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Determination of protein levels and amino acid composition of bee pollen collected from different geographical regions of Turkey

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ABSTRACT

In this study, determination of the amino acid profile of bee pollen produced in different parts of Turkey and performed to determine the protein level. The research was carried out with a total of 90 specimens from East-Southeast Anatolia, Central Anatolia, Black Sea, Marmara, Aegean, and Mediterranean. LC / MS-MS for bee pollen amino acid levels and Bradford method for protein level were preferred. According to the analysis results, the average on mg/g basis in bee pollen are alanine (143.40 ± 0.00), arginine (34.45 ± 0.00), histidine (32.55 ± 0.09), isoleucine (20.94 ± 1.24), leucine (23.75 ± 1.27), lysine (31.15 ± 0.43), methionine (39.10 ± 1.32), phenylalanine (20.09 ± 0.95), proline (309.05 ± 28.56), and valine (15.79 ± 0.88) amino acids were detected respectively. According to the analysis results, protein level of bee pollen in mg / g basis in Black Sea region (127.27 ± 0.31), Marmara region (117.56 ± 0.31), Mediterranean region (115.66 ± 0.31), Central Anatolia (115.09 ± 0.31), Aegean region (110.06 ± 0.31) and East-South East Anatolia (124.9 0 ± 0.31). The differences between the regions were found in protein and amino acid levels (P <0.01). In this study, the protein content and amino acid composition of bee pollen collected from plants growing in various regions were determined. We believe that this research may help with the manufacture, inspection, and standardization of healthy bee products.

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Introduction

Bee products have been used for centuries for the prevention and treatment of diseases and for a healthy life (Cherbuliez, 2013; Aker and Nisbet, 2020). With the introduction of new biological activities, these natural foodstuffs occupy an important place among research topics in recent years. Honeybee pollen, a product of apitherapy, is the main food source used for colony development, consisting of a mixture of flower pollen and glucose oxidase enzyme from bee secretion (Gerigelmez, 2003). In many studies and research based on clinical experience, bee pollen has been found to be effective on antioxidant (LeBlanc et al., 2009), antibacterial (Proestos et al., 2005), antifungal (Garcia et al., 2001), stress reducer (Seven et al., 2011), stomach disorders (Wang et al., 2007), immunomodulatory (Oliveira et al., 2013) and anti-inflammatory (Xiaozhi et al., 2018) properties. However, it is not correct to rely on these properties for all bee pollen (Nisbet et al., 2018). The reason is that the chemical content of pollen varies depending on the plant source. Honeybees not only collect pollen from different kinds of flowers, but also add different

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chemicals found in this plant to their pollen content (Nisbet et al., 2009; Denisow and Wrzesien, 2015). The chemical composition and biochemical properties of pollen depend on the plant species from which the pollen was collected (Szczęsna, 2006), its geographical origin (Morgano et al., 2012), by season (Morgano et al., 2012; Denisow and Wrzesien, 2015), soil type, and storage method (Feás et al., 2012; Siuda et al., 2012. These factors also change the composition of the active substances of the pollen. Therefore, the protein, carbohydrate, mineral, oil, vitamin and phenolic compounds contained in bee pollen are different. In other words, the food quality and use of each bee pollen produced in apitherapy should be revealed with laboratory data. In this study, which we think may contribute to the production, inspection and standardization of healthy bee products, the protein levels and amino acid components of bee pollen obtained from plants grown in different regions were determined.

Materials and Methods

Sampling: A total of 90 pollen samples from different regions of Turkey, including Central and Eastern Black Sea (23), Marmara (14), Central Anatolia (12), Mediterranean (12), Aegean (17), East-South Anatolia (12), were used. Pollen samples were dried in the laboratory by baking at 40°C for 48 hours. The dried samples were ground.

Extraction: A solution was prepared by weighing 1 g of the ground samples and using 10 ml of 0.2 mmol acetic acid. The prepared solutions were vortexed for 2 minutes. After the vortex application, it was centrifuged at 5000 rpm at -4°C for 10 minutes. After centrifugation, the supernatant part of the solution under the oil layer was taken and filtered with disposable filters of 0.45 nmol, and 5 ml solutions were prepared (Ulusoy, 2010).

Biochemical analysis: Amino acids to be analyzed in pollen samples were determined as aspartic acid, glutamic acid, proline, alanine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, phenylalanine, lysine and arginine, glutamine, tryptophan, glycine, histidine, serine, threonine and asparagine. The method reported by Szczesna (2006) was preferred for the analysis of the identified amino acids. High Performance Liquid Chromatography-Tandem Mass Spectrometer (LC/MS-MS 8040, SHIMADZU) device was used for amino acid analyses. In this study, ready-made commercial standard solution was used for qualitative and quantitative analyses of amino acids (Amino acids Mix Solution., Product No: 79248-BCBS9675V). A modified Bradford method was used to determine the protein concentration in pollen (Lu et al., 2010).

Statistical analysis: The data obtained from the study were evaluated with the analysis of variance (ANOVA) technique in factorial order and the differences between the means were determined by Duncan multiple comparison test. Statistical evaluations were made using the SPSS statistical program (SPSS,2004).

Results

Amino acid analysis results: Amino acid types and levels determined in pollen samples were presented in Table 1 and 2. As a result of the analysis of variance performed according to the randomized plot design, a possibility difference was determined between the regions ($P<0.01$).

Table 1. Mean, minimum and maximum free amino acid levels compared to Turkey's average (mg/g pollen) (n=90)

Amino acid	Mean (mg/g pollen)	Minimum (mg/g pollen)	Maximum (mg/g pollen)
Alanine	143.40 ± 0.0 0	143.40	143.40
Arginine	34.45 ± 0.0 0	34.45	34.45
Histidine	32.55 ± 0.09	32.22	33.73
Isoleucine	20.94 ± 1.24	12.58	62.76
Leucine	23.75 ± 1.27	16.30	58.50
Lysine	31.15 ± 0.43	30.04	31.87
Methionine	39.10 ± 1.32	37.78	40.43
Phenylalanine	20.09 ± 0.95	13.27	48.11
Proline	309.05 ± 28.56	9.50	1503.41
Valine	15.79 ± 0.88	10.60	21.83

Table 2. Free amino acid mean values by region (mg/g pollen)

	Mediterranean	East-Southeast	Central Anatolia	Black Sea	Marmara	Aegean
Alanine	143.40±0.00	-	-	-	-	-
Arginine	34.45±0.00	-	-	-	-	-
Histidine	32.46±0.17	32.45±0.09	32.64±0.24	32.53±0.11	32.53±0.30	32.66±0.35
Isoleucine	18.01±0.94	22.98±3.28	19.66±1.19	21.22±4.65	21.52±3.89	22.12±1.71
Leucine	20.59±1.06	23.95±2.15	22.47±1.24	29.33±7.81	24.15±4.04	23.04±1.34
Lysine	30.04±0.00	-	-	31.85±0.00	-	31.35±0.51
Methionine	-	-	-	37.78±0.00	40.43±0.00	-
Phenylalanine	30.04±0.00	20.81±2.01	20.75±1.29	20.66±4.16	20.05±3.45	18.83±0.95
Proline	374.88±74.20	424.69±91.64	408.78±60.03	207.00±66.88	293.99±47.57	254.65±55.20
Valine	16.02±1.31	18.76±3.07	-	14.13±1.61	16.61±2.10	14.45±2.45

Table 3. The total protein and total amino acid amounts by regions (mg/g)

Regions	Total protein (mg/g)	Total amino acid (mg/g)	Total essential amino acid (mg/g)	Total non-essential amino acid (mg/g)
Marmara	117.56 ± 0.31	103.36 ± 22.96	22.47 ± 1.93	310.26 ± 53.93
Aegean	110.06 ± 0.31	92.19 ± 22.19	22.56 ± 1.04	253.55 ± 65.47
Mediterrenian	115.66 ± 0.31	113.8 ± 29.18	20.81 ± 1.14	374.88 ± 74.2 0
Central Anatolia	115.09 ± 0.31	135.18 ± 32.63	21.96 ± 1.95	408.78 ± 60.03
Black Sea	127.27 ± 0.31	100.29 ± 30.25	24.57 ± 2.56	119.40 ± 45.68
East-Southeast Anatolia	124.90 ± 0.31	123.51 ± 33.70	22.32 ± 1.53	424.69 ± 91.64

Table 4. Differences between regions according to protein analysis results (mg/g)

Regions	Mean (mg/g)	Minimum (mg/g)	Maksimum (mg/g)
Black Sea	127,27 ± 0.31^{a}	120.97	133.57
Marmara	117.56 ± 0.31^{bc}	111.25	123.86
East-Southeast Anatolia	124,90 ± 0.31^{ab}	118.60	131.20
Aegean	110.06 ± 0.31^{c}	103.75	116.36
Central Anatolia	115.09 ± 0.31^{c}	108.78	121.39
Mediterrenian	115.66 ± 0.31^{bc}	109.35	121.96

Table 5. Differences in protein content between regions (mg/g)

Aegean region	110.06c		
Central Anatolia region	115.09c		
Mediterranean region	115.66c	115.66b	
Marmara region	117.56c	117.56b	
East-Southeast region		124.90b	124.90a
Black Sea region			127.27a
Sig.	1.27	0.53	5.93

Table 6. Comparison of the amino acid profile of Turkey with the amino acid profiles of other countries

Amino Acid	Turkey	China (Yang, 2013)	Spain (Gonzalez, 2006)	South Africa (Nicolson, 2013)	Brazil (Negrao, 2018)
Alanine	143.40 ± 0.0	12.5 ± 0.6	10.68 ± 1.10	53.7 ± 4.60	11.54 ± 0.50
Arginine	34.45 ± 0.0	14.00 ± 0.9	5.03 ± 1.50	41.8 ± 2.60	8.75 ± 1.70
Histidine	32.55 ± 0.10	8.10 ± 0.5	6.84 ± 7.20	56.6 ± 5.50	4.58 ± 0.40
Isoleucine	20.94 ± 1.20	11.10 ± 0.7	9.22 ± 2.00	38.8 ± 2.50	7.17 ± 0.70
Leucine	23.75 ± 1.3	170.00 ± 1.1	10.81 ± 1.70	63.2 ± 3.80	12.59 ± 9.50
Lysine	31.15 ± 0.40	15.30 ± 0.8	10.97 ± 1.97	62.9 ± 2.70	11.69 ± 0.10
Methionine	39.10 ± 1.3	4.20 ± 0.1	4.10 ± 1.57	-	5.18 ± 0.30
Phenylalanine	20.09 ± 10	1.80 ± 0.6	9.65 ± 2.2	39.0 ± 2.60	7.15 ± 0.60
Proline	309.05 ± 28.60	15.70 ± 0.8	22.88 ± 3.5	61.9 ± 5.00	21.24 ± 2.90
Valine	15.79 ± 0.80	12.9 0 ± 0.8	7.26 ± 1.90	43.4 ± 3.20	8.42 ± 1.20

Protein Analysis Results: The verage of protein concentration determined in pollen samples in Turkey was presented in Table 4. As a result of the analysis of variance performed according to the randomized plot design, a possibility difference was determined between the regions (P<0.01).

Discussion and Conclusion

Today, pollen and pollen products are used in the fields of health and cosmetics as well as nutrition (Morais et al., 2011; Denisowand and Denisow-Pietrzyk, 2016). The high nutritional value of pollen is completely dependent on the chemical structure and biochemical properties of bee pollen. The therapeutic activity of bee pollen is not equally valid for all pollen. In other words, the chemical structure of pollen differs from region to region and country to country. The plant composition shaped by each flora offers honey bees a source of pollen from different species (Aker and Nisbet, 2020). On the other hand, it is known that honey bees do not use all flowering plant species as pollen source in the natural flora where they work, there is a preference, and the number of flowering species preferred for bees in the flora in general has a very low share among all flowering plant species (Baydar and Gurel, 1998).

According to the results we obtained, alanine, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, and valine amino acids were found in the amino acid profile of the pollen samples included in the study. On the other hand, threonine, glycine, aspartate, cysteine, glutamate, tyrosine and serine amino acids could not be detected because they were at very low levels. Studies conducted in different countries (Poland, Belgium, Korea, South Africa, Spain, China) differ in terms of amino acid diversity in pollen (Gonzalez et al., 2006; Yang et al., 2013). In the obtained data, the amino acid concentration in the samples is Proline > alanine > methionine > arginine > histidine > lysine > leucine > isoleucine > phenylalanine > valine , respectively. It originates from lysine, histidine, threonine, phenylalanine, leucine, isoleucine and valine, which are very important essential amino acids for the honey bee (Cook et al., 2003). In the present study, except for threonine and tryptophan, other essential amino acids were detected in pollen. When the minimal essential levels required for honey bees are compared with data obtained, Turkish pollen seems to be in a good position (Nicolson et al., 2013). In this study, proline and alanine are the predominant amino acids that make up 45% of the total amino acids in Turkey.

The results of the analysis reveal that Turkish pollen (689.18 mg/g) has a high nutritional value as a food substance compared to countries such as China (238.96 mg/g), Poland (201.52 mg/g) and Korea (181.10 mg/g) in terms of total amino acid concentration. When compared between regions in this study, it seems that the amino acid content and concentration of pollen obtained from different regions are different from each other

According to the results obtained, the protein level in the pollen is 127.27±0.31 mg/g in the Black Sea region, 117.56±0.31 mg/g in the Marmara region, 115.66±0.31 mg/g in the Mediterranean region, 115.09±0.31 mg/g in the Central Anatolia region, 110.06±0.31 mg/g in the Aegean region, 124.9±0.31 mg/g in the East-South East Anatolia region. In the study conducted by DeGrandi-Hoffman et al. (2018) in the Sonoran region of Arizona, the total protein level was determined as 425 ± 30 mg/g.

In a study conducted in eastern Saudi Arabia, the total protein level was reported as 202.3 mg/g (Taha et al., 2019). Ketkar et al. (2014) looked at the potential values of bee pollen obtained from Indian mustard monoflorally, they determined the amount of protein is 182.2±5.9 mg/g. When compared with the data of other countries, it is thought that the difference in the amount of protein in the results obtained from the bee pollen in Turkey is due to the multifloral plant diversity. At this point, it has been confirmed by the results of the study that the chemical composition of pollen is variable due to the fact that it is obtained from flower products, it differs according to the plant species and the geographical structure of the plant, season and soil structure. The emergence of different data is an indication that it is not considered possible for the pollen samples to be standard.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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