ACTA ALIMENTARIA POLONICA

Vol. XVII/XLI/ No. 2 1991

ALINA KRAUZE
JERZY KRAUZE

CHANGES IN CHEMICAL COMPOSITION OF STORED HONEYDEW HONEYS*

Institute of Commodity Science
Academy of Economics, Poznań

Key words: honey sugars, acidity, invertase, glucose oxidase, catalase

Changes in sugars, acidity and activity of enzymes and other components were studies in honeydew honeys stored at room temperature for over two years. The glucose, fructose and saccharose contents decreased, while the content of disaccharides other than saccharose increased. Accumulation of lactone of gluconic acid was higher than of free acids. Glucose oxidase and catalase activities and the inhibin value as well as invertase activity decreased to a greater extent than diastase activity.

INTRODUCTION

White et al. [16] demonstrated considerable changes in chemical composition of stored honeys. Rybak and Achremowicz [10] recently described the effects of one-year storage on selected chemical and physical properties of natural and adulterated nectar honeys. In this research we studied the effect of two-year storage on honeydes honey, paying special attention to changes in contents of glucose, fructose, saccharose and other oligosaccharides, and also to the activity of enzymes, particularly glucose oxidase and catalase playing a major role in the bacteriostatic properties of honey.

MATERIAL AND METHOD

14 samples of unheated honeydew honey were obtained from the District Apiarian Cooperatives in Cracow and Poznań. Each 1-kg sample was divided into two portions placed in closed glass jars. One jar was stored at about -15°C pending analyses (fresh honey) and the other kept in darkness at room temperature for 26-30 months.

The contents of water, reducing sugars, apparent saccharose (saccharose + melezitose), 5-hydroxymethylfurfural (5-HMF), pH values, electric conductivity and diastase number were determined according to the Polish Norm

* Research supported by Project RPBP III.41/05.2.1.

PN-75/A-77626. The contents of free acids, gluconic acid lactone, and ash were determined using AOAC methods [1]. Invertase activity was determined by Siegnethaler's method [15], while glucose oxidase and catalase activities according to Schepartz and Subers [12, 14]. Before determinations of glucose oxidase and catalase activities, honey solutions were dialyzed against running tap water for ca. 20 h. The absence of glucose in the dialyzed honey was tested with Gluco Merckognost paper (Merck).

Glucose and fructose were determined with the specific enzymatic Test-Combination UV-Method (Boehringer). Di- and higher oligosaccharides were separated on Bio-Gel P2 -440 mesh (Bio-Rad) according to [9]. Saccharose content in the disaccharides fraction was determined using the glucose oxidase-peroxidase reagent to measure glucose before and after enzymatic hydrolysis of the fractions [1].

The antibacterial effect of honey was studied using the Dold and Witzenhausen test modified by Shade et al. [11]. The media with nutrient agar contained 4.5, 8.9, 13.1, 17.3 and 21.3% honey. Each plate was inoculated with 0.1 cm^3 suspension of *S. aureus* or *E. coli* prepared from 24-h cultures. Bacteria growth was checked after 24 h of incubation at 37°C.

RESULTS AND DISCUSSION

The effects of two-year storage at room temperature on sugars content in honeydew honeys are presented in Table 1. The water content decreased in the stored honey to various extents in different samples, and hence the contents of sugars and other components were expressed for dry substance before and after storage. As can be seen in Table 1, the content of reducing sugars increased while that of glucose and fructose decreased, with glucose losses exceeding those of fructose which led to changes in the ratio of these sugars (F/G). In contrast, the content of disaccharides (separated on a column with Bio-Gel P2) increased as a result of accumulation of disaccharides other than saccharose. The average content of saccharose determined by the specific enzymatic method in the disaccharides fraction decreased considerably but variously in different samples. For example, in one honeydew honey sample the initial saccharose content of 4.41% decreased to 0.39% while in others a small initial content (e.g. 0.60 or 0.88%) remained practically unchanged after storage (0.49 and 0.80% respectively). The relatively high mean percentage of changes in the content of saccharose determined by the specific enzymatic method was similar to the mean percentage of changes in apparent saccharose (saccharose + melezitose) measured as the increase of reducing sugars content after acid hydrolysis. The effect of storage of tri- and higher oligosaccharides contents was negligible.

In their studies of sugars content in honeys stored at room temperature for two years White et al. [16] observed decreases in the contents of glucose (13%) and fructose (5.5%) and a substantial increase of reducing disaccharides or "maltose" content (69%). In the honeydew honeys we studied the analogous

T a b l e 1. Changes in sugars contents in stored honeydew honeys

	Reducing sugars %	Glucose (G) %	Fructose (F) %	F/G	Total G + F	Disaccharides %	Disaccharides other than saccharose %	Saccharose %	Apparent saccharose %	Tri-and-higher oligosa charide %
Range before storage	72.92-88.20	27.91-33.16	29.93-40.47	0.98-1.30	57.84-71.68	5.33-12.77	4.59-12.26	0.35-4.41	2.56-12.87	1.82-6.22
Range after storage	75.19-91.13	25.63-31.96	28.50-39.91	1.00-1.30	57.71-70.50	7.50-15.58	7.06-15.03	0.35-1.06	0.86-5.56	1.80-6.30
Mean value before storage	82.84	30.82	34.40	1.12	65.21	7.86	6.84	1.02	5.84	3.99
Mean value after storage	85.15	28.87	33.20	1.15	62.07	10.54	9.93	0.61	3.12	3.93
% Change	+ 2.79	− 6.33	− 3.49	− 2.68	− 4.82	+ 34.10	+ 45.17	− 40.20	− 46.57	− 1.50

changes went in the same direction but the losses of glucose and fructose and the increments of disaccharides other than glucose were not as great. According to White et al. [16] there occur in stored honeys relatively high increases of small amounts of saccharose and higher oligosaccharides. Deifel et al. [5] found that during 10-month storage at 30°C of the products of feeding bees with sugar syrup there was a decrease of the content of saccharose and the trisaccharide erlose.

The changes in sugars composition during long storage of honey are seen as due to honey invertase (α-glucosidase). The activities of invertase and dastase decrease depending on temperature and time of storage [18, 19]. The decrease of invertase (SN) and diastase (DN) activities in shown in Table 2. In some samples DN did not changes, while in others it decreased by ca. 20 and over 30%. Invertase is more susceptible to temperature than diastase [18, 19]. Drops in invertase activity were observed in all samples of honeydew honey, and the mean percentage of changes was higher than for diastase. The correlation coefficent of diastase and invertase activities in fresh honeys was 0.7382 and it decreased to 0.6315 after storage. The correlation between the activities of these enzymes was lower than that reported for nectar honeys by White [18] and other authors [8].

Tabele 2. Effect of storage on distase and invertase activity in honeydow honey

Sample	Saccharose Number (SN)			Diastase Number (DN)		
	before storage	after storage	% of changes	before storage	after storage	% of changes
1	18.30	14.23	−22.24	10.9	8.3	−23.85
2	16.78	13.78	−17.88	17.9	17.9	0
3	10.76	9.66	−10.22	13.9	13.9	0
4	6.02	5.91	−1.83	17.9	17.9	0
5	22.12	13.58	−38.61	23.8	17.9	−24.79
6	29.39	21.54	−26.71	38.5	29.4	−23.64
7	37.83	19.18	−49.30	23.8	17.9	−24.79
8	0.68	0.49	−24.94	8.3	8.3	0
9	23.39	16.95	−27.53	23.8	23.8	0
10	31.85	20.17	−36.67	29.4	23.8	−19.05
11	28.61	19.96	−30.23	29.4	17.9	−39.12
12	0.95	0.76	−20.00	17.9	17.9	0
13	21.94	17.98	−18.05	29.4	23.8	−19.05
14	30.29	22.07	−27.14	29.4	23.8	−19.05
Mean	19.92	14.02	−29.62	22.45	18.75	−16.48

The initially low 5-HMF content (0.07-1.04 mg/100 g dry substance) increased substantially in stored honeys (to 0.59-3.69 mg/100 g). The increase was uneven however: in some samples it was over 30-fold while in others it was less than 2-fold. The accumulation of 5-HMF did not exceed the level accepted in standards for honey [4]. The effect of long storage on pH and electric

conductivity, important criteria in differentiating nectar and honeydew honeys, was negligible. Mean pH and electric conductivity values in fresh honeys were 4.45 and 9.19 $\times$ $10^{-4}S \times cm^{-1}$ respectively, and after storage — 4.43 and 8.95 $\times$ $10^{-4}S \times cm^{-1}$.

Free acids and lactone were greatly affected by storage, and their contents in stored samples were higher than in fresh honeydew honeys (Table 3). Lactone accumulation was different in various samples but its mean increment was higher than free acids increment. The content of lactone in fresh honeys was not correlated with this content after storage. On the other hand, a significant correlation ($r = 0.9570$) was found between the free acid content before and afer storage. The principal acid in honey is gluconic acid, and its lactone is due to the action of glucose oxidase [17-19]. The contents of both free acids and lactone was not correlated with glucose oxidase activity in fresh and stored honeys.

Table 3. Effect of storage on acidity of honeydew honey

	Free acids miliequiv./kg	Lactone miliequiv./kg	Total acidity miliequiv./kg
Range of content before storage	28.63–59.60	4.86–10.47	34.57–69.01
Range of content after storage	36.79–71.21	9.57–14.80	48.43–85.91
Mean value before storage	42.63	7.40	50.03
Mean value after storage	38.37	11.83	60.21
% Change	+13.46	+59.86	+20.33

The properties of glucose oxidase and catalase and the colorimetric method of their determination were described by Scheparts and Subers [12-14]. These methods were used to determine the activities of both enzymes in the studies honeydew honeys. In fresh honeys these activities varied greatly (Table 4). Schepartz and Subers [14] quote a similar range of catalase activity (0-17.8 $k_f \times 10^{-3}$) in American honeys. In European heather and rhododendron honeys catalase activity amounted to 119-241 $k_f \times 10^{-3}$) g/min [6]. There are no reports in the literature of glucose oxidase activity determinations in Polish honeys. The catalase activity in Polish honeys was determined by the titration method and the obtained results cannot be compared with the activity expressed by Schepartz and Subers as k_f. After over two years of storage the glucose oxidase and catalase activities in honeydew honey decreased considerably. As can be seen in Table 4, in several samples (4, 8 and 12) the low initial glucose oxidase activity was practically unaffected by storage.

It is by now clear that inhibin, an antibacterial agent, is connected with the presence of hydrogen dioxide in honey. The products of glucose oxidation by glucose oxidase are gluconic acid and hydrogen dioxide which is decomposed by the catalase present in honey [17]. The changes in glucose oxidase and catalase activities were compared with changes of antibacterial properties of the stored honeydef honeys. Using the modified Dold's microbiological test we assessed the

Table 4. Effect of storage glucose oxidase, catalase and inhibin activitin honeydew honey

Sample	Glucose oxidase mU/g			Catalase $k_f \times 10^{-3} \times g^{-1} \times min^{-1}$			Inhibin value	
	1985	1988	% Change	1985	1988	% Change	1985	1988
1	138.1	47.9	- 65.31	13.07	9.87	- 24.48	3	1
2	206.5	88.5	- 57.14	20.49	10.97	- 46.46	4	2
3	105.2	90.5	- 13.97	11.71	7.91	- 32.45	5	2
4	43.4	43.8	+ 0.92	0	0	0	2	1
5	94.7	80.4	- 15.10	18.85	9.01	- 52.20	3	1
6	262.4	253.8	- 3.28	26.19	10.40	- 60.29	4	3
7	263.0	105.1	- 60.04	14.83	10.83	- 26.97	4	3
8	28.3	25.5	- 9.89	12.48	6.60	- 47.11	0	0
9	280.1	217.5	- 22.35	6.91	4.78	- 30.82	5	2
10	462.9	274.4	- 40.72	22.50	10.06	- 55.29	4	2
11	254.8	196.4	- 22.92	9.12	6.94	- 23.90	4	3
12	25.6	25.7	+ 0.39	3.12	1.42	- 54.49	0	0
13	209.1	139.3	- 33.38	6.38	3.60	- 43.57	5	3
14	391.9	139.3	- 64.45	20.02	12.41	- 38.01	5	3
Mean	197.6	123.4	- 29.09	13.26	7.49	- 38.29		

honeys' inhibin value on a 5 to 0 scale in which inhibin value 5 is that for the plate with the lowest honey concentration inhibiting the growth of the test organism. The antibacterial properties of honeydew honeys varied, with two samples failing to inhibit bacteria growth altogether. The inhibin value decreased after storage but the antibacterial properties of honey were not eliminated completely. Bogdanov [2] reported two antibacterial factors in honey, namely the heat- and light-sensitive inhibin (i.e. the H_2O_2 system) and substances belonging mainly to the group of heat-resistant flavonoids. This author observed considerable reductions of the capacity to produce hydrogen dioxide during 3- and 6-month storage of honey (light honey especially). Dustmann [7] studied the effect of light on the peroxide value of honey and found that glucose oxidase is destroyed by visible light more rapidly in nectar honey than in the dark honeydew honey. The honeydew honeys studied in this research were stored in darkness and their glucose oxidase and catalase were not inactivated completely. According to Bogdanov et al. [3], the H_2O_2 system of honey is better correlated with invertase and diastase activity than the thermostable inhibin. The glucose oxidase activity

in the studied honeydew honeys prior to storage was correlated with this activity after storage (r = 0.8281). No correlation was found between glucose oxidase and catalase activities. Glucose oxidase activity was correlated with invertase activity (0.8251 in fresh honeys, and 0.7755 after storage). The correlation coefficient for glucose oxidase/diastase before storage was 0.6985 and after storage it was 0.7623.

Colorimetric determination of invertase activity using p-nitrophenyl--D-glucoside as substrate is simpler and far less tediouds than determinations of glucose oxidase and catalase activities. The changes in activities of these enzymes suggest that invertase activity may serve as an approximate indicator of glucose oxidase activity and H_2O_2 production capability even in the case of long storage of the dark-coloured honeydew honeys.

CONCLUSIONS

The following changes take place in unheated honeydew honeys stored for over two years in darkness at room temperature:

1. Reduction of glucose, fructose and saccharose contents; increase of the content of disaccharides other than saccharose, with the tri-and higher oligosaccharides contents remaining virtually unchanged.
2. Increase of acidity accompanied by greater accumulation of lactone than of free acids.
3. Diverse increases of 5-hydroxymethylfurfural content.
4. Negligible pH and electric conductivity changes.
5. Losses of glucose oxidase, catalase and invertase activities exceeding those of diastase activity.

LITERATURE

1. AOAC Official Methods of Analysis, Washington 1984.
2. Bogdanov S., Lebensm.-Wiss. u. Technol., 1984, **17,** 74.
3. Bogdanov S., Rieder K., Ruegg M.: Apidologie 1987, **18,** 11.
4. Codex Standard for Honey (European Regional Standard), Codex-Stan 12-1981.
5. Deifel A., Gierschner K., Vorwohl G.: Deutsche Lebensmittel-Rundschau 1985, **81**, 356.
6. Dustmann J.H.: Z. Lebensm. Unters. Forsch., 1971, **145,** 194.
7. Dustmann J.H.: Z. Lebensm. Unters. Forsch., 1972, **148,** 263.
8. Klemarewski J.: Pszczelnicze Zeszyty Naukowe 1976, **20,** 171.
9. Krauze A.: Acta Alimentaria Polonica 1991, **17.**
10. Rybak H., Achremowicz B.: Pszczelnicze Zeszyty Naukowe 1987, **31,** 19.
11. Schade J.E., Marsh G. L., Eckert J. E.: Food Research 1958, **23,** 446.
12. Schepartz A.I., Subers M. H.: Biochim. Biophys., Acta 1964, **85,** 228.
13. Schepartz A.I.: J. Apicultural Research 1866, **5,** 167.
14. Schepartz A.I., Subers M. H.: J. Aplicultural Research 1966, **5,** 37.
15. Siegenthaler U.: Mitt. Gebiete Lebensm. Hyg., 1977, **68,** 251.
16. White J.W., Riethof M. L., Kushnir I.: J. Food Sci., 1961, **26,** 63.
17. White J.W., Subers M. H., Schepartz A. I.: Biochem. Biophys. Acta 1963, **73,** 57.

18. White J.W.: Advances in Food Research 1978, **24,** 287.
19. White J.W.: "Composition of Honey", in: "Honey. A. Comprehensive Survey", ed. E. Crane, Heinemann, London 1976, 157.

Manuscript received: May, 1990
Authors address: 60-967 Poznań, Al. Niepodległości 19

A. Krauze, J. Krauze

ZMIANY SKŁADU CHEMICZNEGO PRZECHOWYWANYCH MIODÓW SPADZIOWYCH

Instytut Towaroznawstwa Akademia Ekonomiczna, Poznań

Streszczenie

Miody spadziowe przechowywano ponad 2 lata w temperaturze pokojowej, w ciemności. Po tym okresie następowały znaczne zmiany składu cukrów, który badano stosując rozdział na Bio-Gel P2 i metody enzymatyczne. Zawartość glukozy obniżyła się średnio o 6.33%, fruktozy o 3,49%, a sacharozy oznaczonej eznymatycznie w rozdzielonej frakcji disacharydów o ok. 40%. Ten ubytek sacharozy był zbliżony do zmian sacharozy łącznie z melezytozą oznaczonych na podstawie zawartości cukrów redukujących przed i po hydrolizie kwasowej. Podwyższyła się natomiast zawartość disacharydów innych niż sacharoza (średnio o 45,17%) i praktycznie nie zmieniła ilość tri- i wyższych oligosacharydów.

Po przechowywaniu wzrosła kwasowość miodów, a średni wzrost zawartości laktonu był większy niż wolnych kwasów. 5-hydroksymetylofurfural nagromadzał się w różnych ilościach, a przewodność elektryczna i pH utrzymywały się na poziomie zbliżonym do wartości wyjściowych.

Długookresowe przechowywanie miodów spadziowych nie spowodowało całkowitej utraty aktywności enzymów. Spadek aktywności inwertazy, który był większy niż diastazy, wynosił średnio ok. 30%. Aktywność oksydazy glukozowej i katalazy, jak również działanie bakteriostatyczne świeżych miodów wahały się w szerokich granicach. Po przechowywaniu obniżenie aktywności oksydazy glukozowej nie było dużo większe niż inwertazy, przy czym aktywności obu enzymów były znacznie skorelowane. Wydaje się, że aktywność inwertazy może w przybliżeniu wskazywać, czy w przechowywanym miodzie spadziowym zachowała się aktywność oksydazy glukozowej i zdolność wytwarzania nadtlenku wodoru, z którą jest związane działanie bakteriostatyczne.