

Physical-Chemical Composition of Fresh Bee Pollen from Transylvania

Rodica MĂRGĂOAN, Liviu Al. MĂRGHITAȘ, Daniel S. DEZMIREAN,
Otilia BOBIȘ, Cristina M. MIHAI

University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Science and
Biotechnologies, 3-5 Mănăstur Street, 400372 Cluj-Napoca, Romania;
rodica_margaoan2005@yahoo.com

Abstract. Sixteen samples of fresh bee pollen were collected from different regions in Transylvania and analyzed regarding their moisture, lipids, proteins, ash and major carbohydrates. The obtained results showed a variability of the moisture from 17.59 to 29.55%, proteins 16.27 to 26.50%, ash from 1.75 to 3.25%, fructose from 8.44% to 15.39% and glucose from 4.37% to 16.14%. Floral origin, collection area and distinct methods of sample preparation and techniques of analysis give the variability of physical-chemical composition.

Keywords: fresh bee pollen, physical composition, chemical composition, moisture, ash, lipids, proteins, total carbohydrate, techniques of analysis.

INTRODUCTION

Bee pollen is the result of the agglutination of flower pollens, carried out by worker honeybees, with nectar (and/or honey) and salivary substances, collected at the hive entrance. This definition has been reported by Campos *et al.* in 2008 when an international technical regulation for identity and quality of bee pollen standard was proposed (Melo and Muradian, 2011).

The harvesting of pollen from a variety of plant species assures bees a balanced diet and reduction of the toxic potential from alkaloids and other toxins (Schmidt and Buchmann, 1993 cited by Martins *et al.*, 2011).

Bee collected pollen contains nutritionally essential substances like carbohydrates, proteins, amino acids, lipids, vitamins, mineral substances but also polyphenols, flavonoids, carotenoids. Pollen preparations are distributed worldwide for human dietary purpose and as diet supplement (Kroyer and Hegedus, 2001).

Knowledge of the botanical source of pollen, as well as their chemical composition, it is important to classify the product obtained in the different regions and add value to this product (Campos, 2008; Muradian, 2005).

Worldwide interest in bee pollen has recently developed as more information on their chemical composition and physiological effects has become highlighted.

A standard for the quality of pollen has been proposed for the Swiss Food Manual with composition quality criteria for the content of protein (10-40g/100g), lipid (1-10g/100g), carbohydrates (13-55 g/100g), dietary fibers (0.3-20 g/100g), minerals (0.5-3 g/100g) and vitamins (0.02-0.1 g/100g)(Bogdanov, 2004).

This work aimed to obtain analytical data on the nutritional composition of fresh bee pollen from different regions in Transylvania, Romania.

MATERIALS AND METHODS

The study was carried out in APHIS Laboratory at University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. The research had been made using sixteen samples of fresh bee pollen collected from different regions in Transylvania. The samples after collection were cleaned of impurities and packed in plastic bags then were frozen (-18 °C) until further analysis.

Moisture content was determined by drying 1g samples at 105°C, using conventional oven (Binder, Germany) to a constant weight (Popescu and Meica, 1997).

Lipid content was determined using Soxhlet (Soxtherm Gerhardt coupled with a Multistat Gerhardt) automated method having diethyl ether as solvent (80 ml). The analyzed sample was weighted (2g) and subjected to hot extraction for 5 hours. Results for total lipid content are expressed as percentage of dry weight $\pm$ standard deviation.

The nitrogen content was determined by Kjeldal method (1g of sample). It was used a Buchi Digesion Unit -424 and a Buchi Scrubber B-414 for homogenization and digestion of the sample, a Büchi Kjeldahl distillation unit KjelFlex K-360 for distillation and for titration a TitroLine easy (Schott) (Buchi Application Note).

1 g of sample was placed in glass tubes for digestion for 2 hours and the resulting product was subjected to distillation and then to titration with sulphuric acid 0.25M up to 4.65 pH. The results are expressed as percentage of weight of nitrogen (% N). For calculating the protein content we used a conversion factor of 6.25 to compare our results with those of other studies (Martines, 2011).

The ash content was determined by incineration of 2g of each sample in a muffle furnace at 550°C (Popescu and Meica, 1997). Results are expressed as percentage $\pm$ standard deviation.

Fructose and glucose content were determined by HPLC technique as described by Bonta *et al.* (2007) for honey and adapted to pollen. A Shimadzu VP series liquid chromatograph equipped with a LC-10AD pump, DGU-14A degasser, SLC-10A system controller, CTO-10AS column oven, SIL-10AD auto-injector and a RID-10A refractive index detector was used to analyze the sample solutions. Chromatographic separation of the saccharides was performed on Altima Amino 100A 5 μ m column, containing amino modified silica gel, 250mm x 4,6mm (Alltech) using acetonitrile/water (75:25,v/v) as the mobile phase, at a flow-rate of 1,3 ml/min. Mobile phase was filtered through membrane filter, pore size 0,45 μ m (Technochroma) prior injection. The injection volume was 10 μ l. Calibration curves were plotted for each standard using 5 different concentrations of each reference compound (glucose and fructose), which helped quantify the sugars in bee pollen samples.

RESULTS AND DISCUSSIONS

The physical-chemical composition of the sixteen samples analyzed is presented in Table 1. Fresh bee collected pollen contains about 20-30g water/100g. This high humidity content is an ideal culture medium for microorganisms like bacteria and yeast. For prevention of spoilage and preservation of a maximum quality, the pollen has to be harvested daily and immediately placed in a freezer (Campos, 2008; Moosbeckhofer and Ulz, 1996 cited by Bogdanov, 2004).

Moisture content of collected bee pollen is a parameter of quality for this product and the regulations on the quality of bee pollen do not have clear recommendation for this

determination (Melo and Muradian, 2011). The moisture of the fresh collected bee pollen varied from 17.59 to 35.85g /100g.

Tab. 1

Physical-chemical composition of fresh bee pollen from Transylvania

Samples	Moisture ±SD* [%]	Ash ±SD* [%]	Lipid ±SD* [%]	Protein ±SD* [%]	Fructose ±SD* [%]	Glucose ±SD* [%]	F/G
P1	20.70±0.09	2.54±0.02	7.29±0.72	23.21±0.42	13.20±0.66	16.14±0.45	0.81
P2	27.39±0.40	2.75±0.09	3.75±1.01	25.95±3.4	11.96±0.43	8.53±0.16	1.40
P3	22.09±0.05	2.84±0.07	4.81±0.20	23.79±2.35	12.92±0.19	11.08±2.07	1.16
P4	22.31±0.16	2.80±0.06	5.22±0.61	23.20±1.68	13.13±0.44	14.12±0.19	0.92
P5	35.85±0.04	2.80±0.09	4.37±0.79	24.93±0.40	8.44±0.13	4.37±0.04	1.92
P6	21.95±0.26	3.25±0.07	5.22±0.99	26.25±1.69	12.72±1.75	13.19±1.79	0.96
P7	26.48±0.20	2.28±0.04	3.79±0.78	19.50±1.81	10.93±0.38	12.76±1.09	0.85
P8	17.59±0.41	1.75±0.01	8.93±0.72	17.15±1.21	11.80±0.72	14.94±0.95	0.78
P9	24.37±0.16	2.62±0.00	6.33±1.16	24.47±1.95	12.67±0.63	11.14±1.70	1.13
P10	26.91±0.01	1.95±0.00	4.67±0.72	18.99±1.70	12.63±0.04	12.41±0.18	1.01
P11	27.02±0.01	2.37±0.01	4.24±1.15	26.50±2.10	14.03±1.07	12.04±0.95	1.16
P12	29.55±0.06	2.42±0.00	3.59±0.87	19.54±0.03	11.85±0.33	9.71±0.30	1.22
P13	26.26±0.09	2.91±0.00	7.91±0.02	24.14±0.61	13.04±0.96	9.59±0.11	1.35
P14	27.16±0.31	2.49±0.21	2.13±0.30	20.78±0.32	12.33±0.17	8.51±0.44	1.44
P15	22.77±0.09	2.38±0.01	4.22±0.02	16.27±0.13	15.39±0.85	14.67±0.22	1.04
P16	17.76±0.05	2.69±0.04	2.89±0.61	21.41±0.79	14.85±0.73	11.70±0.07	1.26

* mean values of three independent determinations ± standard deviation

** ash, lipid, protein, fructose and glucose value are reported to dry weight

Ash level for the samples ranged from 1.75 to 3.25%. The ash content reported by Baldi *et al.* in 2004 varied from 0.96 to 6.7 %. The Brazilian and Argentinean regulatory specification shows the ash content in bee pollen should be 4% maximum from dry weight (Muradian, 2005).

Total lipids content varies from 2.13 to 8.93%. The minimum lipids content was 1.76g/100g reported by Baldi *et al.* (2004) and maximum 14g/100g (Bastos *et al.*, 2003). The Brazilian regulatory suggested minimum of 1.8% from dry weight but the Argentinean regulatory does not specify a value (Muradian, 2005)

Total protein content ranged from 16.27 to 26.50%. The lowest value of protein content was reported by Orzaez Villanueva *et al.* (2002) as 9.89g/100g and the highest value 27.8 g/100g was reported by Bastos *et al.* (2003). The Brazilian regulatory specification shows that the minimum amount of protein content should be 8% from dry weight and Argentinian regulatory specification shows that the value should be between 15 and 28% from dry weight (Muradian, 2005).

Fructose content from analyzed samples ranged between 8.44% and 15.39% showing a tendency for higher values compared with those obtained for glucose in most of the cases, which ranged between 4.37% and 16.14%.

The reducing sugars have limits between 9.74 g/100g (Szczesna, 2007) and 23.62 g/100g (Martins *et al.*, 2011) for fructose and 6.99 g/100g (Martins *et al.*, 2011) and 22.4 g/100g (Serra Bonvehí and Jorda, 1997) for glucose. Our lowest values obtained for sample P5 (4.37% glucose and 8.44% fructose) are much lower than the ones shown by literature data.

According to literature, F/G value is related to the glycemic index in honey (Ishayek and Kern, 2006). In the present study, glycemic index of bee pollen was investigated in order to see its glycemic value. In the current paper the glycemic index of bee pollen ranged between 0.78 and 1.92, values that could be considered low compared with 2.24 obtained by Martins et al. (2011) for pollen from Sergipe (Brazil).

CONCLUSION

The variability of physical-chemical composition of fresh bee pollen samples shows great variation among the samples from the different region and period of harvesting.

This variability is given by floral origin, collection area, and distinct methods for sample preparation and techniques of analysis.

The values of the physical-chemical parameters obtained in this paper are within the requirement of the standard proposed for the Swiss Food Manual, Brazilian and Argentinean regulations.

The quality of fresh bee pollen from Transylvania is comparable to countries, which have a tradition in production and marketing of bee products.

Acknowledgments. This paper was supported by APHIS laboratory, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. In addition, we would like to thank Oltica Stanciu who helped with the sugars analysis.

REFERENCES

1. Almeida-Muradian, L.B., L.C. Pamplona, S. Coimbra and O. M. Barth (2005). Chemical composition and botanical evaluation of dried bee pollen pellets. *Journal Food Comp. and Anal.* 18 (1): 105-111.
2. Baldi, C.B., D. Grasso, S.C. Pereira and G. Fernandez (2004). Caracterizacion bromatologica Del polen apicola argentine. *Cienc. Docencia Tecnol.* 29: 145-181.
3. Bastos, D.H.M., C.L. Rocha, I.B.S. Cunha, P.O. Carvalho, and E.A.S. Torres (2003). Composição e qualidade de polen apicola comercializado em algumas cidades nos estados de São Paulo e Minas Gerais-Brasil. *Rev. Inst. Adolfo Lutz.* 62: 239-244.
4. Bogdanov, S. (2004), Quality and Standards of Pollen and Beeswax. *Apiacta* 38: 334-341.
5. Bonta, Victorita, L.Al. Marghitas, O. Bobis and D.S. Dezmirean (2007). HPLC sugar profile in quality and authenticity determination of Romanian honey, *Apiculture—from Science to Agribusiness and Apitherapy*, Cluj-Napoca, 59-64.
6. Campos, M.G.R., S. Bogdanov, L.B.Almeida-Muradian, T. Szczesna, Y. Mancebo, C. Frigerio and F. Ferreira (2008). Pollen composition and standardization of analytical methods. 47(2): 156-163.
7. Ishayek, J.I. and M. Kern (2006). US honey varying in glucose and fructose content elicit similar glycemic indexes. *J. Am. Diet. Assoc.* 106: 1260-1262.
8. Kroyer, G. and N. Hegedus (2001). Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. *Innovative Food Science & Emerging Technologies* 2:171-174.
9. Martins, M.C.T., M.A. Morgano, E. Vicente, S.R. Baggio and D.B. Rodriguez-Amaya (2011). Physicochemical composition of bee pollen from eleven Brazilian states, *Journal of Apicultural Science* 55(2): 107-116.
10. Melo, I.L.P. and L.B. Almeida-Muradian (2011). Comparison of methodologies for moisture determination on dried bee pollen samples. *Cienc. Tecnol. Aliment., Campinas.* 31(1): 194-197.

11. Moosbeckhofer, R. and J. Ulz (1996). Der erfolgreiche Imker. Leopold Stocker, Graz, Stuttgart: 129-134.
12. Orzaez Villanueva, M.T., A. Diaz Marquina, S.R. Bravo and A.G. Blazquez (2002). The importance of bee collected pollen in the diet: a study of its composition. *Int. J. Food Sci. Nutr.*, 53: 217-224.
13. Popescu, N. and S. Meica (1997). *Produsele apicole si analiza lor chimica*. Ed. Diacon Coresi. Bucuresti.
14. Schimdt, J.O. and S.L. Buchmann (1993). Other products of hive. *Dadant and Sons Hamilton*. Ed. Grahman. 927-988.
15. Serra Bonvehi, J. and R. Jorda Escola (1997). Nutrient composition and microbiological quality of honeybee-collected pollen in Spain. *J. Agric. Food Chem.* 45: 725-732.
16. Szczesna, T. (2007). Study on the sugar composition of honeybee-collected pollen. *J. Apic. Scie.* 51(1): 15-21.
17. ***, Buchi Application Note.