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Original research article

Nutritional Compositional Characterization on Five Diets for Development of Pollen Substitute Diet

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Abstract The western honey bee, *Apis mellifera* L. is an essential pollinator for wild plant and commercial crops in the world. High honey bee colony population losses are occurring globally due to effect of multiple stressors. Beekeepers need to provide pollen substitute diets regularly to maintain healthy colony and continuity of bee-related products in apiculture. This study focuses on development of pollen substitute diets, through investigation of different contents in nutritional components on five samples, namely canola pollen, mixed pollen, bee bread, MegaBee, and Test A. Among them, Test A was developed as pollen substitute diet and was compared with other samples. The five samples were analyzed on pH, mineral (ions), total phenol, vitamin B_6 and vitamin C. The value of pH for the Test A was 4.30 and it was similar with beebread and Megabee with pH 4.03 and 4.05, respectively. For analysis of mineral (ions), the elements chlorine (Cl), hydrogen sulfate (SO_4), monohydrogen phosphate (PO_4), nitrogen dioxide (NO_2), bromine (Br), lithium (Li), sodium (Na), ammonium (NH_4), potassium (K), magnesium (Mg), and calcium (Ca) was detected. The total phenol content of five samples was ranked from high to low as following: Canola pollen (16230 mg/kg), Bee bread (15660 mg/kg), Mixed pollen (9588 mg/kg), Megabee (4093 mg/kg), and Test A (3392 mg/kg). Among the five samples the only test A and Canola pollen were found to contain vitamin B_6 and C with 83 mg/kg and 479 mg/kg, respectively. Nutritional content, balance and efficiency needs to be considered for the development of pollen substitute diets. This study will contribute to provide future directions on development of pollen substitute diets.

Keywords Pollen substitute diets, pH, Minerals (ions), Total phenol, Vitamin

INTRODUCTION

The Western honeybee, *Apis mellifera* provides crucial pollination services for wild plant and commercial crops in the world (Williams, 1994; Eilers *et al*., 2011). High honey bee colony losses are occurring globally due to effect of multiple stressors (vanEngelsdorp and Meixner, 2010; Steinhauer *et al*., 2018). It has been proposed that the combination of nutritional stress, infections by pathogens and pesticide exposure are important forces (Goulson *et al*., 2015). Good colony nutrition, such as adequate protein and carbohydrate stores, is believed to help bees to resist or tolerate many of the stressors associated with modern apiculture (Brodschneider and Crailsheim, 2010). Honey bee nutrition is highly dependent on foodstuffs stored within the hive (Fleming *et al*., 2015). Worker bees do not have substantial protein reserves in their bodies therefore, they require a daily diet of about 3.4 to 4.3 mg of pollen, depending upon their age, to make up this nutritional deficiency (Fleming *et al*., 2015). A typical 10-frame colony consumes between 13.4 and 17.8 kg of pollen annually (Crailsheim *et*

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al., 1992).

In pollen, 200 different components have been detected in chemical composition. It mainly constitutes of proteins, amino acids, carbohydrates, fatty acids, phenolic compounds, enzymes-coenzymes, and vitamins (Komosinska-Vassev *et al*., 2015; Mayda *et al*., 2020; Ecem Bayram, 2021). Bee pollen contains different types of phenolic acids such as catechin, epicatechin, quercetin, rutin gallic, protocatechuic, p-hydroxybenzoic, chlorogenic, vanillic, cafeic, syringic, p-coumaric, ferulic, benzoic, o-coumaric, abscisic and trans-cinnamic acid in varying proportions (Ulusoy and Kolayli, 2014; Ecem Bayram, 2021). Phenolic compounds have been shown to induce changes in flavor release and aroma characteristics (Guichard, 2002).

Vitamins are essential for healthy growth and development and are involved in various biological functions of all organisms (Ecem Bayram, 2021). Bee pollen, which contains almost all of the vitamins, is called "vitamin bomb" (Kieliszek *et al*., 2018). It is rich in vitamin B complexes (thiamine, niacin, ribofavin, pyridoxine, pantothenic acid, folic acid and biotin) and caretenoids, but is poor in vitamin C and fat-soluble vitamins (de Arruda *et al*., 2013; Ecem Bayram, 2021). Likewise, minerals are essential for proper regulation of metabolic pathways and physiological processes. For this reason, they should be consumed daily in appropriate amounts (Ecem Bayram, 2021). Many minerals such as K, P, Mg, Ca, Na, S, Fe, Cu, Mn, Zn, Cr and Se have been detected in bee pollen samples from different regions around the world (Ecem Bayram, 2021).

Beekeepers feed colonies with pollen substitute diets when they believe bees are experiencing a nutrition dearth or if the incoming resources are believed to be of low or insufficient quality (Fleming *et al*., 2015). Therefore, pollen substitute diet development is of vital importance for maintaining a healthy colony and increasing the productivity in apiculture. The purpose of this study is to investigate the nutritional value of canola pollen which is a representative pollen that is widely used, mixed pollen, bee bread, MegaBee (commercial bee diet supplement) and our developed product which is named Test A. (Fig. 1). The Test A will be useful for replacing pollen substitute diet. The nutritional value was analyzed by measuring the levels of pH, mineral (ions), total phenol, and vitamin B_6, C. This study will contribute towards providing a future direction on development of better pollen substitute diets.

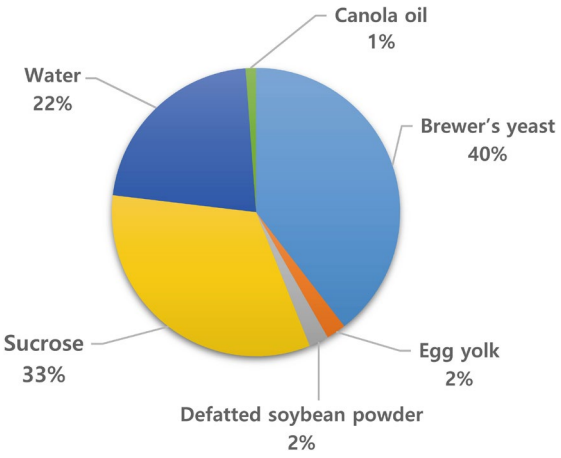

Fig.1. Composition of Test A diet containing constant ingredients (%).

MATERIALS AND METHODS

1. pH measurement

The pH of samples was measured with pH-meter Thermo Scientific Orion Star A211 (Thermo Scientific Inc) with glass electrode. 2 g of each sample was dissolved in 15 mL of distilled water for 24 h at room temperature before analysis (Adaškevičiūtė *et al*., 2019). Calibration of pH-meter was performed with three different buffer solutions having pH values of 4, 7 and 10.

2. Mineral (Ion) analysis

A Dionex ICS-3000 Reagent-Free Ion Chromatograph (Dionex Corporation, Sunnyvale, CA, USA) and column and Dionex IonPac (250 mm×4 mm) was used to identify the mineral levels using Ecem Bayram (Ecem Bayram, 2021). 0.6 g of sample was weighed and 7 mL of suprapur nitric acid (Sigma Aldrich, Germany) (65%) and 1 mL of hydrogen peroxide (Sigma Aldrich, Germany) (30%) were added. After that, the digestion procedures were carried out in a microwave digestion system (Milestone, Ethos Easy, Italy) according to instrumental parameters. The final volume of the samples removed from the microwave was completed to 50 mL with ultra-pure water. The column oven temperature was set at 30°C.

The flow rate of 1 mL/min and injection volume 25 µL was used. The eluent used was 24 mM KOH for negative ion and 20 mM methanesulfonic acid (MSA) for positive ion (Huang *et al*., 2021). Detection system was set on suppressed conductivity, ASRS-URTRA II (4 mm) with recycle mode.

3. Total phenol analysis

Total phenols content was determined by modified Folin-Ciocalteu colorimetry method (Kujala *et al*., 2000). 5 g samples were dissolved in 10 mL of distilled water, and 125 µL folin-Ciocalteu was added to the 125 µL of all samples. The mixture was incubated for 2 min at room temperature, and 1.25 mL of 7% Na_2CO_3 was added. Then, distilled water was added up to 3 mL of the final volume. This mixture was incubated for 90 min at room temperatuere then, centrifuged at 150 g for 10 min. The specific absorbance at 765 nm was immediately measured with UV/VIS Spectrophotometer (Perkin-Elmer Lambda 10). The standard curve was established with gallic acid.

4. Measurement of vitamin content

Vitamin analysis is carried out for vitamin B_6 and vitamin C. For vitamin B_6 analysis, samples were prepared as follows (Aslam *et al*., 2008). For buffer preparation, 1.08 g of hexane sulphonic acid sodium salt and 1.36 g of potassium dihydrogen phosphate were dissolved in 940 mL of HPLC water and 5 mL of triethylamine was added to it and the pH was adjusted to 3.0 with orthophosphoric acid. Extraction solution was made by mixing 50 mL of acetonitrile with 10 mL of glacial acetic acid and the volum was finally made up to 1000 mL with double distilled water. 10 g of each sample was homogenized, and transferred into conical flasks and 25 mL of extraction solution was added, kept on shaking water bath at 70°C for 40 min. Then, the sample was cooled down, filtered and finally the volum was made up to 50 mL with extraction solution. For vitamin C analysis, samples were prepared as follows. Homogenized by adding 10% methane phosphate solution to 10 g of sample, and extracted at 5°C in the dark. This mixture was centrifuged at 12,500 × g for 10 min and the supernatant was filtered with 0.45 µm filter (Hong *et al*., 2017). The samples were then analyzed using an Agilent 1200 series HPLC instrument (Agilent Technologies) under a 20°C-controlled column chamber. For HPLC analysis, 60 µL of each sample was injected into a waters symmetry C18 column, 4.6 mm × 150 mm, 5 µm (Innopia Technologies, Korea). For vitamin B_6 analysis, the mobile phase was used with a linear gradient of Buffer/methanol (96 : 4) and filtered through 0.45 µm membrane filter. The flow of injection into the syetem of HPLC was 1 mL/min, using two channels simultaneously at a wavelength of 210 nm and 280 nm. For vitamin C analysis, the mobile phase was 0.05 M KH_2PO_4/ Acetonitrile (95/5) at pH 6.8. The flow rate of injection into the system of HPLC was 0.5 mL/min, and detection UV 254 nm.

RESULTS AND DISCUSSION

1. pH

The values of pH of the tested samples were identified (Fig. 2). Among them, bee pollen samples which were Canola pollen and Mixed pollen distinguished by the highest pH values with 5.12 and 5.18, respectively, while the lowest pH values showed beebread with pH 4.03 and Megabee with pH 4.05. The Test A was similar with beebread and Megabee with pH 4.30. Other studies in literature showed that the pH values of the bee pollen varied 4.30 to 6.30 and beebread from 4.11 to 4.44 (Siksna *et al*., 2014; Adaškevičiūtė *et al*., 2019). We found the results to be consistent with the data published by other authors. The royal jelly is crucial for the development of a bee larva into a queen (Kurth *et al*., 2019). In the natural system, hypopharyngeal gland secretion has a pH of 5.1,

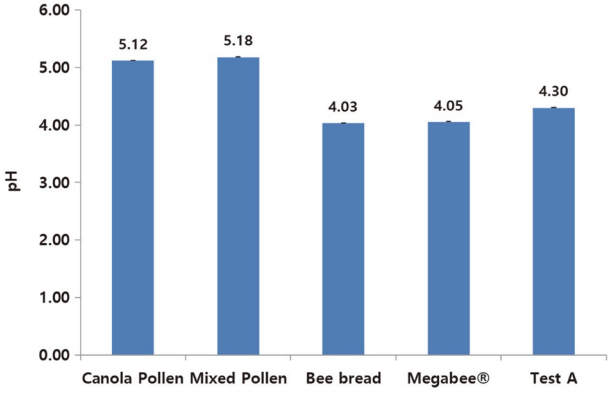

Fig.2. Measurement of pH on five diets.

and just after addition of the mandibular gland secretion (pH 3.9) the pH of the final product is lowered to around pH of 4.0 (Hoffmann, 1960; Buttstedt *et al*., 2018). Thus, royal jelly is the mixture of the hypopharyngeal and mandibular gland secretions that reduces pH levels around 4.0 (Buttstedt *et al*., 2018). In addition, other research showed that the pH value of royal jelly varied from 3.6 to 4.1 (Adaškevičiūtė *et al*., 2019). The pH value of honey ranges from 3.4 to 4.1 (Jantakee and Tragoolpua, 2015). Since the pH of beebread, royal jelly, and honey, the main food for honey bee, is around pH 4.0, it is important to adjust the pH to around 4.0 when developing pollen substitute diets (Mureşan and Buttstedt, 2019).

2. Mineral (Ions)

The elements chlorine (Cl), hydrogen sulfate (SO_4), monohydrogen phosphate (PO_4), nitrogen dioxide (NO_2), bromine (Br), lithium (Li), sodium (Na), ammonium (NH_4), potassium (K), magnesium (Mg), and calcium (Ca) was detected in the present study (Table 1).

The minerals that make up the body are essential micronutrient like amino acid, and it has a variety of physiological functions in the body. However, it is a substance that must be ingested through food because it is not synthesized in the body (Kim *et al*., 2021). In this study, five samples were examined in terms of concentrations of 11 different elements in total. The detailed mineral profile of five samples is presented in Table 1. Of these minerals, K was detected at the highest concentration with 5960.17 mg/kg, 6023.18 mg/kg, 5686.04 mg/kg, 9511.15 mg/kg in Canola pollen, Mixed pollen, Beebread and Megabee, respectively. The K with the highest content is involved in cell growth and helps to excrete sodium to maintain blood pressure and prevent osteoporosis (Ophir *et al*., 1983). In other study, K detected at the highest concentraion with 7400 mg/kg among the detected minerals on the pollen of *Castanea sativa* Mill (Chestnut) (Horcinova Sedlackova *et al*., 2021). Na acts as a water regulator and nerve stimulant in the body (Forbes, 1984). It contains 118.49 mg/kg, 148.53 mg/kg, 281.63 mg/kg, 1087.81 mg/kg, and 290.70 mg/kg in Canola pollen, Mixed pollen, Beebread, Megabee, and Test A, respectively.

3. Total phenol

The phenolic compound content of foods has become the focus of many studies due to their positive efects on health, especially antioxidant activity. The high antioxi-

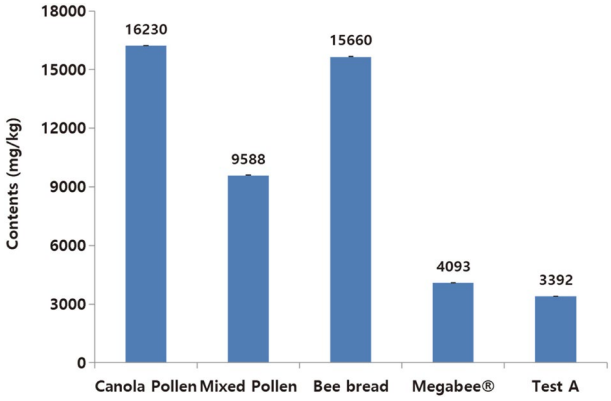

Fig. 3. Contents of total phenol on five diets.

Table 1. Minerals contents on five diets (unit: mg/kg)

Mineral	Canola-pollen	Mixed-pollen	Beebread	Megabee	Test A
Br	70.34	27.74	16.36	31.03	0.00
Ca	1758.30	765.19	1153.67	968.89	918.19
Cl	31.90	89.65	57.17	98.00	100.75
K	5960.17	6023.18	5686.04	9511.15	790.51
Li	1.23	3.65	4.95	0.00	0.00
Mg	1182.65	1282.34	871.5	1734.87	16427.40
Na	118.49	148.53	281.63	1087.81	290.70
NH_4	753.73	528.31	465.76	893.21	318.13
NO_2	0.00	12.81	10.48	9.80	11.46
PO_4	1431.87	1142.95	732.83	714.60	1314.45
SO_4	30.71	31.59	30.83	130.07	86.36

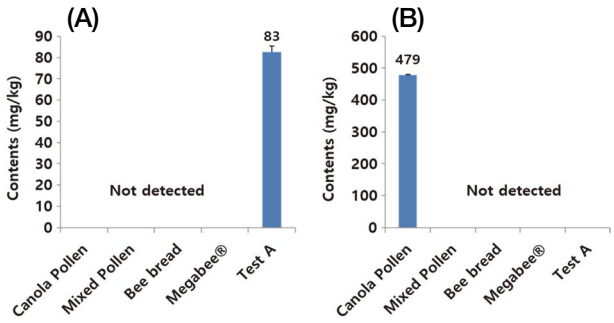

Fig.4. Composition of vitamin B_6 (A) and vitamin C (B) on five diets.

dant capacity of bee pollen is associated with its high content of phenolic compounds (Leja *et al*., 2007). In this study, the total phenol content of five samples was ranked from high to low as following: Canola pollen (16230 mg/kg), Bee bread (15660 mg/kg), Mixed pollen (9588 mg/kg), Megabee (4093 mg/kg), and Test A (3392 mg/kg) (Fig. 3).

4. Vitamin

Bee pollen contains fat-soluble with 0.1% (A, E, D), water-soluble with 0.6% (B_1, B_2, B_6, C) vitamins, along with significant amounts of pantothenic acid, folic acid, rutin, and inositol (Komosinska-Vassev *et al*., 2015; Ecem Bayram, 2021). Although the vitamin content of bee pollen varies between 0.02 and 0.7% (Kieliszek *et al*., 2018). In this study, vitamin B_6 and C were analyzed (Fig. 4). Among the five samples only Test A and Canola pollen were detected vitamin B_6 and C with 83 mg/kg and 479 mg/kg, respectively. Average vitamin C contents of twelve domestic and three imported pollens from Spain, Vietnam and China were 554.7 mg/kg and 62.2 mg/kg, respectively (Lee and Ahn, 2019).Vitamin C content showed 9 times higher in domestic pollens than imported pollens. Chestnut trees are major honey plants in many countries and the vitamin C contents of its pollen was 95.0 ± 2.10 mg/kg (Kim *et al*., 2020; Horcinova Sedlackova *et al*., 2021).

CONCLUSION

Development of pollen substitute diets is important in order to build and maintain healthy colonies and consequently increase the productivity. In bee pollen nutritional contents such as mineral (ions), total phenol, and vitamin are of vital importance. Along with the nutritional contents, economic efficiency also needs to be considered for the development of artificial bee feed. However, the development of a super pollen substitute diet, depends on considering several aspects and requires multicentric studies.

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LITERATURE CITED

Adaškevičiūtė, V., V. Kaškonienė, P. Kaškonas, K. Barčauskaitė and A. Maruška. 2019. Comparison of Physicochemical Properties of Bee Pollen with Other Bee Products. Biomolecules 9(12). DOI: 10.3390/biom9120819.

Aslam, J., M. Mohajir, S. Khan and A. Khan. 2008. HPLC analysis of water-soluble vitamins (B_1, B_2, B_3, B_5, B_6) in in vitro and ex vitro germinated chickpea (*Cicer arietinum* L.). Afr. J. Biotechnol. 7: 2310-2314.

Brodschneider, R. and K. Crailsheim. 2010. Nutrition and health in honey bees. Apidologie 41(3): 278-294. DOI: 10.1051/apido/2010012.

Buttstedt, A., C. I. Mureşan, H. Lilie, G. Hause, C. H. Ihling, S.-H. Schulze, M. Pietzsch and R. F. A. Moritz. 2018. How Honeybees Defy Gravity with Royal Jelly to Raise Queens. Curr. Biol. 28(7): 1095-1100.e1093. DOI: 10.1016/j.cub.2018.02.022.

Crailsheim, K., L. H. W. Schneider, N. Hrassnigg, G. Bühlmann, U. Brosch, R. Gmeinbauer and B. Schöffmann. 1992. Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): Dependence on individual age and function. J. Insect Physiol. 38(6): 409-419. DOI: 10.1016/0022-1910(92)90117-V.

de Arruda, V. A. S., A. A. S. Pereira, A. S. de Freitas, O. M. Barth and L. B. de Almeida-Muradian. 2013. Dried bee

pollen: B complex vitamins, physicochemical and botanical composition. J. Food Compos. Anal. 29(2): 100-105. DOI: 10.1016/j.jfca.2012.11.004.

Ecem Bayram, N. 2021. Vitamin, mineral, polyphenol, amino acid profile of bee pollen from *Rhododendron ponticum* (source of "mad honey"): nutritional and palynological approach. J. Food Meas. Charact. 15(3): 2659-2666. DOI: 10.1007/s11694-021-00854-5.

Eilers, E. J., C. Kremen, S. Smith Greenleaf, A. K. Garber and A.-M. Klein. 2011. Contribution of Pollinator-Mediated Crops to Nutrients in the Human Food Supply. PLoS One 6(6): e21363. DOI: 10.1371/journal.pone.0021363.

Fleming, J. C., D. R. Schmehl and J. D. Ellis. 2015. Characterizing the Impact of Commercial Pollen Substitute Diets on the Level of *Nosema* spp. in Honey Bees (*Apis mellifera* L.). PLoS One 10(7): e0132014. DOI: 10.1371/journal.pone.0132014.

Forbes, R. M. 1984. Use of laboratory animals to define physiological functions and bioavailability of zinc. Fed. Proc. 43(13): 2835-2839.

Goulson, D., E. Nicholls, C. Botías and E. L. Rotheray. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347(6229): 1255957. DOI: 10.1126/science.1255957.

Guichard, E. 2002. Interactions between flavor compounds and food ingredients and their influence on flavor perception. Food Rev. Int. 18(1): 49-70. DOI: 10.1081/FRI-120003417.

Hoffmann, I. 1960. Untersuchungen über die Herkunft der Komponenten des Königinnenfuttersaftes der Honigbienen. Naturwissenschaften 47(10): 239-240.

Hong, I., S. Woo, S. Han and M. Lee. 2017. Chemical Composition and Antioxidant Activity of Korean Buckwheat (Fagopyrum esculentum) Pollen Grain Collected by Honey Bee, *Apis mellifera*. J. Apic. 32: 261-268.

Horcinova Sedlackova, V., O. Grygorieva, K. Šramková, O. Shelepova, I. Goncharovska and E. Mňahončáková. 2021. The chemical composition of pollen, staminate catkins, and honey of Castanea sativa Mill. Potr. S. J. F. Sci. 15: 433-444. DOI: 10.5219/1627.

Huang, W., M. Yan, R. Mulvaney, Z. Qian, L. Liu, C. An, C. Xiao and Y. Zhang. 2021. Spatial Variability of Glaciochemistry along a Transect from Zhongshan Station to LGB69, Antarctica. Atmosphere 12: 393. DOI: 10.3390/atmos12030393.

Jantakee, K. and Y. Tragoolpua. 2015. Activities of different types of Thai honey on pathogenic bacteria causing skin diseases, tyrosinase enzyme and generating free radicals. Biol. Res. 48: 4. DOI: 10.1186/0717-6287-48-4.

Kieliszek, M., K. Piwowarek, A. M. Kot, S. Błażejak, A. Chlebowska-Śmigiel and I. Wolska. 2018. Pollen and bee bread as new health-oriented products: A review. Trends Food Sci. Technol. 71: 170-180. DOI: 10.1016/j.tifs.2017.10.021.

Kim, S. G., S. O. Woo, H. Y. Kim, H. M. Choi, H. J. Moon and S. M. Han. 2021. Quantitative Analysis of Amino Acids and Minerals in Bee Pollen Collected from Castanea crenata by *Apis mellifera*. J. Apic. 36(1): 31-34. DOI: 10.17519/apiculture.2021.04.36.1.31.

Kim, Y. K., S. Lee, J. H. Song, M. J. Kim, U. Yunusbaev, M. L. Lee, M. S. Kim and H. W. Kwon. 2020. Comparison of Biochemical Constituents and Contents in Floral Nectar of *Castanea* spp. Molecules 25(18). DOI: 10.3390/molecules25184225.

Komosinska-Vassev, K., P. Olczyk, J. Kaźmierczak, L. Mencner and K. Olczyk. 2015. Bee Pollen: Chemical Composition and Therapeutic Application. Evid. Based Complement. Alternat. Med. 2015: 297425. DOI: 10.1155/2015/297425.

Kujala, T. S., J. M. Loponen, K. D. Klika and K. Pihlaja. 2000. Phenolics and Betacyanins in Red Beetroot (*Beta vulgaris*) Root: Distribution and Effect of Cold Storage on the Content of Total Phenolics and Three Individual Compounds. J. Agric. Food Chem. 48(11): 5338-5342. DOI: 10.1021/jf000523q.

Kurth, T., S. Kretschmar and A. Buttstedt. 2019. Royal jelly in focus. Insectes Soc. 66(1): 81-89. DOI: 10.1007/s00040-018-0662-3.

Lee, G.-R. and M.-R. Ahn. 2019. The Characteristics and Analysis of Nutritional Compositions of Bee Pollen from Korea. J. Apic. 34(1): 73-86. DOI: 10.17519/apiculture.2019.04.34.1.73.

Leja, M., A. Mareczek, G. Wyżgolik, J. Klepacz-Baniak and K. Czekońska. 2007. Antioxidative properties of bee pollen in selected plant species. Food Chem. 100(1): 237-240. DOI: 10.1016/j.foodchem.2005.09.047.

Mayda, N., A. Özkök, N. Ecem Bayram, Y. C. Gerçek and K. Sorkun. 2020. Bee bread and bee pollen of different plant sources: determination of phenolic content, antioxidant activity, fatty acid and element profiles. J. Food Meas. Charact. 14(4): 1795-1809. DOI: 10.1007/s11694-020-00427-y.

Mureşan, C. I. and A. Buttstedt. 2019. pH-dependent stability of honey bee (*Apis mellifera*) major royal jelly proteins. Sci. Rep. 9(1), 9014. DOI: 10.1038/s41598-019-45460-0.

Ophir, O., G. Peer, J. Gilad, M. Blum and A. Aviram. 1983. Low blood pressure in vegetarians: the possible role of potassium. Am. J. Clin. Nutr. 37(5): 755-762. DOI: 10.1093/ajcn/37.5.755.

Siksna, S., I. Daberte and I. Barene. 2014. Investigation of Bee Bread and Development of Its Dosage Forms. Med. Teor. Prat. 21(1): 16-22. DOI: 10.15591/mtp.2015.003.

Steinhauer, N., K. Kulhanek, K. Antúnez, H. Human, P. Chantawannakul, M.-P. Chauzat and D. vanEngelsdorp. 2018. Drivers of colony losses. Curr. Opin. Insect Sci. 26: 142-148. DOI: 10.1016/j.cois.2018.02.004.

Ulusoy, E. and S. Kolayli. 2014. Phenolic Composition and

Antioxidant Properties of Anzer Bee Pollen. J. Food Biochem. 38(1): 73-82. DOI: 10.1111/jfbc.12027.

vanEngelsdorp, D. and M. D. Meixner. 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J. Invertebr. Pathol. 103: S80-S95. DOI: 10.1016/j.jip.2009.06.011.

Williams, I. H. 1994. The dependence of crop production within the European Union on pollination by honey bees. Agricultural Zoology Reviews 6: 229-257. http://europepmc.org/abstract/AGR/IND20502445.