

STUDY ON THE SUGAR COMPOSITION OF HONEYBEE-COLLECTED POLLEN

T e r e s a S z c z ę s n a

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S u m m a r y

The aim of this study was to investigate the sugar composition in bee pollen collected in different countries (Poland, South Korea and China). A total of 27 pollen samples collected by bees as pollen loads was included in the study. The collected material was assayed for dry matter content and sugar composition using HPLC technique.

The study showed that sugar content of pollen loads dry matter averages 40%. Fructose to be the sugar occurring in the greatest amounts. It accounted for 46% of the total sugar content of the examined samples. The second highest sugar content – 37% - was that of glucose. Monosaccharides expressed as total fructose and glucose accounted for about 83% of the carbohydrate fraction of pollen. Of the assayed disaccharides sucrose accounted for 8% and maltose for 7%, and the remaining disaccharides (trehalose and turanose) for about 1% each. Chemical composition of bee-collected pollen varied substantially with regard to saccharide content which was probably due to its different botanical origin. As compared to pollen loads samples from South Korea and China those collected in Poland had a significantly higher fructose to glucose ratio.

Keywords: pollen loads, sugars, composition, botanical origin, HPLC.

INTRODUCTION

Carbohydrate fraction constitutes an essential part of honeybee-collected pollen dry matter. The first investigations on the subject were concerned with total sugar content, the distinction being made between reducing and non-reducing sugars (Cirnu et al. 1969, Rosenthal et al. 1969, Youssef et al. 1978, Herbert and Shimanuki 1978, Solberg and Remedios 1980, Szczesna et al. 1995a). According to those authors both total carbohydrate content and the content of individual sugars of bee pollen show extensive variation among samples which is related to the plant of origin. According to the study by Szczesna et al. (1995 a) pollen loads collected at the blooming period of raspberry (*Rubus*), hawthorn (*Craetegus*), rowan (*Sorbus aucuparia*) and buckthorn

(*Rhamnus frangula*) had a significantly higher content of sucrose (5.2% on average). Instead, the pollen loads collected at the blooming period of lime tree (*Tilia*), buckwheat (*Fagopyrum esculentum*) and red clover (*Trifolium pratense*) had a significantly lower content of this sugar (3.7% on average). The content of reducing sugars was the highest for samples collected at the blooming time of black locust (*Robinia pseudoaccacia*), white clover (*Trifolium repens*) and meadow and field weeds (42.5% on average). As in the case of sucrose it was the lowest at the blooming time of lime tree, buckwheat and red clover (39.8% on average). The study also showed that the basic sugar composition changes as the product is further processed, during its preservation (drying) and storage (Szczesna et al. 1995 b).

Substantial differences in sugar content

were found for pollen collected directly from the flowers vs. that collected by bees or bee bread (Herbert and Shimanuki 1978, Solberg and Remedios 1980). The content of non-reducing sugars as determined by the latter investigators in hand-collected pollen varied from 7.9% for pollen from broadleaf trees to 10.7% for pollen from conifers. On the other hand, the content of reducing sugars was 4.7 and 1.7%, respectively. The total sugar content of pollen from both tree groups as determined by those authors was therefore exactly the same. Much higher sugar content was found by those authors in pollen loads samples. In thirteen of them the content of reducing sugars was higher (30.8% on average) than that of non-reducing sugars (12.6% on average) and only in three samples (pollen loads from fruit trees) the reverse was true: 17.1% of reducing sugars and 26.9% of non-reducing sugars. The total sugar content was 48.4% in pollen loads and only 12.4% in hand-collected pollen. In the comparative study of pollen loads and bee bread Herbert and Shimanuki (1978) found bee bread to be higher in reducing sugars (27.9%), pollen loads containing 20.7% of those sugars.

A detailed analysis of the carbohydrate fraction of bee pollen, made possible owing to the routine deployment of chromatograph techniques (TLC, GC, HPLC) in pollen studies allowed the identification of as many as 14 different sugars from among which fructose, glucose, sucrose and maltose were determined at the highest amounts (Echigo et al. 1986, Serra Bonvehi et al. 1986, Serra Bonvehi and Escola Jorda 1997, Szczęśna et al. 2002).

Echigo et al. (1986) used TLC to determine the following composition of basic sugars in bee pollen: fructose 44%, glucose 35%, and sucrose 21% of the total sugar content. The data differ but slightly from those obtained by Spanish investigators

(Serra Bonvehi et al. 1986, Serra Bonvehi and Escola Jorda 1997). The fructose to glucose ratio as determined by Serra Bonvehi and Escola Jorda (1997) was from 1.13 to 1.53. The remaining sugars investigated by the above-mentioned authors such as arabinose, ribose, trehalose, turanose, coibiose, gentiobiose, mellibiose, and melezitose occurred in pollen at below 0.5% each.

The dietary fiber content of bee-collected pollen as determined by Serra Bonvehi and Escola Jorda (1997) was 13.7% DM (dry matter) on average (10.6-15.9) of which the soluble fraction accounted for 2.42% DM (1.59-3.66) and the non-soluble fraction for 11.3% DM (9.0-13.1). Starch, a constituent of the carbohydrate fraction of bee pollen according to various investigators, varied from 2 to 17% and was also dependent on botanical origin of pollen (Herbert and Shimanuki 1978, Youssef et al. 1978, Serra Bonvehi and Escola Jorda 1997).

The objective of this study was to examine the sugar composition of pollen loads collected in different countries (Poland, South Korea and China). Those investigations are very necessary since they complement the description of the product with basic chemical composition. The results will find application in setting down the requirements for bee pollen as part of the international standard for that product which is now under development by the International Honey Commission (IHC).

MATERIAL AND METHODS

The material for the study consisted of multifloral bee-collected pollen samples coming from Poland (13 samples), South Korea (9 samples) and China (5 samples). The samples of domestic pollen were collected in June and July of 1997 in the apiary of the Institute of Pomology and Flori-

culture, Apiculture Division (located at the backyard of the building of the Division in Puławy) and in a private apiary located in close proximity of the Division's building. The park, an extensive area of garden plots and home gardens were within the reach of bees' flights. The samples of the Korean pollen were collected in the months of April to August of 1997 in the apiary of the Insect Biotechnology Laboratory, Department of Industrial Entomology, National Institute of Sericulture and Entomology, Suwon, and samples of Chinese pollen were harvested in April of 1997 in Anhui province of China. The study comprised a total of 27 samples of pollen collected as pollen loads. Once collected, the samples were immediately dried at 40°C and stored at ca. -21°C until analyzed.

The collected material was analysed for dry matter content and for the composition of the carbohydrate (sugar) fraction using HPLC technique. The sugar assays were conducted at Korea Bee Product's Research Institute, Seoul, Korea, and the dry matter determinations were done at Kon-Kuk University, Animal Resources Research Center, Seoul, Korea.

Dry matter content was determined by drying at 105°C to constant weight (Serra Bonvehi and Casanova 1987).

Sugar content assays were done by HPLC protocol according to Bogdanov et al. (1997) as modified by the author using Waters liquid chromatograph equipped with an isocratic pump, manual injector with 20 µl loop, Refractive Index Detector (RID) and Waters High Performance Carbohydrate Column, 250 x 4.6 mm, 4 µm. Chromatographic separation was performed under the following conditions: flow rate – 1.5 ml/min, mobile phase – acetonitrile : water (65:35 V/V), column and detector temperature – ambient. A weighted portion of 2 g of pollen was dissolved in a small amount of deionized water and then transferred to a 50 ml volumetric flask. The vo-

lume of 12.5 ml of methanol was added to the flask and brought up to notch with water. The solution was passed through a 0.45 µm membrane filter and the filtrate was injected onto the column.

The identification of sugars in bee pollen samples was performed by comparing retention times of individual sugars in the reference vs. analyzed solution. The quantitative assays were done by comparing peak surfaces corresponding to those sugars. The data on the content of individual sugars were converted to dry matter of bee pollen. The detectability limit for the individual sugars was set at 0.5% DM for fructose and glucose, 0.2% DM for sucrose, maltose, turanose and trehalose.

Pollen samples from Poland were additionally subjected to melissopalynological tests to determine their botanical origin (Szczęsna 2006). Pollen analysis was done according to recognized methods used in melissopalynological studies recommended by the International Commission of Bee Botany of International Union of Biological Sciences (Louveaux et al. 1978).

The data on the content of individual sugars in the tested samples of multifloral pollen from three different countries (Poland, South Korea and China) were analyzed using one-way ANOVA with an unequal number of replications. Significance of differences between means was evaluated using Duncan's test at a significance level of $\alpha = 0.05$. The variability among pollen samples from Poland, South Korea and China for the sugars tested were evaluated by determining standard deviation for each country.

RESULTS

In the tested samples of multifloral pollen from different countries assays were made of monosaccharides – fructose and glucose and disaccharides – sucrose,

turanose, maltose and trehalose. Of all the assayed sugars fructose was found to occur in the highest concentration. It accounted for 46% of the total sugar content in the tested pollen samples (Fig. 1). Glucose was the second highest accounting for 37%. Monosaccharides expressed as the combined concentration of fructose and glucose accounted for 83% of the carbohydrate fraction of pollen. Of the tested disaccharides sucrose accounted for 8%, maltose for 7% and the remaining disaccharides (trehalose and turanose) for about 1% each.

The content of fructose ranged from 15.51 to 19.22% DM for the samples from Poland, from 12.09% to 22.06% for the samples from Korea and from 9.74% to 17.90% for the samples from China, an average of 17.39% DM (Table 1). Compared

with the content of fructose the content of glucose was lower by several percentage points. The samples from Korea were characterized by the highest content of that sugar, an average of 15.84% DM and those from Poland by the lowest, averaging 12.79% DM. Statistically significant differences in the contents of fructose occurred between the samples from Korea and China and in the case of glucose between the samples from Korea and Poland.

Of the tested disaccharides sucrose occurred in the highest concentrations ranging from 2.98% DM for the pollen samples from China to 3.39% DM for the samples from Korea followed by maltose – from 2.22% DM for samples from Korea to 2.75% DM for the samples from Poland. The country averages for sucrose and maltose contents were not significantly different.

Table 1
Sugars content of multifloral pollen samples originating from different countries (% DM).

Sugar	Origin								
	Poland (n=13)			South Korea (n=9)			China (n=5)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Fructose	15.51-19.22	17.39 a,b	1.14	12.09-22.06	18.85 b	3.29	9.74-17.90	15.94 a	3.51
Glucose	9.85-14.29	12.79 a	1.29	9.94-20.06	15.84 b	3.81	8.45-15.10	13.44 a,b	2.84
Sucrose	2.14-4.62	3.22 a	0.81	2.19-4.59	3.39 a	0.83	1.13-4.84	2.98 a	1.48
Maltose	2.11-3.51	2.75 a	0.45	1.07-2.98	2.22 a	0.63	1.43-3.50	2.43 a	0.85
Trehalose	0.21-1.11	0.54 b	0.36	0.31-0.65	0.48 a,b	0.11	0.20-0.38	0.24 a	0.08
Turanose	0.2-0.58	0.31 a,b	0.09	0.2-0.32	0.24 a	0.04	0.21-0.57	0.35 b	0.18
Sum of fructose and glucose	25.95-33.51	30.18 a,b	2.11	22.03-42.58	34.70 b	6.85	18.19-33.00	29.38 a	6.35
Fructose/Glucose ratio (F/G)	1.18-1.63	1.37 b	0.13	1.02-1.44	1.21 a	0.15	1.15-1.21	1.18 a	0.02
Total disaccharides*	5.28-9.24	6.82 a	1.20	4.12-8.19	6.33 a	1.33	3.3-8.68	5.99 a	2.37
Total sugars	32.11-41.90	36.99 a,b	2.93	28.59-48.44	41.03 b	1.21	26.87-40.06	35.37 a	5.35

Explanations:

a,b – significant statistical differences between the mean values compared in the rows at the significance level of $\alpha = 0.05$.

* – sum of sucrose, maltose, trehalose and turanose.

Table 2

Sugars content of pollen samples from Poland depending on the botanical origin (% DM).

Sugar	Botanical origin								
	Brassicaceae (n=6)			<i>Artemisia</i> (n=2)			Multifloral (n=5)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Fructose	15.51-18.33	16.93	0.97	18.67-19.22	18.95	0.39	16.10-18.87	17.69	1.05
Glucose	11.98-15.01	12.94	1.06	12.74-14.29	13.52	1.10	9.85-13.97	12.61	1.72
Sucrose	2.14-4.62	2.94	0.98	3.19-3.24	3.22	0.04	2.58-4.25	3.57	0.70
Maltose	2.09-3.51	2.80	0.50	2.83-2.86	2.85	0.02	2.09-3.08	2.67	0.52
Trehalose	0.21-1.11	0.48	0.36	1.07-1.08	1.08	0.01	0.21-1.02	0.50	0.30
Turanose	0.20-0.30	0.28	0.04	0.29-0.30	0.30	0.01	0.28-0.58	0.35	0.13
Sum of fructose and glucose	29.10-32.66	29.87	1.81	31.41-33.51	32.46	1.48	25.95-32.02	30.30	2.48
Fructose/Glucose ratio (F/G)	1.18-1.42	1.31	0.08	1.34-1.47	1.41	0.09	1.27-1.63	1.42	0.17
Total disaccharides*	5.36-9.24	6.49	1.59	7.38-7.48	7.43	0.07	6.16-8.00	7.08	0.82
Total sugars	34.46-41.90	36.36	2.93	38.89-40.89	39.89	1.41	32.11-39.93	37.38	3.15

Explanations:

* – sum of sucrose, maltose, trehalose and turanose.

The content of trehalose was from 0.24% DM for the pollen samples from China to 0.54% for the samples from Poland. Statistically significant differences in trehalose content were found between samples from Poland vs. those from China. The content of turanose ranged from 0.24% to 0.35% DM. In the majority of pollen samples the content of that sugar was at the detectability limit which for HPLC was determined at 0.20% DM.

The highest content of monosaccharides expressed as a combined content of fructose and glucose was found in the samples from Korea, an average of 34.70% DM, and the lowest in the samples from China, an average of 29.38% DM. The fructose to glucose ratio ranged from 1.18 to 1.37. The highest value of that parameter was found in the samples from Poland, the ratio being

at the same level in the samples from Korea and China.

The content of disaccharides expressed as the total sum of sucrose, turanose, maltose and trehalose ranged from 5.99% DM for the samples from China to 6.82% DM for the samples from Poland. Total sugar content varied from 35.37% DM to 41.03% DM, being the highest for the pollen from Korea. The total sugar content of the samples from Poland was lower by 4% DM and of the samples from China by 6% DM.

All the sugars assayed in this study showed substantial sample-to-sample variation within the samples from each particular country (Poland, South Korea and China) which was probably related to their botanical origin. The measure of this variation is provided by the standard deviation for the sugars assayed as determined for in-

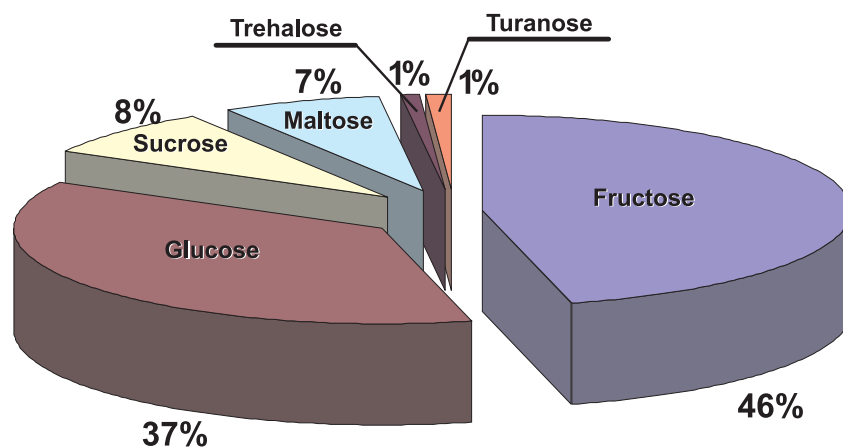


Fig. 1. Percentages of sugar content expressed as total sugars content in honeybee-collected pollen.

dividual countries (Table 1). For fructose, the standard deviation over the samples from each individual country was from 1.14 to 3.51, for glucose – from 1.29 to 3.81, for sucrose from 0.81 to 1.48 and for maltose – from 0.45 to 0.85.

Melissopalynological analysis of the samples from Poland allowed their division into three groups: group I – samples with the predominance of pollen from Brassicaceae family (over 65%), group II – samples with the predominance of *Artemisia* pollen (over 64%) and group III – samples of multifloral pollen in which pollen originated from other ruderal plants (*Rumex*, Coryphyllaceae, *Ranunculus*, *Centaurea cyanus*, *Majorana* type) as well as agricultural and horticultural plants (*Rubus* type, *Fragaria*, *Trifolium* type), trees and shrubs (*Syringa*, *Cornus*, *Robinia*, *Salix*) (Szczęsna 2006).

No substantial differences were found in the content of individual sugars among the sample groups under comparison (Table 2). The samples belonging to the group with the predominant pollen of Brassicaceae family had only a slightly lower fructose to glucose ratio.

DISCUSSION

The data on the content of monosaccharides and of sucrose in pollen loads as obtained in this study are at substantial variance from those obtained earlier by other investigators (Herbert and Shimanuki 1978; Youssef et al. 1978; Szczęsna et al. 1995a, 1995b). The differences are probably due to different floristic and geoclimatic conditions at pollen loads collection sites as well as to different sugar assaying methods used by those authors. It is because in earlier studies reducing properties of sugars were employed which did not allow the precise determination other than of sugar groups (Lane and Eynon method). In this study separation and identification of sugars by high performance liquid chromatography (HPLC) was employed. This technique allowed the qualitative and quantitative analysis of individual sugars (fructose, glucose, sucrose, maltose, trehalose, turanose) as they occurred in the pollen samples tested.

The averages of total sugar content and of individual sugars content of pollen obtained in this study differ from those obtained by Serra Bonvehi et al. (1986) and Serra Bonvehi and Escola Jorda

(1997). It should be noted that those investigators used gas chromatographic technique (GC) to determine those sugars. The differences can be probably attributed to the fact that the material examined by the Spanish investigators involved pollen loads containing predominantly pollen from *Cistus landaniferus* whereas the material in this study consisted of samples of multifloral pollen collected in different countries (in Poland, South Korea, and China). Hence a different range of F/G ratio values (1.18-1.37) obtained in this study when compared to that reported by the above mentioned investigators (1.13-1.53).

When comparing the results from this study with those from earlier investigations (Szcześna et al. 2002) it can be seen that the data on the contents of individual sugars in the pollen samples differ considerably. Among other things, in the earlier study a broader range of the F/G ratio (1.05-2.51) as compared to those in this study (1.18-1.37) and a higher sucrose content were obtained. The reason behind those differences is that this study was performed on pollen samples that came from different forage plants (different beekeeping seasons, different dates, different countries).

The chromatographic-based investigations of sugars in pollen loads showed that their minimum content was ca. 30% DM, monosaccharides expressed as the sum of fructose and glucose accounting for at least 20% DM. The development of quality-related indicators for bee pollen should take into account the results on pollen content of that product obtained in this study.

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CONCLUSIONS

1. Sugar content of bee-collected pollen dry matter averages 40%.
2. The percentages of individual sugars in total sugars of pollen loads are as follows: fructose – 46%, glucose – 37%, sucrose – 8%, maltose – 7%, trehalose and turanose – 1% each.
3. Carbohydrate fraction of bee-collected pollen shows large variability which is related to the varied botanical origin of pollen.
4. Compared to those from South Korea or China samples of pollen loads collected in Poland showed a significantly higher fructose to glucose ratio.

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BADANIA NAD SKŁADEM CUKRÓW W OBNÓŻACH PYŁKOWYCH

S z c z ę s n a T .

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

Celem badań przedstawionych w niniejszej pracy było poznanie składu cukrów w pyłku pszczelim pozyskanym w różnych krajach (Polsce, Korei Południowej i Chinach). Łącznie badaniami objęto 27 próbek pyłku kwiatowego zebranego przez pszczoły w postaci obnóży. W zebranym materiale badawczym wykonano oznaczenia zawartości suchej masy oraz składu cukrów metodą HPLC.

Przeprowadzone badania wykazały, że zawartość cukrów w suchej masie pyłku kwiatowego (obnóży) wynosi średnio około 40%. Fruktaza okazała się cukrem występującym w najwyższej ilości. Procentowy udział tego cukru w ogólnej zawartości cukrów w badanych próbkach pyłku wynosił około 46%. Drugim co do poziomu zawartości była glukoza, stanowiąca około 37%. Cukry proste wyrażone jako suma fruktozy i glukozy stanowiły około 83% frakcji węglowodanowej pyłku. Spośród oznaczonych dwucukrów sacharoza stanowiła około 8%, maltoza – około 7%, a pozostałe dwucukry (trehaloza i turanoza) – po około 1% każdy. Skład chemiczny pyłku zbieranego przez pszczoły dotyczący zawartości cukrów, wykazywał dużą zmienność co było prawdopodobnie związane z jego zróżnicowanym pochodzeniem botanicznym. Różnice istotne statystyczne w zawartości fruktozy wystąpiły między próbkami pochodzącymi z Korei Południowej i Chin, a w przypadku glukozy – między próbkami z Korei Południowej i Polski. Zawartość sacharozy i maltozy nie różniła się istotnie między porównywanymi krajami. Stosunek fruktozy do glukozy wahał się w zakresie od 1,18 do 1,37. Najwyższą wartość tego parametru posiadały próbki z Polski, natomiast dla próbek z Korei i Chin wartość stosunku fruktozy do glukozy była na tym samym poziomie.

Słowa kluczowe: obnóża pyłkowe, cukry, skład, pochodzenie botaniczne, HPLC.