PROTEIN CONTENT AND AMINO ACIDS COMPOSITON OF BEE-COLLECTED POLLEN ORIGINATING FROM POLAND, SOUTH KOREA AND CHINA

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Summary

The objective of the study was to determine the crude protein content and amino acid composition in bee-collected pollen from selected areas of Poland, South Korea and China. A total of 27 samples of pollen were examined after collection by bees. The dry substance, crude protein and amino acids were investigated in the study material. Amino acids were determined by ion exchange chromatography using of an Automatic Amino Acid Analyser (Pharmacia LKB Alpha Plus) after the protein in the study material had been hydrolysed.

As the study showed, regardless of which part of the world it comes from, be-collected pollen contains high content of such amino acids as: glutamic acid, proline, aspartic acid, leucine and lysine. These amino acids account for about 50% of the total amino acids. Strong fluctuations were observed between the samples in terms of the crude protein content and amino acids composition, which may have been due to their various botanical origin. Compared to the pollen from Korea, the pollen from China contained a higher concentration of crude protein and most of the determined amino acids, except for aspartic acid and arginine, whose concentration did not differ in the three compared countries. Essential amino acids accounted for about 37% of the total amino acids in bee-collected pollen. The pollen from Poland contained higher content (by about 3%) of essential amino acids compared to that from Korea and China. Because it is rich in highly nutritious proteins (CS=80%, EAAI=110%), honeybee-collected pollen is recommended as a dietary supplement.

Keywords: Honeybee-collected pollen, crude protein, amino acids, Poland, South Korea, China.

INTRODUCTION

Because of high protein content and rich amino acid composition, pollen has been the subject of numerous studies, mostly conducted by foreign researchers. In these studies, the crude protein content has been determined to be close to 25% (Stanley and Linskens 1974; Herbert and Shimanuki 1978; Talpai 1978; Youssef et al. 1978; Vachonina and Bodrova 1979; Solberg and Remedios 1980; Standifer et al. 1980; Rabie et al. 1983; Echigo et al. 1986; Loper and Cohen 1987; Ceksterite 1988; Ceksteritie 1991; Sommerville 1997). In Poland, pollen protein content has been the subject of studies conducted by Syrocka and Zalewski (1985), Zalewski and Kosson (1985), and Szczęsna et al. (1995 a). Their results were higher than the findings of Spanish scientists, e.g. in a study conducted by Szczęsna et al. (1995 a), the average content of the substance was about 30%; comparable results were obtained by Somerville (1997), however, the crude protein level in Spanish studies was as low as 16% (Serra Bonvechi et al. 1986; Muniategui et al. 1990; Serra Bonvechi et al. 1991; Serra Bonvehi

and Escola Jorda 1997).

Many researchers have found in bee-collected pollen the protein content of 15 to 19 amino acids, including all exogenous amino acids (Bosi and Ricciardelli D'Albore 1975; Zalewski and Kosson 1985). The human body cannot synthesise eight amino acids and they must be delivered with food. These are known as essential amino acids and pollen can be one of their important source. Essential amino acids for bees include these eight as well as arginine and histidine (De Groot, after: Gilliam et al. 1980). Essential amino acid content, expressed as the procentage of the total amino acids in bee-collected pollen shown in the study by Szczęsna et al. (1995 b) was to be close to 40%.

The highest content of amino acids found in pollen protein has been found for glutamic acid, aspartic acid, proline, leucine and lysine (Bosi and Ricciardelli D'Albore 1975; McLellan 1977; Kauffeld 1980; Gilliam et al. 1980; McCaughey et al. 1980, Kim 1986, Kim and Son 1991), and according to Szczęsna et al. (1995 b) and Naumkin (1984), also serine. Solberg and Remedios (1980) found a high level of arginine, with considerable differences in particular amino acids contents depending on the plant species that the pollen was obtained from. High fluctuation was found for serine, cysteine, histidine, proline, lysine, glycine, phenylalanine, valine, leucine, isoleucine and lysine (McLellan 1977, Kaufeld 1980, Somerville 1997).

The objective of the study was to determine the crude protein content and the amino acid compostion of pollen collected by bees from selected areas of Poland, South Korea and China. The subject has not been given extensive coverage in these countries and the product, obtained by beekeepers on a regular basis, is recommended as a dietary supplement rich in high quality protein.

MATERIALS AND METHODS

The experiment was conducted at Kon-Kuk University, Animal Resources Research Center, Seoul, Korea. The material used in the study was comprised of pollen samples from Poland (13 samples), South Korea (9 samples) and China (5 samples). The Polish samples of pollen were obtained in June and July 1997 at the apiary of the Research Institute of Pomology and Floriculture, Apiculture Division, situated in Puławy, and at a private apiary in the neighbourhood. The bees' flight range covered a park, a vast garden area and home gardens. Pollen samples from Korea were collected in April – August 1997 at the apiaries of the Insect Biotechnology Laboratory, Department of Industrial Entomology, National Institute of Sericulture and Entomology, Suwon. The samples from China were taken in April 1997, in private apiaries in the eastern province of Anhui, situated in the Yangtze and Huang He river basins. A total of 27 pollen samples collected by bees were examined. Immediately following the collection, the samples were dried at 40°C, and then kept at about -21°C until they were analysed.

Dry matter content was determined by drying to a constant weight at 105°C (Serra Bonvehi and Casanova 1987). Dry matter content was recorded as percentage of the total samples.

The nitrogen content was determined by Kjeldahl method and crude protein was calculated by multiplying the nitrogen content by a factor of 5.60 (Rabie et al. 1983).

A qualitative and quantitative analysis of the amino acids was performed by ion exchange chromatography with the use of an Automatic Amino Acid Analyser (Pharmacia LKB Alpha Plus), having pre-

viously hydrolysed the protein in the pollen samples (Szczęsna et al. 1995 b). The hydrolysis was performed with 6 N HCl at 110°C for 24 h. Before the hydrolysis, the pollen samples were treated with performic acid in order to avoid the decomposition of sulphur-containing amino acids. After hydrolysis, the solution was evaporated in a vacuum evaporator (EYELA Rotary Vacuum Evaporator) under reduced pressure at 40°C. The dry residue was transferred to a 50 ml measuring flask. The obtained solution was filtered through a 0.45 μm membrane filter and 100 μl of filtrate was injected into the apparatus.

Amino acids in pollen were identified by comparing the retention time of particular amino acids in the reference solution and in an examined solution (qualitative analysis); the quantitative analysis was performed by comparing the peak area which corresponded to those amino acids. The standard solution containing a mixture of amino acids, with each one present at a concentration of 2.5 μM/ml as a reference solution.

The data concerning crude protein, total amino acids and particular amino acids content were converted into dry matter. Essential amino acid content was also expressed as a percentage of the total concetration of all amino acids in the tested samples.

The nutritional value of the protein in the samples of bee-collected pollen was assessed with the chemical index of protein quality CS (Chemical Score), also known as the limiting amino acid index and essential amino acids index EAAI (FAO 1973). 1 mg/g DM was adopted for the calculations for cysteine.

The results were analysed statistically with an ANOVA one-way analysis of variance. The significance of differences between the mean values was assessed by Duncan's test, using a level of significance of α=0.05.

RESULTS

The crude protein content in the samples of bee-collected pollen ranged from 15.80% DM for the pollen from Poland to 26.13% DM for the pollen from China (Table 1). The mean crude protein content in the pollen from Poland was similar to that in the pollen from Korea (20.71% DM), while the pollen from China contained significantly higher content of this component (23.69% DM).

The samples of bee-collected pollen from the selected countries contained 17 amino acids, including all the essential amino acids except for tryptophan, which was not determined in the study. The cysteine content in all the samples was lower than 1 mg/g DM. The pollen from China contained significantly higher concentration of total amino acids, an average of 238.96 mg/g DM, as compared to that from Poland (201.52 mg/g DM) and from Korea (181.10 mg/g DM).

Of all the identified amino acids, glutamic acid, aspartic acid and proline were found in the highest concentration, with their content ranging from 12.08 mg/g DM (proline) to 39.31 mg/g DM (aspartic acid). The mean content of aspartic acid and glutamic acid was the highest in the samples from China, with values of 27.81 and 30.32 mg/g DM, respectively, and the lowest was found in the samples from Poland: 23.10 and 24.08 mg/g DM, respectively. Proline content was similar in the samples from Poland and China, with an average of 24.98 and 25.95 mg/g DM, respectively, while this amino acid content in the Korean samples was much lower – 17.35 mg/g DM, on average. These three amino acids together accounted for about 36% of the total amino acids in the examined pollen samples. Relatively high concentration was also found for leucine and lysine. Leucine was found in content ranging from 14.25 mg/g DM (samples from

Table 1

Crude protein content and amino acids composition of pollen samples from selected areas of Poland, South Korea and China (mg/g DM).

Amino acid	Origin								
	Poland (n=13)			Korea (n=9)			China (n=5)		
	from - to	mean	SD	from - to	mean	SD	from - to	mean	SD
ASP	16.38 - 30.27	23.10 a	4.82	17.96 - 39.31	25.65 a	7.45	19.32 – 34.97	27.81 a	6.04
THR	6.77 - 12.50	9.74 a	1.93	7.05 - 9.64	8.21 a	0.99	9.27 – 13.37	11.61 b	1.72
SER	8.75 - 14.16	11.59 a,b	2.02	7.76 – 11.70	9.74 a	1.32	10.26 – 15.61	12.97 b	1.93
GLU	16.75 - 29.90	24.08 a	4.25	17.42 – 37.15	25.43 a,b	6.12	20.28 – 37.75	30.32 b	7.43
PRO	16.71 - 32.84	24.98 b	4.7	12.08 – 21.98	17.35 a	3.55	14.39 – 30.24	25.95 b	6.52
GLY	7.60 - 12.73	10.24 a	1.79	7.23 – 12.70	9.13 a	1.73	9.62 – 13.96	12.12 b	1.75
ALA	8.86 - 14.14	11.73 a	1.79	8.68 – 12.44	10.59 a	1.44	12.26 – 17.29	14.87 b	2.15
VAL	7.32 - 13.07	10.19 a	1.88	7.88 – 12.20	9.38 a	1.41	9.77 – 14.91	12.29 b	2.31
CYS	0.59 – 0.87	0.73 a	0.12	0.35 – 0.62	0.51 a	0.1	0.58 – 0.84	0.62 a	0.14
MET	2.45 - 4.80	3.52 a	0.78	1.62 – 4.55	2.99 a	0.9	4.24 – 6.66	5.58 b	0.97
ILE	5.01 - 11.03	8.59 a,b	1.84	5.87 – 9.35	7.51 a	1.21	7.31 – 12.78	10.23 b	2.19
LEU	14.44 - 27.82	19.22 b	5.58	11.24 - 20.92	14.25 a	3.11	12.28 – 22.25	17.95 a,b	4.01
TYR	3.17 - 6.00	4.43 a,b	0.92	3.04 – 5.30	4.04 a	0.74	3.62 – 6.05	5.11 b	1.03
PHE	7.03 - 12.39	9.79 a,b	1.68	6.92 – 10.50	8.39 a	1.16	7.56 – 14.22	11.48 b	2.82
HIS	2.56 - 5.85	4.93 a	1.07	3.95 – 5.50	4.77 a	0.54	7.07 – 13.01	10.67 b	2.44
LYS	12.27 - 24.07	17.39 ab	4.65	11.63 – 17.94	14.15 a	2.23	12.92 – 21.40	18.18 b	3.44
ARG	6.10 - 12.10	9.11 a	1.93	6.72 – 11.82	9.42 a	1.65	7.23 – 13.97	11.11 a	2.83
Total	144.64 - 259.03	201.52 a	36.14	148.22 – 233.83	181.10 a	29.86	182.08 – 279.91	238.96 b	39.12
Protein (Nx5.6) (% DM)	15.80 – 24.14	20.71 a	2.86	17.63 – 24.51	20.71 a	2.2	17.83 – 26.13	23.69 b	3.36
Sum AE*	55.31-105.68	78.43 a,b	17.25	54.31 – 83.38	64.88 a	10.3	63.55 – 104.39	87.32 b	16.54
Sum AE* (%)	35.83 – 42.14	38.69 b	1.89	34.59 – 37.24	35.87 a	0.93	34.90 – 37.41	36.41 a	1.04

Explanations: ASP – aspartic acid, THR – threonine, SER – serine, GLU – glutamic acid, PRO – proline, GLY – glycine, ALA – alanine, CYS – cysteine, VAL – valine, MET – methionine, ILE – isoleucine, LEU – leucine, TYR – tyrosine, PHE – phenylalanine, HIS – histidine, LYS – lysine, ARG – arginine.
* – The sum of exogenous amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine).
a,b – significant statistical differences between the mean values compared in lines at the significance level of α=0.05.

Table 2

Nutritional value of bee-collected pollen.

Amino acid	Reference protein FAO (mg/g N)	Poland		Korea		China		Total mean vale	
		Contents (mg/g N)	CS* (%)	Contents (mg/g N)	CS* (%)	Contents (mg/g N)	CS* (%)	Contents (mg/g N)	CS* (%)
Isoleucine	175	232	133	203	116	242	138	225	129
Leucine	412	520	126	385	93	424	103	456	111
Lysine	362	470	130	382	106	430	119	433	120
Methionine + cysteine	156	122	78	108	69	155	99	125	80
Phenylalanine + tyrosine	393	384	98	336	85	392	100	370	94
Threonine	212	263	124	222	105	275	130	252	119
Tryptophan	69	-	-	-	-	-	-	-	-
Valine	218	275	126	253	116	290	133	271	124
Total exogenous amino acids	1997/1928**	2266**	118	1889**	98	2208**	115	2132**	111

* – chemical index of protein quality CS (Chemical Score) - (the limiting amino acid index).
** – total exogenous amino acids except tryptophan, which was not determined.

Korea) to 19.22 mg/g DM (pollen from Poland); for lysine, the values ranged from 14.15 mg/g DM (samples from Korea) to 18.18 mg/g DM (samples from China). The above-mentioned amino acids (proline, glutamic acid, aspartic acid, leucine and lysine) together accounted for about 50% of the total amino acids in the examined samples.

Such amino acids as threonine, serine, glycine, alanine, valine, isoleucine and arginine were found to be present in the samples in medium concentrations, ranging from 7.51 mg/g DM (isoleucine), to 14.87 mg/g DM (alanine). These amino acids together accounted for about 40% of the total amino acids, whereas the concentration of methionine, tyrosine and histidine was found to be the lowest content, the average values ranging from 2.99 mg/g DM of the Korean samples to 10.67 mg/g DM in the pollen from China. These three amino acids did not exceed 10% of the total amino acids in the examined samples.

Statistical analysis show that the pollen from China contained significantly higher concentration of crude protein, total amino acids and most of the determined amino acids, except aspartic acid and arginine, than the pollen from Korea. The levels of these two amino acids in pollen samples originated from these two countries were similar. The content of such amino acids as aspartic acid, serine, proline, isoleucine, tyrosine, phenylalanine and lysine in the samples from Poland did not differ significantly from those values for the pollen from China, while the content of threonine, glutamic acid, glycine, alanine, valine, methionine and histidine was significantly lower. The pollen from Poland contained significantly higher content of proline than that from Korea.

The content of essential amino acids ranged from 54.31 mg/g DM for the pollen from Korea to 105.68 mg/g DM for the pollen from Poland. The mean content of

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the essential amino acids in the samples from Poland was not significantly different from that in the samples from Korea and China. Statistically significant differences in essential amino acids content were found between the samples from Korea and China. The content of essential amino acids, expressed as a percentage of the total amino acids concentration in the pollen samples was significantly higher in the samples from Poland (about 39%) than in those from Korea and China.

The sum of essential amino acids (except tryptophan) in the samples from the pollen in the three countries accounted for 98% (Korea) to 118% (Poland) of the sum of the amino acids in the protein adopted by FAO as a reference protein (Table 2). Isoleucine content was equal to 116 – 138% of the contents of the amino acid in the reference protein; for leucine the value was – 93 – 126%, for lysine – 106 – 130%, for threonine – 105 – 130%, for valine – 116 – 133%, for the sum of methionine and cysteine – 69 – 99%, and for phenylalanine and tyrosine – 85 – 100%. Essential amino acids index EAAI reached value in the range from 97% for pollen from Korea to 116% for pollen from China (110% on average).

DISCUSSION

Most studies into the amino acids composition of pollen collected by bees concerned monofloral pollen, which is obtained under strictly established conditions. However, as a rule, in large apiaries, which are not only indented for honey production, but which also produce bee-collected pollen, it is collected by bees from various plant species. The results achieved in this study corroborate earlier reports by foreign (Bosi and Ricciardelli D'Albore 1975; McLellan 1977; Kauffeld 1980; Gilliam et al. 1980; McCaughey et al. 1980; Kim 1986; Kim, Son 1991), and Polish researchers (Szczęsna et al. 1995 b, Szczęsna 2006), claiming that the level of aspartic acid, glutamic acid, proline, leucine and lysine in pollen is on the highest level. Other Polish authors, Zalewski and Kosson (1985) determined 15 amino acids in pollen, except proline. Earlier studies by Naumkin (1984) and Szczęsna et al. (1995 b) showed that pollen contains relatively high concetration of serine, and by Solberg and Remedios (1980) also arginine. This study has shown that these two amino acids were present in medium concentration in the pollen from selected countries (Poland, South Korea, China), similar to those of threonine, glycine, alanine, valine, isoleucine and phenylalanine – about 5.0%.

The quantitative composition of amino acids in the pollen collected in Korea, which was determined in this study, confirms earlier results received by Korean researchers (Kim 1986, Kim and Son 1991), who also found lower concentration of amino acids in the pollen obtained in various regions of the country. What is noteworthy, in this study was the relatively lower (compared to the pollen collected in Poland) concentration of proline. The literature on the subject does not provide any information concerning the amino acids composition in bee-collected pollen from China, despite the fact that considerable quantity of the product are produced in this country. The results presented in this study enrich the available information on a product which, as seen in the results presented in this paper, contains protein of higher nutritional value than that present in the pollen from Korea or Poland. Using a limiting amino acid index (Chemical Score-CS) the following values can be adopted: 100% for the pollen from China, 80% for the pollen from Poland and 70% for the pollen from Korea. In all the pollen samples, methionine and cysteine have proven to be the limiting amino acids,

which was also found in earlier studies by other authors (Naumkin 1991; Rogala and Szymaś 2004). What is still debatable is the concentration of phenylalanine and tyrosine, whose sum has been shown by this study to account for 85 – 94% of their sum in the FAO reference protein. Naumkin (1991) assessed the nutritional value of pollen from different varieties of buckwheat (*Fagopyrum esculentum*) in relation to the two amino acids to as high as 130 – 150%. High value of essential amino acids index (EAAI=110%) confirmed high nutritional value of pollen samples collected in Poland, Korea and China. The similar results for CS and EAAI values was received by Szczęsna (2006) for pollen from selected botanical origins.

CONCLUSIONS

1. Regardless of the country where it was obtained, bee-collected pollen contained high concentration of glutamic acid, proline, aspartic acid, leucine and lysine. These five amino acids accounted for about 50% of the total amino acids.
2. Strong fluctuations have been shown to exist in crude protein content and amino acids composition of bee-collected pollen, which may be due to their different botanical origin.
3. The pollen from China contained higher level of crude protein and most of the determined amino acids, except for aspartic acid and arginine. The concentration of these two amino acids was on the same level in the pollen from all three countries.
4. Essential amino acids accounted for about 37% of the total amino acids in the bee-collected pollen. The pollen from Poland contained higher concentration (by about 3%) of essential amino acids compared to that obtained in Korea and China.
5. The limiting amino acid index (CS) and the essential amino acids index (EAAI) values of the examined pollen samples were high, which may indicate the high nutritional value of the honeybee-collected pollen.

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ZAWARTOŚĆ BIAŁKA ORAZ SKŁAD AMINOKWASOWY OBNÓŻY PYŁKOWYCH POCHODZACYCH Z POLSKI, KOREI POŁUDNIOWEJ I CHIN

Szczęsna T.

S t r e s z c z e n i e

Celem pracy było określenie zawartości białka oraz składu aminokwasowego obnóży pyłkowych pozyskanych z Polski, Korei Południowej i Chin. Łącznie badaniami objęto 27 próbek pyłku kwiatowego zebranego przez pszczoły w postaci obnóży. W zebranym materiale badawczym wykonano oznaczenia suchej masy, białka ogólnego oraz aminokwasów białkowych. Oznaczenia aminokwasów wykonano metodą chromatografii jonowymiennej za pomocą Automatycznego Analizatora Aminokwasów (Pharmacia LKB Alpha Plus) po uprzedniej hydrolizie białka w materiale badawczym.

Przeprowadzone badania wykazały, że obnóża pyłkowe, niezależnie od rejonu świata, charakteryzowały się wysokim poziomem takich aminokwasów jak: kwas glutaminowy, prolina, kwas asparaginowy, leucyna i lizyna. Aminokwasy te stanowiły około 50% ogólnej zawartości aminokwasów białkowych. Zawartość białka oraz skład aminokwasowy w pyłku pszczelim wykazywały duże wahania między próbkami co mogło być związane z ich różnym pochodzeniem botanicznym. Pyłek kwiatowy z Chin charakteryzował się wyższą w porównaniu z pyłkiem z Korei zawartością białka ogólnego, ogólną zawartością aminokwasów oraz większości oznaczonych aminokwasów, za wyjątkiem kwasu asparaginowego i argininy. Zawartość tych dwóch aminokwasów nie różniła się między trzema porównywanymi krajami. Aminokwasy egzogenne stanowiły około 37% ogólnej zawartości aminokwasów białkowych w pyłku pszczelim. Pyłek pochodzący z Polski charakteryzował się wyższą o około 3% zawartością aminokwasów egzogennych w porównaniu z pyłkiem pozyskanym w Korei i Chinach. Pyłek kwiatowy, że względu na wysoką wartość odżywczą białka (CS=80%, EAAI=110%), może być rekomendowany jako suplement naszej diety.

Słowa kluczowe: Obnóża pyłkowe, białko, aminokwasy, pochodzenie, Polska, Korea Południowa, Chiny.