

PROXIMATE COMPOSITION AND ANTIOXIDANT ACTIVITY OF HONEY BEE COLLECTED POLLEN IN THE MAIN FLOWERING SEASON, IN WEST SHEWA ZONE, CENTRAL ETHIOPIA

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Abstract

This study aimed to assess the effect of botanical and geographical differences on the protein, minerals, total phenolic content and the antioxidant activity of bee pollen collected from five districts in West Shewa Zone, Ethiopia. Pollens of *Guizotia* sp., *Brassica* sp., *Trifolium* sp., *Eucalyptus* sp., *Plantago lanceolata* and *Vicia faba* were color-sorted from multifloral pollens and subjected to chemical analysis. The results revealed the mean content of moisture 10.6% of fresh weight (FW), protein 19.4% of dry weight (DW) and ash 2.4% (DW); the most dominant minerals, K (9765.6), Ca (963.4), Mg (960.5), Fe (142.8) and P (126.5) in mg/kg DW. The average of the total phenolic content (TPC) and the antioxidant activity (DPPH radical) were 40.3 GAE mg/g FW and 13.7 AAE mg/g FW (gallic and ascorbic acid equivalent, respectively). *V. faba* pollen had the highest protein, ash, P and Cu content. Four pollen taxa had the highest Ca values, while multifloral pollens contained the highest Fe content. The samples from Ejere had the highest ash, Ca, Na and Cu content. The results showed a high content of the tested chemicals and a strong antioxidant capacity of bee pollen, varying with botanical and geographical origins. These findings would also serve as inputs for further assessment of the nutritional values of pollen for honey bee colonies and potential human consumption. However, comprehensive chemical investigations of larger number of samples representing diversified flora and locations are crucial to developing a broad regional information base on the nutritional profile of bee pollen.

Keywords: bee pollen, botanical and geographical origins, Ethiopia, minerals, proteins

INTRODUCTION

Honey bees collect pollen from a wide range of flowering plants, which usually fulfills their dietary requirements for proteins, lipids, minerals and vitamins (Avni et al., 2014). Pollen is essential for brood rearing, maturation, development and enhancing colony health (Alaux et al., 2010; Brodschneider & Crailsheim, 2010; Wright et al., 2018). There is variation in nutritional 'quality' of pollen from different plant families, species and even individual plants within a population (Roulston & Cane, 2000). The nutritional values of pollen to bees are affected by the diversity of pollen source plants. Studies revealed that honey bees fed on polyfloral pollens showed enhanced immune responses, a lower level of

pathogen infection, a higher brood and adult bee population and an increased survival rate (Alaux et al., 2010; Di Pasquale et al., 2013; Branchiccela et al., 2019). Therefore, land use intensification and the expansion of monoculture agricultural areas may deprive bees of the pollen diversity needed to fulfill their nutritional requirements, despite the seasonal abundance of pollen from mass-flowering crops (Naug, 2009). Seasonal variation in availability and diversity of pollen sources forces forager bees to collect whatever is available to satisfy these demands, without a preference for pollen of high protein content or even accepting pollen of poor nutritional value (Pernal & Currie, 2001; Danner et al., 2017). Bee pollen is a mix of bee-collected floral pollens from different species, widely varying in

composition that comprises about 250 substances (Komosinska-Vassev et al., 2015). These include proteins and free amino acids, carbohydrates, lipids, including fatty acids and their esters, vitamins, coenzymes and minerals (Margaoan et al., 2019). Besides the floral origin, the chemical composition of pollen depends on geographical factors, climatic conditions, soil types, seasonal variations and harvesting and processing techniques (Liolios et al., 2019; Al-Kahtani & Taha, 2020; Castagna et al., 2020). Pollen contains different proteins that vary in the range of 1-40%, while the content of carbohydrates, lipids, crude fiber and ash lies at 13-60%, 1-20%, 0.3-20% and 2-6%, respectively (Campos et al., 2008; Ares et al., 2018). Moreover, it is rich in phenolic compounds that amount to 1.6% on average, mainly comprising flavonoids (1.4%) and phenolic acids (0.2%), and is an important source of both fat-soluble (0.1%) and water-soluble (0.6%) vitamins (Komosińska-Vassev et al., 2015; Margaoan et al., 2019). Pollen also contains over twenty-five different minerals, including both macroelements (K, Na, P, S, Ca and Mg) and microelements (Cu, Mn, Fe, Zn and Se) (Kieliszek et al., 2018). The high nutritional profile of pollen and its positive influence on health have led people to recognize it as “the complete food” and promote it as a functional food for human consumption (Prdun et al., 2021). Various investigations have demonstrated the anti-inflammatory, antimicrobial, antioxidative, anticancerogenic and immunostimulant functional properties of bee pollen, which are mainly attributed to its multiple bioactive compounds (Margaoan et al., 2019).

The very complex physiographic features and climatic heterogeneity of Ethiopia, varying from arid lowland plains to moist Afroalpine, have created favorable conditions for the existence of about 7,000 plant species (Mengesha et al., 2020). The significant majority of them provide surplus nectar and pollen to honey bees (Nuru, 2007). The country is the number one honey and beeswax producer in Africa and among the top ten the world, with an estimated 7.5 million managed honey bee colonies in different hive types (Gratzer et al., 2021). West Shewa Zone in Oromia Regional State is one of the areas with high potential for diversity

and abundance of honey bee forage plants, beekeeping practices and honey production (Fichtle & Admasu, 1994). However, most of the investigations related to pollen in Ethiopia in general and in this zone in particular have focused on the melissopalynological analysis of honey and the identification and documentation of pollen grain references of common pollen source plants (Fichtl & Adi, 1994; Nuru, 2007). However, Debissa et al. (2008) reported the botanical compositions and N, P, and K content of bee pollen samples collected from a site in Menagesha State Forest. Therefore, information on the chemical composition and how it is affected by the floral and geographical origins of bee pollen in the region is lacking. Based on this, proximate and mineral compositions and the antioxidant activity of bee pollen were determined in samples collected from five apiaries in five districts in the West Shewa Zone from October to December 2022.

MATERIAL AND METHODS

The apiaries were located in five districts in West Shewa Zone, Oromia Regional State: Welmera (09° 03' 19" N, 038° 30' 35" E), Ejere (09° 03' 16" N, 038° 26' 48" E), Dendi (09° 01' 19" N, 038° 11' 17" E), Toke Kutaye (08° 58' 11" N, 037° 45' 14" E) and Cheliya (09° 01' 50" N, 037° 26' 11" E) (Fig. 1). The zone is located between 8° 17' - 8° 57' N latitude and 37° 08' - 38° 07' E longitudes in central Ethiopia, with an elevation of 1,050-3,500 m above sea level. It has an annual average rainfall of 1,115 mm (Abera et al., 2023). The meteorological data show that Welmera has a mean annual rainfall of about 1,041 mm and mean minimum and maximum temperatures of 6.7 and 23.7°C, respectively (Obsa, 2022). Ejere receives an annual average rainfall ranging from about 900 mm to 1,200 mm and has mean minimum and maximum temperatures of 9 and 28°C, respectively (Addisu & Chernet, 2023). Dendi gets a mean annual rainfall of 1,140 mm and has mean minimum and maximum temperatures of 8.4 and 24.6°C, respectively (Obsa, 2022), while Toke Kutaye receives a mean annual

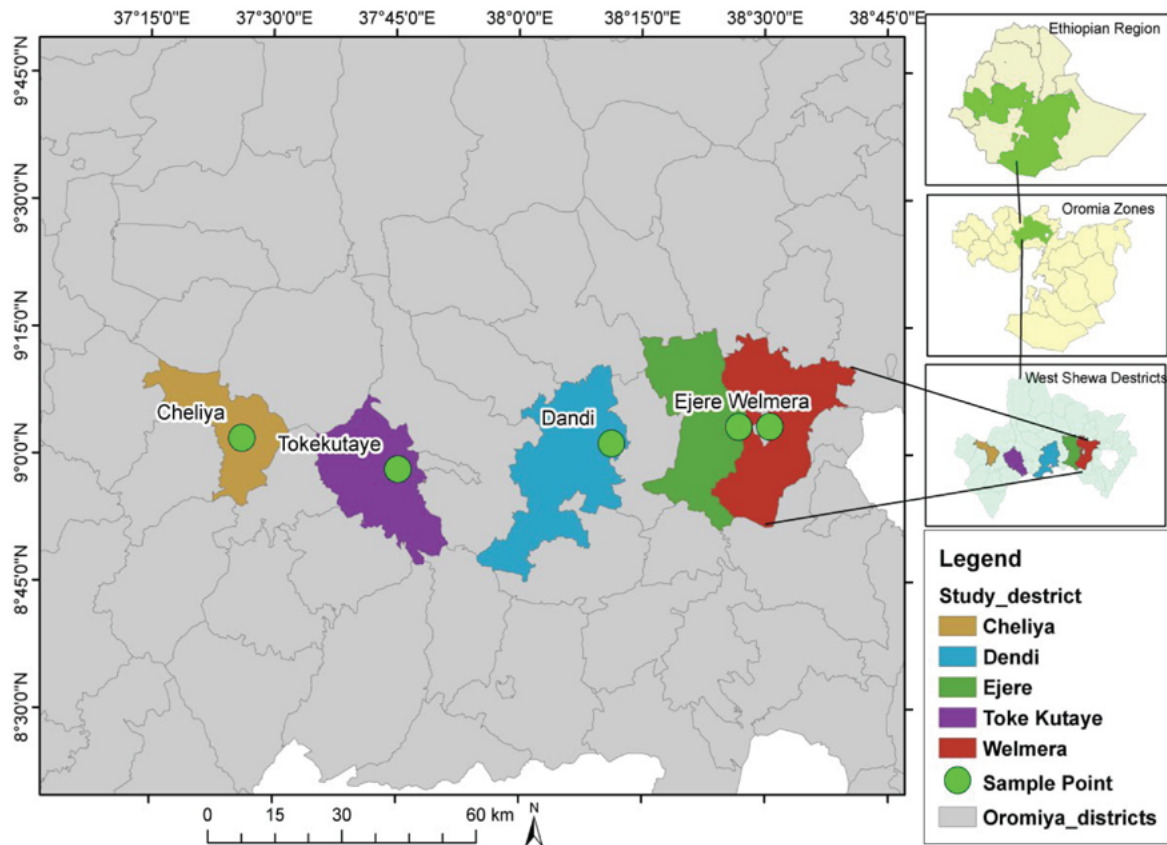


Fig. 1. Map of pollen sample collection apiaries in five districts of West Shewa Zone, Oromia, Ethiopia.

rainfall of 1,293 mm and has mean minimum and maximum temperatures of 8.9 and 27.4°C, respectively (Tolera et al., 2016). In Cheliya, the annual precipitation ranges from 800 to 1,200 mm. The mean minimum and maximum temperatures are 8.7 and 24°C, respectively (Achal & Emiru, 2023). Pollen traps were fixed at the entrance of three Zander hives per apiary occupied by *Apis mellifera simensis* colonies (Meixner et al., 2011). Bee pollen was collected every fifteen days, starting from mid-October to mid-December 2022. After removal from the pollen traps, the samples were stored in plastic bottles at -18°C until analyzed as fresh pollen. A total of seventy-five pooled bee pollen samples, five from each site, were collected from five rounds of harvesting (replicated with the number of pollen-collecting colonies).

Sample preparation

All the samples collected directly from the pollen traps were multifloral pollens, as the pollen frequency from a single plant taxon was <80%

(Campos et al., 2008). Therefore, all the pollens hereafter referred to as “monofloral” were prepared by handpicking based on their pellet color. The original samples were thoroughly homogenized to avoid unintentional bias from color and pellet size and to maintain the original botanical composition, weighed on an analytical balance (JA-SERIES, JA203H, 0.001 g). Then, exactly half of the original weight was scooped to equally split the samples into two parts (Topitzhofer et al., 2021); half of the pollen was used for the preparation of the monofloral samples, and the other half was used to prepare composite multifloral samples.

The monofloral samples were prepared by visually sorting the pollen pellets to match their color with the color classes of a comprehensive printed color identification chart (The Aqua and Velvet Pigment, 2013) in bright daylight and recording the color (Kirk, 2018). As suggested by Almeida-Muradian et al. (2005), the botanical source of the color-sorted pollen was melissopalynologically confirmed following the Barth et al. (2010) method with the use of a light microscope (Zeiss,

2010) fitted with an Axiocam 105 color camera. Then, the subsamples of single botanical origin were pooled together to prepare monofloral samples that weighed at least 15 g. Therefore, the monofloral samples were pollen with a uniform color that qualified to have a pollen frequency of >90% originating from a single plant taxon (Freitas et al., 2013). The monofloral samples were selected based on the weight contribution of each taxon in the original bee-collected multifloral pollens. When a sufficient amount (in weight) of the color-sorted monofloral pollen samples were not obtained, homogenizing and further dividing of the remaining half of the original multifloral pollens into two equal parts by weight were repeated once or twice to gather more pellets and attain the minimum required weight. In total, seventeen monofloral samples in three replicates were obtained and considered for further analysis. The color description of the monofloral samples is presented in Tab. 1.

The multifloral samples were obtained by preparing a composite sample from half (or quarter) of the original samples. The original samples were well homogenized, and 3 g from each of the five samples (rounds of harvest) were scooped and pooled to make a composite multifloral pollen sample that weighed 15 g. Therefore, one composite multifloral sample in three replications from each district was considered for chemical analysis. Their botanical composition was also analyzed following the same procedure as described above.

Determination of proximate and mineral composition

The water content expressed as a percentage of fresh weight was determined as a weight difference after 1 g of pollen was dried to a constant weight at 70°C. The total nitrogen content was determined by wet-digesting 1 g of pollen with the use of the Kjeldahl method in a DK 20 heating digester (VELP SCIENTIFICA, Italy) and multiplying by a factor of 5.6 to get the crude protein content (Rabie et al., 1983). About 2 g of pollen sample in a porcelain crucible was incinerated in a muffle furnace (Jaberthem, Model 150) at 550°C until a constant weight was reached to determine the

ash content. Both protein and ash content were expressed as a percentage of dry weight of pollen. The ashes obtained from the above procedure were wetted in 5 mL of 6 M HCL to extract the minerals. Elements K, Na, Ca, Mg, Fe, Mn, Cu and Zn at their respective wavelengths were measured on an Atomic Absorption Spectrophotometer (Agilent Technologies 200 Series AA, Malaysia). The analysis of all the above chemicals followed AOAC (2000) procedures. The P content was determined by the molybdovanadate method AOAC (1990) with the use of a 5 mL wet-digested sample prepared for protein analysis, and the developed color was measured at $\lambda=410$ nm by a spectrophotometer (JENWAY 6300, UK). The concentration of minerals was expressed as mg/kg of dry-weight pollen.

Determination of total phenolic content (TPC) and antioxidant activity

The determination of TPC and antioxidant activity was started through the preparation of a pollen ethanol extract by dissolving 2 g of fresh pollen in 20 mL of ethanol (95%) by agitation for 60 minutes at 40°C, followed by centrifuging (BIORIDGE, TGL-20M), filtering and re-extraction (Mayda et al., 2020). The extracts were pooled together and then diluted with 95% ethanol to obtain 5 mg/mL test solutions. TPC was spectrophotometrically determined by the Folin-Ciocalteu (FC) method with the use of gallic acid (GA) as a reference standard and microtiter 96-well plates (Magalhaes et al., 2010). Concentrations and volumes of the samples, standard solution and blank were prepared and added into microplate wells (Mayda et al., 2020), placed in a microplate reader (Multiskan FC 357; Thermo Scientific) and incubated for 30 minutes. The absorbance was measured at 765 nm. The GA solutions at concentrations between 0.004-0.02 mg/mL were used to develop a calibration curve, $y=0.0066x-0.0422$, $R^2=0.997$. The TPC was determined and expressed as mg GA equivalents per g of fresh pollen (mg GAE/g FW). The antioxidant activity was assessed with the use of DPPH assay on the 96-well microplate with some modifications of

the methods described by Herald et al. (2012). A total of 15 µL of the diluted sample was added to 185 µL of DPPH solution (150 µmol/L) in ethanol. After 45 minutes in the dark at room temperature, the absorbance was measured at 517 nm by the same microplate reader used above. Ascorbic acid (AA) at concentrations of 0.004-0.02 mg/mL was used as a standard to develop a calibration curve, $y = -0.0025x + 0.0855$, $R^2 = 0.992$. The results were expressed as mg AA equivalents per g of sample (mg AAE/g FW). Both assays were performed in triplicates.

Statistical analysis

All the determinations were carried out in triplicate and the results were reported as mean $\pm$ standard error of mean (SE). The differences between samples were analyzed with the use of

ANOVA followed by Tukey's HSD. Student's t-test was used to compare measurements between monofloral and multifloral pollens from the same location. Correlation between TPC and the antioxidant activity was assessed by Pearson's correlation efficient. A difference was considered statistically significant when $p < 0.05$. All the statistical analyses were performed using IBM SPSS Statistics 20.

RESULTS

Pollen analysis through color sorting and microscopic identification showed that honey bees collected pollen grains from a total of 52 pollen source plant taxa (species or identified to genus level) that belong to twenty-three families during the main flowering season (mid-October to mid-December) in the study

Table 1.

Color-sorted monofloral pollen samples, their color description and composition of taxa

Pollens	Districts	Color	Purity	Other microscopically identified pollen grains (%) out of 500 pollen/count
Brassica N=15	A, B, C, D, E	Brow mod (707)	94.6-97.4%	<i>Eucalyptus</i> ($\leq 1.6\%$), <i>Lepidium sativum</i> ($\leq 0.9\%$), <i>Rumex</i> ($\leq 0.7\%$), <i>Trifolium</i> ($\leq 0.5\%$), <i>Guizotia</i> ($\leq 0.4\%$), <i>Bidens</i> ($\leq 0.4\%$), others ($\leq 1.0\%$)
Eucalyptus N=6	A, E	Grey vanish (639)	93.3-96.8%	<i>Brassica</i> ($\leq 2.1\%$), <i>Guizotia</i> ($\leq 1.4\%$), <i>Plantago</i> ($\leq 0.9\%$), <i>Trifolium</i> ($\leq 0.6\%$), <i>Apodytes dimidiata</i> ($\leq 0.6\%$), others ($\leq 1.2\%$)
Guizotia N=15	A, B, C, D, E	Neon orange mod (610)	93.7-97.8%	<i>Bidens</i> ($\leq 2.6\%$), <i>Trifolium</i> ($\leq 1.4\%$), <i>Eucalyptus</i> ($\leq 1.2\%$), <i>Scenecio</i> ($\leq 0.6\%$), others ($\leq 1.0\%$)
Plantago N=6	A, E	Yellow orange (313)	90.4-94.5%	<i>Trifolium</i> ($\leq 3.3\%$), <i>Eucalyptus</i> ($\leq 1.8\%$), <i>Cyanotis barbata</i> ($\leq 1.0\%$), <i>Brassica</i> ($\leq 0.7\%$), <i>Rumex</i> ($\leq 0.4\%$), <i>Andropogon abyssinicus</i> ($\leq 0.3\%$), others ($\leq 2.1\%$)
Trifolium N=6	B, D,	Sandalwood (260)	91.1-95.3%	<i>Eucalyptus</i> ($\leq 3.0\%$), <i>Plantago</i> ($\leq 1.8\%$), <i>Rumex</i> ($\leq 1.1\%$), <i>Brassica</i> ($\leq 0.8\%$), <i>Guizotia</i> ($\leq 0.5\%$), others ($\leq 1.8\%$)
Vicia N=3	C	Latte (235)	92.5-96.2%	<i>Trifolium</i> ($\leq 1.8\%$), <i>Brassica</i> ($\leq 1.5\%$), <i>Eucalyptus</i> ($\leq 0.9\%$), <i>Guizotia</i> ($\leq 0.6\%$), <i>Acacia abyssinica</i> ($\leq 0.6\%$), <i>Pisum sativum</i> ($\leq 0.5\%$), <i>Glycine wightii</i> ($\leq 0.3\%$), others ($\leq 1.3\%$)

A=Welmera, B=Cheliya, C=Ejere, D=Toke Kutaye, E=Dendi

Table 2.
Results of content of moisture (FW), ash, protein and minerals (DW) and total phenolics (FW);
and antioxidant activity (FW) of pollens in relation to botanical origin

Pollens	Moisture (%)	Ash (%)	Protein (%)	TPC (mg GAE/g)	DPPH (mg AAE/g)	K (mg/kg)	Ca (mg/kg)
<i>Brassica</i>	11.8±0.4 ^a	2.6±0.1 ^{bc}	20.9±0.4 ^{bc}	45.5±1.8 ^a	14.7±0.8 ^{abc}	8965.7±552.8 ^a	1003.6±57.6 ^a
<i>Eucalyptus</i>	9.4±0.7 ^{ab}	2.9±0.2 ^{ab}	26.8±1.0 ^a	44.0±2.9 ^a	18.1±0.2 ^a	10657.8±206.4 ^a	857.6±174.8 ^{ab}
<i>Guizotia</i>	11.9±0.4 ^a	2.0±0.1 ^c	15.5±0.3 ^d	43.0±1.4 ^a	11.4±0.8 ^{bc}	10374.5±603.3 ^a	1088.1±52.2 ^a
<i>Multifloral</i>	9.8±0.4 ^{ab}	2.5±0.1 ^{bc}	18.5±0.6 ^c	37.8±1.3 ^a	14.6±0.2 ^{abc}	9243.9±771.8 ^a	922.0±49.5 ^{ab}
<i>Plantago</i>	9.6±0.3 ^{ab}	2.3±0.4 ^{bc}	14.5±0.9 ^d	41.3±2.1 ^a	15.3±0.3 ^{ab}	11161.5±802.8 ^a	616.3±34.8 ^b
<i>Trifolium</i>	9.9±0.4 ^{ab}	2.0±0.1 ^c	21.8±0.6 ^b	23.8±1.4 ^b	11.8±0.9 ^{bc}	9321.6±583.8 ^a	1064.2±40.6 ^a
<i>Vicia</i>	8.0±0.7 ^b	3.3±0.4 ^a	26.4±4.0 ^a	26.8±1.3 ^b	10.9±1.3 ^c	9642.3±251.3 ^a	1050.7±86.4 ^a
Mean±SE	10.6±0.2	2.4±0.1	19.4±0.5	40.3±0.9	13.7±0.3	9765.6±294.2	963.4±30.5
Min	6.6	0.4	12.1	19.0	0.4	1007.2	360.3
Max	14.7	4.1	34.4	69.0	25.5	14375.0	1448.2
P	0.000	0.000	0.000	0.000	0.000	0.381	0.002

Pollens	Mg (mg/kg)	Fe (mg/kg)	P (mg/kg)	Na (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)
<i>Brassica</i>	893.2±91.7 ^a	105.5±9.2 ^{bc}	125.9±12.1 ^{bc}	109.9±13.0 ^a	30.8±3.2 ^a	15.7±1.2 ^a	3.3±0.5 ^{bc}
<i>Eucalyptus</i>	978.4±92.6 ^a	101.2±14.3 ^{bc}	185.4±3.6 ^a	122.4±9.0 ^a	24.5±6.4 ^a	18.5±1.6 ^a	3.6±0.9 ^{bc}
<i>Guizotia</i>	1048.2±73.6 ^a	112.4±8.6 ^{bc}	99.8±5.8 ^c	118.5±11.5 ^a	30.3±2.2 ^a	19.1±1.3 ^a	3.7±0.7 ^{bc}
<i>Multifloral</i>	930.6±76.5 ^a	229.3±30.0 ^a	113.5±3.9 ^c	127.3±8.8 ^a	30.8±3.5 ^a	22.6±1.5 ^a	4.3±0.3 ^{ab}
<i>Plantago</i>	1002.3±125.9 ^a	62.6±4.5 ^c	109.6±3.5 ^c	116.4±11.7 ^a	20.3±4.6 ^a	15.2±2.0 ^a	1.0±0.3 ^c
<i>Trifolium</i>	847.2±93.7 ^a	206.3±51.8 ^{ab}	155.6±6.5 ^{ab}	123.1±14.8 ^a	25.1±5.1 ^a	19.8±2.0 ^a	4.9±0.4 ^{ab}
<i>Vicia</i>	1114.2±392.0 ^a	165.3±38.2 ^{abc}	185.3±5.9 ^a	162.8±18.6 ^a	26.8±8.2 ^a	21.6±4.4 ^a	7.2±1.5 ^a
Mean±SE	960.5±38.6	142.8±11.2	126.5±4.7	121.2±4.9	28.4±1.5	18.9±0.7	3.8±0.3
Min	456.9	48.9	13.1	54.9	8.3	9.2	0.4
Max	1884.2	560.7	195.7	207.0	60.5	32.3	10.1
P	0.774	0.000	0.000	0.557	0.530	0.016	0.002

FW=fresh weight, DW=dry weight. Mean ± standard error (Brassica, n=15; Eucalyptus, n=6; Guizotia, n=15; Multifloral, n=15; Plantago, n=6; Trifolium, n=6; Vicia, n=3).

Values within each column followed by different letters are significantly different at (p<0.05)

area. The lowest number of taxa (41) was from Ejere, while 52 occurred in Cheliya. Asteraceae, Fabaceae, Brassicaceae, Myrtaceae and Lamiaceae were the dominant families in terms of the number of occurring taxa. However, only six monofloral pollen taxa with sufficient weight (≥ 15 g) and >90% pollen frequency from a single taxon were selected for chemical analysis (Tab. 1).

The results revealed that, across all samples, the moisture content in pollen varied between 6.6 and 14.7 g/100 g FW (fresh weight), while the protein content ranged between 12.1 and

34.4 g/100 g DW (dry weight). The ash content was found between 0.4 and 4.1 g/100 g DW. The analysis of nine elements (in dry pollen) determined that the most abundant elements were K (1007.2-14,375.0 mg/kg), Ca (360.3-1448.2 mg/kg), Mg (456.9-1884.2 mg/kg), Fe (48.9-560.7 mg/kg), P (13.1-195.7 mg/kg), followed by Na (54.9-207.0 mg/kg), Zn (8.3-60.5 mg/kg), Mn (9.2-32.3 mg/kg) and Cu (0.4-10.1 mg/kg). The total phenolic content (TPC) ranged from 19.0 to 69.0 mg GAE/g FW, while in the DPPH assay, the antioxidant activity changed between 0.4 and 25.5 mg AAE/g

Table 3.

Results of content of moisture (FW), ash, protein and minerals (DW) and total phenolics (FW); and antioxidant activity (FW) of all pollen samples from different locations

Districts	Moisture (%)	Ash (%)	Protein (%)	TPC (mg GAE/g)	DPPH (mg AAE/g)	K (mg/kg)	Ca (mg/kg)
A	11.3±0.5 ^a	2.6±0.1 ^{ab}	19.5±1.0 ^a	43.6±1.4 ^a	13.7±0.9 ^a	9455.6±656.6 ^a	1051.7±69.7 ^a
B	11.0±0.6 ^a	2.1±0.2 ^b	18.7±0.9 ^a	44.7±2.0 ^a	13.8±0.7 ^a	9133.1±501.1 ^a	1025.2±36.9 ^a
C	9.7±0.4 ^a	2.9±0.2 ^a	20.3±1.5 ^a	30.9±1.8 ^b	12.4±0.7 ^a	11170.2±692.6 ^a	1047.3±37.5 ^a
D	11.2±0.7 ^a	2.2±0.1 ^b	19.0±1.1 ^a	42.1±1.7 ^a	13.7±0.5 ^a	9379.6±420.1 ^a	1099.7±47.0 ^a
E	9.8±0.4 ^a	2.2±0.2 ^b	19.5±1.4 ^a	33.4±1.6 ^b	15.1±0.5 ^a	9766.8±784.9 ^a	649.6±50.6 ^b
Mean±SE	10.6 ±0.2	2.4±0.1	19.4±0.5	40.1±0.9	13.7±0.3	9765.6±294.2	963.4±360.3
Min	6.6	0.4	12.1	19.0	0.4	1007.2	360.3
Max	14.7	4.0	34.4	69.0	25.5	14375.0	1448.2
P	0.064	0.002	0.912	0.000	0.284	0.237	0.000

Districts	Mg (mg/kg)	Fe (mg/kg)	P (mg/kg)	Na (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)
A	922.4±92.8 ^a	112.8±15.5 ^b	129.1±8.6 ^a	92.5±8.3 ^c	22.8±2.6 ^a	17.1±1.8 ^a	2.4±0.5 ^b
B	871.3±83.1 ^a	218.6±45.4 ^a	121.7±7.8 ^a	118.2±11.6 ^{bc}	32.9±3.5 ^a	20.5±1.8 ^a	3.9±0.6 ^b
C	1100.6±88.9 ^a	154.5±17.6 ^{ab}	145.6±8.9 ^a	160.8±9.9 ^a	34.4±3.2 ^a	22.1±1.5 ^a	6.3±0.6 ^a
D	912.5±70.1 ^a	118.3±12.6 ^b	114.8±13.3 ^a	100.3±8.2 ^c	25.1±2.9 ^a	16.6±0.9 ^a	4.1±0.4 ^b
E	996.0±87.0 ^a	102.9±13.6 ^b	121.7±11.9 ^a	137.1±6.4 ^{ab}	28.0±3.6 ^a	18.7±1.2 ^a	2.7±0.5 ^b
Mean±SE	960.5±38.6	142.8±11.2	126.5±4.7	121.2±4.9	28.4±1.5	18.9±0.7	3.8±0.3
Min	456.9	48.9	13.1	54.9	8.3	9.2	0.4
Max	1884.2	560.7	195.7	207.0	60.5	32.3	10.0
P	0.407	0.005	0.328	0.000	0.060	0.074	0.000

A=Welmera, B=Cheliya, C=Ejere, D=Toke Kutaye, E=Dendi. FW=fresh weight, DW=dry weight. Mean ± standard error (A, n=15; B, n=12; C, n=12; D, n=12; E, n=15).

Values within each column followed by different letters are significantly different at (p<0.05)

FW (Tab. 2).

As shown in Tab. 2, the botanical origin of the pollen significantly influenced the content of moisture, ash, protein, Ca, Fe, P, Mn, Cu and TPC. The DPPH radical quenching ability of the samples also varied depending on this factor (p<0.05). The highest moisture content was measured in *Brassica* sp. and *Guizotia* sp., while *Eucalyptus* sp. and *Vicia faba* had the highest protein and P content. *V. faba* pollen also contained the highest ash and Cu content (p<0.05). *Brassica* sp., *Guizotia* sp., *Trifolium* sp. and *V. faba* pollens had the highest Ca content, while multifloral pollens contained the highest concentration of Fe. *V. faba* and *Trifolium* sp. pollens had the lowest TPC values, while *Eucalyptus* sp. showed the highest antioxidant activity against DPPH radical.

Table 3 summarizes the findings by providing the mean values of the measurements by the areas of origin. The content of ash, Ca, Fe, Na, Cu and total phenolics significantly varied depending on geographical origin (p<0.05). Overall, significantly the highest amounts of ash, Ca, Na and Cu were found in samples from Ejere, while pollens from Cheliya contained the highest Fe content. The lowest Ca content was measured in samples from Dendi, while those from Welmera, Cheliya and Toke Kutaye had the highest TPC values. Moreover, comparisons of the monofloral pollens from different locations showed that ash, protein, K, Ca, Mg, Fe, Na, Mn, Cu and TPC in *Brassica* sp. pollen varied significantly (p<0.05) (Tab. 4). *Guizotia* sp., also had significantly different values of moisture, ash, protein, P, Na, Cu, TPC and DPPH radical scavenging activity depending on the areas

Table 4.

Results of content of moisture (FW), ash, protein and minerals (DW) and total phenolics (FW); and antioxidant activity (FW) in *Brassica* sp. pollen samples from different locations

Districts	Moisture (%)	Ash (%)	Protein (%)	TPC (mg GAE/g)	DPPH (mg AAE/g)	K (mg/kg)	Ca (mg/kg)
A	12.7±0.2 ^a	2.6±0.2 ^{ab}	21.0±0.6 ^{ab}	46.4±4.6 ^a	15.7±1.9 ^a	6647.9±438.2 ^b	1063.2±34.5 ^a
B	12.3±0.7 ^a	2.8±0.2 ^{ab}	22.1±0.6 ^a	53.5±1.5 ^a	15.5±1.6 ^a	7142.5±429.7 ^b	1129.2±44.8 ^a
C	10.7±0.3 ^a	2.8±0.2 ^a	18.9±0.6 ^b	42.5±0.6 ^a	16.1±2.3 ^a	11373.9±785.0 ^a	1026.9±81.8 ^a
D	12.9±1.0 ^a	2.5±0.1 ^{ab}	20.5±0.9 ^{ab}	43.6±1.9 ^a	12.4±1.3 ^a	8954.3±460.3 ^{ab}	1186.0±54.7 ^a
E	10.2±1.1 ^a	2.1±0.1 ^b	22.1±0.3 ^a	27.5±0.2 ^b	15.2±0.2 ^a	10709.8±850.3 ^a	612.6±17.9 ^b
Mean±SE	11.8±0.4	2.6±0.1	20.9±0.4	45.5±1.8	14.7±0.8	8965.7±552.8	1003.6±57.6
Min	8.8	1.9	17.7	27.1	12.4	6130.5	586.4
Max	14.5	3.3	22.9	69.0	16.1	12937.5	1294.2
P	0.104	0.046	0.028	0.002	0.495	0.001	0.000

Districts	Mg (mg/kg)	Fe (mg/kg)	P (mg/kg)	Na (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)
A	495.2±20.7 ^b	57.0±5.0 ^b	139.7±6.5 ^a	58.8±3.7 ^b	16.8±1.2 ^a	10.0±0.5 ^c	1.1±0.2 ^b
B	517.3±29.4 ^b	142.4±22.2 ^a	144.0±4.5 ^a	70.0±7.3 ^b	25.4±2.5 ^a	13.8±0.7 ^{bc}	1.1±0.3 ^b
C	1126.8±127.5 ^a	104.2±5.1 ^{ab}	150.2±1.6 ^a	167.6±12.7 ^a	35.1±6.8 ^a	21.1±1.0 ^a	5.4±0.4 ^a
D	1025.3±40.5 ^a	123.7±18.2 ^a	87.2±37.3 ^a	89.6±14.9 ^b	33.9±9.3 ^a	14.0±1.7 ^{bc}	4.4±0.5 ^a
E	1301.2±77.7 ^a	100.2±4.1 ^{ab}	108.4±46.6 ^a	163.8±3.9 ^a	42.6±3.8 ^a	19.6±2.4 ^{ab}	4.6±0.8 ^a
Mean±SE	893.2±91.7	105.5±9.2	125.9±12.1	109.9±13.0	30.8±3.2	15.7±1.2	3.3±0.5
Min	456.9	49.0	13.1	54.9	14.6	9.2	0.6
Max	1441.9	175.9	161.2	183.9	51.8	23.0	6.2
P	0.000	0.012	0.450	0.000	0.065	0.002	0.000

A=Welmera, B=Cheliya, C=Ejere, D=Toke Kutaye, E=Dendi. FW=fresh weight, DW=dry weight.

Mean ± standard error (n=3); values within each column followed by different letters are significantly different at (p<0.05).

of origin (p<0.05) (Tab. 5). Similarly, variations in location significantly impacted the ash, P, Na and Mn content in *Trifolium* sp.; Ca, Fe and TPC in *Eucalyptus* sp. and TPC in *Plantago lanceolata* pollen.

DISCUSSION

This study showed that honey bees collected only multifloral pollens in all collections in the study area from mid-October to mid-December. As recommended by Almeida-Muradian et al. (2005), all the monofloral samples utilized for chemical analysis in this study were thus prepared through color sorting. However, the microscopic analysis revealed that the visually uniform pollens were mixtures of pollen grains

from different plant taxa (Tab. 1). A similar investigation revealed that pollens with monofloral labels were composed of up to five chromatic subsamples and different source plants (Almeida-Muradian et al., 2005). This might have come from contamination during collection by bees and preparation of samples in the laboratory, or a given pellet color may consist of pollen grains of multiple taxa (Almeida-Muradian et al., 2005). It is known that a single pellet color cannot guarantee the uniflorality of the source, as pollen grains of multiple taxa are commonly found in a given pellet color, or one plant species causes more than one pollen color (Almeida-Muradian et al., 2005; Kirk, 2018).

The microscopic analysis of bee pollen collected in the specified season showed that the total

Table 5.

Results of content of moisture (FW), ash, protein and minerals (DW) and total phenolics (FW); and antioxidant activity (FW) in *Guizotia* sp. pollen samples from different locations

Districts	Moisture (%)	Ash (%)	Protein (%)	TPC (mg GAE/g)	DPPH (mg AAE/g)	K (mg/kg)	Ca (mg/kg)
A	13.7±0.5 ^a	2.0±0.1 ^{ab}	16.9±0.5 ^a	41.5±1.4 ^{ab}	7.3±2.1 ^b	7583.4±699.4 ^a	1239.2±111.1 ^a
B	12.0±0.4 ^{ab}	1.5±0.1 ^b	14.4±0.1 ^b	47.3±2.7 ^a	11.1±1.4 ^{ab}	10396.3±705.2 ^a	1020.6±80.0 ^a
C	9.8±0.4 ^b	2.6±0.4 ^a	15.8±0.4 ^{ab}	31.4±4.0 ^b	10.8±0.9 ^{ab}	12612.4±448.8 ^a	1079.1±124.6 ^a
D	13.6±1.1 ^a	1.9±0.1 ^{ab}	15.2±0.4 ^{ab}	48.3±2.2 ^a	15.0±0.5 ^a	11277.5±736.5 ^a	1234.0±64.4 ^a
E	10.2±0.3 ^b	1.8±0.2 ^{ab}	15.0±0.7 ^b	36.8±2.6 ^b	13.0±1.0 ^{ab}	10002.8±2032.7 ^a	867.5±90.4 ^a
Mean±SE	11.9±0.5	2.0±0.1	15.5±0.3	43.0±1.4	11.4±0.8	10374.5±603.3	1088.1±52.2
Min	9.2	1.5	14.0	27.4	0.4	6468.3	712.8
Max	14.7	3.2	17.8	58.1	17.0	13758.9	1448.2
P	0.002	0.049	0.023	0.000	0.006	0.076	0.099

Districts	Mg (mg/kg)	Fe (mg/kg)	P (mg/kg)	Na (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)
A	663.5±57.9 ^a	120.6±24.0 ^a	103.2±2.3 ^{ab}	70.4±6.1 ^c	26.7±2.9 ^a	15.8±1.5 ^a	2.3±0.3 ^b
B	1121.2±128.7 ^a	118.9±23.9 ^a	85.9±1.1 ^b	109.6±8.5 ^{bc}	30.9±4.4 ^a	17.7±2.0 ^a	3.1±0.8 ^b
C	1118.2±27.4 ^a	121.1±14.6 ^a	134.0±17.5 ^a	194.1±8.2 ^a	36.8±6.6 ^a	26.1±3.0 ^a	8.2 ±1.0 ^a
D	1205.5±68.3 ^a	124.0±20.5 ^a	89.1±1.0 ^b	115.4±14.5 ^b	26.8±3.0 ^a	18.4±2.8 ^a	3.5±0.3 ^b
E	1132.5±262.6 ^a	77.3±5.6 ^a	86.9±4.2 ^b	103.2±9.1 ^{bc}	30.5±7.2 ^a	17.7±2.4 ^a	1.2±0.4 ^b
Mean±SE	1048.2±73.6	112.4±8.6	99.8±5.8	118.5±11.5	30.3±0.7	19.1±1.3	3.7±0.7
Min	566.6	77.3	79.9	58.1	18.9	13.4	0.6
Max	1534.0	124.0	168.4	207.0	47.5	31.8	10.0
P	0.108	0.421	0.009	0.000	0.647	0.082	0.000

A=Welmera, B=Cheliya, C=Ejere, D=Toke Kutaye, E=Dendi. FW=fresh weight, DW=dry weight.

Mean ± standard error (n=3); values within each column followed by different letters are significantly different at (p<0.05).

number of pollen taxa was 46, 41, 49, 43 and 52 in Welmera, Eejre, Dendi, Toke Kutaye and Cheliya, respectively. However, the major pollen source plants were similar in all locations, and more than 78% of the total collected pollen originated from the top eleven plant taxa. This similarity in pollen diversity could be due to the resemblance in the amount and pattern of rainfall and temperature variabilities in the region, which also resulted in a similarity in the blooming season of the source plants. The fact that all the apiaries were situated at altitudes between 2000 and 2200 m above sea level may also explain this scenario. However, variations in the botanical composition of polyfloral pollens from the five districts have caused differences in the

diversity of the monofloral pollen recovered for chemical analysis. For instance, *Guizotia* sp. and *Brassica* sp. were obtained from all the districts, while a sufficient amount of *Vicia faba* pollen was obtained only from Ejere (Tab. 1). The moisture and protein content varied in response to the botanical origin of the pollen. The results showed that the moisture content in some of the samples was lower than records in many similar studies and did not comply with the standards proposed for fresh bee pollen (20-30%) (Campos et al., 2008). However, Roulston & Cane (2000) argued that fresh pollens generally contained less than 20% water. Comparable protein content was reported in pollen collected from Welmera (Debissa et al., 2008) and other regions of the world (Somerville & Nicol, 2006; Carpes et al., 2009; Mayda et al., 2020;

Salonen et al., 2021; Castiglioni et al., 2022). In agreement with this study, Roulston & Cane (2000), Pernal & Currie (2001), and Somerville & Nicol (2006) also identified *V. faba*, *Trifolium* sp. and *Brassica* sp. pollens as high protein sources. The impact of floral differences on the moisture and protein content was observed not only among all samples but also among pollens of different botanical origins from a single location, in agreement with other results (Prdun et al., 2021; Castiglioni et al., 2022). In contrast to reports by Carpes et al. (2009), Mayda et al., (2020) and Castiglioni et al. (2022), the data showed that both moisture and protein content did not vary in response to the areas of origin of pollen. These two measurements revealed variations only when *Brassica* sp. and *Guizotia* sp. pollen from the five districts were compared in Tab. 4 and Tab. 5, respectively. Both the botanical and geographical origins affected the ash content of the pollen. The average ash content was 2.4%, while the highest, 3.3%, was measured in *V. faba* pollen. Thus, it meets the standards (2-6%) suggested by Campos et al. (2008). These results corroborate those that reported comparable ash content in pollens of different botanical and geographical origins (Almeida-Muradian et al., 2005; Mayda et al., 2020; Prdun et al., 2021). The variations in ash content in pollens from different areas were very high, and all sample types from Ejere contained the highest amount of ash (Tab. 3, 4 and 5). This implies that such geographical factors as soil type influence the ash content in bee pollen, besides the plant's ability to accumulate minerals (Prdun et al., 2021).

The mineral analysis revealed the predominance of K, Ca, Mg, Fe and P. Similarly, the dominance of K, followed by Ca, Mg and Fe in their concentration levels, was also reported in Serbia (Kostic et al., 2015). However, Morgano et al. (2012) and Liolios et al. (2019) reported different orders of dominance of these minerals. The amount of K in pollen observed in this study was higher, while the P content was by far lower than what was previously reported (Somerville & Nicol, 2002; Morgano et al., 2012; Liolios et al., 2019; Mayda et al., 2020). However, Debissa et

al. (2008) revealed lower concentrations of K and P in pollen collected from apiaries in Welmera, close to the present one. On the other hand, the present results showed that bee pollen contained a far lower Ca content than others (Somerville & Nicol, 2002; Morgano et al., 2012; Liolios et al., 2019). However, comparable

Ca concentrations were reported by Carpes et al. (2009), while Mayda et al. (2020) revealed lower Ca values. A higher amount of Mg than what Carpes et al. (2009) demonstrated was observed in pollen. However, Liolios et al. (2019) reported comparable results, while Morgano et al. (2012) and Mayda et al. (2020) found lower Mg concentrations. Similarly, lower quantities of Fe and Na in bee pollen than the current ones were reported earlier (Somerville & Nicol, 2002; Morgano et al., 2012). On the other hand, the content of Zn, Mn and Cu in bee pollen was lower than previously reported (Morgano et al., 2012; Liolios et al., 2019; Mayda et al., 2020). The results showed that the botanical origin of pollen impacted the concentrations of Ca, Fe, P and Cu. For instance, *Guizotia* sp., *V. faba*, *Trifolium* sp. and *Brassica* sp. pollens had the highest Ca content, while multifloral pollen contained the highest amount of Fe. The highest P and Cu values were measured in *V. faba* pollen. *Eucalyptus* sp. pollen also had the highest P amount. On the other hand, the pollen of *Plantago lanceolata* contained the lowest of Ca, Fe, P and Cu (Tab. 2). Elements such as K, Mg, Na, Zn and Mn were not significantly different depending on the floral origin of pollen, though investigations by Somerville & Nicol (2002) and Liolios et al. (2019) showed otherwise.

The evaluation of the influence of areas of origin on the mineral compositions of pollen showed that the concentrations of Na, Ca, Fe and Cu were significantly different. When compared across locations, the total samples from Ejere had the highest amounts of Na and Cu, while those from Cheliya contained the highest Fe content. The lowest amount of Ca was measured in samples from Dendi (Tab. 3). According to Liolios et al. (2019), studying monofloral pollen from different areas helps to attribute variations found in mineral composition solely to the soil and climate

conditions by excluding the influence of botanical origin. In this study, the geographical origin significantly affected the concentrations of K, Mg, Fe, Na, Mn and Cu in *Brassica* sp. pollen, while it caused variations in P, Na and Cu content in *Guizotia* sp. pollen (Tab. 4 and 5). Kostic et al. (2015) also argued that only the analysis of monofloral pollen of a single plant taxon from different areas could demonstrate variations in the mineral content of pollen due to its geographical origin.

The variations in mineral compositions of bee pollen collected from different locations are mainly associated with differences in soil and geographic origin (Carpes et al., 2009; Liolios et al., 2019). Geochemical and geological features of the soil affect the concentrations of minerals in plant products as they enter a plant from the soil through the root system (Kostic et al., 2015). The soils in the study area have variable characteristics. The dominant soil types in Welmera, Ejere, Toke Kutaye and Cheliya are Eutric nitosols with high clay texture and low amounts of P, K, Ca and Mg (Tolera et al., 2016; Achalu & Emiru, 2023; Addisu & Chernet, 2023). According to Obsa (2022), the soils in Dendi are Pellic Vertisol, Vertic Cambisol and Nitosol, with high exchangeable bases, Ca being the most dominant and deficient in P (Tekalign et al., 2002). Therefore, neither the high concentrations of K, Ca and Mg in bee pollen nor their pattern of locational variations in the study area reflected the properties of the soils. Despite the dominance of Ca in its soils, the samples from Dendi had the lowest amount of the element. However, the low P content in pollens across all districts better explained its deficiency in both Nitosol and Vertisol soils. It is worth mentioning that, in addition to soil sources, plants bioaccumulate minerals from water and air (Kostic et al., 2015). Furthermore, the sample collection did not consider the representation of soil types but the accessibility of the apiaries.

This study showed that both botanical and geographical origins significantly impacted the total phenolic content (TPC) in bee pollen, which agrees with findings that observed similar associations and comparable TPC values (Mayda

et al., 2020; Salonen et al., 2021). Others reported higher (Araújo et al., 2017; de Arruda et al., 2021) and lower TPC values (Ceksteryte et al., 2016; Rocchetti et al., 2019). However, the antioxidant activity assessed by the DPPH quenching ability of the pollen extracts was affected only by the floral sources of the pollen, except when *Guizotia* sp. pollen from different locations was compared, as opposed to the results reported by de Arruda et al. (2021) and Castiglioni et al. (2022).

The data of the antioxidant activity of pollen in literature are highly inconsistent and difficult to compare because of variations in the usage of standard compounds, extracting solvents and analysis techniques (Ares et al., 2018). In the present study, assessing the antioxidant activity of the ethanol pollen extracts with the use of ascorbic acid as a reference standard in the DPPH assay also caused limitations in finding and making comparisons with similar results. However, in the current study, the pollen extracts showed higher antioxidant activity against DPPH radical than the results expressed as Trolox equivalents (TE/g of pollen) (Ceksteryte et al., 2016; Mayda et al., 2020; Castiglioni et al., 2022). Even though both the TPC and the activity against DPPH radical indicated the significance of the antioxidant capacities of pollen, there was no strong correlation between the two ($r=0.247$). Investigations also indicated that the antioxidant capacity of pollen could be due to the contribution of other constituents than the total phenolics and their synergetic effects (Ares et al., 2018; Rocchetti et al., 2019).

In summary, the botanical and geographical origins affected the chemical composition of bee pollen differently. For instance, the significant variation in ash, Ca, Fe, Cu and TPC depended on both the botanical and geographical origins. On the other hand, moisture, protein, P and the antioxidant activity in the DPPH assay significantly differed on a botanical basis, while Na varied based on only areas of origin. Therefore, the analysis of the chemical compositions of bee pollen singled out distinct pollen source species and collection locations.

In conclusion, this study showed that bee pollen from the study area is a good source

of protein, minerals and total phenolics. The antioxidant activity was also of high significance. Considering this, it is possible to suggest that the bee pollen meets the standards of protein, ash and many of the minerals nutritionally required by honey bee colonies. Moreover, the results also indicated that the bee pollen fulfills the standards set targeting human consumption, except in their very low P content. Therefore, this could be a starting point for the establishment of the bee pollen's quality standards geared towards pollen harvesting and processing. However, this study is based on samples only from a few apiaries, one blooming season and limited number of chemical analysis. Therefore, further comprehensive investigations of chemical compositions that address as many pollen source plant taxa as possible over extended seasons and areas are crucial to developing a broad information base and narrowing the information gap on the nutritional profile of the bee pollen in the region.

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