ATTEMPT TO ASSAY MALTODEXTRINS OCCURRING IN STARCH SYRUP AND IN WINTER STORES MADE BY BEES FROM THAT SYRUP

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Summary

A method was developed to make quantitative assays of maltodextrins: maltotetraose (Dp4), maltopentaose (Dp5), maltohexaoze (Dp6) and maltoheptaose (Dp7). The maltodextrins occur in starch syrup and in the winter stores processed by bees from that syrup. The method was checked for its suitability to detect honey adulteration with starch syrup additions. The precision and repeatability of the method when used for maltodextrins assays was satisfactory. The detectability threshold for the maltodextrins was 0.05%. Additionally, the tests of carbohydrate composition of crystallized winter store samples processed by bees from starch syrup were aimed at explaining substantial losses of colonies in apiaries fed with that syrup under the conditions of long winter of 2005/2006.

The HPLC device manufactured by Shimadzu with a refractometric detector and a column recommended to assay oligosaccharides Luna 5 µm NH_2 100Å 250 x 4.60 mm (Phenomenex) was used to assay maltodextrins. The 65 : 35 acetonitrile-water system was used as an eluent, flow rate 3 ml/min, analysis time – 10 min, temperature 40°C. Maltodextrin contents (%) were assayed based on the comparing peak areas obtained in the examined samples with those from the reference solution (external standard method). The method described above is not used to identify oligosaccharides characteristic of honey. It can be used to assess the extent to which honey was adulterated with centrifuged stores hoarded by the bees in the combs following their feeding with syrup or with the syrup itself. Using that method it is possible to detect as small an addition as 10% of starch syrup inverted by bees.

Following the analysis of carbohydrate composition of crystallized winter store samples it was established that high glucose content of the winter stores in apiaries which were fed that syrup was the major cause of substantial bee colony losses sustained under the conditions of the long winter of 2005/2006. The glucose content was as high as 38.0% with a relatively low fructose content (22.1%), what explains the crystallization of glucose in the honeycomb cells. An excessive load of maltodextrins in the bee rectum can also be a hazard for the wintering bees. In the samples an average maltodextrine content was ca. 2%, the method being used to assay only a part of those compounds: associations of 4 to 7 glucose molecules.

Keywords: Honey, starch syrup, maltodextrins, adulteration, method, HPLC, bee wintering.

INTRODUCTION

In Poland, for several years various types of starch syrup have been produced and utilized chiefly by food industry. They have been commonly referred to as starch or maltose syrups. Some of those syrups have risen the interest of beekeepers due to their relatively low price and a convenient, ready-to-use presentation in the form of solutions. Reports by German investigators (Ohe von der and Schönberger

2000, 2002; Liebig 2005) who were somewhat earlier to start investigations into the use of starch syrups in beekeeping indicate that of syrup can be used for winter feeding of bees. The investigators emphasized, however, that the studies (usually one-year long) did not provide sufficient basis to issue an unequivocal opinion on the suitability of such syrups. The long and severe winter of 2005/2006 in Poland seems to have largely confirmed those concerns. Samples of heavily crystallized stores processed by the bees from starch syrup fed to them in autumn of 2005 were sent to the Laboratory of Bee Products in Puławy with a note that the bees had been unable to use up the syrup during the winter.

The aim of the study was to develop a quantitative method to assay maltodextrins in samples sent to the laboratory with an idea to use that method and also to identify adulterations of honey with admixtures of starch syrups. The problem of methods to be used for verification of honey authenticity is still a current issue and the characteristic sugar pattern is mentioned in many reports and standards as helpful in that assessment (Low and Sporns 1988; Swallow and Low 1994; Low and South 1995; Bogdanov et al. 2002; Cotte et al. 2003; PN-88/A-77626; Codex Alimentarius Commission 2001). A full description of carbohydrate composition using the draft method to assay maltodextrins will allow the determination of the cause of substantial bee losses in apiaries during the long winter of 2005/2006.

MATERIAL AND METHODS

The material to develop and verify the malto-compounds assaying method was comprised of: 4 samples of starch syrup purchased by beekeepers from the same source (producer) and alongside with that (from the same beekeepers) 4 samples of crystallized winter stores (processed syrup that was stored in combs by the bees).

In addition, 2 honey samples with an admixture of syrup (9:1) were prepared in the laboratory. Analogous samples were prepared from honey and from the winter stores i.e. from the syrup processed by bees.

Water content of the samples was assayed using a refractometric method according to the standard PN-88/A-77626 „Miód pszczeli" (Honeybee honey).

Assays of mono- di- and trisaccharides in honey were made using HPLC according to Bogdanov et al. (1997) as modified by the authors (Rybak-Chmielewska and Szczęsna 2003).

Detection of starch dextrins (qualitative analysis) was made by precipitation with ethanol according to the standard PN-88/A-77626 „Miód pszczeli" (Honeybee honey): item 5.3.17. Detection of disqualifying traits.

In the study aimed at the development of the method to identify and assay maltodextrins the apparatus used was an HPLC manufactured by Shimadzu with a refractometric detector and a column recommended to assay oligosaccharides Luna 5 µm NH_2 100Å 250 x 4.60 mm (Phenomenex). The 65 : 35 acetonitrile-water system was used as an eluent, flow rate 3 ml/min, analysis time – 10 min, temperature 40°C. To make a reference solution maltodextrins made up of 4 to 7 glucose molecules were used: maltotetraose (Dp4), maltopentaose (Dp5), maltohexaose (Dp6) and maltoheptaose (Dp7), by preparing 1% solutions of each of them. Reference solutions of the maltodextrins thus prepared were mixed in equal parts and loaded onto the chromatograph column.

The quantitative assays of those compounds in syrup samples and in the winter stores processed thereof were made using the external standard method by preparing aqueous solutions of the samples using the

same protocol as that used to assay sugars in honey (Rybak-Chmielewska and Szczęsna 2003). After being passed through membrane filters 20 µl of each sample, solution was loaded onto the column. The contents of those compounds were assayed based on the comparing peak areas obtained in the examined samples with those from the reference solution.

The data obtained were subjected to one-way ANOVA. Duncan's test was used to measure significance of differences between means at α=0.05.

RESULTS AND DISCUSSION

The precision and repeatability of the method as applied to maltodextrins was satisfactory. Under chromatograph separation conditions as described above the following average retention times were obtained: for Dp4 – 2.600 min with variation coefficient of 0.05%; for Dp5 – 2.540 min with variation coefficient of 0.05%; for Dp6 – 2.836 min with variation coefficient of 0.04% and for Dp7 – 3.170 min with variation coefficient of 0.06%. The chromatogram of maltodextrin separation was shown in Fig. 1.

The variation coefficient of the assays of the individual maltodextrins was 2.16; 0.94; 1.56 and 2.39%, respectively. The detection threshold for those maltodextrins was 0.05%.

Examples of chromatograms of maltodextrin separation in the syrup and in the stores are shown in Fig. 2 and 3. Apart from the identified associations of 4 to 7 glucose molecules there were other compound peaks in the samples of increasingly longer chains – probably made up of 8, 9, 10 etc. molecules of the monosaccharide. The lack of suitable reference maltodextrins made their identification impossible. Nor were any assays made of isomaltoglucose associations such as isomaltotetraose, isomaltopentaose etc. in which ring forms of D-glucose are bound with 1-6 rather than 1 – 4 glicoside bonds. None-the-less, in starch syrup samples and in the winter stores processed from those syrups four-molecule and higher glucose associations are numerous enough (3.29% in the syrup, 2.02% in the stores) to lend themselves to be used as indicators in honey authenticity studies (Table 1). The above described method is not used to identify oligosaccharides characteristic of honey. Fig. 4 shows an example chromatogram of a honey sample of guaranteed quality from the monitored apiary of the Apiculture Division. The identification of the malto-compounds under investigation and their quantitative assay is possible even following 10-fold dilution of syrup (Fig. 5) and of the stores (Fig. 6) samples of honey. At that dilution the identification of dextrins using the qualitative method according to the standard PN-88/A-77626 „Miód pszczeli" (Honeybee honey) was already unreliable and in the case of honeydew honeys – unfeasible.

The percent composition of the maltodextrins in the syrups and in the stores processed thereof was shown in Table 1 along with the content of other carbohydrates. The comparison of the makeup of those products before and after the inversion by the bees furnishes an answer to the question: to what extent are the secretions of bee pharyngeal glands capable of breaking down starch syrup into monosaccharides and why did the post-hydrolytic product undergo crystallization? Another question is whether the enzymes are capable of poly-carbohydrate hydrolysis, especially the hydrolysis of malto-compounds occurring in α-dextrin?

Based on the comparison of the data on the carbohydrate contents before and after the syrup was processed by the bees it can be said that there was a six-fold increase in fructose content, from 3.7 to 22.1% on average, and near two-fold increase in the

content of the other simple sugar – glucose – from 22.2 to 38.0%. It occurred primarily at the expense of the hydrolysis of disaccharides – of sucrose (27% of which in the syrup was reduced to less than 5% in the stores) and of maltose (from 18% down to 8%). Unlike in the syrup, in the stores small amounts of other disaccharides were identified – 0.7% of turanose and trehalose and 0.5% of isomaltose. Given the suitable conditions, the pharyngeal gland enzymes: α- and β-amylase, maltase and invertase (including the transglucosidase nature of maltase activity) are theoretically capable of breaking down all bonds not only between di- and polysaccharides (Kubota et al. 2004, Takenaka 1980, Serra Bonvehi et al. 2000) by forming transitionally small amounts of other accidental di- and trisaccharides. From the report of Hrassnigg et al. (2005) it appears that worker bees, as opposed to drones, can use soluble starch as the energy source to fly. According that investigator, it gives evidence that the enzyme α-amylase is also in the gland secretion of older worker bees and that those bees are capable of using it to generate energy. They hydrolyze very long chains of amylose and amylopectin into glucose. Amylose has no ramifications and is made up of glucose subunits that are bound together with α-1,4-glycoside bonds. Amylopectin is a ramified form of starch in which there is one α-1,6-glycoside bond per ca. thirty α-1,4-glycoside bonds (Stryer 2003). An essential problem is to preserve conditions that promote the activity of those enzymes. However, there is a contradiction. Under optimum conditions for the hydrolysis of the carbohydrates sucrose and maltose there occurs such a high concentration of glucose that the sugar undergoes crystallization in the combs. The critical saturation point of glucose in the solution is 32 g/100g (Ohe von der and Schönberger 2000). The average glucose content of the winter stores was as much as 38% and the fructose to glucose ratio was 0.60. It accounts for the crystallization of the sugar in the comb cells.

From the data in the table under discussion it becomes evident that the individual malto-compounds underwent a significant reduction in the stores (by ca. 1/3).

However, some of those compounds may be an excessive burden for the bee rectum. At the same time, conditions favouring the activity of the enzymes which break down the 1-4 glycoside bond will also promote the decomposition of the disaccharide maltose and increase the content of glucose. In the winter stores samples examined in this study there was still ca. 2% of assayed 4-7-molecule associations of glucose, whereas more complex saccharides and maltotriose were not determined. Some of those associations may be difficult to digest by the digestive tract of bees. It is thus a second hazard for bees wintering on the starch syrup. A long winter and no possibility to make foraging flights may be a cause for high colony losses. In this particular case, the losses will be caused by an excessive load of the rectum in bees wintering on that food.

CONCLUSIONS

1. The method to assay maltodextrins described in this study can be used to evaluate the extent to which honey was adulterated with centrifuged winter stores from starch syrup or with the syrup per se. Using that method an addition of the syrup as low as 10% can be detected.
2. Under the climatic conditions of this country where long and severe winters recur every several years wintering of bees on the starch syrup of the makeup as described in this study carries a high risk of bee losses.

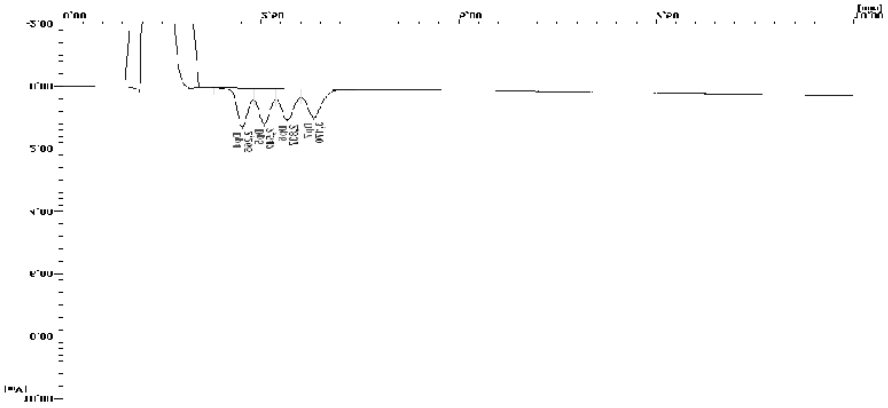

Fig. 1. Chromatogram of the reference maltodextrin solution (Dp4, Dp5, Dp6, Dp7).

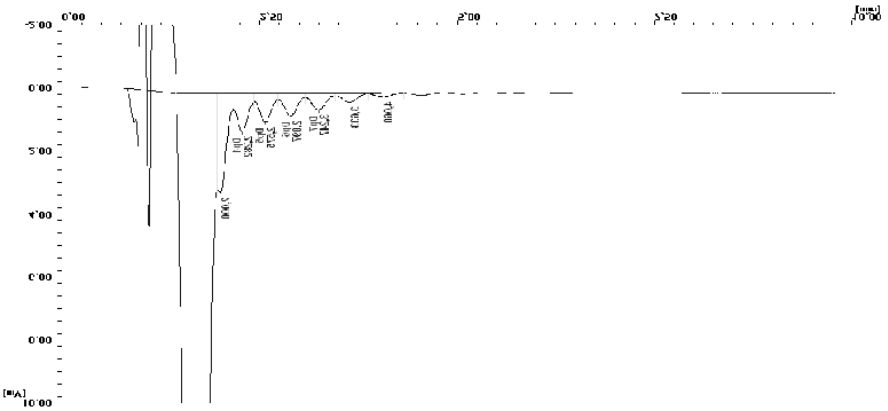

Fig. 2. Chromatogram of maltodextrins in starch syrup.

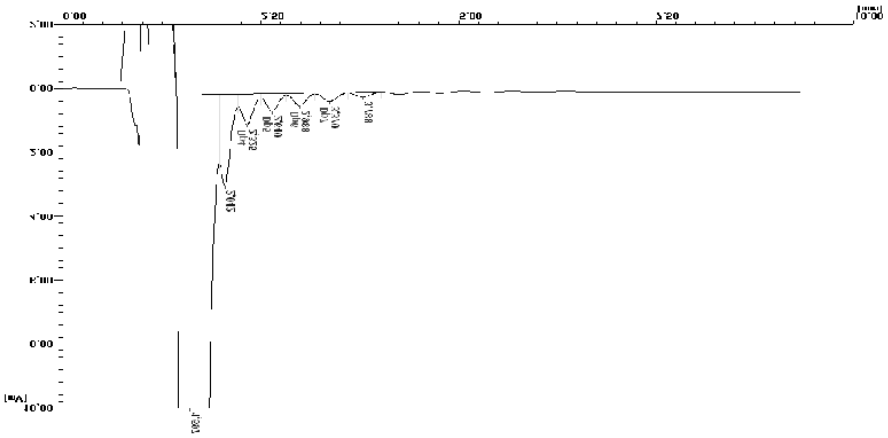

Fig. 3. Chromatogram of maltodextrins in crystallized winter store processed by the bees from starch syrup.

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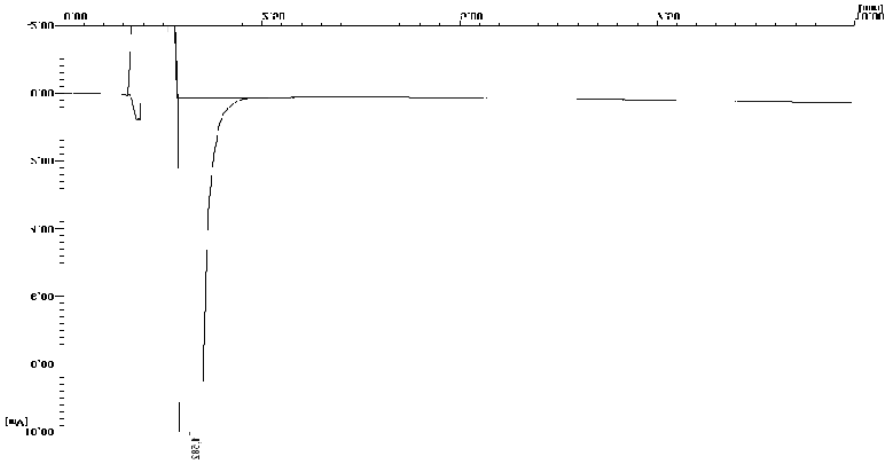

Fig. 4. Example chromatogram for honey (no maltodextrins).

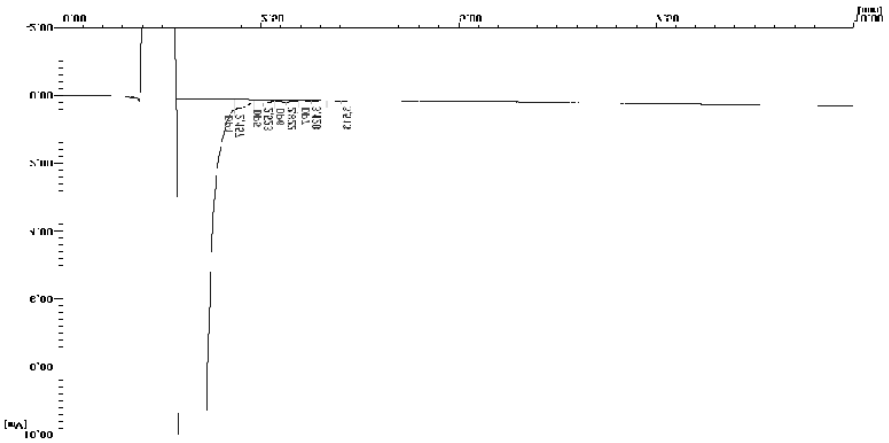

Fig. 5. Chromatogram of maltodextrins in syrup following dilution with honey (1:9).

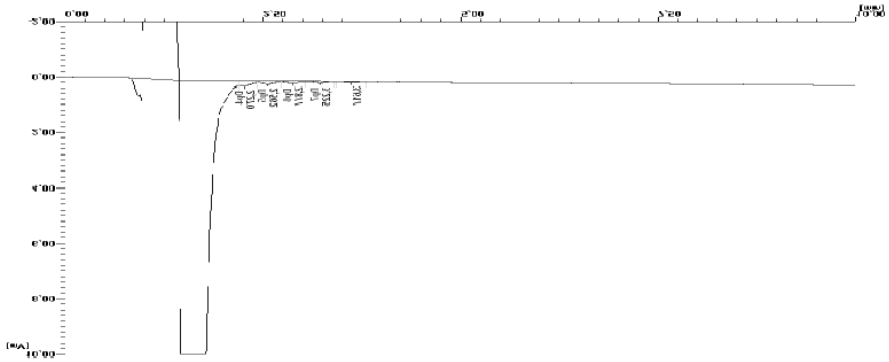

Fig. 6. Chromatogram of maltodextrins in winter store following dilution with honey (1:9).

Table 1

Carbohydrate contents of starch syrup and of winter stores made from that syrup by the bees.

	Content (%)	Starch syrup						Winter stores					
		1	2	3	4	SD	Mean	1	2	3	4	SD	Mean
1	Water	19.8	20	21.2	20	0.6	20.3 a*	14.2	14.8	20.8	17.8	3	16.9 a
2	Fructose	3.7	3.6	3.3	4.1	0.3	3.7 a	23.1	16.6	27.8	20.8	4.7	22.1 b
3	Glucose	22.4	22.4	22	22.2	0.2	22.2 a	39.3	42.7	30.9	39.1	5	38.0 b
4	Monosaccharides (G + F)	26.1	26	25.3	26.3	0.4	25.9 a	62.4	59.3	58.7	59.9	1.6	60.1 b
5	Fructose/Glucose	0.17	0.16	0.15	0.19	0.01	0.17 a	0.59	0.39	0.9	0.53	0.22	0.60 b
6	Sucrose	27.5	27.3	29.3	25.6	1.5	27.4 b	2.2	5.5	7.6	3.7	2.3	4.8 a
7	Turanose	nd	nd	nd	nd	-	0	0	0.6	1.4	0.9	0.6	0.7
8	Maltose	17.3	20.5	16.7	18.5	1.7	18.3 b	7.7	10.5	6	7.8	1.9	8.0 a
9	Trehalose	nd**	nd	nd	nd	-	0	1	0.6	0.6	0.6	0.2	0.7
10	Izomaltose	nd	nd	nd	nd	-	0	0.7	0.6	0.3	0.4	0.2	0.5
11	Raffinose and melezitose	nd	1.5	nd	nd	-	-	nd	1.2	0.3	0.8	0.5	0.6
12	Total saccharides	70.9	75.2	71.3	70.4	2.2	72.0 a	74	78.7	75	74.2	2.2	75.5 a
13	Dextrins												
a	Dp 4 (made up of 4 glucose molecules)	0.96	0.99	0.87	1.22	0.15	1.01 a	0.66	0.83	0.32	0.97	0.28	0.70 a
b	Dp 5 (made up of 5 glucose molecules)	0.82	0.86	0.72	1.07	0.15	0.87 b	0.57	0.67	0.23	0.67	0.21	0.54 a
c	Dp 6 (made up of 6 glucose molecules)	0.74	0.79	0.64	0.96	0.13	0.78 b	0.52	0.54	0.18	0.5	0.17	0.44 a
d	Dp 7 (made up of 7 glucose molecules)	0.61	0.67	0.53	0.71	0.08	0.63 b	0.37	0.55	0.13	0.35	0.17	0.35 a
e	Total Dp 4 to Dp 7	3.13	3.31	2.76	3.96	0.5	3.29 b	2.13	2.58	0.86	2.49	0.79	2.02 a

* a,b – differences statistically significant between means in rows at α=0.05.

** nd – below detectability threshold which for turanose, trehalose, raffinose and melezitose is 0.2%.

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PRÓBA ILOŚCIOWEGO OZNACZENIA MALTODEKSTRYN WYSTĘPUJĄCYCH W SYROPIE SKROBIOWYM I W ZAPASIE NA ZIMĘ WYTWORZONYM PRZEZ PSZCZOŁY Z TEGO SYROPU

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Streszczenie

Opracowano metodę ilościowego oznaczania maltodekstryn: maltotetraozy (Dp4), maltopentaozy (Dp5), maltohexaozy (Dp6) i maltoheptaozy (Dp7), występujących w syropie skrobiowym i w pokarmie na zimę, wytworzonym przez pszczoły z tego syropu. Sprawdzono jej przydatność do identyfikacji zafałszowań miodu domieszkami syropów skrobiowych. Obliczona precyzja i odtwarzalność metody w stosunku do oznaczanych maltodekstryn były

zadowalające. Wyznaczona granica wykrywalności tych maltodekstryn była na poziomie 0,05%. Ponadto badania składu węglowodanów próbek skrystalizowanego w plastrach zapasu wytworzonego przez pszczoły z syropu skrobiowego miały na celu ustalenie przyczyn dużych strat rodzin pszczół w pasiekach karmionych tym syropem w warunkach długiej zimy 2005/2006.

Do oznaczania maltodekstryn wykorzystano HPLC firmy Shimadzu z detektorem refraktometrycznym i kolumnę zalecaną do oznaczania oligosacharydów - Luna 5 µm NH_2 100Å 250 x 4,60 mm (Phenomenex). Jako eluent zastosowano układ acetonitryl : woda w stosunku 65 : 35; przepływ - 3 ml/min; czas analizy – 10 minut; temperatura 40°C. Zawartości maltodekstryn (w %) oznaczono na podstawie porównania powierzchni pików związków uzyskanych w próbkach badanych i w roztworze wzorcowym (metoda standardu zewnętrznego). Opisaną wyżej metodą nie identyfikuje się oligocukrów charakterystycznych dla miodu. Może być ona użyta do oceny stopnia jego zafałszowania odwirowanym zapasem złożonym przez pszczoły w plastrach, po podkarmieniu ich syropem skrobiowym lub też samym syropem. Za pomocą tej metody możliwe jest wykrycie w miodzie już 10% dodatku zinwertowanego przez pszczoły syropu skrobiowego.

Po przeprowadzeniu analizy składu węglowodanowego próbek skrystalizowanego zapasu ustalono, że główną przyczyną dużych strat rodzin pszczół w warunkach długiej zimy 2005/2006, w pasiekach karmionych tym syropem była wysoka zawartość glukozy w zapasach. Wynosiła ona aż 38% przy stosunkowo niskiej zawartości fruktozy (22,1%). Tłumaczy to jej krystalizację w komórkach plastrów. Zagrożeniem dla zimujących pszczół na omawianym syropie skrobiowym może być też zbyt duże obciążenie maltodekstrynami jelita prostego pszczół. W analizowanych próbkach średnia zawartość oznaczonych maltodekstryn wynosiła około 2%, a opracowaną metodą oznaczono tylko część tych związków - połączenia złożone z 4 do 7 cząsteczek glukozy.

Słowa kluczowe: Miód, syrop skrobiowy, maltodekstryny, zafałszowanie, metoda, HPLC, zimowanie pszczół.