MELLIFERA RESEARCH ARTICLE

Protein Analysis of Anzer Bee Pollen by Bradford Method

Ömür GENÇAY ÇELEMLİ[1,2*], Nezahat Pınar BARKAN[1,2], Çiğdem ÖZENİRLER[1,2], Duygu ÖZEL DEMİRALP[3], Kadriye SORKUN[1,2]

[1]Hacettepe University, Science Faculty, Department of Biology, Beytepe, 06800, Ankara-Turkey

[2]Hacettepe University Bee and Bee Products Applied and Research Center, Beytepe, 06800, Ankara-Turkey

[3] Ankara University, Engineering Faculty, Department of Biomedical Engineering, Ankara- Turkey

Corresponding author e-mail: gencay@hacettepe.edu.tr

Received; February 27,2017 accepted; September 14, 2017; published; October 31, 2017

A B S T R A C T

The aim of this work is to determine the plants that are foraged by honeybees in Anzer Plateau and to evaluate the protein content of the pollen samples. Within the context of the study, pollen samples were collected from two different bee farms located in the Anzer Plateau during the period June-August, 2013 by using traps in honey bee hives.
The pollen loads of the bees were firstly classified according to their color. The origin of plants was determined in family and/or genus level according to microscopical analyses. Total protein content of pollen samples was determined by using Bradford method. As a result of palynological analysis, it was found that the protein content of the Anzer pollen samples were found between the range of 3.2-17.6 g/100g. Geraniaceae family was found as the mostly preferred taxon as a foraged plant. According to our findings, its protein content is determined to be found between 5-7.8 g/100g according to the collection date and location.

Key words: Bee pollen, protein analysis, Bradford method, palynological analysis, Geraniaceae

Introduction

Bee pollen which is an important source of protein in bee colonies are in fact male reproductive microspores of flowering plants collected by honey bees that are later on mixed with nectar and bee salivary glands secretions [1-2]. The pollen is used for feeding, especially for larvae growth and development [3]. Honey bees use pollen as their nutritional source of protein, fatty acids, lipids, sterols, vitamins, minerals and certain carbohydrates [4].
Pollen is a fine powder-like material [5]. Depending of the plant species pollen grains, differ in shape, color, size, and weight. The color of pollen varies, ranging from bright yellow to black [6].

Chemical composition of pollen depends on the availability of bee pastures and species of plants visited by bees. Pollen is an essential source of compounds with health protective potential like phytosterols and phytochemicals such as phenolic compounds [7]. Bee pollen is rich in sugar, protein, lipid, vitamin and flavonoid [1,8]. Besides, it includes minerals, some antioxidant vitamins, namely C, E, β-carotene, and also vitamins from the B-complex [9] and it is also a rich source of

flavonoid glycosides [10]. Almaraz and Naranjo (2004) reported that this bee hive product can be an important source of natural flavonol antioxidants [11]. However, the composition of bee pollen depends strongly on the plant source and its geographic origin, together with other factors such as climatic factors, soil type, and beekeeper activities [12] .

Bee pollen has antimicrobial, antifungal, antioxidant,anti-radiation,hepatoprotective, chemopreventive, antioxidant, anticancer and anti-inflammatory effects [12]. Such uses suggest that bee pollen could be useful in the prevention of diseases associated with free radicals. These therapeutic and protective effects, especially antioxidant activities, may be related to polyphenols. Antioxidant activities of bee pollen may be associated with the floral species [13]. According to the results in a study with 200mg/kg pollen fed white New Zealand rabbits, the body weight had increased and biochemical profiles of blood had improved [14]. It has been shown that bee pollen fed chickens revealed a better development of small intestine villi from the duodenum, jejunum and ileum. These findings suggest that bee pollen could promote the early development of the digestive system [15].
Honey bee collected pollen can be considered as a potential source of energy and proteins for human consumption [4]. Bee pollen has also been used for many years in traditional medicine and supplementary nutrients, primarily because bee pollen has nutritional and health benefits [13]. In recent years people's interest in natural foods have gained increased attention. Due to its rich content, bee pollen is preferred by people as a natural food supplement [16].

Pollen provides a rich source of easily digestible protein and essential amino acids for humans. Protein content in pollen depends on the origin of the plant. These values vary between a large range from 3.8 to 40.8%, with 25% being the average [17]. Cane and Buchmann (2000) indicates these values as 2.5% to 61% [18-20]. According to Bonvehi et al., pollen from insect-pollinated species are richer in protein content compared to that from anemophilous plants [21]. Quite the contrary, Cane and Buchmann (2000) supported that zoophilous species are not statistically richer in protein content than anemophilous species and both mass of protein per pollen grain and pollen grain volume were correlated with stigma-ovule distance [22]. They suggested that the need for growing pollen tubes probably plays a more important role in determining pollen protein content than rewarding pollinators. are essential for life and the organism cannot synthesize them by itself [6].

Bogdanov, (2012) reported the that total protein content of the pollen to be no less than 15g/100g [18].

Anzer plateau is located in Rize province at the Eastern Black Sea region at an altitude above 2300m. With its rich flora, this plateau is famous for its honey which provides beekeepers as an important source of income. Anzer honey is the most popular and expensive honey in Turkey, and it is traditionally used for medical purposes [23], explaining why so much attention has been paid to this honey, [23-25] but not on other bee products, with only one study conducted on pollen so far [26].

The aim of this work is to determine the plants foraged by honeybees in Anzer Plateau and also to evaluate their protein content by using Bradford method.

Cite as: ÇELEMLİ, Ö G; BARKAN, P; ÖZENİRLER, Ç; DEMİRALP D Ö; SORKUN, K (2017) Protein Analysis of Anzer Bee Pollen by Bradford Method. Mellifera, 17(1): 21-32

Materials and Methods

Collection of Pollen samples

Pollen samples were collected from two different bee farms located in the Anzer Plateau during a period of June-August of 2013. The samples were kept at -18°C until the analysis.

A total of nine mixed samples were obtained for investigation. The collection times and the locality of the samples are given in Table 1.

Table 1. Sample no, code, collection time, location, plant family, plant taxon, pollen color and protein content of pollen samples

Sample No	Sample code	Collection time	Location	Plant Family	Plant Taxon	Protein content (g/100g)
Sample 1	1-1	25.06.13	Plateu 1	Campanulaceae		7.6
	1-2			Rosaceae	*Sarcopoterium* spp.	4.4l
	1-3			Geraniaceae		5
	1-4			Fabaceae	*Onobrychis* spp.	6
	1-5			Ranunculaceae	Type1	7
	1-6			Asteraceae	*Bellis* spp.	5.4
	1-7			Brassicaceae		11.8
	1-8			Asteraceae	*Carduus* spp.	5.4
Sample 2	2-1	11.07.13	Plateu 1	Ericaceae		7.8
	2-2			Cistaceae		3.2
	2-3			Geraniaceae		6.2
	2-4			Ranunculaceae	Type 2	5.6
	2-5			Fabaceae	Trifolium repens	9.2
	2-6			Caryophyllaceae		17.6
	2-7				*Buxus sempervirens*	10
Sample3	3-1	28.07.13	Plateu 1		*Astragalus* spp.	7
	3-2			Cistaceae		5.4
	3-3			Cistaceae	*Cistus* spp.	4.2
	3-4			Asteraceae	*Taraxacum* spp.	3.6
Sample 4		14.08.13	Plateu 1			
	4-1			Geraniaceae		5
	4-2			Poaceae		9.6
	4-3			Campanulaceae		6.4
	4-4			Dipsecaeae	*Scabiosa* spp	7.8
	4-5			Asteraceae	*Carduus* spp	7.4
	4-6			Cistaceae	*Cistus* spp.	6
Sample 5	5-1	28.08.13	Plateu 1	Geraniaceae		5
	5-2			Gentaniaceae		11.2
	5-3			Dipsecaeae	*Scabiosa* spp	8
	5-4			Asteraceae	*Taraxacum* spp	1.6
	5-5			Asteraceae	*Carduus* spp	11.2
	5-6			Campanulaceae		7.4
Sample6	6-1	11.07.13	Plateu 2	Caryophyllaceae		7.8
	6-2			Rosaceae	Type 1	12.4
	6-3			Geraniaceaae		7.2
	6-4			Asteraceae	*Taraxacum* spp	4.6
	6-5			Rosaceae	Sarcopoterium	10.2

Cite as: ÇELEMLİ, Ö G; BARKAN, P; ÖZENİRLER, Ç; DEMİRALP D Ö; SORKUN, K (2017) Protein Analysis of Anzer Bee Pollen by Bradford Method. Mellifera, 17(1): 21-32

Sample 7	7-1	28.07.13	Plateu 2	Geraniaceae		7.8
	7-2			Asteraceae	*Taraxacum* spp.	6.4
	7-3			Caryohyllaceae		6.2
	7-4			Campanulaceae		14.2
	7-5			Buxaceae	*Buxus sempervirens*	11.4
Sample 8	8-1	14.08.13	Plateu 2	Asteraceae	Centaurea spp	7
	8-2			Geraniaceae		7.8
	8-3			Gentaniaceae		1.8
	8-4			Campanulaceae		11.4
	8-5			Ranunculaceae	Type1	7.6
Sample 9	9-1	28.08.13	Plateu 2	Gentianaceae	*Centaurium* spp.	5.8
	9-2			Geraniaceae		7.6
	9-3			Campanulaceae		5.4
	9-4				*Scabiosa* spp	12.4
	9-5			Asteraceae	*Carduus* spp	4.8
	9-6			Ranunculaceae	Type 1	10.4
	9-7			Asteraceae	*Taraxacum* spp.	6.6

Microscopic Analysis of Pollen samples

Analyses were carried out assuming that a pollen load was collected from only one plant species [27-29]. The mixed pollen samples were firstly classified according to their color. After this process, pollen slides were prepared for examination by light microscopy according to the Wodehouse method [30].

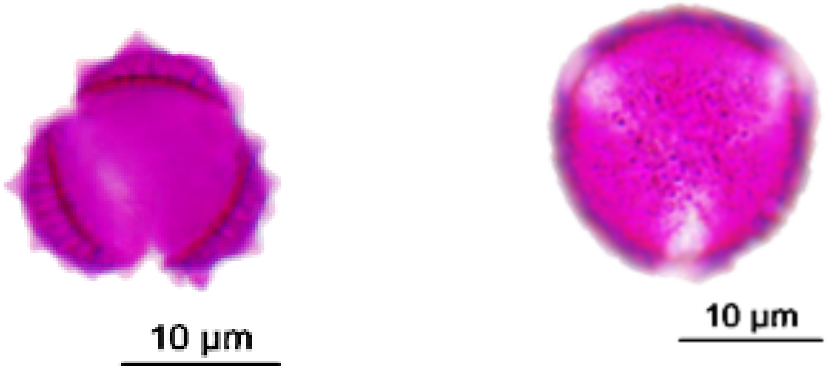
Protein Concentration Measurement

Protein content of pollens were determined by using Bradford [31]. Three replicates from each pollen sample was pooled. Briefly, 4 mg of each pollen sample was deffatted in 80 µl of dichloromethane for two hours and this step was repeated. Defatted pollens were grinded by using liquid N_2. The obtained pollen powder was mixed with 80 µl of urea lysis buffer [(7 M urea (Bio-Rad, ABD), 2 M thiourea (Bio-Rad, USA), % 4 CHAPS (Amresco, Ohio), % 1 DTT (Fluka, Switzerland), % 2 carrier ampholytes (pH 3-10, Fluka, Switzerland), 1 tablet protease inhibitor cocktail (Roche, Switzerland)] and were homogenized by sonication. Following homogenization, each pollen lysate was centrifuged at 17000 g for 1 hour at +4°C. The supernatant was used for further analysis while the pellet was discarded. 200, 400, 600, 800 ve 1000 µg/ml of Bovine Serum Albumin (BSA) were used as standards. BSA standards and 1:10 diluted samples were loaded on 96-well microplate as 4 replicates. 245 µl of Coommassie Brilliant Blue G-250 dye was added on each well and the microplate was incubated for 15 minutes in dark at room temperature. Lastly, the samples were measured spectrophometrically at 595 nm. Values were obtained in g/ 100g.

Results and Discussion

Microscopical analysis of pollen samples

According to the results from microscopic analysis, it is determined that the pollen samples collected by honey bees belong to 15 different families (Asteraceae, Brassicaceae, Campanulaceae, Cistaceae, Rosaceae, Geraniaceae, Fabaceae, Ranunculaceae,Ericaceae,Caryophyllaceae, Poaceae, Campanulaceae, Dipsecaceae, Gentianaceae, Buxaceae) (Table 1, Figure1-4).

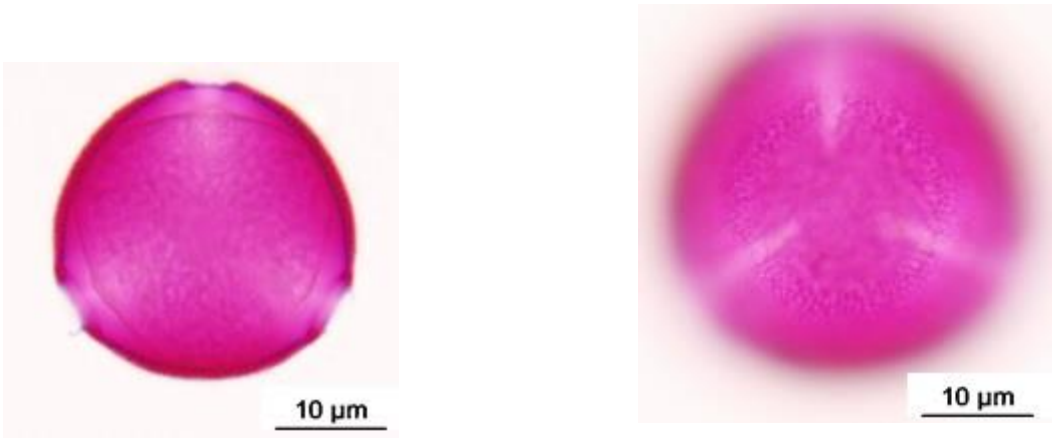


a) b)

Figure 1. Microphotograph of a) *Carduus* spp. pollen (x100) b) Ranunculaceae pollen (x100)

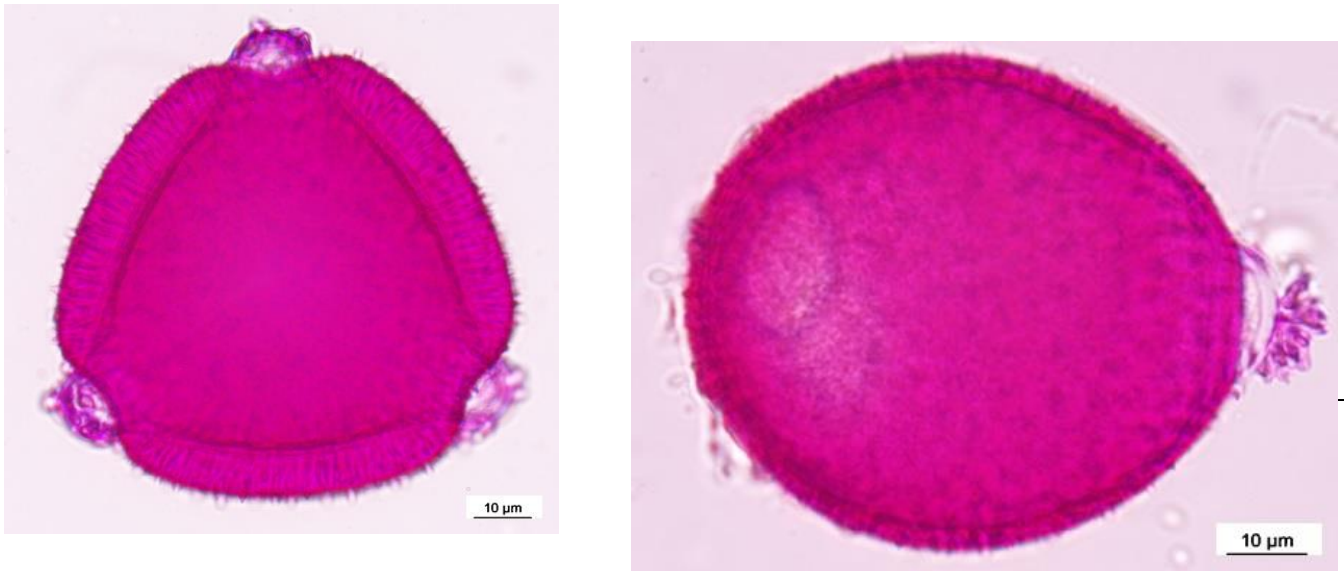
a) b)

Figure 2. Microphotograph of a-b) Geraniaceae pollen (x100)



a) b)

Figure 3. Microphotograph of a-b) Cistaceae pollen (x100)



a) b)

Figure 4. Microphotograph of a) Dipsacaceae pollen- polar view b) Dipsacaceae pollen-equatorial view (x100)

Protein content analysis of pollen samples

According to the results obtained by using Bradford method, the protein content of the pollen samples were found between the range 3.2-17.6 g/100g. The lowest value was found for the Cistaceae pollen (sample code 2-2), collected from first bee farm in July, 2013.

The highest value was found for the pollen sample (sample code 2-6) collected from the first bee farm in July, 2013, which belongs to the Caryophyllaceae family.

Bee pollen is the result of the agglutination of flower pollens; it is made by worker honey bees with nectar and salivary substances and stored at the hive entrance [12].The pollen should not contain impurities like bee parts, wax, plant

articles or other extraneous matter. Some countries as Brazil, Bulgaria, Poland and Switzerland have national standards about pollen but there is no international standard [18]. Also Turkey has a pollen standard but it is not detailed so much.

In this study we have determined the protein content of nine different pollen samples collected from hives of honey bees foraging in Anzer plateau. There have been two main approaches for quantifying the protein content of pollen samples. The micro-Kjeldahl method which was developed back in the late 1880`s, is based on the estimation of the nitrogen content of the sample of interest. However due to limiting factors such as quantification of nitrogen containing compounds of nonprotein origin, colorimetric techniques are preferred over this method [32]. One of the most commonly used colorimetric methods in determining protein concentration is Bradford (1976). Compared to Lowry method, it is faster, more accurate and shows minimum interference with reagents [33]. It is based on the color transition of the red Coomassie Brilliant Blue G-250 dye to the color blue, upon binding to protein. Bradford method is a widely used for protein quantification of pollen samples [19,34-35].

According to the results from microscopical analysis,, honey bees have mostly preferred gathering pollen of Geraniaceae family, since we identified this pollen in eight of the nine investigated samples. Following Geraniaceae, the second mostly preferred pollens are from is the Campanulaceae family (six of nine samples). *Taraxacum* spp., Cistaceae and *Scabiosa* spp. are following preferences. Kaya et al. examined the pollens of Anzer honey and similar to our results they found the pollen of Campanula, as secondary pollen, *Geranium*, *Lotus*, *Salvia*, *Heracleum*, *Myosotis*, *Lamium*, *Thymus* as minor and *Cardamine*, *Silene*, *Centaurea*, *Veronica*, *Helianthemum*, *Rumex*, *Scabiosa*, *Tragopon*, *Teucrium*, *Anemone*, *Draba*, *Chaerophyllum*, *Onobrychis* as rare pollen in the honey samples according to microscopic analysis [36].
Sorkun and Doğan (1995) also investigated the Anzer honey microscopically. They had investigated 28 honey samples and identified the pollen from 35 plant taxa mostly in minor (6-20%) or rare (0-5%) ratios. The only pollen that was identified in all 28 samples belonged to the taxa of Myosotis which was observed as minor or rare ratios in the investigated samples [25].

The only research about Anzer pollen was conducted by Ulusoy and Kolaylı [26]. They investigated the phenolic composition and antioxidant properties of Anzer bee pollen. They found the mean content of identified total phenolics from 0.5 mg/100 g pollen to 2.6 mg/100 g pollen. They also declared that the antioxidant activities showed a marked correlation with total phenolics.

Previous studies have shown that the protein content of pollen from the same species may vary depending on environmental factors (climatic conditions [37].

According to the results, the protein content of the Anzer pollen samples were found between the range 3.2-17.6 g/100g. As there is no data available on the protein content of Anzer pollen, we compared our results with previous studies conducted in different regions. We have found that our results show similarities with previous findings.Szczęsna, 2006 found the protein content of Caryophyllaceae pollen as 21.02%, Ranunculus as 17.83%, Brassica 24.08%, Campanula patula 23.60% [17]. In the study it is pointed out that pollen belonging to ruderal plants, especially *Sinapis arvensis*, *Sinapis alba* and *Chelidonium maius* is an important source of protein and amino acids for bees and for humans. Bonvehi and Jorda investigated

the protein content of 20 pollen samples collected from Spain and they found the protein content values between 12.6and 18.2g/100g [4]. Szczęsna,(2006) investigated the protein content of pollen samples collected from Poland (13 samples), South Korea (9 samples) and China (5 samples). For Poland samples they found the protein content values between 15.8-24.14%, for Korea 17.63-24.51%, for China 17.83-26.13% [17].

Feás et al. (2012) examined the botanical origin, and nutritional value of some bee pollens. According to their results, pollens of pine, corn and bulrush contain 13.45; 20.32 and 18.90% proteins respectively. It is also reported that many factors are known to affect the nutrient content of bee pollen, including climate, geography, apicultural practices and the genetic composition of the plant species [12].
Nutritional value of the bee pollen can be influenced from the storage conditions [38].
As seen from Table 2, the protein content of the pollen samples collected on the same date and belonging to the same taxa, varies quite much according to the location. This may indicate that climatic factors can be one of the parameters that effect the protein content of pollen. Besides, harvesting methods and storage conditions may have an impact on the protein content too. Further researches are necessary to say certainresults.

Table 2 .Comparing of protein contents pollen samples collected in the same time and belong to the same taxa according to the location

Collection time	**Plant Family**	**Plant Taxa**	**Code of Plateau 1 samples**	**Protein content of Plateu 1 samples(g/100g)**	**Code of Plateau 2 samples**	**Protein content of Plateu 2 samples(g/100g)**
11.07.13						
	Geraniaceae		2-3	6.2	6-3	7.2
	Caryophyllaceae		2-6	17.6	6-1	7.8
28.07.13						
	Asteraceae	*Taraxacum* spp.	3-4	3.6	**7-2**	6.4
14.08.17						
	Geraniaceae		4-1	5	**8-2**	7.8
	Campanulaceae		4-3	6.4	**8-4**	11.4
28.08.13						
	Geraniaceae		5-1	5	9-2	7.6
	Gentaniaceae		5-2	11.2	9-1	5.8
	Dipsecaeae	*Scabiosa* spp	5-3	8	9-4	12.4
	Asteraceae	*Taraxacum* spp	5-4	1.6	9-7	6.6
	Asteraceae	*Carduus* spp	5-5	11.2	9-5	4.8
	Campanulaceae		5-6	7.4	9-3	5.4

Conclusion

Results of the present study suggest that honey bees have preferred to collect the pollen of taxa belonging to Asteraceae, Brassicaceae, Campanulaceae, Cistaceae, Rosaceae, Geraniaceae, Fabaceae, Ranunculaceae, Ericaceae, Caryophyllaceae, Poaceae, Campanulaceae, Dipsecaceae, Gentianaceae, Buxaceae families in Anzer Plateu. In contrast to previous findings, it

can be concluded that honeybees may not always prefer the pollen types that have higher protein content, owing to the fact that the highly preferred Geraniaceae pollen has a protein content between the range 5-7.8 g/100g, while Caryophyllaceae pollen which is the least preferred pollen sample has a relatively higher protein content level (7.8-17.6 g/100g) Another observation will be the fact that locality may be an effective
parameter for the protein content of pollen samples.

Anzer Poleninin Bradford Metoduyla Protein Analizi

ÖZ
Bu çalışmanın amacı Anzer yaylasında bal arıları tarafından tercih edilen bitkileri saptamak ve bu bitkilerin polenlerinin protein içeriklerinin değerlendirilmesidir. Bu kapsamda, polen örnekleri Anzer yaylasının iki farklı arılığından 2013 yılı Haziran-Ağustos zaman peryodunda kovanlara konulan tuzaklarla toplanılmıştır.

Polen topakları öncelikle renklerine göre sınıflandırılmıştır. Mikroskobik analiz ile polenler familya ve /veya cins düzeyinde tanımlanmıştır. Polen örneklerinin toplam protein miktarı ise Bradford metodu kullanılarak saptanmıştır.

Protein analizlerin sonucunda, Anzer polen örneklerinin protein içerikleri 3.2-17.6 g/100g değerleri arasında bulunmuştur. Geraniaceae familyası en çok tercih edilen takson olarak saptanmıştır. Bulgularımıza göre, protein içeriği ise 5-7.8 g/100g değerleri arasında toplanma tarihi ve yerine göre değişiklik göstermektedir.

Anahtar Kelimeler: Arı poleni, protein analizi, Bradford metodu, palinolojik analiz, Geraniaceae

R E F E R E N C E S

[1]DONG, J., GAO, K., WANG, K., XU, X. & ZHANG, H. 2015. Cell Wall Disruption of Rape Bee Pollen Treated with Combination of Protamex Hydrolysis and Ultrasonication. *Food Research International,* 75**,** 123-130.

[2]ALMEIDA-MURADIAN, L., PAMPLONA, L. C., COIMBRA, S. L. & BARTH, O. M. 2005. Chemical composition and botanical evaluation of dried bee pollen pellets. *Journal of food composition and analysis,* 18**,** 105-111.

[3]FREIRE, K. R., LINS, A., DÓREA, M. C., SANTOS, F. A., CAMARA, C. A. & SILVA, T. 2012. Palynological origin, phenolic content, and antioxidant properties of honeybee-collected pollen from Bahia, Brazil. *Molecules,* 17**,** 1652-1664.

[4]SERRA BONVEHI, J. & ESCOLÀ JORDÀ, R. 1997. Nutrient composition and microbiological quality of honeybee-collected pollen in Spain. *Journal of Agricultural and Food Chemistry,* 45**,** 725-732.

[5]ZHANG, H., WANG, X., WANG, K. & LI, C. 2015a. Antioxidant and tyrosinase inhibitory properties of aqueous ethanol extracts from monofloral bee pollen. *Journal of Apicultural Science,* 59**,** 119-128.

[6]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,* 2015.

[7]ALIČIĆ, D., ŠUBARIĆ, D., JAŠIĆ, M., PAŠALIĆ, H. & AČKAR, Đ. 2014. Antioxidant properties of pollen. *Hrana u zdravlju i Bolesti,* 3**,** 6-12.

[8]SILVA, T. M. S., CAMARA, C. A., DA SILVA LINS, A. C., MARIA BARBOSA-FILHO, J., DA SILVA, E. M. S., FREITAS, B. M. & DE ASSIS RIBEIRO DOS SANTOS, F. 2006. Chemical composition and free radical scavenging activity of pollen loads from stingless bee Melipona subnitida Ducke. *Journal of Food Composition and Analysis,* 19**,** 507-511.

[9]SATTLER, J. A. G., DE MELO, I. L. P., GRANATO, D., ARAÚJO, E., DA SILVA DE FREITAS, A., BARTH, O. M., SATTLER, A. & DE ALMEIDA-MURADIAN, L. B. 2015. Impact of origin on bioactive compounds and nutritional composition of bee pollen from southern Brazil: A screening study. *Food Research International,* 77**,** 82-91.

[10]ZHOU, J., QI, Y., RITHO, J., ZHANG, Y., ZHENG, X., WU, L., LI, Y. & SUN, L. 2015. Flavonoid glycosides as floral origin markers to discriminate of unifloral bee pollen by LC–MS/MS. *Food Control,* 57**,** 54-61.

[11]ALMARAZ-ABARCA, N. 2004. Variability of antioxidant activity among honeybee-collected pollen of different botanical origin. *Interciencia,* 29**,** 574-578.

[12]FEÁS, X., VÁZQUEZ-TATO, M. P., ESTEVINHO, L., SEIJAS, J. A. & IGLESIAS, A. 2012. Organic bee pollen: botanical origin, nutritional value, bioactive compounds, antioxidant activity and microbiological quality. *Molecules,* 17**,** 8359-8377.

[13]ZHANG, Y., BIELORY, L., MI, Z., CAI, T., ROBOCK, A. & GEORGOPOULOS, P. 2015b. Allergenic pollen season variations in the past two decades under changing climate in the United States. *Global change biology,* 21**,** 1581-1589.

[14]ATTIA, Y., AL‐HANOUN, A., TAG EL‐DIN, A., BOVERA, F. & SHEWIKA, Y. 2011. Effect of bee pollen levels on productive, reproductive and blood traits of NZW rabbits. *Journal of animal physiology and animal nutrition,* 95**,** 294-303.

[15]WANG, S., LÜ, H., LIU, J. & NEGENDANK, J. F. 2007. The early Holocene optimum inferred from a high-resolution pollen record of Huguangyan Maar Lake in southern China. *Chinese Science Bulletin,* 52**,** 2829-2836.

[16]KROYER, G. & HEGEDUS, N. 2001. Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. *Innovative Food Science & Emerging Technologies,* 2**,** 171-174.

[17]SZCZÊSNA, T. 2006. Protein content and amino acid composition of bee-collected pollen from selected botanical origins. *Journal of Apicultural Science,* 50**,** 81-90.

[18]BOGDANOV, S. 2012. Pollen: Collection, harvest, composition, quality. *The bee pollen book***,** 1e13.

[19]CANE, J. H. & BUCHMANN, S. L. 2000. What governs protein content of pollen: pollinator preferences, pollen–pistil interactions, or phylogeny? *Ecological monographs,* 70**,** 617-643.

[20]ROULSTON, T. A. H. & CANE, J. H. 2000. Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution,* 222**,** 187-209.

[21]SERRA BONVEHÌ, J. & LÒPEZ ALEGRET, P. 1986. Microbiological studies on honeybee-collected pollen produced in Spain: Total bacteria, coliforms, Escherichia coli, Staphylococcus, Streptococcus “D” of Lancefield,

Cite as: ÇELEMLİ, Ö G; BARKAN, P; ÖZENİRLER, Ç; DEMİRALP D Ö; SORKUN, K (2017) Protein Analysis of Anzer Bee Pollen by Bradford Method. Mellifera, 17(1): 21-32

sulfite reducing Clostridia, Bacillus, yeasts, moulds, and the detection of aflatoxins by thin layer chromatography (TLC). *Ann. Falsif. Expert. Chim,* 849**,** 259-266.

[22]T'AI, H. R. & CANE, J. H. 2000. Pollen nutritional content and digestibility for animals. *Pollen and pollination.* Springer.

[23]GOK, S., SEVERCAN, M., GOORMAGHTIGH, E., KANDEMIR, I. & SEVERCAN, F. 2015. Differentiation of Anatolian honey samples from different botanical origins by ATR-FTIR spectroscopy using multivariate analysis. *Food Chem,* 170**,** 234-40.

[24]ŞEKERDEN, Ö., KıLıÇ, M. & KAPLAN, Ö. 1992. Türkiye'de Anzer Balı Üretim Bölgesinin Florası, Coğrafik ve klimsel Özellikleri ile Bu Şartlarda Arıcılığın Yapılma Şekli. *Doğu Anadolu Bölgesi 1. Hayvancılık Semineri***,** 3-4.

[25]SORKUN, K. & DOGAN, C. 1995. Pollen analysis of Rize-Anzer (Turkish) honey. *Apiacta,* 30**,** 75-82.

[26]ULUSOY, E. & KOLAYLI, S. 2014. Phenolic composition and antioxidant properties of Anzer bee pollen. *Journal of Food Biochemistry,* 38**,** 73-82.

[27]PELLETT, F. C. 1947. *American honey plants*, Orange Judd.

[28]ANDRADA, A. C. & TELLERÍA, M. C. 2005. Pollen collected by honey bees (Apis mellifera L.) from south of Caldén district (Argentina): botanical origin and protein content. *Grana,* 44**,** 115-122.

[29]ÖZENIRLER, Ç., BARKAN, N. P. & SORKUN, K. 2016. Aspir (Carthamus tinctorius L.) Remzibey-05 Bitkisi Poleninin Bal Arılarının Beslenme Ekonomileri Açısından İncelenmesi. *Sinop Üniversitesi Fen Bilimleri Dergisi,* 1**,** 69-80.

[30]WODEHOUSE, R. P. 1935. Pollen grains. Their structure, identification and significance in science and medicine. *Pollen grains. Their structure, identification and significance in science and medicine*

[31]BRADFORD, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry,* 72**,** 248-254.

[32]VANDERPLANCK, M., LEROY, B., WATHELET, B., WATTIEZ, R. & MICHEZ, D. 2014. Standardized protocol to evaluate pollen polypeptides as bee food source. *Apidologie,* 45**,** 192-204.

[33]KRUGER,NJ.1994. The Bradford method for protein quantitation. Methods Mol.Biol., 32,9-15.

[34]SHAHALI, Y., MAJD, A., POURPAK, Z., TAJADOD, G., HAFTLANG, M. & MOIN, M. 2007. Comparative study of the pollen protein contents in two major varieties of Cupressus arizonica planted in Tehran. *Iranian Journal of Allergy, Asthma and Immunology,* 6**,** 123-127.

[35]SHAHALI, Y., POURPAK, Z., MOIN, M., MARI, A. & MAJD, A. 2009. Instability of the structure and allergenic protein content in Arizona cypress pollen. *Allergy,* 64**,** 1773-1779.

[36]KAYA, Z., BINZET, R. & ORCAN, N. 2005. Pollen analyses of honeys from some regions in Turkey. *Apiacta,* 40**,** 10-15.

[37]SZCZĘSNA, T. 2007. Concentration of selected elements in honeybee-collected pollen. *Journal of Apicultural Science,* 51**,** 5-13.

Cite as: ÇELEMLİ, Ö G; BARKAN, P; ÖZENİRLER, Ç; DEMİRALP D Ö; SORKUN, K (2017) Protein Analysis of Anzer Bee Pollen by Bradford Method. Mellifera, 17(1): 21-32

[38]HUMAN, H. & NICOLSON, S. W. 2006. Nutritional content of fresh, bee-collected and stored pollen of Aloe greatheadii var. davyana (Asphodelaceae). *Phytochemistry,* 67**,** 1486-1492.

Cite as: ÇELEMLİ, Ö G; BARKAN, P; ÖZENİRLER, Ç; DEMİRALP D Ö; SORKUN, K (2017) Protein Analysis of Anzer Bee Pollen by Bradford Method. Mellifera, 17(1): 21-32