005. Chemical composition and botanical evaluation of dried bee pollen pellets. *Journal of Food Composition and Analysis,* 18 (1): 105–111.

Altunatmaz, S.S., Tarhan, D., Aksu, F., Barutçu, U.B., Or, M.E., 2017. Mineral element and heavy metal (Cadmium, lead and arsenic) levels of bee pollen in Turkey. *Food Science and Technology*, 37: 136–141.

Alvarez-Suarez, J.M., 2017. Bee products - chemical and biological properties. *The dried chemicals.* Products - Chemical and Biological Properties, Springer Cham, Germany, p. 1–306.

Ares, A.M., Valverde, S., Bernal, J.L., Nozal, M.J., Bernal, J., 2018. Extraction and determination of bioactive compounds from bee pollen. J*ournal of Pharmaceutical and Biomedical Analysis,* 147: 110–124.

Aylanc, V., Falcão, S.I., Ertosun, S., Vilas-Boas, M., 2021. From the hive to the table: Nutrition value, digestibility and bioavailability of the dietary phytochemicals present in the bee pollen and bee bread. *Trends in Food Science and Technology*, 109: 464–481.

Baky, M.H., Abouelela, M.B., Wang, K., Farag, M.A., 2023. Bee pollen and bread as a super-food: a comparative review of their metabolome composition and quality assessment in the context of best recovery conditions. *Molecules*, 28(2): 715.

Barth, O.M., Freitas, A.S., Oliveira, É.S., Silva, R.A., Maester, F.M., Andrella, R.R. S., Cardozo, G.M.B.Q., 2010. Evaluation of the botanical origin of commercial dry bee pollen load batches using pollen analysis: A proposal for technical standardization. A*nais Da Academia Brasileira de Ciencias,* 82(4): 893–902.

Bridi, R., Echeverría, J., Larena, A., Nuñez Pizarro, P., Atala, E., De Camargo, A.C., Oh, W.Y., Shahidi, F., Garcia, O., Ah-Hen, K.S., Montenegro, G., 2022. Honeybee pollen from southern chile: phenolic profile, antioxidant capacity, bioaccessibility, and ınhibition of DNA Damage. *Frontiers in Pharmacology*, 13:1–12.

Çelik, S., Kutlu, N., Gerçek, Y.C., Bayram, S., Pandiselvam, R., Bayram, N.E., 2022. Optimization of ultrasonic extraction of nutraceutical and pharmaceutical compounds from bee pollen with deep eutectic solvents using response surface methodology. *Foods,* 11:3652.

Çobanoğlu, D.N., 2024. Assessing monofloral bee pollens from Türkiye: Palynological verification, phenolic profile, and antioxidant activity. *Journal of Food Science*, 89: 1711–1726.

Çobanoğlu, D.N., Kizilpinar Temizer, İ., Candan, E.D., Yolcu, U., Güder, A., 2023a. Evaluation of the nutritional value of bee pollen by palynological, antioxidant, antimicrobial, and elemental characteristics. *European Food Research and Technology*, 249: 307–325.

Çobanoğlu, D.N., Şeker, M.E., Temizer, İ.K., Erdoğan, A., 2023b. Investigation of botanical origin, phenolic compounds, carotenoids, and antioxidant properties of monofloral and multifloral bee bread. *Chemistry & Biodiversity*, 20: e202201124.

De-Melo, A.A.M., Estevinho, M.L.M.F., Sattler, J.A.G., Souza, B.R., Freitas, A. da S., Barth, O.M., Almeida-Muradian, L.B., 2016. Effect of processing conditions on characteristics of dehydrated bee-pollen and correlation between quality parameters. *Lwt,* 65: 808–815.

De Souza, R.R., de Abreu, V.H.R., de Novais, J.S., 2019. Melissopalynology in Brazil: a map of pollen types and published productions between 2005 and 2017. *Palynology*, 43: 690–700.

Dimou, M., Tananaki, C., Liolios, V., Thrasyvoulou, A., 2014. Pollen foraging by honey bees (*Apis mellifera* L.) in Greece: Botanical and geographical origin. *Journal of Apicultural Science*, 58: 11–23.

Dulger Altiner, D., Sandikci Altunatmaz, S., Sabuncu, M., Aksu, F., Sahan, Y., 2020. In-vitro bioaccessibility of antioxidant properties of bee pollen in Turkey. *Food Science and Technology*, 41: 133–141.

Estevinho, L.M., Rodrigues, S., Pereira, A.P., Feás, X., 2012. Portuguese bee pollen: Palynological study, nutritional and microbiological evaluation. *International Journal of Food Science and Technology*, 47: 429–435.

Çobanoğlu, D.N., Felek, İ., Dündar, O., 2021. Palynological, Antioxidant and Physicochemical Properties of Pollen Loads from Eastern Anatolia. *Bee Studies*, 13: 31–38.

Gercek, Y.C., Celik, S., Bayram, A.S., 2021. Screening of plant pollen sources, polyphenolic compounds, fatty acids and antioxidant/antimicrobial activity from bee pollen. *Molecules*, 27;117.

Giovanetti, M., 2018. Do bees like olive? A preliminary analysis of honey bee behaviour on flowers of the wind-pollinated species Olea europaea. *Acta Horticulturae,* 1199: 121–126.

Görhan, K., 2021. Mersin merkez ilçelerinin (Akdeniz, Toroslar, Yenişehir, Mezitli) arı bitkileri üzerine bir araştırma. *MAS Journal of Applied Sciences*, 8:518–523.

Halbritter, H., Ulrich, S., Grímsson, F., Weber, M., Zetter, R., Hesse, M., Buchner, R., Svojtka, M., Frosch-Radivo, A. 2018. Illustrated pollen terminology. Springer Nature, Cham, Germany, p1-483.

Hsu, P.S., Wu, T.H., Huang, M.Y., Wang, D.Y., Wu, M.C., 2021. Nutritive value of 11 bee pollen samples from major floral sources in taiwan. *Foods*, 10: 1–15.

Karkar, B., Şahin, S., Güneş, M.E., 2018. Antioxidative effect of turkish chestnut bee pollen on dna oxidation system and ıts phenolic compounds. *Gida / The Journal of Food*, 43: 34–42.

Kıvanç, M., Yapıcı, E., 2015. Kefir as a Probiotic Dairy Beverage: Determination Lactic Acid Bacteria and Yeast. *ETP International Journal of Food Engineering*, 1:55–60.

Komosinska-Vassev, K., Olczyk, P., Kafmierczak, J., Mencner, L., Olczyk, K., 2015. Bee pollen: chemical composition and therapeutic application. *Evidence-Based Complementary and Alternative Medicine,* 2015: 1-6.

Kostić, A.T., Barać, M.B., Stanojević, S.P., Milojković-Opsenica, D.M., Tešić, Ž.L., Šikoparija, B., Radišić, P., Prentović, M., Pešić, M.B., 2015. Physicochemical composition and techno-functional properties of bee pollen collected in Serbia. *Lwt,* 62: 301–309.

Küçükaydın, S., Tel-Çayan, G., Çayan, F., Taş-Küçükaydın, M., Eroğlu, B., Duru, M.E., Öztürk, M., 2023. Characterization of Turkish Astragalus honeys according to their phenolic profiles and biological activities with a chemometric approach. *Food Bioscience,* 53(2): 102507.

Li, Q.Q., Wang, K., Marcucci, M.C., Sawaya, A.C.H.F., Hu, L., Xue, X.F., Wu, L.M., Hu, F.L., 2018. Nutrient-rich bee pollen: A treasure trove of active natural metabolites. J*ournal of Functional Foods*, 49: 472–484.

Liolios, V., Tananaki, C., Dimou, M., Kanelis, D., Goras, G., Karazafiris, E., Thrasyvoulou, A., 2015. Ranking pollen from bee plants according to their protein contribution to honey bees. *Journal of Apicultural Research,* 54(5): 582–592.

Maha, S., Haddad, N., 2012. Utılızatıon of forest bıodıversıty: rewards of ceratonıa sılıqua l. for apıs mellıfera, *Uludag Bee Journal*, 12(2):62–67.

Mărgăoan, R., Özkök, A., Keskin, Ş., Mayda, N., Urcan, A.C., Cornea-Cipcigan, M., 2021. Bee collected pollen as a value-added product rich in bioactive compounds and unsaturated fatty acids: A comparative study from Turkey and Romania, *Lwt*,149: 111925.

Mayda, N., Özkök, A., Ecem Bayram, N., Gerçek, Y.C., Sorkun, K., 2020. Bee bread and bee pollen of different plant sources: determination of phenolic content, antioxidant activity, fatty acid and element profiles. *Journal of Food Measurement and Characterization*, 14 (4): 1795–1809.

Mutlu, C., Erbas, M., 2023. Turkish bee pollen: Composition, regional discrimination and polyphenol bioaccessibility. *Food Bioscience,* 53: 102805.

Oroian, M., Dranca, F., Ursachi, F., 2022. Characterization of Romanian Bee Pollen—An Important Nutritional Source. *Foods*, 11(17): 2633.

Rocchetti, G., Castiglioni, S., Maldarizzi, G., Carloni, P., Lucini, L., 2019. UHPLC-ESI-QTOF-MS phenolic profiling and antioxidant capacity of bee pollen from different botanical origin. I*nternational Journal of Food Science & Technology*, 54: 335–346.

Şahin, H., Kemal, M., 2020. Antioxidant capacity of a bee pollen sample obtained from Giresun, Turkey. *Journal of Apitherapy and Nature*, 2: 46–51.

Silici, S., Gökceoglu, M., 2007. Pollen analysis of honeys from Mediterranean region of Anatolia. *Grana*, 46: 57–64.

Somerville, D.C., 2001. Nutritional Value of Bee Collected Pollens. Rural Industries Research and Development Corporation, 1–166.

Sultana, S., Foster, K., Lim, L.Y., Hammer, K., Locher, C., 2022. A Review of the Phytochemistry and Bioactivity of Clover Honeys (*Trifolium* spp.). *Foods*, 11: 1–17.

Taha, E.K.A., Al-Kahtani, S., Taha, R., 2019. Protein content and amino acids composition of bee-pollens from major floral sources in Al-Ahsa, eastern Saudi Arabia. *Saudi Journal of Biological Sciences*, 26: 232–237.

Temizer, İlginç Kızılpınar, 2023. Elemental composition and evaluation of noncarcinogenic risks of bee pollen from different Turkish areas. I*ntegrated Environmental Assessment and Management,*00: 1-11*:*

Temizer, İlginc Kizilpinar, 2023. Diversity in bee pollen from three phytogeographical regions in Turkey. *Grana*, 62: 331–341.

Yang, K., Wu, D., Ye, X., Liu, D., Chen, J., Sun, P., 2013. Characterization of chemical composition of bee pollen in China. J*ournal Of Agricultural And Food Chemistry*, 61: 708–718.

To Cite Çobanoğlu, D.N., 2024. Palynological, Protein, and Phenolic Profiling of Bee Pollen from Mersin: An Investigation. *ISPEC Journal of Agricultural Sciences*, 8(2): 461-473. DOI: https://doi.org/10.5281/zenodo.11305923.