Original Article

BOTANICAL ORIGIN AND BIOCHEMICAL COMPOSITION OF DIFFERENT COLOR FRACTIONS OF BEE POLLEN

Sema Anık ORCID: 0000-0002-4932-8862
Filiz Vardar* ORCID: 0000-0002-1051-5628

Department of Biology, Science Faculty, Marmara University 34722, İstanbul, Turkey

*corresponding author: filiz.vardar@gmail.com
Received: 30 May 2024; accepted: 12 September 2024

Abstract

Bee pollen is a good nutrient storage and a natural source of phenolic acids and flavonoids with antimicrobial and antioxidant effects. Pollen color is related to flavonoids and carotenoids groups of structurally different plant pigments which are also non-enzymatic antioxidants. This study aims to indicate the biochemical differences between bee pollen of different color fractions concerning antioxidant properties. We separated the different colored bee pollens as light and dark to determine their botanical origins and biochemical compositions. According to their botanical origins, seventeen genera belonging to fourteen families were identified in the different colored bee pollen samples. *Scandix* sp. (Apiaceae), *Arbutus* sp. (Ericaceae), *Trifolium* sp., *Caesalpinia* sp. (Fabaceae), and *Morus* sp. (Moraceae) pollen were only encountered in dark pollen grains, whereas *Betula* sp. (Betulaceae), *Quercus* sp. (Fagaceae), and *Crataegus* sp. (Rosaceae) were only seen in light pollen grains. Total phenolics and carotenoids showed higher values in light color fraction however total flavonoids were higher in dark color. Four different antioxidant assays (ABTS, CERAC, CUPRAC, and DPPH) were performed for better comprehension. Although the bioactive ingredients were different based on pollen color fractions, there were no significant differences in antioxidant activities.

Keywords: antioxidant capacity, bee pollen, carotenoids, flavonoids, phenolic acids

INTRODUCTION

Pollen is the male gametophyte of gymnosperms and angiosperms carrying two sperm cells transferred to the female gametophyte where fertilization occurs. The mature pollen grain also serves as a nutrient storage site (proteins, sugars, lipids, mineral salts, and vitamins) for germinating and elongating the pollen tube (Vardar & Ünal, 2011). Pollen has also been considered to be a significant dietary supplement due to its rich nutritional constituents and its widespread applications in cosmetics, food and modern/alternative medicine. Its therapeutic properties are directly related to its protective properties against oxidative damage. Pollen's anti-inflammatory, antioxidant, antimicrobial, anti-carcinogenic, and immune-enhancing features are studied extensively (Oroian et al., 2022). It aids in recovery from chronic illness, slows aging, lowers cholesterol levels and regulates intestinal functions depending on their free radical scavenging potential. The numerous health benefits of pollen are mainly due to its abundance of nutrients and such functional compounds as polyphenols, particularly phenolic acids and flavonoids contributing to its bioactive properties (Aylanc et al., 2021; Qiao et al., 2024). Such phenolic acids as p-coumaric, ferulic, gallic, chlorogenic, vanillic, caffeic and syringic acids, are aromatic secondary plant metabolites that spread throughout the plants. The consumption of phenol-containing foods prevents DNA and tissue damage induced by oxidative stress caused by a variety of endogenous and exogenous factors. Flavonoids, a group of natural substances with variable phenolic structures, are essential in plants' growth, development and defense. Furthermore, they have subgroups that include flavanonols, flavanols (catechins), anthocyanins, chalones, isoflavones, and neoflavanoids (Kocot et al., 2018; Kieliszek et al., 2018).

The nutritional, chemical, and functional properties of bee pollen are greatly impacted by its botanical origin. Monofloral pollen from a particular plant source tends to have consistent physicochemical, functional, and sensory properties, while polyfloral pollen loads exhibit variations in these properties. Even pollen from the same plant source may have different compositions due to seasonal and regional conditions (Thakur & Nanda, 2020). The botanical origin also affects the color of the pollen grain ranging from white (e.g. *Clematis* sp.) and yellow (e.g. *Brassica* sp.) to orange (e.g. *Calendula* sp.), red (e.g. *Daucus* sp.), green (e.g. Corylus sp.), gray (e.g. *Papaver* sp.), dark blue (e.g. *Scilla* sp.) and dark brown (e.g. *Trifolium* sp.) (Bleha et al., 2021). Pollen pigments both preserve DNA from harmful short-wavelength radiation through the absorption of UV light and affect the visible color of pollen for UV-sensitive flower visitors. Such visitors as hoverflies (Syrphidae, Diptera) and bees (Apoidea, Hymenoptera), which feed on pollen, exhibit behavioral reactions triggered by visual pollen signals (Lunau, 2000). Pollen color is related to flavonoids and carotenoids, groups of structurally different plant pigments. Carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, lycopene) also called tetraterpenoids, are yellow, orange, and red plant pigments and are accessory pigments in photosynthesis and one of the non-enzymatic antioxidants (Bleha et al., 2021). Both carotenoids and flavonoids cause yellow pollen, but yellow color tones vary from species to species. Carotenoids have been suggested to appear only in the yellow pollen of entomophilous flowers, although flavonoids have appeared in the yellow pollen of anemophilous and entomophilous flowers. Several analyses of pollen demonstrated that pigments were located in pollenkitt, exine or cytoplasm of pollen grains (Lunau, 1995).

Only <5% of pollen has been reported to reach the stigma for reproduction. Although it is the main element for sexual reproduction in plants, a huge amount is collected as bee food and consumed by other animals and humans (Oroian et al., 2022). Pollen is one of the essential food sources of honeybees (*Apis mellifera* L.) and is collected by foraging worker bees with their hind legs (Dolezal & Toth, 2018) combined with honeybee secretions and nectar. Traps attached to the entrance of the hive have been designed to collect bee pollen loads directly from the legs of the returning bees to be sold to consumers (Mayda et al., 2020; Bayram et al., 2021). As with other apicultural products, bee pollen is highly nutritious and extensively used as a preventive and supplementary product in the well-known alternative medicine field all over the world (Oroian et al., 2022), because it is a strong antioxidant substance with a rich amount of phenolic substances, flavonoid and carotenoid contents. Physical, chemical, and biological properties of bee pollen vary depending on the geographical location, botanical origin, climatic characteristics, and bee secretions (Karataş et al., 2000).

There is little available research on the relation to color and other biochemical properties of pollen. Yang et al. (2013) revealed the correlation between color values and Ca, Mg, and Fe contents. Likewise, de Melo et al. (2016) compared processing conditions during bee pollen dehydration. The researchers determined the color values after the two dehydrating methods and correlated their color and antioxidant-antimicrobial potentials by revealing the color effectiveness on biochemical parameters. Salazar-González et al. (2022) characterized the carotenoid profile and α-tocopherol content of Andean pollen by considering botanical origin and seasonal conditions. They also correlated their results with pollen antioxidant capacity. Flavonoids and carotenoids are responsible for both pollen's color and antioxidant properties, but, phenolic substances also have antioxidant properties. One of the remarkable questions is whether bee pollen of different color fractions makes a difference in terms of antioxidant properties. If antioxidant capacity varies depending on color difference, it will offer an option for the separate presentation of bee pollens with the desired color majority or for pollen production through the consideration of the botanical origin (e.g. monofloral) in the food industry. Therefore, we aimed to indicate the

biochemical differences between bee pollen of different color fractions especially in terms of antioxidant properties from different botanical origins.

MATERIAL AND METHODS

Bee pollen (BP) samples were provided commercially from Baldağı Food Industry and Trade Limited Company (Ankara, Türkiye). They had collected by *Apis mellifera* in the provinces of Afyon, Burdur, Denizli, Isparta and Kütahya (Inner Aegean Region, Türkiye; Fig. 1). BP granules were separated and named according to the pollen color chart (https://www.mybeeline.co/en/p/pollen-identification-color-guide) under a stereo microscope providing bright LED illumination (Leica EZ4). To determine the taxa in every different colored pollen granule, the preparations were performed according to Wodehouse (1935). At least 500 pollen grains were counted in each preparation to determine the frequency classes of pollen types. Frequency classes were determined (Jones & Bryant, 1996) using the terms 'very frequent' (>50%), 'frequent' (20-50%), 'low frequent' (10-20%) and 'rare' (<10%). For the botanical identification, each pollen type was compared with the palynological database PalDat (www.paldat.org) and the data of several atlases (Bicakci, 1996; 1999; Sorkun, 2008). Pollen slides were examined and measurements (Polar and equatorial axes) were performed with an Olympus BX-51 microscope and photographed with a KAMERAM camera and software (Argenit, Türkiye). Then, pollen granules were combined in two main fractions as light (yellow-orange and their tones) and dark (red-brown-green-purple-blue and their tones) color, and color analysis, total carotenoid, phenolic, flavonoid contents and antioxidant activities were determined. Physico-chemical analysis of BP moisture (method no: 925.40) and ash (method no: 950.49) contents were performed according to the standard methods of the Association of Official Analytical Chemists (2000).

Determination of Surface Color

The surface color measurement of bee pollen samples (light fractions-LF and dark fractions-DF) was carried out by NH 300 colorimeter (3nh, China). The results were expressed with the use of the CIE L*, a*, b* color system. Before analysis, the instrument was calibrated with a white ceramic plate. The following parameters were determined: L* defines lightness (L*=0 black, L*=100 white), a* denotes the red/green value (a*<0 green, a*>0 red), and b* the yellow/blue value (b*<0 blue, b*>0 yellow).

Determination of Total Carotenoid Content

Total carotenoid content (TCC) in BP (LF and DF) was quantified according to the methodology described by Szydłowska-Czerniak et al. (2011). Samples (1.0 g) were homogenized in

Fig. 1. Provinces (marked with orange) where bee pollen samples were collected. Latitude and longitude coordinates of Afyon: 38°45' N 30°32' E; Burdur: 37°43' N 30°16' E; Denizli: 37°46' N 29°5' E; Isparta: 37°46' N 30°33' E; Kütahya: 39°25' N 29°58' E.

n-hexane (10 mL). After centrifugation at 7500 rpm for 10 min, the diluted (1:1 v/v) supernatant was measured against n-hexane at 450 nm spectrophotometrically and expressed as mg carotenoids per g of samples (mg/g) using a β-carotene curve 1-20 mM (R^2=0.999).

Preparation of Bee Pollen Extracts for Total Phenolic, Flavonoid Content and Antioxidant Activities

Aliquots of 2 g BP samples (LF and DF) were dissolved in 10 mL 95% (v/v) ethanol by agitation on a vortex followed by ultrasonic-assisted extraction in an ultrasonic cleaning bath for 60 min at 40°C. The mixture was centrifuged at 2772 g for 30 min. at 40°C. The extraction procedure was repeated twice combining into a 25 mL volumetric flask. The volume was made up of ethanol (95% - v/v). A portion of the supernatant was filtrated through a 0.45 µm membrane and diluted to the appropriate concentration (Mayda et al*.,* 2020). The filtrated supernatant is used for determining total phenolic and flavonoid content and antioxidant activity assays.

Determination of Total Phenolic Content

To determine the total phenolic compounds (TPC), the Folin-Ciocalteu method was performed using gallic acid as the reference standard (Magalhães et al., 2010). A mixture of 50 µL of BP extract, 50 µL of Folin-Ciocalteu reagent (1:5, v/v), and 100 µL of 0.35 M NaOH solution (w/v) was added into each well. The absorbance of the blue complex formed was measured after three min at 760 nm spectrophotometrically. The results were expressed as mg gallic acid equivalent (mg GAE/g) compared to a gallic acid standard curve 1-8 mM (R^2=0.9957).

Determination of Total Flavonoid Content

Total flavonoid (TFC) analysis was performed according to Zhishen et al*.* (1999). 1 mL of BP extract was mixed with 0.3 mL of 10% $AlCl_3$ (w/v), 0.3 mL of 5% $NaNO_2$ (w/v), 2 mL of 1 M NaOH (w/v) and 2.4 mL of water and the mixture was stirred. The absorbance was measured at 510 nm spectrophotometrically. The results were expressed as mg quercetin equivalent (mg QE/g) compared to a quercetin standard curve 1-10 mM (R^2=0.9994).

Radical Cation Decolorization (2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid-ABTS) Assay

The 7 mM ABTS reagent (radical cation, w/v) was reacted with 2.45 mM $K_2S_2O_8$ (w/v) as a stock solution and left in the dark at room temperature for 12-16 h before use. The working ABTS solution was diluted from stock solution with ethanol to an absorbance of 0.70 at 734 nm and equilibrated at 30°C. The 1 ml BP extracts were mixed with 1 ml of ABTS and 2 mL methanol. The tubes were kept at room temperature (RT) for six minutes, and the absorbances of the samples were measured at 734 nm (Re et al., 1999). The results were expressed as mg Trolox equivalent antioxidant capacity (TEAC) per g of samples (mg TEAC/g) compared to a Trolox standard curve 1-20 mM (R^2=0.9984).

Ce (IV)-Based Reducing Capacity (CERAC) Assay

1 mL 2.0 mM Ce(IV) solution (w/v) and 1 mL BP extract were mixed and diluted to 10 mL with distilled water. The solution was kept for thirty minutes at RT. The absorbance of the mixture was measured at 320 nm (Ozyurt et al., 2010). The results were expressed as mg Trolox equivalent antioxidant capacity (TEAC) per g of samples (mg TEAC/g) compared to a Trolox standard curve 1-10 mM (R^2=0.9995).

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay

1 mL of 0.1 mM copper (II) chloride (w/v), 1 mL of 7.5 mM neocuproine (w/v), and 1 mL of 1 M ammonium acetate (w/v) buffer (pH=7.0) were mixed through agitation for five seconds by a vortex (Apak et al., 2007). Then, 0.1 mL of BP extract and 1 mL of distilled water were added to these solutions with a final volume of 4.1 mL. Tubes were kept at RT with the cover closed for thirty minutes. The absorbances of the solutions were measured at 450 nm and expressed as mg Trolox equivalent antioxidant capacity (TEAC) per g of samples (mg TEAC/g) compared to a Trolox standard curve 1-10 mM (R^2=0.9976).

Free Radical Scavenging (2,2-diphenyl-1-picrylhydrazyl-DPPH) Assay

15 µL BP extract and 185 µL of DPPH solution (150 µmol/L, w/v) in methanol were mixed and vortexed for ten seconds. After being kept for forty-five minutes in the dark at RT, the absorbance was measured at 515 nm (Kumaran & Karunakaran, 2006) and expressed as mg Trolox equivalent antioxidant capacity (TEAC) per g of samples (mg TEAC/g) compared to a Trolox standard curve 1-4 mM (R^2=0.9991).

Statistical Analysis

Statistical analysis was performed with the use of one-way analysis of variance (ANOVA), (SPSS 21.0 software) between LF and DF of BP parameters. The significance of the applications was designated at the p<0.05 level with the use of Tukey's test. All data presented are means ±SD. All experiments were conducted three times. Linear regression coefficient (R^2) for total phenolic, flavonoid, and carotenoid contents with antioxidant activities was analyzed by Graph Pad Prism for Windows, Version 7 (Graph Pad Software, San Diego, CA, USA). A p-value <0.05 was considered significant.

RESULTS

Baldağı BP contains mostly blue crocus color (L1) with a rate of 28.39%, followed by ivy color (L2) with 28.21% (Tab. 1). Additionally, poplar-colored (L7) BP is the third most abundant with 23.59%. While the most abundant BP forms the LF with 92.74%, the dark fraction presented a ratio of 7.26%. According to their botanical origins, seventeen genera belonging to fourteen families were identified in the different colored bee pollen samples (Tab. 2). *Chenopodium* sp.

Table 1.

Microscopic and colorimetric analysis of BP color fractions

Samples		Color Name	Weight (%)	L* (Lightness)	a* (Red/Green Value)	b* (Blue/Yellow Value)	
LIGHT COLORS	L1	Blue Crocus	28.39	61.69 ±4.00	+6.92 ±0.31	+16.49±1.47	
	L2	Ivy	28.21				
	L3	Wall Flower	7.58				
	L4	Ling Heather	2.49				
	L5	Yellow Crocus	0.18				
	L6	Willow	2.30				
	L7	Poplar	23.59				
DARK COLORS	D1	Berberis	0.53	48.12 ±0.72	+7.01 ±0.18	+8.26 ±0.52	
	D2	Pear/Crab Apple	0.43				
	D3	White Horse Chestnut	0.08				
	D4	Lupin	0.10				
	D5	Red Deadnettle	0.09				
	D6	Blackthorn	0.44				
	D7	False Acacia	0.03				
	D8	Rose Bay Willow Herb	0.02				
	D9	Sycamore	0.74				
	D10	Gooseberry	3.53				
	D11	Red/White Clover	0.35				
	D12	Red Horse Chestnut	0.92				

D: Dark color fraction, L: Light color fraction (https://www.mybeeline.co/en/p/pollen-identification-color-guide)

(Amaranthaceae), *Carduus* sp. (Asteraceae), *Cistus* sp. (Cistaceae), *Convolvulus* sp. (Convolvulaceae), *Stachys* sp. (Lamiaceae), *Pinus* sp. (Pinaceae), *Rosa* sp. (Rosaceae), *Salix* sp. (Salicaceae) pollen were common in both LF and DF of BP samples. Besides *Carduus* sp., *Cistus* sp., *Convolvulus* sp. and *Salix* sp. were very prevalent in both samples. *Scandix* sp. (Apiaceae), *Arbutus* sp. (Ericaceae), *Trifolium* sp., *Caesalpinia* sp. (Fabaceae), and *Morus* sp. (Moraceae) pollen were only encountered in DF of BP. Moreover, *Betula* sp. (Betulaceae), *Quercus* sp. (Fagaceae), and *Crataegus* sp. (Rosaceae) were only seen in LF of BP.

Based on the CIE color measurement system based on a three-coordinate diagram (L*, a*, b*), colors in numeric values are evaluated as the following colors: L*=100 white, L*=0 black; positive a* red, negative a* green; positive b* yellow and

Table 2.

Botanical origins of bee pollen

Samples	Amaranthaceae	Apiaceae		Asteraceae	Betulaceae	Cistaceae	Convolvulaceae	Ericaceae	Fabaceae		Fagaceae	Lamiaceae	Moraceae	Pinaceae	Rosaceae		Salicaceae
	Chenopodium sp.	*Anthriscus sp.*	*Scandix* sp.	*Carduus* sp.	*Betula* sp.	*Cistus* sp.	*Convolvulus* sp.	*Arbutus* sp.	*Trifolium* sp.	*Caesalpinia* sp.	*Quercus* sp.	*Satchys* sp.	*Morus* sp.	*Pinus* sp.	*Rosa* sp.	*Crataegus* sp.	*Salix* sp.
L1	R	-	-	F	-	F	LF	-	-	-	-	R	-	-	R	R	R
L2	-	-	-	-	R	VF	R	-	-	-	-	R	-	-	-	-	R
L3	R	-	-	F	-	-	LF	-	-	-	-	LF	-	R	-	-	R
L4	-	-	-	R	-	VF	-	-	-	-	-	R	-	R	-	-	LF
L5	R	-	-	LF	-	VF	-	-	-	-	-	F	-	-	-	-	R
L6	-	-	-	F	-	F	R	-	-	-	LF	-	-	-	-	-	LF
L7	-	-	-	F	F	F	R	-	-	-	-	-	-	-	-	-	R
D1	-	-	-	-	-	VF	LF	-	-	-	-	-	-	-	-	-	LF
D2	-	-	-	-	-	LF	LF	-	-	-	-	-	-	-	-	-	F
D3	-	-	-	R	-	F	F	-	-	-	-	-	-	-	-	-	-
D4	-	-	-	VF	-	LF	x	-	-	-	-	-	-	LF	-	-	-
D5	-	-	-	R	-	VF	LF	-	-	-	-	-	-	-	-	-	F
D6	-	-	-	R	-	VF	R	-	-	-	-	-	-	-	-	-	F
D7	-	-	-	R	-	R	LF	-	LF	-	-	F	LF	-	-	-	R
D8	-	-	-	LF	-	R	-	-	-	-	-	-	-	-	-	-	VF
D9	R	LF	LF	R	-	R	VF	-	-	-	-	-	-	-	R	-	R
D10	-	-	-	-	-	-	R	-	VF	-	-	-	-	-	R	-	R
D11	-	F	-	LF	-	-	VF	-	-	-	-	-	-	-	-	-	LF
D12	R	-	-	-	-	-	VF	R	-	R	-	-	-	-	-	-	R

Botanical origins of BP. VF: Very Frequent; F: Frequent; LF: Low Frequent; R: Rare.

negative b* blue. CIA measurement values for the bee pollen samples are shown in Tab. 1. Mean L*, a*, and b* values were 61.69, +6.92, and +16.49 in light and 48.12, +7.01 and +8.26 in dark BP, respectively. Color measurement values revealed L (white-black), and b (yellow-blue) values were significantly different between LF and DF.

According to physicochemical analyses, the moisture was 4.78% in LF and 4.69% in DF, and the ash was 2.01% in LF and 2.55% in DF of BP (Tab. 3). TPC was 8.85 mg/g in LF and 7.11 mg/g in DF of BP. However, TFC was 1.73 mg/g in LF and 2.15 mg/g in DF of BP. These results showed that LF of BP has higher phenolics and lower flavonoids and vice versa for DF of BP. Similarly, total carotenoid content results revealed that LF of BP contains higher carotenoids than DF. TCC was 8.57 mg/g in LF and 6.97 mg/g in DF of BP. Antioxidant activity results revealed that there was no significant difference in antioxidant enzyme activities between LF and DF of BP. The CUPRAC results were 9.76 mg/g in LF and 9.58 mg/g in DF. The ABTS was 0.84 mg/g in LF and 0.86 mg/g in DF. The DPPH was 9.13 mg/g in LF and 8.62 mg/g in DF. The CERAC was 25.52 mg/g in LF and 27.37 mg/g in DF of BP.

Coefficient analysis indicated a strong positive correlation between ABTS-CERAC and TPC in LF; however, a strong positive correlation between DPPH-CERAC and TPC in DF was observed (Tab. 4). A strong positive correlation was also determined between ABTS-CERAC and TFC in LF, but on the other hand, a strong positive correlation between all antioxidant capacity tests and TFC in DF was detected. Additionally, CUPRAC-ABTS-CERAC and TCC in LF showed a strong positive correlation; however, a positive correlation between DPPH-CERAC and TCC in DF was noticed.

Table 3.

Results of moisture, ash, TPC, TFC, TCC, and antioxidant activities

	Moisture (%)	Ash (%)	TPC (mg GAE/g)	TFC (mg QE/g)	TCC (µg/mL)	ABTS (mg TEAC/g)	CERAC (mg TEAC/g)	CUPRAC (mg TEAC/g)	DPPH (mg TEAC/g)
LF	4.78	2.01	8.85±0.52	1.73±0.04	8.57±0.17	0.84±0.01	25.52±1.42	9.76±0.58	9.13±0.57
DF	4.69	2.55	7.11*±0.23	2.15*±0.11	6.97*±0.14	0.86±0.01	27.37±0.89	9.58±0.21	8.62±0.30

The data with asterisks (*) is significantly different from the light color fraction according to Tukey's test at P<0.05 for independent samples. Results are expressed as mean±SD.

Table 4.

The correlation coefficient values (R2) of TPC, TFC, TCC, and antioxidant capacity methods (P<0.05)

		TPC	TFC	TCC
LF	**ABTS**	0.964*	0.942*	0.871*
	CERAC	0.959*	0.937*	0.879*
	CUPRAC	0.183	0.145	0.709*
	DPPH	0.003	0.001	0.328
DF	**ABTS**	0.185	0.535*	0.166
	CERAC	0.978*	0.681*	0.999*
	CUPRAC	0.387	0.750*	0.037
	DPPH	0.978*	0.750*	0.989*

DISCUSSION

Türkiye is as rich as the European continent in terms of plant diversity. The polyfloral BP identifications in Afyon, Burdur, Denizli, Isparta and Kütahya matched in previous studies performed in the mentioned cities. Taşkın (2016) reported fifty-eight taxa belonging to thirty-three different families in Burdur region honey. Their microscopic analysis showed similar taxa to those in our results, including Asteraceae, Ericaceae, Fabaceae (*Trifolium* spp.), Rosaceae and Apiaceae (*Anthriscus* spp.). Bicakci et al. (1996) reported similar taxa in the airborne pollen of Isparta: Pinaceae (*Pinus* spp.; 32.8%), Fagaceae

(*Quercus* spp., 10.84%), Moraceae (2.66%), Rosaceae (2.34%), Salicaceae (*Salix* spp., 0.52%), Cistaceae (*Cistus* spp., 0.07%), Amaranthaceae (2.38%), Asteraceae (1.36%). Besides Bicakci et al. (1999) reported Cistaceae (*Cistus* spp., 0.05%), Ericaceae (0.29%), Moraceae (0.27%), Pinaceae (*Pinus* spp., 35.82%), Fagaceae (*Quercus* spp., 4.32%), Rosaceae (1.34%) and Salicaceae (*Salix* spp., 1.15%) in the airborne pollen of Kütahya. To identify the plant origin of BP, it is very important to standardize the biochemical content and antioxidant capacity between color fractions. According to our results, *Anthriscus* sp., *Arbutus* sp., *Trifolium* sp., *Caesalpinia* sp., and *Morus* sp. probably contribute to the formation of the DF.

BP contains a wide variety of pollen types and is used as a food source for all stages of bee development (Campos et al., 2008). Its rich chemical structure of proteins (10% to 40%), free amino acids, carbohydrates (13% to 55%), lipids (1% to 10%), fatty acids, phenolic compounds (1.6%), vitamins (including B-complex vitamin E, D, and C), minerals, carotenoids (provitamin A as β-carotene), flavonoids (1.4%), sterols and terpenes highlights BP as a good nutritional supplement (Kieliszek et al., 2018). BP also contains such acids as pantothenic, nicotinic, folic, biotin, rutin, and inositol (Komosinska-Vassev et al., 2015).

BP has some critical bioactive ingredients, especially flavonoids, phenolic acids, and carotenoids (Qiao et al., 2024). These substances affect the antimicrobial, antioxidant, antifungal, and anti-inflammatory effects of pollen, as well as such physicochemical properties as color, taste and odor (Kieliszek et al., 2018). Furthermore, free amino acids are an important part of nectar taste as an attractant to pollinators and the pleasant smell of pollen (Nicolson et al., 2007). Flavonoids have a role as antioxidants eliminating the free radicals directly, interacting with enzymes or binding the metal cations chelatically. Phenolic compounds neutralize free radicals mainly by quenching oxygen or decomposing peroxides. BP also contains carotenoids which are naturally occurring pigments responsible for yellow and orange color and can scavenge the radicals in such ways as electron transfer, addition reactions and hydrogen elimination (Aličić et al., 2014; Fatrcovà-Šramková et al., 2016). Many studies have revealed that the individual composition as well as the antioxidant activity of BP samples collected from different locations were differently correlated with the botanical and genetic sources, soil type, bee secretions and climatic conditions (Kostićaž-Milinčič et al., 2020). These differences lead to the necessity of its effective identification and classification.

Previous research also showed that BP had different amounts of bioactive compounds and antioxidant capacities (Tutun et al., 2021). Özcan et al. (2019) reported that TCC were 12.78 and 98.62 mg/g in the BP of Türkiye and Russia, respectively. Mayda et al. (2020) demonstrated that TFC values varied between 2.62-4.44 mg QE/g and TPC varied between 26.69-43.42 mg GAE/g in BP samples from Türkiye. Yesiltas et al. (2014) indicated that the TPC of nine BP samples collected from different regions of Türkiye was between 12.0-36.7 mg GAE/g. Ulusoy & Kolayli (2014) also reported that the TPC was between 44.07-124.10 mg/g in Anzer pollen from Türkiye. Similarly, Özkök & Silici (2017) revealed that TPC was 2340.07 mg GAE·100/g in BP from Türkiye. Moreover, Kanar & Mazı (2019) measured the TPC as 14.42 mg GAE/g in multifloral fresh BP from Türkiye. Dulger-Altiner et al. (2020) reported that the TPC of BP from İstanbul-Türkiye varied between 147.10-462.02 mg GAE/g.

Many assays are used to determine the antioxidant capacity of BP based on different mechanisms of the scavenging of free radicals or chelation of metal ions (Aličić et al., 2014). Several results also revealed that the antioxidant activity of BP may vary depending on its bioactive compounds (Kocot et al., 2018; Saral et al., 2019). Yesiltas et al. (2014) indicated that the antioxidant capacities of the nine BP samples collected from different regions of Türkiye according to ABTS, CUPRAC, DPPH, and FRAP methods were 15.2-33.6 mg TE/g, 20.7-89.4 mg TE/g, 5.7-15.2 mg TE/g and 5.2-15.7 mg TE/g,

respectively. Similarly, Ulusoy & Kolaylı (2014) reported the results of FRAP as 11.77-105.06 µmol Trolox/g, CUPRAC as 33.1-86.8 mmol/g, DPPH as 0.65-8.20 mg/mL in Anzer pollen. Özkök & Silici (2017) also demonstrated the DPPH value as 42.37 mg AAE/g in monofloral BP samples from Türkiye. Karkar et al. (2020) evaluated the antioxidant capacities of chestnut BP, and they were between 3.70-34.18 mg TE/g and 3.15-20.24 mM TE/g by CHROMAC and FRAP methods, respectively. On the other hand, Özcan et al. (2019) measured the DPPH as 60.35% and 81.41% in BP from Türkiye and Russia, respectively. Moreover, Kanar & Mazı (2019) reported the DPPH results as 0.29 mg dry pollen in multifloral fresh BP from Türkiye. Dulger-Altiner et al. (2020) also revealed the results of CUPRAC as 6.25-257.27 µmol TE/g, ABTS as 6.20-111.40 µmol TE/g and DPPH as 0.44-83.84 µmol TE/g in BP from Türkiye. Mayda et al. (2020) reported that DPPH was between 3.08-3.85 mg TE/g and ABTS was between 1.80-5.98 mg TE/g in BP from Türkiye.

The assays used to measure antioxidant capacity can be divided into the categories of hydrogen atom transfer (HAT) and single electron transfer (SET) methods. SET assays measure the antioxidant's ability to donate an electron to stabilize a radical, while HAT assays measure the antioxidant's ability to donate hydrogen atoms. Although the end products of both mechanisms may be the same, their reaction rates are different. No single method is entirely reliable for determining antioxidant activity, since their chemical structures and effectiveness are not easily categorized as HAT or SET mechanisms. The ABTS and DPPH methods that we used are scavengers of stable free radicals. They are also popular assays for antioxidant activity based on hydrogen atom transfer (HAT). While CERAC and CUPRAC are reducing antioxidant power assays by single electron transfer (SET) (Jaganjac et al., 2021).

According to our results, TPC and TCC were higher in LF and TFC was higher in DF. For better comprehension, the four different antioxidant assays ABTS, CERAC, CUPRAC and DPPH were performed to reveal the relation between biochemical contents and antioxidant activity. The results indicated that whereas the bioactive ingredients were different based on pollen color fractions, there were no significant differences in antioxidant activities in different color BP. However, correlation coefficient values revealed that ABTS and CERAC assays were more significantly correlated to TPC, TFC, and TCC in LF. Furthermore, DPPH and CERAC assays were more significantly correlated to TPC, TFC, and TCC in DF. Additionally, TFC values were highly correlated with four antioxidant assays in DF. Similarly, TCC results showed a high correlation to CUPRAC-ABTS-CERAC in LF.

In conclusion, the correlation coefficient results revealed that both LF and DF pollen grains have antioxidant capacity for antioxidant and TPC-TFC-TCC assays. In these tests, the CERAC assay came forward due to a strong significant correlation both in LF and DF. Besides the strong correlation of TFC with four antioxidant assays in DF is noteworthy. Results can be interpreted TPC, TFC, and TCC do not have direct or single effects on antioxidant activity, even in different amounts in different colored BP. The food industry can evaluate these results by packaging pollen in different colors and presenting it to the consumer. For example, to obtain DF pollen with high TFC and antioxidant properties, beekeeping can be carried out in areas with certain flowers (such as *Scandix* sp., *Arbutus* sp., *Trifolium* sp., *Caesalpinia* sp., *Morus* sp.) and be evaluated in the future as a functional food product. Similarly, LF containing high TCC and antioxidant properties may be preferable by consumers.

ACKNOWLEDGMENT

This study is part of a Ph.D. dissertation by Sema Anık at Marmara University and supported by the Research Foundation of Marmara University (BAPKO) project number FEN-C-DRP-090517-0290. We special thanks to Dr. Yusuf Can Gercek (İstanbul University, Plant and Herbal Products Application and Research Center) for conducting our TPC-TFC-TCC and antioxidant capacity analysis.

REFERENCES

Aličić, D., Šubarić, D., Jašić, M., Pašalić, H., Ačkar, Đ. (2014). Antioxidant properties of pollen. *Hrčak, 3*(1), 6-12. DOI: 10.31467/uluaricilik.896045

Apak, R., Güçlü, K., Özyürek, M., Karademir, S.E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, Using their cupric ion reducing capability in the presence of neocuproine: CUPRAC Method. *Journal of Agricultural and Food Chemistry, 52*(26), 7970-7981. DOI: 10.1021/jf048741x

Aylanc, V., Larbi, S., Calhella, R., Barros, L., Rezouga, F., Rodríguez-Flores, M.S. … Falcão, S.I. (2023). Evaluation of antioxidant and anticancer activity of mono- and polyfloral Moroccan bee pollen by characterizing phenolic and volatile compounds. *Molecules, 28*(2), 835. DOI: 10.3390/molecules28020835

Bayram, N.E., Gercek, Y.C., Çelik, S., Mayda, N., Kostic, A.Z., Dramicanin, A.M., Özkök, A. (2021). Phenolic and free amino acid profiles of bee bread and bee pollen with the same botanical origin - similarities and differences. *Arabian Journal of Chemistry 14*(3), 103004. DOI: 10.1016/j.arabjc.2021.103004

Bicakci, A., Akkaya, A., Malyer, H., Ünlü, M., Sapan, N. (1996). Airborne pollen grains of Isparta. *Medical Faculty Journal SDU, 3*(4), 37-51.

Bicakci, A. (1999). Airborne pollen concentration of Kütahya. *Turkish Journal of Botany, 23*(2), 75-81.

Bleha, R., Shevtsova, T.V., Živčková, M., Korbářová, A., Ježková, M., Saloň, I., … Synytsya, A. (2021). Spectroscopic discrimination of bee pollen by composition, color, and botanical origin. *Foods, 10*(8), 1682. DOI: 10.3390/foods10081682

Campos, M.G., Bogdanov, S., De Almeida-Muradian, L.B., Szczesna, T., Mancebo, Y., Frigerio, C., Ferreira, F. (2008). Pollen composition and standardization of analytical methods. *Journal of Apicultural Research, 47*(2), 154-161. DOI: 10.1080/00218839.2008.11101443

De-Melo, A.A.M., Estevinho, L.M., Moreira, M.M., Delerue-Matos, C., Freitas, A.d.S.d., Barth, O.M., Almeida-Muradian, L.B.d. (2018). Phenolic profile by HPLC-MS, biological potential, and nutritional value of a promising food: Monofloral bee pollen. *Journal of Food Biochemistry, 42*(16), e12536. DOI: 10.1111/jfbc.12536

Dolezal, A.G., & Toth, A.L. (2018). Feedback between nutrition and disease in honey bee health. *Current Opinion in Insect Science, 26*, 114-119. DOI: 10.1016/j.cois.2018.02.006

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*(Supp. 1)*,* 133-141. DOI: 10.1590/fst.10220

Fatrcová-Šramková, K., Nôžková, J., Máriássyová, M., Kačániová, M. (2016). Biologically active antimicrobial and antioxidant substances in the *Helianthus annuus* L. bee pollen. *Journal of Environmental Science and Health B, 51*(3), 176-181. DOI: 10.1080/03601234.2015.1108811

Jaganjac, M., Sredoja T.V., Zarkovic, N. (2021). Short overview of some assays for the measurement of antioxidant activity of natural products and their relevance in dermatology. *Molecules, 26*(17), 5301. DOI: 10.3390/molecules26175301

Jones, G.D., & Bryant, V.M.J. (1996). Melissopalynology. In: Jansonius J, McGregor DC, editors, Palynology: Principles and Applications 3, (pp. 933-938), AASP, USA.

Kanar, Y., & Mazı, B.G. (2019). Effect of different drying methods on antioxidant characteristics of bee pollen. *Meas, 13,* 3376-3386. DOI: 10.1007/s11694-019-00283-5

Karataş, F., Munzuroğlu, Ö., Gür, N. (2000). Investigation of A, E and C vitamins, and selenium levels of bee pollen. *Fırat University Journal of Science and Engineering, 12,* 219-224.

Karkar, B., Şahin, S., Güneş, M.E. (2021). Evaluation of antioxidant properties and determination of phenolic and carotenoid profiles of chestnut bee pollen collected from Turkey. *Journal of Apicultural Research, 60*(5), 765-774. DOI: 10.1080/00218839.2020.1844462

Kieliszek, M., Piwowarek, K., Kot, A., Blazejak, S., Chlebowska-Smigiel, A., Wolska, I. (2018). Pollen and bee bread as new health-oriented products: A review. *Trends in Food Science and Technology, 71*, 170-180. DOI: 10.1016/j.tifs.2017.10.021

Kocot, J., Kiełczykowska, M., Luchowska-Kocot, D., Kurzepa, J., Musik, I. (2018). Antioxidant potential of propolis, bee pollen, and royal jelly: Possible medical application. *Oxidative Medicine and Cell Longevity, 2018,* Article ID 7074209. DOI: 10.1155/2018/7074209

Komosinska-Vassev, K., Olczyk, P., Kaźmierczak, J., Mencner, L., Olczyk, K. (2015). Bee pollen: Chemical composition and therapeutic application. *Evidence-based Complementary and Alternative Medicine: eCAM, 2015*, 297425. DOI: 10.1155/2015/297425

Kostićaž-Milinčič, D.D., Barač, M.B., Shariarti, M.A., Tesic, Ž.L., Pešić, M.B. (2020). The application of pollen as a functional food and feed ingredient - the present and perspectives. *Biomolecules, 10*(1), 84. DOI: 10.3390/biom10010084

Kumaran, A., & Karunakaran, R.J. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus. Food Chemistry, 97*(1), 109-114. DOI: 10.1016/j.foodchem.2005.03.032

Lunau, K. (1995). Notes on the colour of pollen. *Plant Systematics and Evolution, 198*, 235-252. DOI: 10.1007/BF00984739

Lunau, K. (2000). The ecology and evolution of visual pollen signals. *Plant Systematics and Evolution, 222,* 89-111. DOI: 10.1007/BF00984097

Magalhães, L.M., Santos, F., Segundo, M.A., Reis, S., Lima, J.L.F.C. (2010). Rapid microplate high-throughput methodology for assessment of Folin-Ciocalteu reducing capacity. *Talanta, 83*(2), 441-447. DOI: 10.1016/j.talanta.2010.09.042

Mayda, N., Özkök, A., Bayram, N.E., Gercek, 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*, 795-1809. DOI: 10.1007/s11694-020-00427-y

Nicolson, S., & Thornburg, R.W. (2007). Nectar Chemistry. In: Nicolson SW, Nepi M, Pacini E, editors, Nectaries and Nectar 5, (pp. 215-264), Springer, Netherlands.

Oroian, M., Dranca, F., Ursachi, F. (2022). Characterization of Romanian bee pollen - an important nutritional source. *Foods, 11*(7), 2633, DOI: 10.3390/foods11172633

Özcan, M.M., Aljuhaimi, F., Babiker, E.E., Uslu, N., Ceylan, D.A., Ghafoor, K., … Alsawmahi, O.N. (2019). Determination of antioxidant activity, phenolic compound, mineral contents, and fatty acid compositions of bee pollen grains collected from different locations. *Journal of Apicultural Research, 63*(1), 69-79. DOI: 10.2478/jas-2019-0004

Özkök, D., & Silici, S. (2017). Antioxidant activities of honeybee products and their mixtures. *Food Science and Biotechnology 26*(1), 201-206. DOI: 10.1007/s10068-017-0027-0

Ozyurt, D., Demirata, B., Apak, R. (2010). Modified cerium(IV)-based antioxidant capacity (CERAC) assay with selectivity over citric acid and simple sugars. *Journal of Food Composition and Analysis, 23*(3), 282-288. DOI: 10.1016/j.jfca.2009.09.005

Qiao, J., Zhang, Y., Haubruge, E., Wang, K., El-Seedi, H.R., Dong, J., Xu, X., Zhang, H. (2024). New insights into bee pollen: nutrients, phytochemicals, functions and wall disruption. *Food Research International, 178*, 113934. DOI: 10.1016/j.foodres.2024.113934

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237. DOI: 10.1016/s0891-5849(98)00315-3

Salazar-González, C.Y., Stinco, C.M., Rodríguez-Pulido, F.J., Díaz-Moreno, C., Fuenmayor, C., Heredia, F.J., González-Miret, M.L. (2022). Characterization of carotenoid profile and α-tocopherol content in Andean bee pollen influenced by harvest time and particle size. *LWT, 170,* 114065. DOI: 10.1016/j.lwt.2022.114065

Saral, Ö., Kilicarslan, M., Şahin, H., Yildiz, O., Dincer, B. (2019). Evaluation of antioxidant activity of bee products of different bee races in Turkey. *Turkish Journal of Veterinary and Animal Science, 43*(4), 441-447. DOI: 10.3906/vet-1901-3

Sorkun, K. (2008). *Türkiye'nin Nektarlı Bitkileri, Polenler ve Balları,* Palme Yayınevi, Türkiye (in Turkish).

Szydłowska-Czerniak, A., Trokowski, K., Karlovits, G., Szłyk, E. (2011). Effect of refining processes on antioxidant capacity, total contents of phenolics and carotenoids in palm oils. *Food Chemistry, 129*(3), 1187-1192. DOI: 10.1016/j.foodchem.2011.05.101

Taşkın, D. (2016). Pollen analysis of Burdur Region honeys. (Isparta, Türkiye). [Thesis]. Isparta University, Institute of Pure and Applied Science.

Thakur, M., & Nanda, V. (2020). Composition and functionality of bee pollen: A review. *Trends in Food Science and Technology, 98*, 82-106. DOI: 10.1016/j.tifs.2020.02.001

Tutun, H., Kaya, M.M., Usluer, M.S., Kahraman, H.A. (2021). Bee pollen: It's antioxidant activity. *Uludağ Bee Journal, 21*(1), 119-131.

Ulusoy, E., & Kolayli, S. (2014). Phenolic composition and antioxidant properties of Anzer bee pollen. *Journal of Food Biochemistry, 38*, 73-82. DOI: 10.1111/jfbc.12027

Vardar, F., & Ünal, M. (2011). Cytochemical and ultrastructural observations of anthers and pollen grains in *Lathyrus undulatus* Boiss. *Acta Botanica Croatica, 70*(1), 53-64. DOI: 10.2478/v10184-010-0014-5

Wodehouse, R.P. (1935). Pollen Grains, McGraw-Hill Book Company, New York, USA.

Yang, K., Wu, D., Ye, X., Liu, D., Chen, J., Sun, P. (2013). Characterization of the chemical composition of bee pollen in China. *Journal of Agricultural and Food Chemistry, 61*(3), 708-718. DOI: 10.1021/jf304056b

Yesiltas, B., Capanoglu, E., Firatligil-Durmus, E., Sunay, A.E., Samanci, T., Boyacioglu, D. (2014). Investigating the in-vitro bioaccessibility of propolis and pollen using a simulated gastrointestinal digestion system. *Journal of Apicultural Research, 53*(1), 101-108. DOI: 10.3896/IBRA.1.53.1.10

Zhishen, J., Mengcheng, T., Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*, 555-559. DOI: 10.1016/S0308-8146(98)00102-2