

## CHANGES IN THE CARBOHYDRATE COMPOSITION OF HONEY UNDERGOING DURING STORAGE

Helena Rybak-Chmielewska

Research Institute of Pomology and Floriculture, Apiculture Division, Department of Bee Products, 24-100 Puławy, Kazimierska 2, Poland. E-mail: helena.chmielewska@man.pulawy.pl

*Received 05 November 2006; accepted 25 February 2007*

### S u m m a r y

Changes undergoing in the composition of honey during a half-year storage time of that product at temperatures of  $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$  were determined. The samples were also checked for any possible changes that could have been brought about by the thermal stabilization treatment which was performed according to the standard PN-88/A-77626 „Miód pszczeli” (Honeybee Honey) at a temperature of  $100^{\circ}\text{C}$  (in a boiling water bath) for 15 minutes.

The study indicates that a honey sample can be protected against changes in carbohydrate contents for half a year by storing it in a refrigerator (in this particular experiment at  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ). At room temperature, during a half-year storage period of honey samples the greatest changes were recorded in sucrose content. Sucrose content dropped by as much as 79% compared to its initial value. Thermal stabilization process itself did not bring about any change in the content of the sugars tested. The stabilization of honey samples to be assayed for sugar contents proved to be warranted if the samples were to be exposed to uncontrolled ambient temperatures during storage and transport.

Based on the results obtained the preparation of the reference material from honey stabilized and stored at a temperature of  $4^{\circ}\text{C}$  for up to 24 weeks was adopted for the HPLC - based research protocol to determine sugar contents.

Storage of honey samples to be assayed for sugar contents and as well as that of check samples should be also performed at a low temperature (of ca.  $4^{\circ}\text{C}$ ).

A prerequisite to obtain comparable results of sugar content assays of honey is to observe constant and low temperatures during storage and transport of honey samples to be tested. It is of particular importance when samples for sucrose assays are handled. The assays of fructose, glucose, sucrose, turanose, maltose, isomaltose, trehalose and melezitose with erlose were run using HPLC with a refractometer detector according to Bogdanov et al. (1997).

**Keywords:** honey, carbohydrate, thermal stabilization, storage, reference material, changes.

### INTRODUCTION

Of the studies concerning changes in carbohydrate contents of honey under different storage conditions the reports by White et al. (1961, 1964) and by White (1980) are particularly informative. According to those reports in various unifloral honeys stored for two years at  $23^{\circ}\text{C}$  to  $29^{\circ}\text{C}$  ca. 9% of monosaccharides were converted to oligosaccharides. Those investigators also observed that glucose declined faster than fructose. In other reports of

1964 and 1980 White and his associates dealt with the problem of 5-hydroxymethylfurfural (HMF) an aldehyde arising in honey under different storage conditions into which monosaccharides are gradually converted as the storage time increases, especially at temperatures of above  $20^{\circ}\text{C}$ . The problem was investigated, among others, by Curyło (1972), Skowronek et al. (1994), Rybak-Chmielewska and Szczęśna (1998), Szczęśna and Rybak-Chmielewska

(1999). Earlier reports were concerned also with changes in sucrose content (Bornus et al. 1966, Gontarski 1960, Fedorowska 1964, Čepurnoj and Artemiev 1981, Rybak and Achremowicz 1986). Bornus et al. (1966) reported on a rapid decline of sucrose content during the storage of acacia honey. Gontarski (1960) drew attention to adulterated honeys which, despite high initial sucrose content, showed an enzymatic activity high enough to break down that sugar down to a 5% level i.e. to a level that complied with the standard for that parameter. Fedorowska (1964) in her study on the stabilization of honey samples to be tested for sugar contents also confirmed a rapid process of the breakdown of that sugar in stored honey when conditions favoured enzymatic activity. Čepurnoj and Artemiev (1981) while testing a mixture of honey with winter stores processed by bees from sucrose (at a ratio of 1:1) found that as early as after 7 days of incubation at 36°C sucrose content conformed to the honey standard's requirements (did not exceed 5%). Similarly, in the study of Rybak and Achremowicz (1986) sucrose content declined from a dozen or so percent in the fresh material to a few percentage points after two months of storage at 20°C.

The objective of the study was to check if the conditions of the thermal stabilization of honey samples do not change the sugar profile of honey samples subjected to the treatment as well as to determine what changes occur in the sugar composition of honey during a half-year storage period of stabilized and non-stabilized samples of that product at temperatures of 20°C and ca. 4°C.

## MATERIAL AND METHODS

Preparation of the material for tests: In the autumn of 2002, from the experiment apiary a honey sample of ca. 1 kg was col-

lected and mixed with 1 kg of winter store after it had been deposited by bees from the syrup (sucrose : water at a ratio of 3 : 2) fed to them. The two ingredients were blended with a purpose to obtain a product of a higher sucrose content so that changes in sucrose content under different storage conditions could be more easily recorded and analyzed. From the starting material thus prepared four samples were subsequently formed. Two of them were subjected to stabilization treatment (in order to destroy the enzymes): the one was stored at a temperature of ca. 20°C, the other at ca. 4°C. The other two samples to be stored at respective temperatures of ca. 20°C and ca. 4°C (in the refrigerator) were left without thermal stabilization. Thus prepared material was kept under conditions as described for 24 weeks. In the samples of higher sucrose contents sugar assays were done after 3, 6, 12 and 24 weeks of storage. Alongside, under the same storage conditions sugars were analyzed in two honey samples: one of nectar and another of honeydew honey, the tests for carbohydrate contents being repeated after 12 and 24 weeks.

The tests included 6 samples of honey or of a fresh blended product, the assays being repeated after 12 and 24 weeks, in the blended product (4 samples) the assays being done also after 3 and 6 weeks and in 3 samples previously subjected to thermal stabilization treatment. Each analysis covered: fructose, glucose, sucrose, turanose, maltose, isomaltose, trehalose and melezitose with erlose.

Methods: Sugar content tests were performed using HPLC according to Bogdanov et al. (1997) on a high-pressure SHIMADZU liquid chromatograph equipped with LC-10ATVP liquid chromatograph pumps, DGU-14A degasser, CTO-10AVP column thermostat, RID-10A refractometric detector, POL-LAB CHROMA 2001 software and KROMASIL-NH2 HPLC (Phenomenex)

25 cm x 4,6 mm, 5  $\mu$ m chromatograph column. The amount of the sample injected onto the column was 20  $\mu$ l. The separation on the KROMASIL-NH<sub>2</sub> column was conducted at a temperature of 30°C with the acetonitrile : water (8:2) binary mobile phase at a flow rate of 1.3 ml/min. The identification of sugars in honey was done by comparing retention times of individual sugars in the standard vs. tested solution (qualitative analysis). The quantitative analysis was done by comparing peak surfaces of individual sugars in the standard solution against those in the tested sample. Stabilization of the samples was performed according to the PN-88/A-77626 „Miód pszczeli” (Honeybee Honey) protocol. The following thermal stabilization conditions were applied: honey sample weight 200 g, temperature (boiling water bath) – 100°C, time – 15 min.

## RESULTS AND DISCUSSION

The results of the sugar content assays of non-stabilized samples: fresh and after 3, 6, 12 and 24 weeks of storage at 4°C in the refrigerator at a room temperature of ca. 20°C are listed in Table 1. The trisaccharides erlose and melezitose did not separate under the analytical conditions applied. The greatest changes occurred in sucrose content. In the samples of the non-stabilized product the concentration of that sugar declined by an average of 14% from its initial value (from 2.3% to 1.9%) following 24 weeks of storage in the refrigerator. At a temperature of ca. 20°C, the decline was as much as by 79% (from 2.3% to 0.5%).

Apart from sucrose, melezitose with erlose (assayed jointly) was also decreased from the initial value of 3.3% to 2.7% at 4°C and to 2.5% at 20°C. The contents of other sugars were not altered to any major extent. They remained at a level close to the initial value throughout the testing period

i.e. for 24 weeks (isomaltose, trehalose) or their content increased by several to a dozen or so percentage points especially at a room temperature (e.g. fructose content increased by 4% compared to the initial value at 4°C and by 7% at 20°C, the increase of glucose content was by 1.1% and 8.8%, respectively, turanose rose by 10.5% and 31.6%, respectively). Maltose content decreased from 4.2% to 3.7% and 3.6% over the first 12 weeks and then rose to 4.1% and 3.9% over the next 12 weeks.

The sugar contents of stabilized samples collected from the same primary batch of the product with the same initial contents of the tested carbohydrates are shown in Table 2. The contents of individual sugars were determined once again following the thermal stabilization treatment and following 3, 6, 12 and 24 weeks of storage. The stabilization treatment alone did not alter the contents of particular sugars, the results after the treatment being only in a few cases lower by 0.1% (sucrose, trehalose, erlose with melezitose) or higher by 0.1% (turanose). Nor did the sugar composition of those samples undergo any changes over the whole 24-week period save for small variations which were most probably related to the accuracy of the method. In the stabilized product no major changes were found in the contents of the tested sugars when the samples were stored at temperatures of 4°C or 20°C, the maximum variations being by 0.2%. Thus stabilization of honey samples to be assayed for sugar contents can be recommended, especially if they have to be transported over long distances or stored under different conditions and assayed again, e.g. as counter samples. In Tables 3 and 4 the results are shown of nectar and honeydew honey samples analyzed for sugar contents after half-year storage period in the refrigerator at a temperature of ca. 4°C. In Table 3 the results of sugar contents of non-stabilized honeys and in Table 4 those of stabilized samples

are listed. At a temperature of ca. 4°C in the check samples a small content of sucrose (0.1%) was maintained at the same level over the whole testing period (half a year) regardless of whether the samples were stabilized or not, departures falling within the error limit of the method. In the case of the content of the remaining

disaccharides the variations were slightly greater and reached 0.5%.

Based on the results obtained it can be stated that the thermal stabilization treatment of the refrigerator-kept samples improved but very slightly the repeatability of results for the contents of analyzed sugars. It seems that in order to protect a honey sample for half a year against carbohydrate

Table 1

Sugars content of fresh (0 weeks), non-stabilized honey samples and after 3, 6, 12, 24 weeks of storage at temperature of 4 and 20°C.

Sugars content %	°C	Storage time (weeks)				
		0	3	6	12	24
Fructose	4	35.5	34.2	35.6	34.6	36.7
	20	35.5	34.8	36.2	36.2	38.0
Glucose	4	29.7	28.3	29.6	28.7	30.8
	20	29.7	28.8	30.2	30.0	32.3
Sucrose	4	2.3	2.1	2.0	1.9	1.9
	20	2.3	1.8	1.3	0.9	0.5
Turanose	4	1.9	1.8	1.9	1.8	2.1
	20	1.9	1.9	2.0	2.1	2.5
Maltose	4	4.2	4.0	4.0	3.7	4.1
	20	4.2	4.2	4.1	3.6	3.9
Trehalose	4	0.8	0.8	1.1	1.2	0.7
	20	0.8	0.8	1.1	1.4	0.9
Isomaltose	4	0.5	0.5	0.6	0.6	0.5
	20	0.5	0.6	0.6	0.7	0.6
Melezitose + Erlöse	4	3.3	3.1	3.2	3.2	2.7
	20	3.3	3.2	3.1	3.1	2.5
Total	4	78.2	74.8	78.2	75.8	79.5
	20	78.2	76.2	78.6	77.8	81.2
Fructose + Glucose (F+G)	4	65.1	62.5	65.3	63.3	67.5
	20	65.1	63.6	66.4	66.0	70.2
Fructose/Glucose (F/G)	4	1.2	1.2	1.2	1.2	1.2
	20	1.2	1.2	1.2	1.2	1.1

Table 2

Sugar contents (%) of stabilized honey samples and after 3, 6, 12, 24 weeks of storage at temperatures of 4 and 20°C.

Sugars	°C	Storage time (weeks)				
		0	3	6	12	24
Fructose	4	35.5	33.6	35.2	34.5	36.0
	20	35.5	34.0	35.3	34.7	36.0
Glucose	4	29.7	27.9	29.3	28.7	30.2
	20	29.7	28.2	29.4	28.8	30.0
Sucrose	4	2.2	2.1	2.2	2.1	2.1
	20	2.2	2.1	2.2	2.1	2.1
Turanose	4	2.0	1.8	1.9	1.8	2.1
	20	2.0	1.8	1.8	1.8	2.1
Maltose	4	4.2	4.1	4.0	3.5	4.3
	20	4.2	4.0	4.0	3.6	4.5
Trehalose	4	0.7	0.7	0.9	1.3	0.7
	20	0.7	0.9	1.0	1.1	0.6
Isomaltose	4	0.5	0.5	0.5	0.6	0.5
	20	0.5	0.5	0.6	0.5	0.5
Melezitose + Erllose	4	3.2	3.0	3.2	3.2	2.7
	20	3.2	3.2	3.2	3.3	2.6
Total	4	77.9	73.7	77.2	75.7	79.1
	20	77.9	74.7	77.5	75.9	78.4
Fructose + Glucose (F+G)	4	65.2	61.5	64.5	63.2	66.2
	20	65.2	62.2	64.7	63.5	66.0
Fructose/Glucose (F/G)	4	1.2	1.2	1.2	1.2	1.2
	20	1.2	1.2	1.2	1.2	1.2

content changes it is sufficient to store it at a temperature of ca. 4°C without the necessity to stabilize it.

The content of monosaccharides in non-stabilized samples stored at a temperature of 20 ±2°C dropped after 12 weeks (when compared to the initial value taken as 100%) by 1% for fructose and by 2.9% for glucose only to rise by 3.6% and 4.2%,

respectively, against the initial value (Table 5). Likewise, in the sample of honeydew honey fructose and glucose contents declined a little only to return to the initial values after the subsequent 12 weeks (fructose), or to exceed them a little (glucose). Somewhat greater changes occurred in the content of disaccharides of the nectar honey sample, especially for maltose the

Table 3

Sugar contents (%) of non-stabilized control honey samples and after 12 and 24 weeks of storage at a temperature of 4°C.

Sugars	Storage time (weeks)					
	Nectar honey			Honeydew honey		
	0	12	24	0	12	24
Fructose	38.7	38.1	39.3	32.9	32.0	32.8
Glucose	30.8	30.4	31.5	29.1	28.3	29.0
Sucrose	0.1	0.2	0.1	0.1	0.1	0.1
Turanose	1.7	1.7	1.8	1.8	1.8	1.8
Maltose	3.4	3.1	3.5	1.4	1.7	2.0
Trehalose	0.8	0.7	0.8	3.1	4.2	3.5
Isomaltose	0.8	0.8	0.8	0.9	0.9	0.8
Melezitose + Erlose	0.1	0.7	0.9	2.5	2.4	2.3
Total	76.3	75.7	78.7	71.8	72.4	72.3
Fructose + Glucose (F+G)	69.5	68.5	70.8	62.0	60.3	61.8
Fructose/Glucose (F/G)	1.3	1.3	1.2	1.1	1.1	1.1

Table 4

Sugar contents (%) of stabilized control honey samples and after 12 and 24 weeks of storage at temperature of 4°C.

Sugars	Storage time (weeks)					
	Nectar honey			Honeydew honey		
	0	12	24	0	12	24
Fructose	38.5	38.8	38.9	33.0	32.7	33.1
Glucose	31.3	31.0	31.1	29.7	29.0	29.3
Sucrose	0.2	0.1	0.1	0.1	0.1	0.1
Turanose	2.0	1.8	1.8	2.0	1.9	1.8
Maltose	4.0	3.3	3.5	1.4	1.6	1.6
Trehalose	1.0	1.3	1.0	3.2	4.3	4.0
Isomaltose	0.9	0.8	0.8	1.0	0.9	1.0
Melezitose + Erlose	0.2	0.7	0.7	2.4	2.3	2.5
Total	78.1	77.8	77.9	72.8	72.8	73.4
Fructose + Glucose (F+G)	69.8	69.8	70.0	62.7	61.7	62.4
Fructose/Glucose (F/G)	1.2	1.3	1.3	1.1	1.1	1.1

Table 5

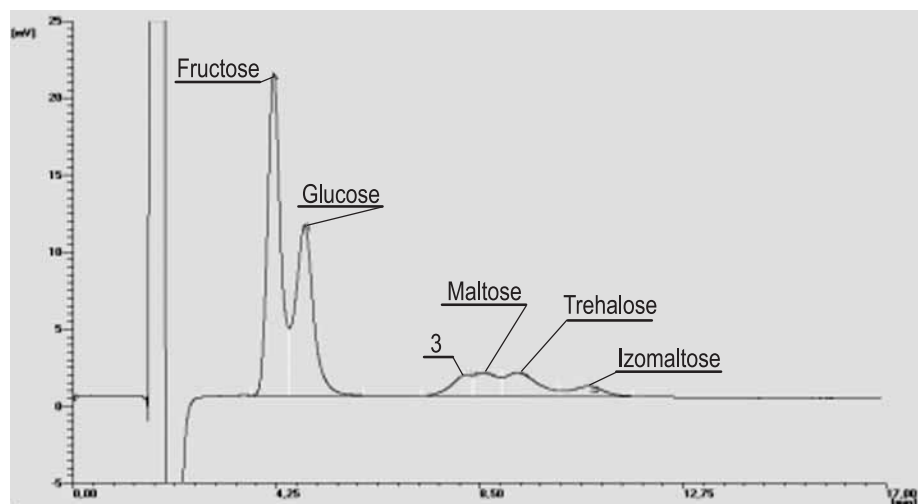
Sugar contents (%) of non-stabilized control honey samples and after 12 and 24 weeks of storage at temperature of 20°C.

Sugars	Storage time (weeks)					
	Nectar honey			Honeydew honey		
	0	12	24	0	12	24
Fructose	38.5	38.1	39.9	32.9	32.0	33.0
Glucose	31.3	30.4	32.6	29.1	28.3	29.7
Sucrose	0.2	0.2	0.0	0.1	0.1	0.1
Turanose	2.0	1.7	2.5	1.8	1.8	1.8
Maltose	4.0	3.1	2.4	1.4	1.7	1.5
Trehalose	1.0	0.7	0.8	3.1	4.2	3.0
Isomaltose	0.9	0.8	0.7	0.9	0.9	0.8
Melezitose + Erlose	0.2	0.7	0.2	2.5	2.4	2.0
Total	78.5	75.7	79.1	71.8	71.4	71.9
Fructose + Glucose (F+G)	69.8	68.5	72.5	62.0	60.3	62.7
Fructose/Glucose (F/G)	1.2	1.3	1.2	1.1	1.1	1.1

Table 6

Sugar contents (%) of stabilized control honey samples and after 12 and 24 weeks of storage at temperature of 20°C.

Sugars	Storage time (weeks)					
	Nectar honey			Honeydew honey		
	0	12	24	0	12	24
Fructose	38.5	38.8	38.9	33.0	32.7	33.1
Glucose	31.3	31.0	31.1	29.7	29.0	29.3
Sucrose	0.2	0.1	0.1	0.1	0.1	0.1
Turanose	2.0	1.8	1.8	2.0	1.9	1.8
Maltose	4.0	3.3	3.5	1.4	1.6	1.6
Trehalose	1.0	1.3	1.0	3.2	4.3	4.0
Isomaltose	0.9	0.8	0.8	1.0	0.9	1.0
Melezitose + Erlose	0.2	0.7	0.7	2.4	2.3	2.5
Total	78.1	77.8	77.9	72.8	72.8	73.4
Fructose + Glucose (F+G)	69.8	69.8	70.0	62.7	61.7	62.4
Fructose/Glucose (F/G)	1.2	1.3	1.3	1.1	1.1	1.1



**Fig. 1.** Chromatogram of sugars in 40-years old honey.

content of which dropped from 4.0% to 3.1% after 12 weeks and to 2.4% after 24 weeks which was 60% of the initial value. After 24 weeks no presence of sucrose was detected. The sugars in non-stabilized honeydew honey stored at room temperature proved to be more stable although also in that sample variation could be observed – upward after 12 weeks and downward after 24 weeks of storage (Table 5). In Table 6 results are listed of sugar contents in the same samples and at the same temperature of ca. 20°C but the samples involved had been subjected to stabilization treatment. Variation occurred both for the contents of monosaccharides and disaccharides which can be partly explained by the accuracy of the method itself and probably also by non-enzymatic processes occurring in samples of stored honey. With the storage times lasting several years the effect of chemical reactions becomes still more conspicuous: the sample gets increasingly darker and its odour and flavour change. The changes which gradually but visibly change the organoleptic traits of the product belong to the group of reactions that cause non-enzymatic darkening of food-stuffs. Theoretically, they can be ranked

among the reactions first described by Maillard (Horubała 1975). The confirmation of such reactions occurring in honey can be provided by the sugar profile of a honey sample stored in a cellar for more than 35 years in a closed jar (Fig. 1). In nectar honey stored for such a long time there was only 14% of fructose, 8.8% of glucose, an unidentified peak no. 3 – ca. 2%, ca. 1.7% of maltose, 2.6% of trehalose and 1.8% of isomaltose. Sugars totalled 31% of the total weight whereas in fresh honeys they account for 70%. The HMF content was 870 mg/kg. It is possible that over all those years 40% of the sugars were converted, via 5-hydroxymethylfurfural, to complex compounds that were dark brown in colour.

## CONCLUSIONS

1. A honey sample can be protected against changes in carbohydrate content for half a year by storing it at a temperature of ca. 4°C.
2. During a half-year storage of honey samples at a temperature of ca. 20°C without their stabilization the greatest changes occurred in sucrose content.



- It decreased by as much as 79% compared to its initial value.
3. Stabilization treatment did not alter the contents of individual sugars in honey.
  4. During 24 weeks the composition of sugars in samples stored at 4°C was not altered, either.
  5. Stabilization treatment of honey samples intended to be assayed for sugar contents is warranted when long storage and transport at uncontrolled ambient temperatures is the case.
  6. Based on the results obtained the protocol developed to analyze sugars using HPLC included in-house preparation of a reference material of stabilized honey and storing of that material at a temperature of ca. 4°C for a period of 24 weeks.
  7. When stored for several weeks honey samples intended to be analyzed for sugar contents should be kept at a low temperature (ca. 4°C).
  8. In inter-laboratory tests, temperatures of transport and storage kept low and constant are prerequisite to obtain comparable results of sugar contents in honey. It is of particular importance in handling samples to be assayed for sucrose content.
- Čepurnoj I.P., Artemiev V. (1981) – Issledovanie sacharoz w miede. *Pčelovodstvo*, 4-5: 55-56.
- Fedorowska Z. (1964) – O stabilizacji próbek miodu. *Pszczeln. Zesz. Nauk.*, 7(1): 20-27.
- Gontarski H. (1960) – Fermentbiologische Studien an Honigen. *Z. f. Bienenforsch.*, 5(2): 30-39.
- Horubała A. (1975) – Podstawy Przechowywania Żywności. *PWN*. Warszawa 417.
- Polska Norma PN-88/A-77626 – Miód pszczeli (1998). *Dziennik Norm i Miar* nr 8, 1998, poz. 19. *Wydawnictwa Normalizacyjne Alfa*.
- Rybak-Chmielewska H., Szczęśna T. (1998) – Changes in the composition and properties of buckwheat honey during storage. *Pszczeln. Zesz. Nauk.*, 42(2): 69-70.
- Rybak H., Achremowicz B. (1986) – Zmiany w składzie chemicznym miodów naturalnych i zafałszowanych ziniwertowaną przez pszczoły sacharozą zachodzące podczas przechowywania. *Pszczeln. Zesz. Nauk.*, 30: 19-35.
- Skowronek W., Rybak-Chmielewska H., Szczęśna T., Pidek A. (1994) – Wpływ czynników opóźniających krystalizację miodu na jego jakość. *Pszczeln. Zesz. Nauk.*, 38: 75-83.
- Szczęśna T., Rybak-Chmielewska H. (1999) – Determination of hydroxymethylfurfural (HMF) in honey by HPLC. *Pszczeln. Zesz. Nauk.*, 43: 219-225.
- White J. W. Jr. (1980) – Hydroxymethylfurfural content of honey as an indicator of its adulteration with invert sugars. *Bee World*, 61(1): 29-37.
- White J. W. Jr, Riethof M. L., Kushnir I. (1961) – The composition of honey VI. The effect of storage on carbohydrates, acidity and diastase content. *J. Food Sci.*, 26: 63-71.
- White J.W.Jr., Kushnir I., Subers M.H. (1964) – Effect of storage and processing temperatures on honey quality. *Fd Technol.*, 18: 153-156.

## REFERENCES

- Bogdanov S. (1997) – Charakterisierung von Schweizer Sortenhonigen. *Agrarforsch.*, 4: 427-430.
- Bornus L., Kalinowski J., Zalewski W. (1966) – Produkcja i skład chemiczny miodu akacjowego w m. Cigacice. *Pszczeln. Zesz. Nauk.*, 10 (1-2-3-4): 113-130.
- Curyło J. (1972) – Zawartość 5-hydroksymetylofurfuralu (HMF) w polskich miodach pszczelich. *Pszczeln. Zesz. Nauk.*, 16: 147-151.

## ZMIANY W SKŁADZIE WĘGLOWODANÓW MIODU ZACHODZĄCE PODCZAS PRZECHOWYWANIA

Rybak - Chmielewska H.

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

Określono zmiany zachodzące w składzie węglowodanów miodu w czasie półrocznego przechowywania próbek tego produktu, w temperaturze  $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$  i  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Sprawdzono też, czy stabilizacja termiczna próbek miodu, którą przeprowadzono wg PN-88/A-77626 „Miód pszczeleli” - temperatura -  $100^{\circ}\text{C}$  (we wrzącej łaźni wodnej); czas – 15 minut, nie zmieniła zawartości cukrów w próbkach poddanych temu procesowi?

Przeprowadzone badania wskazują, że próbkę miodu na pół roku można zabezpieczyć przed zmianami zawartości węglowodanów, przechowując ją w lodówce, (w doświadczeniu  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ). W temperaturze pokojowej, w czasie półrocznego przechowywania próbek miodu największe zmiany odnotowano w zawartości sacharozy. Zawartość ta obniżyła się aż o 79% w stosunku do wartości początkowej. Sam proces stabilizacji termicznej próbek miodu nie zmienił zawartości badanych cukrów. Stabilizowanie próbek miodu przeznaczonych do oznaczania zawartości cukrów okazało się uzasadnione przy ich przechowywaniu i transporcie w niekontrolowanej temperaturze otoczenia.

Na podstawie uzyskanych wyników przyjęto dla procedury badawczej oznaczania cukrów metodą HPLC przygotowanie materiału odniesienia z miodu stabilizowanego i przechowywanego w temperaturze około  $4^{\circ}\text{C}$ , przez okres do 24 tygodni.

Kilkutygodniowe przechowywanie próbek miodu do badań na zawartość cukrów oraz kontrol próbek powinno być także prowadzone w niskiej temperaturze (około  $4^{\circ}\text{C}$ ).

Warunkiem uzyskania porównywalnych wyników zawartości cukrów w miodzie w badaniach międzylaboratoryjnych jest przestrzeganie jednakowej i niskiej temperatury przechowywania i transportu próbek miodu do badań porównawczych. Jest to szczególnie ważne przy postępowaniu z próbkami do badań na zawartość sacharozy. Badania zawartości cukrów fruktozy, glukozy, sacharozy, turanozy, maltozy, izomaltozy, trehalozy i melecytozy z erlozą przeprowadzono metodą HPLC z detektorem refraktometrycznym wg Bogdanova i in. (1997).

**Słowa kluczowe:** miód, węglowodany, stabilizacja termiczna, przechowywanie, materiał odniesienia, zmiany.