

## GAS CHROMATOGRAPH (GC) STUDY OF SUGAR COMPOSITION IN HONEYS AND WINTER STORES PROCESSED BY BEES FROM SUCROSE SYRUPS

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### S u m m a r y

The objective of the study was to compare sugar composition of honey with that of several syrups (bee feeds) before and after they were processed by bees and deposited in honeycombs as winter stores. Another objective was to find an answer to the question of to what extent gas chromatography (GC) as a method to assay individual carbohydrate contents is able to identify "sugar honey" and other honey surrogates made by enzymatically hydrolyzing sucrose to monosaccharides. The study material consisted of ready-to-use inverts from two manufacturers (syrup A and B) and of 70% sucrose solution (sugar syrup) which is conventionally fed to bees in the autumn as a winter feed. The contents of individual sugars in winter stores processed from those feeds were compared to those in spring honey samples collected from the same colonies.

The use of capillary gas chromatography to assay carbohydrate contents of honey and of inverts (following their processing by bees) allowed some important differences to be found among the products under comparison. The differences were those for erlose and sucrose contents and for maltose to isomaltose content ratio (M/IM). It was also observed that the ratio of sucrose to maltose content can also be a distinguisher to be used in the identification of inverts processed by bees from sucrose syrups. In all winter store samples the ratio was close to 1 or higher. Instead, in the examined honeys the ratio did not exceed 1 and averaged 0.46. The observation needs to be confirmed using broader experiment material, especially using a higher number of winter store samples from sucrose syrups examined for carbohydrate composition. The current standards still stipulate for 5% as the admissible sucrose content of nectar honeys. The product processed by bees from sugar (from sugar beet sucrose) will not be disqualified by a requirement laid down in this manner. What is characteristic of and what distinguishes those inverts is an erlose content of several percentage points.

**Keywords:** Honey, sucrose syrup, carbohydrates, capillary gas chromatography, adulteration, identification.

### INTRODUCTION

Honey is a natural sweet substance composed mainly of easily digestible simple sugars and of some small percentage of di- and trisaccharides the qualitative and quantitative composition of which is peculiar to the product. The proper image of carbohydrates in honey may thus be a guarantee of the product's authenticity. Lane-Eynon's

method recommended in older standard documents (PN-88/A-77626; Recommended European Regional Standard for Honey – 1970) did not identify individual honey carbohydrates but only the total content of reducing sugars before and after acid hydrolysis. Ever increasing quality-related requirements laid down for food in EU countries translate to higher demands

relating to quality of honey. Among other things, they entail the necessity to use more precise methods to determine the content of the major constituents of honey: fructose, glucose and sucrose with particular emphasis on chromatograph techniques (GC or HPLC). Those techniques are recommended in the methods to be used in the standard for honey of the Codex Alimentarius Commission of 2001. The qualitative and quantitative saccharide assays mentioned in that document provide a much more accurate image of those compounds, as compared to that obtained by the classic Lane-Eynon's method (Bogdanov et al. 1997), thereby facilitating the identification of honey surrogates and of honey adulteration (Low and Sporns 1988; Swallow and Low 1994; Low and South 1995; Bogdanov and Martin 2002; Cotte et al. 2003). According to many researchers chromatographic assays of sugar composition of honey also allow its classification according to type: nectar, honeydew, as well as according to variety: acaccia, rape (Maurizio 1964; Sabatini et al. 1989, 1990; Ohe W. von der and Ohe K. von der 1996; Bogdanov et al. 1997; Rybak-Chmielewska and Szczesna 2000).

The objective of the study was to compare sugar composition of honey with that of syrups (bee feeds) before and after they were processed by bees and deposited in honeycombs as winter stores. Another objective was to find an answer to the question as to what extent gas chromatography (GC) as a method to assay individual carbohydrate contents is able to identify "sugar honey" and other honey surrogates made by enzymatically hydrolyzing sucrose into monosaccharides.

## MATERIAL AND METHODS

The material for the study consisted of samples of ready-made inverts proceeding

from two manufacturers (syrup A and B) and 70% sucrose solution (sugar syrup) conventionally fed to bees in the autumn as winter feed.

Syrup A was produced from the enzymatic sucrose invert. For the commercial hydrolysis of sucrose the yeast enzymes from *Saccharomyces cerevisiae* and *Saccharomyces uvarum* are used. Once the sucrose was nearly completely broken down into monosaccharides and each of the sugars had reached a 20 – 25% concentration in solution following enzyme de-activation sucrose was added in the production process of that feed to reach an appropriate concentration ca. 30% of that sugar. The invert thus obtained was supplemented with fructose by the manufacturer in order to obtain the prevalence of that sugar which allowed the crystallization rate of the feed in storage to be slowed down.

Syrup B was manufactured in the similar manner. The only difference was that fructose was not added in the production process.

Each of the feeds was fed in the autumn to 5 bee colonies in the apiary of the Apiculture Division. Samples of winter stores were collected late in the autumn – 30<sup>th</sup> October 2000 (store I) and early in the spring – in the beginning of April 2001 (store II). First spring honeys were also sampled, one sample from each experiment colony and from control colonies (of the same apiary). A total of 25 samples of winter stores, 15 samples of honey and three samples of bee feeds were examined for sugar content.

Sugar composition of the experiment material was assayed using gas chromatography according to Bogdanov et al. 1997 as modified by the author (Rybak-Chmielewska and Szczesna 2000). Silil derivatives of honey sugars were loaded onto capillary column FS-SE-54 (25 m x 0.32 mm ID, 0.25 µm). Prior to sililation the honey was carefully dehy-

drated by freeze-drying. In the study the gas chromatograph SHIMADZU GC 14 APF with flame-ionization detector (FID) was used. The identification of individual sugars in honey was carried out by comparing retention time of sugars in a given sample with sugar retention time in the reference solution. The quantitative assay was done using the internal standard – xylose. The so-called mass correction coefficient, constant for the column and chromatograph operation conditions, was used. The above-described method was used to assay the following sugars: fructose, glucose, sucrose, maltose, isomaltose, turanose, erlose, maltotriose and melezitose. The data were subjected to one-way ANOVA. The significance of differences among sugar content values in the treatments under comparison was examined using Duncan's test at  $\alpha=0.05$ .

## RESULTS AND DISCUSSION

In Tables 1 to 5 results are listed concerning sugar content in bee-processed sucrose syrups and in honeys: Tables 1 and 2 – results for 70% sucrose solution (sugar syrup), Tables 3 and 4 – results for enzymatic sucrose hydrolysate supplemented with fructose (syrup A), in Table 5 – results obtained for the enzymatic sucrose invert (syrup B).

Sugar content of individual sugars processed from winter store feeds were compared with those in spring honey samples obtained from the same colonies (Tables 2 and 4) or from control colonies of the same apiary (Tables 1, 3, and 5). In Tables 1 and 2 results were listed concerning sugar contents of winter store bee-processed from 70% sucrose solution. Significant differences in sugar content of the samples under comparison (winter store vs. honey) were obtained for sucrose and erlose contents and for the value described as maltose to isomaltose ratio. When compared to honey

winter store samples collected from the bees in the autumn had also a significantly higher content of the disaccharide maltose (Table 1). In the spring the content of that disaccharide in winter store samples also was higher when compared to that in honey but the differences were no longer significant (Table 2). When the results for winter stores are compared with those for honey, it becomes apparent that the bees, using their pharyngeal glands, process sucrose into a product similar in carbohydrate composition to honey (Tables 1 and 2). What can be clearly seen here is the transglucosidase nature of the enzymatic sucrose-to-monosaccharide processing via trisaccharide erlose, the contents of the latter in the winter store samples averaging 3.34% (Table 1) to 3.87% (Table 2). Enzyme activity also resulted in other associations of glucose molecules: maltose, isomaltose and maltotriose as well as in associations of glucose and fructose – turanose. As early as in 1962 Maurizio stated that in the case of the enzymatic activity of the bee midgut there occurs a typical transglucosidase activity and in the case of pharyngeal glands joint activity of several enzymes is the observed result. A complex activity of honey enzymes is also suggested by the investigations of White and Kushnir (1967). The investigators separated the enzymatic dialysate obtained from several honey samples and from "sugar honey" and in all cases several fractions were obtained from  $\alpha$ -glucosidase preparations. Likewise, Simpson et al. (1968) found a complex nature of  $\alpha$ -glucosidases from bee pharyngeal glands. It was supported by the study of Takenaka (1980) in which the author separated the enzyme maltase from honey and found its activity against maltose and sucrose. He also showed the transglucosidase mechanism of the enzyme's activity. Under the action of that enzyme erlose ( $\alpha$ -maltosyl- $\beta$ -D-fructofuranoside or fructo-

Table 1

Sugar composition of bee-processed syrup and of honey from control colonies (%).

Sugar content:	Syrup (70% sucrose solution)	Winter store I (late autumn)		Spring honey (from control colonies)	
		from - to	mean	from - to	mean
Fructose	–	32.09 – 37.41	34.61 a*	35.22 – 35.59	35.31 a
Glucose	–	28.29 – 30.92	29.33 a	27.52 – 37.41	31.43 a
Sucrose	69.14	1.13 – 3.89	2.48 b	0.08 – 1.32	0.68 a
Maltose	–	1.34 – 2.20	1.86 b	0.47 – 1.84	1.12 a
Isomaltose	–	0.32 – 0.52	0.41 a	0.21 – 0.73	0.51 a
Turanose	–	1.62 – 3.14	2.52 a	1.06 – 3.07	1.92 a
Erlose	–	1.81 – 4.58	3.34 b	0.15 – 0.88	0.55 a
Maltotriose	–	0.03 – 0.08	0.05 a	0.03 – 0.07	0.05 a
Melezitose	–	0.08 – 0.10	0.09 a	0.04 – 0.14	0.09 a
Total	69.14	66.97 – 81.37	74.69 a	67.87 – 74.62	71.66 a
F/G	–	1.13 – 1.25	1.18 a	0.95 – 1.30	1.12 a
M/IM	–	3.95 – 5.35	4.54 b	1.87 – 2.53	2.20 a

a, b\*- significant differences between means in lines at  $\alpha=0.05$ .

Table 2

Sugar composition of the winter store sampled in early spring vs. that of the first honey recovered from the same colonies (%).

Sugar content:	Syrup (70% sucrose solution)	Winter store II (early spring)		Spring honey (from the same colonies)	
		from - to	mean	from - to	mean
Fructose	–	31.54 – 33.82	32.71 a	30.33 – 42.44	36.75 a
Glucose	–	26.92 – 28.93	27.66 a	26.42 – 29.55	27.61 a
Sucrose	69.14	1.85 – 6.22	3.94 b	0.16 – 1.90	0.69 a
Maltose	–	1.69 – 2.33	1.94 a	1.34 – 2.30	1.75 a
Isomaltose	–	0.46 – 0.74	0.54 a	0.65 – 0.76	0.70 b
Turanose	–	0.47 – 4.03	2.69 a	2.61 – 3.61	3.07 a
Erlose	–	2.83 – 5.20	3.87 b	0.63 – 0.95	0.85 a
Maltotriose	–	0.08 – 0.20	0.13 a	0.06 – 0.69	0.32 a
Melezitose	–	0.12 – 0.20	0.15 a	0.13 – 0.20	0.17 a
Total	69.14	72.10 – 78.63	73.64 a	63.79 – 78.06	71.91 a
F/G	–	1.09 – 1.26	1.18 a	1.15 – 1.52	1.33 a
M/IM	–	2.69 – 4.75	3.59 b	2.06 – 3.03	2.50 a

a, b\*- significant differences between means in lines at  $\alpha=0.05$ .

Table 3

Sugar composition of enzymatically inverted syrup (A) and of the winter store bee-processed from that syrup vs. that of honey (%).

Sugar content:	Syrup A (enzymatic hydrolysis of sucrose)	Winter store I (late autumn)		Spring honey (from control colonies)	
		from - to	mean	from - to	mean
Fructose	22.57	32.95 – 40.73	36.21 a	35.22 – 35.59	35.31 a
Glucose	17.15	28.70 – 32.22	30.17 a	27.52 – 37.41	31.43 a
Sucrose	34.19	3.50 – 5.38	4.17 b	0.08 – 1.32	0.68 a
Maltose	–	1.18 – 2.46	1.96 b	0.47 – 1.84	1.12 a
Isomaltose	–	0.16 – 0.40	0.32 a	0.21 – 0.73	0.51 a
Turanose	–	0.34 – 2.68	1.83 a	1.06 – 3.07	1.92 a
Erlose	–	0.35 – 2.26	1.29 a	0.15 – 0.88	0.55 a
Maltotriose	–	0.02 – 0.08	0.05 a	0.03 – 0.07	0.05 a
Melezitose	–	0.04 – 0.14	0.09 a	0.04 – 0.14	0.09 a
Total	73.91	72.82 – 81.73	76.09 a	67.87 – 74.64	71.66a
F/G	1.32	1.10 – 1.30	1.20a	0.95 – 1.30	1.12 a
M/IM	–	5.10 – 7.38	6.13 b	1.87 – 2.53	2.20 a

a, b\*- significant differences between means in lines at  $\alpha=0.05$ .

Table 4

Sugar composition of enzymatically inverted syrup (A) and of the winter store bee-processed from that syrup vs. that of honey (%).

Sugar content:	Syrup A (enzymatic hydrolysis of sucrose)	Winter store II (early spring)		Spring honey (from the same colonies)	
		from - to	mean	from - to	mean
Fructose	22.57	34.27 – 40.07	37.41 a*	31.13 – 40.64	37.47 a
Glucose	17.15	27.17 – 32.74	29.37 a	25.33 – 27.16	26.47 a
Sucrose	34.19	0.04 – 2.18	1.14 a	0.13 – 0.89	0.63 a
Maltose	-	0.65 – 1.96	1.02 a	1.38 – 1.97	1.75 a
Isomaltose	-	0.20 – 0.28	0.23 a	0.47 – 0.79	0.64 b
Turanose	-	0.26 – 2.15	1.15 a	2.39 – 4.19	3.13 b
Erlose	-	0.17 – 2.79	0.86 a	0.58 – 1.38	1.12 a
Maltotriose	-	0.03 – 0.06	0.04 a	0.19 – 0.76	0.41 a
Melezitose	-	0.12 – 0.12	0.12 a	0.14 – 0.20	0.17 b
Total	73.91	65.60 – 78.26	71.34 a	62.30 – 76.07	71.79 a
F/G	1.32	1.11 – 1.44	1.27 a	1.23 – 1.52	1.42 a
M/IM	-	2.33 – 7.84	4.43 a	2.49 – 3.00	2.74 a

a, b\*- significant differences between means in lines at  $\alpha=0.05$ .

Table 5

Sugar composition of enzymatically inverted syrup (B) and of the bee-inverted winter store vs. that of honey (%).

Sugar content:	Syrup B	Winter store II (late autumn)		Spring honey (from control colonies)	
		from - to	mean	from - to	mean
Fructose	22.65	31.89 – 35.53	32.91 a	35.22 – 35.59	35.31 a
Glucose	23.66	28.98 – 33.73	31.18 a	27.52 – 37.41	31.43 a
Sucrose	26.12	2.24 – 4.32	3.15 b	0.08 – 1.32	0.68 a
Maltose	–	1.68 – 2.33	1.97 b	0.47 – 1.84	1.12 a
Isomaltose	–	0.23 – 0.44	0.34 a	0.21 – 0.73	0.51 a
Turanose	–	1.61 – 2.17	1.89 a	1.06 – 3.07	1.92 a
Erlöse	–	0.61 – 1.40	0.96 a	0.15 – 0.88	0.55 a
Maltotriose	–	0.02 – 0.05	0.03 a	0.03 – 0.07	0.05 a
Melezitose	–	0.05 – 0.06	0.05 a	0.04 – 0.14	0.09 a
Total	72.43	68.72 – 79.02	72.48 a	67.87 – 74.64	71.66 a
F/G	0.96	0.98 – 1.12	1.06 a	0.95 – 1.30	1.12 a
F+G	46.31	60.87 – 69.26	64.09 a	62.52 – 73.00	66.74 a
M/IM	–	4.80 – 7.48	5.79 b	1.87 – 2.53	2.20 a

a, b\*- significant differences between means in lines at  $\alpha=0.05$ .

maltose) was formed from sucrose and maltotriose was formed from maltose. Those reports were also confirmed in the study carried out towards the end of the 80's in Canada by a team of investigators (Low et al. 1986, Low and Sporns 1988, Low et al. 1988) who determined the carbohydrate composition of nectars and honeys processed there from using gas chromatography, a much more accurate method than those used in earlier studies. They detected erlose only when in the nectar or in the solution prepared in the laboratory from various sugars sucrose was formed as a result of the action of pharyngeal gland enzymes.

Characteristic of the carbohydrate makeup of the inverts which were bee-processed from sucrose solution is an erlose content of several percentage points. The

trait is characteristic of "sugar honeys". A significantly higher sucrose content as compared to that of honey was also found in those inverts. The latter, however, exceeded 5% only occasionally. On average, it was 2.48 and 3.94% and in honey 0.68 and 0.69%, respectively.

In the current standards for honey admissible sucrose content have remained at 5% in the case of nectar honeys. Even though methods have been introduced which allow the determination of actual sucrose content "sugar honey" (in the case of sugar beet sucrose being used) will not be disqualified by a requirement laid down in this manner.

In Tables 3, 4 and 5 the results concerning carbohydrate content of commercial sucrose syrups (syrups A and B) and in the bee-processed inverts are listed. As was in

the case of the winter stores processed by bees from sucrose solution when the mixture made up of three solutions – fructose, glucose and sucrose (in the ratio of ca. 2:2:3) the total content of which was ca. 70% – was treated with bee enzymes a product arose which was similar in carbohydrate composition to honey. Significant differences between honey and the invert were those for sucrose and maltose content and for maltose – to – isomaltose ratio (Tables 3 and 5). In the spring the samples under comparison (store II vs. honey – Table 4) contained significantly less of some oligosaccharides – isomaltose: 0.23 in the store and 0.64 in honey, and turanose: 1.15 and 3.13%, respectively. In all winter store samples the ratio of sucrose to maltose was close to 1 or higher. Instead, in the honey under comparison the respective ratios were: 0.6, 0.4, 0.36 averaging 0.46. Sucrose to maltose ratio seems to be a good distinguisher to identify inverts processed by bees from sucrose syrups. Calculated from the results prepared for publication concerning the carbohydrate contents of unifloral honeys (60 samples) sucrose to maltose ratio was even lower and varied from 0.1 to 0.24 averaging 0.21. In the winter store samples it ranged from 1.1 to 2.1 and averaged 1.6. The observation requires confirmation using broader experiment material, specifically using a higher number of samples of winter stores bee-processed from sucrose syrups and examined for their carbohydrate content.

### CONCLUSIONS

1. The use of capillary gas chromatography to assay honey and bee-processed inverts for carbohydrate composition allowed the determination of a few important differences between the products compared.
2. The differences for carbohydrate composition between the bee-pro-

cessed 70% sucrose solution and honey were those for erlose and sucrose content and for maltose to isomaltose ratio (M/IM).

3. The ratio of sucrose to maltose content may provide a distinguisher for the identification of bee-processed inverts. In all winter store-derived samples the ratio was close to 1 or higher. Instead, in the honey samples the ratio did not exceed 1 and averaged 0.46. The observation needs to be confirmed using a broader experiment material, and specifically using a higher number of samples of winter stores bee-processed from sucrose syrups and examined for their carbohydrate content.
4. In the current standards for honey admissible sucrose content has remained at 5% in the case of nectar honeys. A product processed by bees from sugar (sugar beet sucrose) will not be disqualified by a requirement laid down in this manner. An erlose content of several percent may be a characteristic trait and may provide a good distinguisher of those inverts.

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## BADANIA CHROMATOGRAFICZNE (GC) SKŁADU CUKRÓW W MIODACH I ZAPASACH NA ZIMĘ WYTWORZONYCH PRZEZ PSZCZOŁY Z SYROPÓW SACHAROZOWYCH

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

Celem badań było porównanie składu cukrów w miodzie z ich składem w kilku syropach (karmach dla pszczół) przed i po ich przetworzeniu przez pszczoły i złożeniu w plastrach w postaci zapasu na zimę, oraz odpowiedź na pytanie - w jakim stopniu metoda chromatografii gazowej (GC) oznaczania zawartości poszczególnych węglowodanów identyfikuje „miód z cukru” i inne namiastki tego produktu wytworzone za pomocą enzymatycznej hydrolizy sacharozy do cukrów prostych. Materiał do badań stanowiły: próbki gotowych inwertów z dwóch firm (syrop A i B) oraz 70% roztwór sacharozy (syrop z cukru) tradycyjnie podawany pszczołom jesienią jako pokarm na zimę. Zawartości poszczególnych cukrów w wytworzonych

z karm zapasów na zimę zostały porównane z zawartościami tych cukrów w próbkach miodu wiosennego z tych samych rodzin.

Zastosowanie kapilarnej chromatografii gazowej do oznaczania składu węglowodanów w miodzie i w inwertach (po ich przetworzeniu przez pszczoły) pozwoliło na odnalezienie w porównywanych produktach kilku istotnych różnic. Różnice w zawartościach węglowodanów pomiędzy przetworzonym przez pszczoły 70% roztworem sacharozy a miodem dotyczyły: zawartości erlozy, zawartości sacharozy i stosunku zawartości maltozy do izomaltozy (M/IM).

Zauważono, że wyróżnikiem przy identyfikacji inwertów wytworzonych przez pszczoły z syropów sacharozowych może być także stosunek zawartości sacharozy do zawartości maltozy. We wszystkich próbkach zapasów stosunek ten był bliski jedności lub wyższy. Natomiast w porównywanych miodach nie przekroczył jedności, a średnio wynosił 0,46. Obserwacja ta wymaga potwierdzenia na szerszym materiale doświadczalnym, zwłaszcza większej liczbie przebadanych pod względem składu węglowodanowego próbek zapasów z syropów sacharozowych. W aktualnych dokumentach normalizacyjnych wymaganie dotyczące dopuszczalnej zawartości sacharozy pozostało w przypadku miodów nektarowych na poziomie 5%. Produkt wytworzony przez pszczoły z cukru (sacharoza z buraka cukrowego) przy pomocy tak ustawionego wymagania nie zostanie zdyskwalifikowany. Cechą charakterystyczną i wyróżnikiem tych inwertów może być kilkuprocentowa zawartość erlozy.

**Słowa kluczowe:** Miód, syrop sacharozowy, węglowodany, kapilarna chromatografia gazowa, erloza, zafałszowanie, identyfikacja.