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SUGAR SPECTRUM OF POLISH NECTAR AND HONEYDEW HONEYS*

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Key words: nectar honeys, honeydew honey, glucose, fructose, saccharose, oligosaccharides

Sugars of various nectar and honeydew honeys were separated on Biol-Gel P2, and glucose, fructose and saccharose were determined using specific enzymatic methods. The fructose/glucose ratio differentiated primarily acacia and rape honeys from all others. The highest amounts of disaccharides were found in acacia honey, while honeydew honeys were richest in tri- and higher oligosaccharides. The amount of enzymatically determined saccharose was more than twice lower than the apparent saccharose content determined as increase of reducing sugars after acid hydrolysis of honey solution.

INTRODUCTION

Over 20 different sacharides have been identified in honey; reviews of literature on the complexity of honey sugars composition were presented by White [16, 17], among others. The honey sugars spectrum is due to the action of invertase and may depend in part on the composition of sugars in nectar and honeydew out of which the bees produces honey. Honey invertase (-glucosidase) catalyzes the hydrolysis of saccharose and oligosaccharides resynthesis [16, 17]. The enzyme transfers the glucosyl remainder to other sugars, and according to Deifl [3] the principal products of transglucosylation are maltose and erlose (-maltosyl- -fructofuranoside). Most oligosaccharides are present in honey in small or trace amounts [16, 17, 20]. The separation and quantitative determination of these compounds is not easy, also when gas chromatography and HPLC are used [15].

White [19] developed a method of sugars separation using charcoal column chromatography, and of quantitative determination of glucose, fructose, saccharose and higher oligosacharides in eluted fractions. These methods, along with HPLC, were accepted by AOAC [1].

The composition of sugars in Polish nectar and honeydew honeys presented in the literature is based mainly on determinations by non-specific methods of

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total reducing sugars, saccharose, aldoses, ketoses and higher oligosacchrides as honey dextrin [5-10]. Recently, gas chromatography was used for separation of sugars of two natural and adulterated nectar honyes [12].

In this research gel permeation chromatography on Bio-Gel P2 of honey sugars, and specific enzymatic methods of determining glucose, fructose and saccharose were used to compare the sugar spectra in diferrent kinds of nectar honey and honeydew on the basis of glucose, fructose, di-,tri- and higher oligosaccharides contents.

MATERIAL AND METHODS

45 samples of nectar and honeydew honeys obtained from the District Apiarian Cooperative in Poznań were studied. The honeys were harvested from October to November in the 1985-1988 seasons, and 0.5 or 1 kg unheated samples were stored pending analyses at -15° C. Types and species of honey were determined organoleptically according to Polish Norm PN-75/a-77626.

A water-jacketed column (K 16/100, Pharmacia) packed with Bio-Gel P2-400 mesh (Bio-Rad) was maintained at 65° C. Elution was performed with deaerated redistilled water, and the eluent was pumped through the column at constant flow rate of 19 cm^3/h; fraction volume was 2 cm^3. Column effluents were monitored continuously with an Aerograph Refractive Index Detector (Varian) coupled with an LKB 2210 recorder; chart paper speed was 0.2 mm/min. 50% honey solution was treated with Carrez reagents I and II and filtered. 20 μl portions of honey solution were appplied to the column. Contents of di-,tri- and higher oligosaccharides were determined by weighing residues of evaporated fractions [1, 19] and estimating peak areas on the elution graph.

Saccharose in the disaccharides fraction was hydrolyzed with yeast -fructofuranosidase (Sigma) and the released glucose was determined using the glucose oxidase-peroxidase method [1, 19].

Glucose and fructose was determined in honey using the Glucose/Fructose UV method nad a Boehringer reagent set.

Sugars in column-separated fractions were analyzed by thin-layer chromatography. Plates (Kieselgel G-60, Merck) were developed in a 30:50:10:2 mixture of n-butanol, isopropanol, boric acid and acetic acid. Sugar spots were detected by spraying with diphenylamine-aniline-phosphoric acid reagent [15].

The contents of total reducing sugars and apparent saccharose (sucrose with melezitose) were determined according to Polish Norm PN-75/A-77626. The activity of honey invertase (-glucosidase) was determined colorimetrically using p-nitrophenyl--D-glucoside (Sigma) as substrate.

RESULTS AND DISCUSSION

The results of enzymatic determinations of glucose and fructose in honey and the fructose/glucose ratio (F/G) are presented in Table 1. Acacia and rape honey stand out from among the studied honeys with respect to the F/G ratio. Glucose

Tabele 1. Glucose and fructose in nectar honeys, honeydew honeys and honey-related products

Honey	Glucose (G) %	Fructose (F) %	Total G+F	F/G	Aldoses[a] %	Ketoses[a] %	K/A
Acacia							
(9 samples)	25.80–	40,67–	66.47–	1.51–			
1987 crop	30.82	48.12	78.47	1.60			
average	28.44	44.15	72.60	1.55	30.27	42.05	1.39
					30.63	42.15	1.38
					30.35	40.45	1.33
Rape							
(4 samples)	38.60–	36.06–	76.23–	0.92–			
1986 crop	39.70	37.63	77.30	0.97			
average	39.29	36.95	76.74	0.94	41.53	37.95	0.91
					42.10	37.76	0.90
					42.68	39.90	0.93
					41.27	37.52	0.91
Linden							
(5 samples)	31.70–	35.92–	71.38–	1.01–			
1986 crop	35.87	40.98	76.54	1.27			
average	34.58	38.93	73.51	1.13	37.57	38.59	1.03
					37.95	39.04	1.03
					37.02	38.28	1.03
Floral[b]							
(6 samples)	30.22–	33.72–	63.94–	1.03–			
1988 crop	35.42	37.70	71.96	1.13			
average	33.21	35.87	69.09	1.08	37.89	37.64	0.99
					36.40	36.54	1.00
					36.72	37.27	1.01
					37.28	37.05	0.99
Heather							
(6 samples)	30.27–	37.12–	67.39–	1.20–			
1988 crop	33.55	40.92	73.94	1.27			
average	31.94	39.55	71.48	1.24	31.0	37.5	1.21
					33.3	38.1	1.14
					33.7	39.3	1.17
Honeydew							
(15 samples)	27.91–	29.93–	57.84–	0.98–			
1985 crop	33.16	40.47	71.68	1.30			
average	30.92	34.04	64.97	1.10	32.35	33.59	1.04
Honey-related products with herb extracts:							
Camomile	30.48	31.73	62.21	1.04			
Nettle	34.13	32.19	66.32	0.94			
Pine	30.98	32.75	63.79	1.06			
Hawthorn	34.24	26.92	66.16	0.79			
average	32.46	30.90	64.62	0.96			

a) the means given by Fedorowska et al. (5–10)

b) nectar from various plants

dominates in rape nectar and F/G ratio in rape honeys was below 1. It is know that acacia honey is rich in fructose [17]. The mean F/G value for Polish acacia honeys (1.55) is comparable with the respective values for Romanian, Hungarian and Chinese (1.4-1.7) and French honeys (1.32-1.56) [14]. A relatively high F/G value (1.24 on average) was found in heather honey. The other studied honeys had more fructose than glucose but the mean F/G values were lower. In only two of the 15 honeydew honeys that were studied there was slightly more glucose than fructose. For comparison, glucose and fructose contents were determined in several sam ples of herbal honeys produced by bees from sugar syrups containing herb extracts. These honeys contained less fructose than nectar honeys and their F/G values hovered around 1.

The anzymatic method of determining glucose and fructose with glucose-6-phosphate dehydrogenase and phosphoglucose isomerase is sensitive and specific. The mean glucose value it gave was generally lower than the mean aldoses content reported by Fedorowska et al. [5-10], and the F/G values higher than the ketoses/aldoses ratio calculated from the data by these authors and listed in Table 1.

The total glucose and fructose content was occasionally a few per cent lower than the total reducing sugars content, but there was considerable correlation between total glucose and fructose on the one hand and reducing sugars on the other. The correlation coefficient for nectar honeys was +0.7673 and for all 45 honey samples +0.7902.

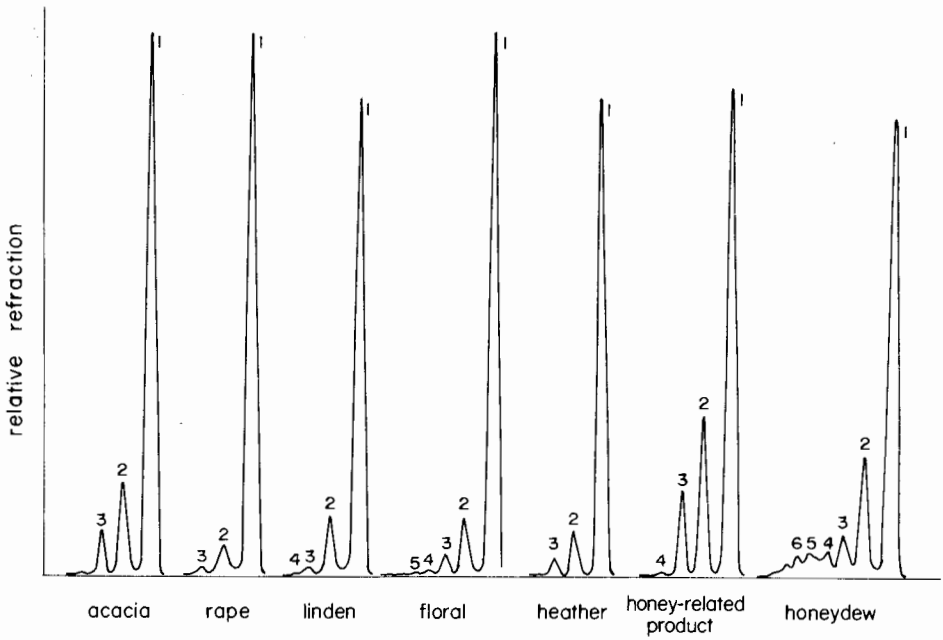

Fig. 1. Fractionation of honeys and honey-related products saccharides on Bio-Gel P2 (-400 mesh) column; 1 — monosaccharides, 2 — disaccharides, 3 — trisaccharides, 4, 5 etc. — higher oligosaccharides

Literature reports on the separation of oligosaccharides of different structures and molecular weights occurring in hydrolyzates of starch and other polysaccharides [11] prompted the use of gel chromatography on Bio-Gel P2 column to study honey sugars.

Exemplary elution profiles of honey sugars from Bio-Gel P2 column are presented in Fig. 1. Peaks corresponding to di-,tri- and higher saccharides can be seen alongside the dominating monosacchrides. The studies honeys differed as to the number of oligosaccharides fractions. Mono-, di- and trisaccharides were consistently separated from rape and heather honeys. A small peak of tetrasaccharides is present in the elution profiles of the remaining honeys. A large number of higher oligosaccharides fractions was separated from some honeydew and polyfleur (nectar from various plants) honeys, with the remaining honeys lacking these fractions. Given the small quantities of tetra- and higher oligosaccharides, they were determined together with trisaccharides.

The nectar honey with the highest di,- tri- and higher saccharides content was the acacia variety, the lowest content being found in rape honey (Table 2). The disaccharides content in linden, polyfleur and heather honeys was more or less the same, with considerable differences between individual samples of each variety. Honeydew honeys differed from nectar honeys in having a higher average conten of tri- and higher oligosaccharides. In herbal honeys the content of di-, tri- and higher oligosaccharides was higher than in nectar honeys, including also acacia honey. The contents of di-, tri- and higher oligosaccharides in the tested honeys are within the ranges given by White [20]: 3.29-18.6% disaccharides, 0.13-3.85% higher sugars. Bogdanov [2] separated honey sugars using thin-layer chromatography and found that trisascharides content in honeydew honey was $4.0 \pm 2.2\%$.

Fig. 2 shows a thin-layer chromatogram illustrating separation of sugars in honeydew honey. A similar spot pattern ocurred on chromatograms of nectar honeys. In most cases the disaccharides fraction produced five spots, one of which corresponded to maltose and another to saccharose, Zürcher et al. [21] separated disaccharides using gas chromatography and also identified maltose and saccharose as three other sugars labelled x_1, x_2 and x_3. The trisaccharides fraction of the studied honeys always featured two spots, and many samples had one or two more. The spot farthest from the start was present on thin-layer chromatograms of nectar and honeydew honeys as well as of herbal honeys. This spot possibly represents erlose, but this could not be checked as the standard for this sugar was not available.

Saccharose quantities were determined in the disaccharides fraction eluted from the Bio-Gel P2 column by enzymatic hydrolysis and measurement of the liberated glucose by the glucose oxidase — peroxidase method. In his discussion of the effect of sugars complexity on the analysis of these compounds White [17, 18] stresses that the procedure of measuring glucose liberated after enzymatic hydrolysis of saccharose in the disaccharides fraction is specific, and that its results are comparable with those obtained with HPLC. The figures in Table 2

Table 2. Di-, tri- and higher oligosaccharides and saccharose in nectar honeys, honeydew honeys and honey-related products

Honey	Disaccharides (%)	Tri-and higher oligosaccharides (%)	Disaccharides other than saccharose (%)	Saccharose in disaccharides fraction (%)	Apparent sucrose (%)
Acacia (5 samples) 1987 crop	7.62-10.25	2.48-4.08	6.56-8.83	1.05-3.56	2.29-7.55
average	9.55	3.48	7.25	2.31	5.04
Rape (4 samples) 1986 crop	1.82-3.77	0.48-1.07	1.37-3.12	0.22-0.66	1.12-1.75
average	2.80	0.70	2.31	0.49	1.38
Linden (5 samples) 1986 crop	4.84-7.47	0.10-1.09	4.62-7.17	0.22-0.54	0.49-1.90
average	6.21	0.66	5.83	0.38	1.43
Floral[a] (6 samples) 1988 crop	3.50-7.99	0.85-2.14	2.61-7.55	0.44-0.89	0.96-6.00
average	5.64	1.67	5.03	0.61	3.30
Heather (6 samples) 1988 crop	4.10-8.42	1.27-3.00	3.77-7.61	0.33-0.86	1.30-3.16
average	6.37	2.00	5.77	0.59	1.90
Honeydew (15 samples) 1985 crop	5.33-12.77	2.15-6.22	4.59-12.26	0.35-4.41	2.07-10.05
average	8.05	4.25	7.07	0.98	4.87
Honey-related products with herb extracts:					
Camomile	15.42	7.11	9.38	6.04	8.40
Nettle	9.16	6.40	8.23	0.93	5.39
Pine	12.25	4.86	9.24	3.01	8.00
Hawthorn	15.02	3.40	8.14	6.88	7.13
average	12.96	5.44	8.75	4.21	7.23

a) nectar from various plants

show that the amount of saccharose in the disaccharides fraction of nectar honeys was at least twice lower than the content of apparent saccharose determined as reducing sugars before and after acid hydrolysis. The largest differences in saccharose contents determined by both methods were observed in honeydew honeys. In herbal honeys the saccharose content determined by the specific method with glucose oxidase was much higher than in natural honeys, acacia honey included. In these products the contents of disaccharides other than saccharose was similar to the contents in most of the honeydew honeys.

Apparent saccharose was more correlated with tri- and higher oligosaccharides ($r = +0.7450$) than with disaccharides ($r = +0.6720$).

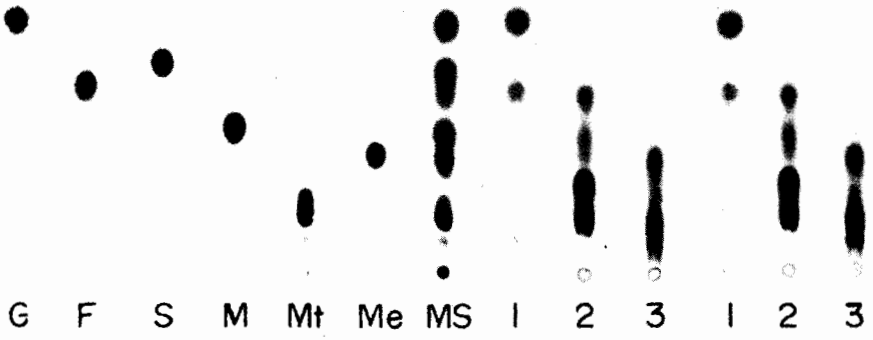

Fig. 2. TLC pattern of mono-, di- and trisaccharides of honeydew honey previously separated on Bio-Gel P2 column; Standard: G — glucose, F — fructose, S — saccharose, M — maltose, Mt — maltotriose, Me — melezitose, MS — mixed sugar standard; Column fractions: 1 — mono-, 2 — di-, 3 — trisaccharides

Invertase (-glucosidase) activity in honeys was investigated alongside sugars composition. This activity in terms of saccharose number (SN) is presented in Table 3. It varied among the various types and varieties of honey, and also

Tabele 3. Invertase activity (SN) in nectar and honeydew honeys

Honey	Number of samples	Mean value of SN	Range of SN values
Acacia	10	7.03	5.03-10.79
Rape	5	11.38	7.98-15.45
Linden	10	21.44	15.00-26.51
Floral[a]	14	17.30	7.28-28.24
Heather	13	16.49	5.66-26.07
Honeydew	15	18.61	0.68-31.84
Honey-related products	4	7.02	2.31-11.56

[a] from various plants nectars

among samples of each variety. The lowest SN values were found in acacia honeys and in herbal honeys containing the largest amounts of oligosaccharides. Values in rape honeys were close to the average figures in acacia honeys, but their di- and higher oligosaccharides contents were the lowest. Nectar sugars probably affected the spectrum of sugars in acacia and rape honeys. No statistical correlation was found between SN and saccharose, di-, tri- and higher

oligosaccharides fractions, and total glucose and fructose. The lack of correlation between invertase activity and saccharose content was recently interpreted by Deifel et al. [4] who found that saccharose content depends on density of bees' feed. High sugar in this feed leads to lower invertase activity. It is being suggested that low water content in mature honey favours transglucosylation catalyzed by invertase [17].

CONCLUSIONS

1. The fructose/glucose ratio sets apart acacia and rape honey from other Polish nectar honeydes honeys.

2. The contents of di, tri- and higher oligosaccharides in acacia honey is much higher and in rape honey lower than in other nectar honeys. Large amounts of these oligosaccharides are present in herbal honeys. Honeydew honey generally contains more tri- and higher oligosaccharides than nectar honeys.

3. The content of saccharose determined by the specific enzymatic method in the disaccharides fraction in Polish honeys is at least twice lower than the content of apparent saccharose determined as reducing sugars before and after acid hydrolysis.

4. It is prossible to compare sugars spectra of various nectar, honeydew and herbal honeys by separating mono-, di-, tri- and higher oligosaccharides on Bio-Gel P2 (-400 mesh).

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OBRAZ CUKRÓW W KRAJOWYCH MIODACH NEKTAROWYCH I SPADZIOWYCH

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Streszczenie

Cukry miodów nektarowych: akacjowego, rzepakowego, lipowego, wielokwiatowego i wrzosowego oraz miodów spadziowych i kilku ziołomiodów rozdzielano metodą chromatografii żelowej na Bio-Gel P2 (-400 mesh) na frakcje mono-, di-, tri- i wyższych oligosacharydów, a zawartość glukozy, fruktozy i sacharozy oznaczano metodami enzymatycznymi.

Miody akacjowy i rzepakowy wyróżniały się skrajnie odmiennym stosunkiem fruktozy do glukozy (F/G). W przypadku pierwszej odmiany średnia wartość F/G wynosiła 1,55, a drugiej 0,94. Inne odmiany miodu nektarowego zawierały więcej fruktozy niż glukozy, ale średnio wartości F/G były niższe w porównaniu z miodem akacjowym. W miodzie akacjowym występowało przy tym najwięcej disacharydów (9.55%), a w miodzie rzepakowym znajdowały się najmniejsze ilości tej frakcji cukrów (2,80%). W pozostałych odmianach zawartość disacharydów kształtowała się na poziomie pośrednim. Największe ilości disacharydów zawierały ziołomiody. Miód spadziowy odznaczał się wyższą średnią zawartością tri- i wyższych oligosacharydów (4,25%) w porównaniu z miodami nektarowymi. Ilości sacharozy oznaczonej metodą enzymatyczną w frakcji diasacharydów były co najmniej dwa razy mniejsze niż ilość sacharozy łącznie z melezytozą, oznaczona na podstawie zwartości cukrów redukujących przed i po hydrolizie kwasowej. Ziołomiody zawierały więcej sacharozy w frakcji diasacharydów niż miody naturalne.

Aktywność inwertazy, zróżnicowana tak w odmianach, jak i poszczególnych próbach miodu nie była skorelowana z sumą glukozy i fruktozy, z zawartością sacharozy oraz disacharydów ogółem, a także tri- i wyższych oligosacharydów