

MONO- AND OLIGOSACCHARIDES COMPOSITION OF SOME POLISH UNIFLORAL HONEYS BY MEANS OF GAS CHROMATOGRAPHY

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S u m m a r y

The aim of our research was determination of Polish unifloral honeys in respect of quality and quantity composition of sugars by means of gas chromatography. Study material was provided by samples of honey collected from the apiaries of the Apiculture Division in Puławy and from private apiaries localized in different regions of Poland during four years (1997-2000). The samples were grouped according to their organoleptic properties as follows: robinia, lime, buckwheat, heather, honeydew, multifloral honeys. The sugar analyses were carried out using GC 14 APF Shimadzu Chromatograph.

Quality and quantity composition of sugars of honeys were dependent on their floral origin. Sugars spectrum of honey can be used for classification of some kinds of honey: robinia (which fructose/glucose ratio is higher than 1.40), buckwheat (which contains trace amounts of trisaccharides: raffinose, maltotriose and melezitose), honeydew (which contains several times higher concentration of raffinose and maltotriose). Results received by gas chromatography for reducing monosaccharides and saccharose are lower than results received by conventional Layne-Eynon method, adopted as the official method by national standard and EU Directive.

Keywords: honey, kinds of honey, sugars composition, gas chromatography, Poland.

INTRODUCTION

Carbohydrates constitute almost 98% of dry matter of honey. Their quality and quantity composition depend on sugars concentration in nectar and honeydew and also on enzymatic changes occurred during ripening and storing of honey (Rybak 1986, Rybak, Achremowicz 1986, Sabatini et al. 1989, Serra Bonvehi, Bosch Callis 1989, Tourn et al. 1989, Ortiz Valbuena et al. 1995, Serra Bonvehi, Ventura Coll 1995).

Results received by means of conventional methods for apparent reducing sugars as well as apparent sucrose (based on Fehling method) were not satisfactory (PN-88/A-77626 „Miód pszczeli“, Council Directive 1974). For this reason chromatographic methods have been adopted to characterise honey quality. These methods have been used for almost 30 years however at present they don't allow a complete separation of all sugars,

particularly oligosaccharides (B o g d a n o v 1999). Carbohydrates profile of natural honey, especially composition of oligosaccharides, differs from that of invert syrups: chemically and enzymatically prepared (S w a l l o w , L o w 1994, L o w , S o u t h 1995). Because of this, in many countries determination of sugar spectrum of unifloral honeys using accurate chromatographic methods became conventional study of its quality.

The aim of this study was to complete characteristics of Polish unifloral honeys regarding to their sugar quality and quantity composition, determined by gas chromatography.

MATERIAL AND METHODS

MATERIAL

Material for analyses was provided by samples of honey collected from the experimental apiaries of the Apiculture Division in Puławy. Some samples were sent by beekeepers from various regions of Poland. Studies were conducted over the years 1997-2000. The honey samples were grouped according to their organoleptic properties as follows: robinia, lime, buckwheat, heather, honeydew, multifloral honeys. In addition some unifloral honey samples were collected from the following botanical sources: *Taraxacum officinale*, *Melilotus* sp. and *Oenothera* sp. and analysed by the same method. All samples until analyses were stored at 4°C ($\pm 2^\circ\text{C}$).

METHODS

Honey variety was determined using organoleptic test according to the Polish Standard for Honey PN-88/A-77626.

The sugars analyses were carried out by gas chromatography according to the method described by B o g d a n o v et. al. (1997) and Méthodes officielles d'analyse du miel (1977), modified by the authors (R y b a k - C h m i e l e w s k a , S z c z e s n a 2000). The honey sugars were silylated and the derivatives separated and quantified by means of gas chromatography using xylose as the internal standard.

Packed and capillary column chromatography was performed on a GC 14 APF Shimadzu Chromatograph.

1. Packed glass column (4 m x 3 mm i.d.) containing 4% Silicon OV-101 on Chromosorb W/HP 100/120 mesh
2. Capillary column FS-SE-54 (25 m x 0,32 mm ID with 0,25 μm particle diameter)
3. Flame Ionisation Detector
4. Pol-Lab Chroma 3,2x Computer Software.

Identification of sugars was accomplished by comparison of the retention times with chromatographed standards. Their concentration was expressed in percent of fresh weight of honey. The following sugars were studied: the monosaccharides - fructose and glucose; from among disaccharides: saccharose, maltose, turanose; and the trisaccharides: raffinose, maltotriose and melezitose. In addition, the total reducing monosaccharide content (fructose + glucose) and fructose/glucose ratio (F/G) were calculated.

RESULTS AND DISCUSSION

The fructose content ranged between 29.11 and 42.31% (Table 1). Among the examined unifloral kinds of honey the lowest fructose content was found in lime honey ($x=31.82\% \pm 2.96$) and the highest in robinia honey ($x=41.17\% \pm 0.66$). Noticeably high fructose content characterised heather ($x=39.17\% \pm 0.53$) and buckwheat ($38.18\% \pm 1.84$) honeys.

Table 1

Reducing monosaccharides (fructose, glucose) content and fructose/glucose (F/G) ratio in Polish unifloral honeys (%)
Zawartość cukrów redukujących (fruktozy, glukozy) i stosunek fruktozy do glukozy (F/G) w polskich miodach odmianowych (%)

Honey Miód	Fructose - Fruktaza (%)		Glucose - Glukoza (%)		Total reducing monosaccharides - Ogólna zawartość cukrów redukujących (%)		F/G ratio Stosunek F/G	
	from - to od - do	mean średnio	from - to od - do	mean średnio	from - to od - do	mean średnio	from - to od - do	mean średnio
Robinia Akacjowy	41.13-42.31	41.17	27.40-29.38	28.70	69.14-70.58	69.86	1.41-1.54	1.44
Lime Lipowy	29.11-34.97	31.82	27.31-33.61	29.86	57.78-68.58	61.68	1.02-1.15	1.07
Buckwheat Gryczany	34.70-40.16	38.18	31.70-37.35	33.71	66.40-76.74	71.90	1.05-1.23	1.14
Heather Wrzosowy	38.79-39.54	39.17	28.49-30.13	29.31	67.28-69.67	68.48	1.31-1.36	1.34
Honeydew Spadziowy	32.65-34.79	33.84	27.95-28.67	28.33	60.60-63.46	62.17	1.17-1.21	1.19
Multifloral Wielokwiatowy	35.03-41.10	37.90	28.42-33.56	30.65	63.38-73.21	68.59	1.14-1.29	1.24

Glucose content varied between 27.31 and 37.35%. Honeydew and robinia honeys were characterised by a low glucose content, always below 30%. Noticeably high values (from 31.70 to 37.35%) characterised the buckwheat honey, with the mean value of 33.71%.

The total content of reducing monosaccharides (fructose + glucose) ranged between 57.78 and 76.74%. All groups, except lime and honeydew honeys, showed values above 62%. The buckwheat honey was characterised by high total reducing monosaccharides content, with the mean value of 71.90%.

The values of the fructose/glucose ratio ranged from 1.02 to 1.54. The lowest values were found in lime honey ($x=1.07 \pm 0.07$), and the highest in robinia honey ($x=1.44 \pm 0.07$). Instead, noticeably high value ($x=1.34 \pm 0.04$) characterised heather honey.

Sucrose content was particularly low in buckwheat and heather honeys where it was present mostly as non-measurable traces and always less than 0,2% (Table 2). The highest content of sucrose was detected in robinia and honeydew honeys, with the mean values of 1.12 and 0.71, respectively.

Table 2

Disaccharides (saccharose, maltose and turanose) content in Polish unifloral honeys (%) - Zawartość dwucukrów (sacharozy, maltozy i turanozy) w polskich miodach odmianowych (%)

Honey Miód	Saccharose - Sacharoza (%)		Maltose - Maltoza (%)		Turanose - Turanoza (%)	
	from - to od - do	mean średnio	from - to od - do	mean średnio	from - to od - do	mean średnio
Robinia Akacjowy	tr-1.52	1,12	2.14-2.98	2.16	2.31-2.42	2.37
Lime Lipowy	0.17-2.11	0,85	1.04-2.15	1.44	0.94-2.07	1.43
Buckwheat Gryczany	tr-0.16	0,11	0.84-2.12	1.64	0.46-1.08	0.87
Heather Wrzosowy	0.05-0.12	0,09	2.18-2.51	2.35	-	-
Honeydew Spadziowy	tr-1.00	0,71	1.40-2.27	1.78	1.64-2.03	1.84
Multifloral Wielokwiatowy	tr-0.88	0,57	0.04-2.38	1.86	tr-2.80	1.83

Maltose was found in all analyzed honey samples in percentages from 0.04 to 2.98. Heather and robinia honeys contain highest concentration of maltose, 2.35 and 2.16%, respectively.

Turanose has been identified in all examined samples from unmeasurable traces to 2.80%. The highest concentration of this sugar was found in robinia honey ($x=2.37\% \pm 0.08$), and the lowest in buckwheat honey ($x=0.87\% \pm 0.36$).

Rafinose was not identified in buckwheat honey, and throughout other analyzed groups ranged from unmeasurable traces to 2.03% (Table 3). The honeydew honey was characterized by highest content of this trisaccharide,

with the mean values of 1.84% \pm 0.28. Maltotriose was found from unmeasurable traces to 1.19%. The same dependence as for raffinose was received also for maltotriose. The buckwheat honey was not contain this sugar, and honeydew honey was characterized by highest content, with the mean value of 1.13% \pm 0.08.

Table 3

Trisaccharides (raffinose, maltotriose and melezitose) content in Polish unifloral honeys (%) - Zawartość trójcukrów (rafinozy, maltotriozy i melecycytozy) w polskich miodach odmianowych (%)

Honey Miód	Raffinose - Rafinoza (%)		Maltotriose - Maltotrioza (%)		Melezitose - Melecycytoza (%)	
	from - to od - do	mean średnio	from - to od - do	mean średnio	from - to od - do	mean średnio
Robinia Akacjowy	0.03-0.31	0.17	0.12-0.14	0.13	0.25-0.28	0.27
Lime Lipowy	0.05-0.54	0.25	0.04-0.07	0.06	0.11-0.18	0.14
Buckwheat Gryczany	tr	tr	tr	tr	tr	tr
Honeydew Spadziowy	1.64-2.03	1.84	1.07-1.19	1.13	0.16-0.22	0.19
Multifloral Wielokwiatowy	tr-0.16	0.14	tr-0.17	0.09	tr-0.29	0.20

Among trisaccharides, melezitose was found in lowest concentration, and buckwheat honey was not contain of this sugar.

Table 4 present results of examined sugar contents (fructose, glucose, sucrose and maltose) of some kinds of honey from: *Taraxacum officinale*, *Melilotus* sp. and *Oenothera* sp. Predominant monosaccharide in *Melilotus* sp. and *Oenothera* sp. honeys was fructose. F/G ratio in this honeys was 1.16 and 1.21, respectively. F/G ratio in *Taraxacum officinale* honey was about 1,00. Sucrose was present in *Taraxacum officinale* and *Oenothera* sp. honeys in trace amounts, and in *Melilotus* sp. honey in the amount of 1%. *Melilotus* sp. and *Oenothera* sp. honeys were characterized by high maltose content averaging about 2.2%. *Taraxacum officinale* honey has lower content of maltose, 1.66%.

Sugar composition of unifloral honeys examined by means of paper chromatography was presented already by Maurizio in 1964. Author formulated a brave contention in her work that sugar spectrum, like pollen analysis, can be one of indicators of honey origin. In her opinion, influence of raw material (nectar, honeydew) on sugar composition of honey appears as a fructose/glucose ratio eg. as large fructose quantities of *Labiata*, *Epilobium* honeys and as large glucose quantities of rape (*Brassica napus*), *Myosotis* sp.

and lime (*Tilia cordata*) honeys. Furthermore the author confirmed results obtained earlier that sucrose content is generally low and rarely exceeds 5% of total quantities of sugars; maltose content is usually higher than saccharose content, often achieves values 5-10% (even 15%); fructomaltose (erlose) occurs usually in measurable quantities and oligosaccharides content accounts for considerable part of total quantities of honey sugars.

Table 4

Basic sugar composition of some kinds of honey (%)
Podstawowy skład cukrów niektórych odmian miodu (%)

Sugar - cukier	Honey - Miód		
	<i>Taraxacum officinale</i> Mniszek lekarski	<i>Melilotus</i> sp. Nostrzyk	<i>Oenothera</i> sp. Wiesiołek
Fructose - fruktoza	37.65	39.09	39.54
Glucose - glukoza	37.29	33.26	30.13
Saccharose - sacharoza	tr	1.00	0.05
Maltose - maltoza	1.66	2.14	2.18
Reducing sugars Cukry redukujące	74.94	72.35	63.46
F/G	1.01	1.16	1.21

Results of recent research conducted using new chromatographic techniques (HPLC, GC) confirm results of investigations of Maurizio (1964) and supplement characteristics of sugars composition of other unifloral honeys (Rybak, Achremowicz 1986, Sabatini et al. 1989, Serra Bonvehi, Bosch Callis 1989, Tourn et al. 1989, Ortiz Valbuena et al. 1995 Serra Bonvehi, Ventura Coll 1995, Bogdanov 1999). Results obtained in our study give evidence of: low saccharose content in studied unifloral honeys (robinia, lime, buckwheat, heather, honeydew and multifloral) usually ranging from traces to 1,5%, content of maltose and turanose about twice as high as that of saccharose, and low quantities of trisaccharides (rafinose, maltotriose and melezitose) that exceed 2% only in honeydew honey. The reason for low sucrose and melezitose contents of honey that were obtained in our study was probably related to analytical conditions of separation and elution of analyzed oligosaccharides.

CONCLUSIONS

1. Quality and quantity of sugars content of honey is dependent on its floral origin.
2. Sugars spectrum and sensorial test, without resorting to very difficult melissopalynological analysis, can be used for classification of studied

kinds of honey:

- robinia - fructose/glucose ratio of which is higher than 1.40,
 - buckwheat - which contains trace amount of trisaccharides: raffinose, maltotriose and melezitose,
 - honeydew - which contains several times higher concentration of raffinose and maltotriose.
3. Sugars determination in honey by gas chromatography method, especially in the case of oligosaccharides content, requires further improvements of analytical conditions of their separation and elution.
 4. Results of honey analyses by means of gas chromatography for reducing monosaccharides show that their contents are lower than results of similar analyses obtained by conventional Layne-Eynon method, adopted as the official national standard method and by EU Directive.

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SKŁAD CUKRÓW PROSTYCH I WIELOCUKRÓW W KILKU POLSKICH MIODACH ODMIANIOWYCH OZNACZANY METODĄ CHROMATOGRAFII GAZOWEJ

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

Cukry stanowią niemal 98% suchej masy miodu. Ich skład jakościowy i ilościowy zależy od składu cukrów w nektarze i spadzi - głównych surowców miodowych, oraz od enzymatycznych przemian, które zachodzą w czasie dojrzewania i przechowywania miodu. Celem przeprowadzonych badań było uzupełnienie charakterystyki krajowych miodów odmianowych o ilościowy i jakościowy skład cukrów, oznaczanych za pomocą chromatografii gazowej.

Materiał do badań stanowiły próbki miodów odmianowych pozyskane w pasiekach Oddziału Pszczelnictwa ISK w Puławach oraz od prywatnych pszczelarzy z terenu całego kraju, w latach 1997-2000. Próbkę te, na podstawie badań organoleptycznych (zgodnie z PN-88/A-77626 „Miód pszczeli“) zaliczono do następujących odmian: akacjowy, lipowy, gryczany, wrzosowy, spadziowy i wielokwiatowy.

Badania cukrów przeprowadzono z wykorzystaniem chromatografu gazowego firmy SHIMADZU GC 14 APF, wyposażonego w kolumnę kapilarną FS-SE-54 (25 m x 0,32 mm ID, 0,25 µm) oraz szklaną kolumnę pakowaną (4 m, faza stacjonarna - 4% Silicon

OV-101, faza nośna - Chromosorb W/HP 100/120 mesh), detektor płomieniowo-jonizacyjny FID i integrator komputerowy Pol-Lab Chroma 3,2x. Oznaczanie cukrów w miodzie metodą chromatografii gazowej polegało na przeprowadzeniu ich w sililowe pochodne, a następnie na rozdzieleniu i identyfikacji tych pochodnych za pomocą chromatografu gazowego. Identyfikację poszczególnych cukrów w miodzie przeprowadzono przez porównanie czasów retencji cukrów w badanej próbce z czasami retencji cukrów w roztworze wzorcowym. Ilościowego oznaczenia cukrów w miodzie dokonano metodą standardu wewnętrznego - ksylozy. Dla każdego cukru określono tzw. współczynnik korekcji masy, stały dla zastosowanej w badaniach kolumny i ustalonych warunków pracy chromatografu.

Z przeprowadzonych badań wynika, że jakościowy i ilościowy skład cukrów w miodzie jest ściśle związany z jego pochodzeniem botanicznym. Obraz cukrów miodu i badania organoleptyczne, bez konieczności przeprowadzania bardzo trudnej analizy pyłkowej, mogą być wykorzystane do klasyfikacji badanych odmian miodu: akacjowego, gdzie stosunek fruktozy do glukozy wynosi powyżej 1,4; gryczanego, w którym nie zidentyfikowano takich trójcukrów jak: rafinoza, maltotrioza i melecytoza; oraz spadziowego, charakteryzującego się, w porównaniu z innymi odmianami, kilkakrotnie wyższą zawartością trójcukrów: rafinozy i maltotriozy. Zawartość cukrów prostych (glukozy i fruktozy) oznaczana metodą chromatograficzną jest o kilka procent niższa w badanych odmianach miodu, niż zawartość tych cukrów oznaczana standardową metodą Lane-Eynona, uznaną w normie krajowej i Dyrektywie UE.

W tabeli 4 przedstawiono podstawowy skład cukrów mało znanych odmian miodów: z mniszka lekarskiego (*Taraxacum officinale*), nostrzyka (*Melilotus* sp.) i wiesiołka (*Oenothera* sp.).

Słowa kluczowe: skład miodu, odmiany miodów, zawartość cukrów, chromatografia gazowa, Polska.