

Composition of Honey. VI. The Effect of Storage on Carbohydrates, Acidity and Diastase Content

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SUMMARY

Quantitative examinations have been made of the changes that occur in honey while stored at room temperature. Though honey is a relatively stable commodity, it has been found to change in composition and biochemical activity even when stored at $26 \pm 3^\circ \text{C}$. During two years of such storage about 9% of the monosaccharides are converted per year into more complex disaccharides and higher sugars. The ratio of fructose to glucose increases markedly as the free glucose content declines more rapidly than the free fructose content. All samples examined showed such changes. Significant increases were noted in acidity during storage, but some samples showed no change. Evidence for the enzymic nature of the change is given. Diastase values of unheated honey decline in storage at room temperature ($23\text{--}28^\circ \text{C}$), with diastase showing a half-life of 17 months under these conditions of storage. Cool or cold storage and expeditious handling are recommended for preservation of diastase in honey for export to Europe.

Honey is considered to be a relatively stable foodstuff, with only minor changes in flavor and color taking place during several years storage. It is well-known that properly ripened honey is not susceptible to spoilage by microorganisms, with the exception of osmophilic yeasts, and then only above moisture contents of 17% (9). Granulation of honey does increase the possibility of such spoilage since it results in an increase in the moisture content of the liquid portion. A comprehensive study of the effect of storage at elevated temperature and of heat processing on the color of honey has been described by Milum (12).

Both physical and chemical actions are involved in transformation of nectar into honey, with the activity of enzymes being most prominent. Since these enzymes remain in the honey, their action may continue at a declining rate. The long-noted (3) decrease in the sucrose content of honey after extraction has been ascribed to a continuing action of the invertase added by the bee. Sucrose content does not reach

zero after several years of storage, however, even though a honey may still contain an active invertase.

It was recently shown (22) that honey contains a transglucosylase which produces several oligosaccharides, including maltose and isomaltose, from sucrose. Austin pointed out (2) that because of this enzymic activity the "maltose" (actually reducing disaccharide) content of a honey will depend to some degree on methods of apiary management, storage temperature, and density of honey. He did not predict the effect of storage in general on the maltose content of honey.

De Boer (5) examined a number of yearly honey samples stored for up to 22 years; nearly all were white clover and all were stored in the unheated state. He pointed out that the same changes in composition that take place on heating of honey also occur in storage. He concluded that polarization is unchanged and the change in sucrose content negligible, implying no changes in the sugars. The amounts of glucose and fructose and their ratio remained unchanged; no relative increase was noted in fructose content, contrary to previous reports (1).

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Diastase was found to decrease with age, 3 Gothe "steps" in 10 years. The acidity was unchanged, but the Fiehe test for hydroxymethylfurfural (HMF) became positive and after 10 years HMF could be determined gravimetrically.

Armbruster, quoted by deBoer (5), reported that aging of as little as 2½ months sometimes causes a noticeable decrease of diastatic activity, while other types of honey show no loss after 2-5 months. After 2½ years a considerable decrease was found in one type of honey.

The recent introduction of a more comprehensive method of carbohydrate analysis of honey (20, 23) has made it possible to learn more about the carbohydrate make-up of honey. Whereas older procedures reported only glucose, fructose, sucrose and dextrin, the newer procedure allows determination of reducing disaccharides, which in itself provides more accurate values for glucose and fructose. Better values for higher sugars ("dextrin") also result because reducing sugar contamination of dextrans, common to older precipitation procedures, is eliminated.

During an extensive analytical survey of the composition of American honey by the newer methods, we have re-examined the effects of storage on the composition of honey. We have studied the effect of room-temperature storage of up to 3 years on unheated and mildly heated honey, determining changes in glucose, fructose, reducing disaccharides, sucrose, higher sugars, diastase, free acidity, lactone and total acidity. Significant changes were found for nearly all of these constituents, contrary to previous beliefs.

MATERIALS AND METHODS

Honey samples used in this work were some of those collected from producers for the analytical survey noted above; they will be described in detail when the results of the survey are published. In general, unheated samples were received, and divided into 3 portions. One was stored at about -20° C within one day of receipt, a second heated in a closed jar in a water bath at 55° C for 30 min and cooled (essential pasteurization without enzyme inactivation), and the remainder left unheated. The latter two portions were stored in the dark at room temperature (23-28° C). Sam-

ples from frozen storage were allowed to reach room temperature overnight before analysis.

Analyses of corresponding samples of a set were carried out on the same day; sets were selected at random.

CARBOHYDRATE ANALYSIS

Where necessary, samples were liquefied by the new AOAC procedure (20). Carbohydrates were determined by the selective adsorption method (20). Moisture was determined by refractive index using the Chataway table (11). Results for each set were calculated to the moisture content of the sample kept in cold storage.

ACIDS

Free acidity, lactone, and total acidity were determined by a recently developed method (24). All samples were unheated; aliquots stored at room temperature and -20° C were analyzed; the cold-storage samples in duplicate and others in triplicate.

DIASTASE

The method of Schade, Marsh and Eckert (14) slightly modified as adopted by the AOAC (20) was used. Samples which were granulated were made as homogeneous as possible by stirring before sampling; no heat was used. Samples upon which carbohydrates and diastase were determined were all liquid and did not require heating before carbohydrate analysis.

RESULTS AND DISCUSSION

CARBOHYDRATES

Table 1 shows the values obtained for each type of storage for 5 honey samples, each set calculated to the moisture content shown for the cold-storage sample. The moisture values in parentheses are those actually found for the samples. The column under "age" gives the number of months of storage for the samples after receipt at the laboratory.

The data in Table 1 were analyzed statistically by the analysis of variance. Each set of 15 values for each sugar was examined and the variability due to sample and storage was calculated and tested statistically. The variance and F values are shown in Table 3. The differences due to storage are shown in all cases to be significant at the 1% probability level, except for the unanalyzed portion, where the change is significant at the 5% probability level.

TABLE 1
 EFFECT OF STORAGE ON HONEY SUGARS¹

No. ²	H ₂ O	Fructose	Glucose	Maltose	Sucrose	Higher Sugars	Unanalyzed ⁴	Age
91F	18.6	35.85	33.87	4.92	0.58	1.28	4.90	20
91H	(17.5) ³	35.07	29.82	8.94	0.93	1.46	5.18	20
91R	(16.6)	34.85	29.44	9.22	0.89	1.45	5.55	20
258F	20.8	35.95	32.31	5.43	0.28	1.71	3.62	22
258H	(19.0)	33.95	27.88	9.59	0.85	1.67	5.26	22
258R	(19.3)	33.84	27.81	10.18	0.92	2.03	4.42	22
94F	17.4	38.22	31.29	7.54	0.73	1.23	3.59	22
94H	(16.2)	36.39	28.54	11.02	0.87	1.36	4.42	22
94R	(16.6)	36.23	28.55	10.51	0.90	1.46	4.95	22
96F	17.7	36.46	29.85	7.64	0.78	1.77	5.79	23
96H	(16.0)	34.19	25.39	13.13	0.85	1.91	6.93	23
96R	(14.2)	34.49	25.24	13.05	0.99	2.05	6.48	23
98F	18.5	37.98	31.02	6.83	0.44	1.84	3.39	23
98H	(17.0)	36.10	28.02	10.95	1.00	1.82	3.61	23
98R	(16.8)	35.73	26.71	11.47	1.16	1.93	4.50	23
Av F	18.8	36.89	31.67	6.47	0.56	1.57	4.26	
Av H		35.14	27.93	10.73	0.90	1.64	5.08	
Av R		35.03	27.55	10.89	0.97	1.78	5.18	
Change in Heated Honey		-1.75	-3.74	+4.26	+0.34	+0.07	-0.82	
Change in Raw Honey		-1.86	-4.12	+4.42	+0.41	+0.21	-0.92	
% Change in Raw Honey		5.0%	13.0%	68%	73%	13.4%	22.2%	

¹ Each set of values calculated to the moisture content of corresponding cold-storage sample.

² The letter following sample number identifies treatment as follows: F = unheated, cold storage; H = heated, room-temperature storage; R = unheated, room-temperature storage.

³ Moisture values in parentheses are actual values found for the samples.

⁴ 100—(sugars plus water).

The mean square resulting from storage conditions was further subdivided and that of frozen samples was compared with that of the two room-temperature storage conditions. The two room-temperature storage sets (heated and unheated) were also compared with each other. A sample calculation is shown in Table 2, and Table 3 gives a summary of the mean squares and the F values obtained therefrom, for each sugar.

It can be seen from the table that the differences between the frozen samples and those stored at room temperature are significant for all sugars at the 1% probability level. It is also apparent that the differences between the average values in Table 1 for the unheated and heated samples, both stored

at room temperature are, in all cases not significant, except for the higher sugar values, which are significant at the 5% probability level.

It may be concluded that when raw honey is stored for two years at temperatures ranging between 23–28° C, the following changes take place in the carbohydrate composition:

1. A decrease of free glucose (averaging 13%) and a decrease of free fructose (averaging 5.5%); an average of 18.5% of the free monosaccharide content of the honey is thus lost.

2. A marked increase of "maltose" or reducing disaccharide sugars, averaging 69% of the amount initially present.

COMPOSITION OF HONEY. VI.

TABLE 2
GLUCOSE
ANALYSIS OF VARIANCE

Source of Variability	S.S.	D.F.	M.S.	F
Total	72.00	14		
Storage	51.79	2	25.89	100 ¹
F vs RT & H	51.43	1	51.43	198 ¹
RT vs H	0.36	1	0.36	1.39
Samples	28.14	4	7.03	27.2 ¹
Error	2.07	8	0.26	

¹ Exceeds .01 probability level.

3. A *relatively* large increase in sucrose content.

4. A small (13%) increase in the higher sugar content of the honey, and

5. An increase averaging 22%, in the amount of unanalyzed material (100 — sugars + water).

The heat treatment given these samples—55° C (130° F) for 30 min—had no effect on these changes, with the possible effect of reducing the extent of increase of the higher sugar values. The changes in the stored samples are in the direction of increased complexity of sugars. This might be expected from a consideration of the conditions within the sample. A high sugar concentration and a considerable acidity over a period of time would promote combination of monosaccharides (reversion, [13]). The presence of an active transglucosylase enzyme (22) in the honey may also result in

accumulation of oligosaccharide material; the heat treatment used was not sufficient to inactivate enzymes. Possible explanations for the changes observed are as follows:

(a) Fructose. This sugar is subject to degradation to hydroxymethylfurfural by long standing in acid solution. Conversion to non-reducing fructose anhydrides is also possible. Fructose-containing oligosaccharides may result from enzyme transfer of glucose to a fructose acceptor.

(b) Glucose. Twice as much glucose disappeared as fructose. This may reflect the specificity of the enzyme transferring glucose from oligosaccharides (honey invertase, a glucoinvertase).

(c) "Maltose." This actually represents reducing disaccharide material, including maltose, isomaltose, maltulose, turanose and nigerose (21). All of these sugars are hydrolyzed by honey α -glucosidase (18). The increase in this category of sugars accounts for most of the decrease in monosaccharides.

(d) Sucrose. Post-harvest ripening has long been known to take place in unheated honey (3). Sucrose is at or reaches a low value within a few months of removal of honey from the hive, but never disappears completely, despite (or probably because of) the presence of an active invertase. The data here show a later stage of sucrose change, where the amount present increases from a low value to approach 1%. Mold

TABLE 3
SIGNIFICANCES OF CHANGES IN HONEY COMPOSITION DUE TO STORAGE

Source of Variability	D.F.	Levulose		Dextrose		Maltose	
		M.S.	F	M.S.	F	M.S.	F
Samples	4	3.18	31.8 ²	7.03	27.0 ²	5.71	22.0 ²
Storage	2	5.46	54.6 ²	25.9	99.6 ²	31.3	120 ²
F vs R & H	1	10.90	109 ²	51.4	198 ¹	62.6	241 ²
R vs H	1	.03	.0	.36	1.4	.06	.2
Error	8	.10		.26		.26	
Source of Variability	D.F.	Sucrose		Higher Sugars		Unanalyzed	
		M.S.	F	M.S.	F	M.S.	F
Samples	4	0.018	0.86	0.217	36.2 ²	3.03	17.8 ²
Storage	2	.240	11.4 ²	.061	10.2 ²	1.33	7.8 ¹
F vs R & H	1	.466	22.2 ²	.073	12.2 ²	2.63	15.5 ²
R vs H	1	.013	.62	.049	8.2 ¹	.02	.1
Error	8	.021		.006		.17	

¹ Exceeds 5% probability level.

² Exceeds 1% probability level.

enzymes have been shown to synthesize sucrose by transfructosylation during their action on sucrose (7).

(e) Higher sugars. The increase in this fraction is further evidence of reversion and transglucosylation.

(f) Unanalyzed. From the point of view of the carbohydrates, this category can contain difructose anhydrides, non-reducing disaccharides (except sucrose), and kojibiose, a very weakly-reducing disaccharide (2-O- α -D-glucosyl-D-glucose) recently discovered in honey by Watanabe and Aso (16). This sugar will not be determined in the analytical procedure used, since it has but about 6% of the reducing power of glucose against copper reagents. The increase in unanalyzed material may represent an increase in the amount of kojibiose (and possibly trehalose) in honey. Both of these compounds have been isolated from hydrol, where it is believed that they arose by reversion from glucose (15).

It is of interest to examine an analysis of a 36-year-old sample of honey and compare it with a corresponding contemporary sample.

Table 4 shows such a comparison. The 1923 sample^b is an alsike clover-white clover honey produced at Delphos, Ohio; it had never been opened and was stored in a dark cupboard and was liquid except for a few coarse crystals at the bottom. Also shown in the table is a similar sample from the 1957 crop. It is alsike and white clover,

^b Donated by C. A. Reese, Department of Entomology, Ohio State University.

TABLE 4
EFFECT OF AGE ON A CLOVER HONEY

	1957 Crop	1923 Crop	Difference	
				% of 1957
Moisture	18.2	(18.2) ¹
Fructose	38.25	35.05	-3.20	-8.3
Glucose	33.58	23.12	-10.29	-30.6
Maltose	5.50	16.41	+10.91	+198
Sucrose	1.68	1.04	-.64	-38.2
Higher Sugars	0.82	2.06	+1.24	+151
Undetermined	2.0	4.1	+2.1	+105

¹ Moisture content of the 1923 sample was 17.6%; data are calculated to the 18.2% shown by the 1957 sample to facilitate comparison.

produced at Columbia City, Indiana, by T. A. Ott. Data were calculated to the same moisture content to facilitate comparison. The differences shown in the table are all similar to those in Table 1 in trend, except that the 1957 sucrose value is higher, though the value for the aged sample (equilibrium?) is close to the average of the 2-year-old samples. In general the changes in monosaccharide and "maltose" shown occurring after 26 years of storage are similar, but larger than those found for the two-year-old samples in Table 1.

Analyses of honey samples after extended storage have been previously reported by de Boer (5) and Auerbach and Bodlander (1). The analytical methods de Boer used would not detect the differences in carbohydrate composition shown here. He did not confirm the earlier conclusion of Auerbach and Bodlander that the ratio of fructose to glucose increased after storage of honey. Auerbach and Bodlander reported the analysis of 13 fourteen-year-old honey samples. Their fructose-glucose ratio ranged from 1.19 to 1.81, averaging 1.40; 10 fresh honey samples ranged from 1.06 to 1.19, averaging 1.11. These values have only relative meaning, since the analytical methods used gave no differentiation between monosaccharide and disaccharide.

The results in Tables 1 and 4 substantiate the views of Auerbach and Bodlander that the amount of free glucose decreases on storage and that the ratio of fructose to glucose increases. They ascribed this to possible enzymic condensation of glucose, which we also believe contributes.

The changes described in the sugar distribution of honey have some practical implications. With the tendency toward increasing complexity, there may be a corresponding loss of nutritive value; some of the disaccharides and higher sugars may not be digestible.

The considerable decrease in glucose content is probably responsible for the gradual liquefaction that is often noted in granulated honey samples as they stand in storage, which was mentioned by de Boer. If the glucose content of a granulated honey is near the lower limit of granulation, the

TABLE 5
EFFECT OF STORAGE ON ACIDITY OF HONEY

Sample	Free Acid		Lactone		Total Acidity	
	F ¹	R ²	F	R	F	R
	meq/kg	meq/kg	meq/kg	meq/kg	meq/kg	meq/kg
91	24.04	27.07	9.87	12.39	33.92	39.46
258	20.56	24.06	6.45	7.73	27.00	31.80
92	19.85	21.66	4.90	5.32	24.35	26.98
94	15.04	15.78	2.55	2.62	17.59	18.40
96	22.28	23.90	6.17	9.21	28.45	33.11
107	23.73	24.88	2.20	2.18	25.93	27.04
97	20.82	20.13	7.00	8.08	27.82	28.21
108	22.88	24.29	1.90	4.21	24.78	28.46
109	25.24	26.45	5.83	7.68	31.05	34.13
98	25.62	26.63	8.33	10.39	33.85	37.02

¹ F = Stored at -20° C; samples 91-96 and 258, 21 months, others 24 months.

² R = Stored at room temperature same times as above.

changes in a year or so will reduce the glucose well below the saturation point so that the crystals will slowly dissolve.

ACIDS

Table 5 shows the free acidity, lactone content and total acidity of ten samples stored under the conditions described above. None of the samples showed visible evidence of fermentation. In Table 6 is given the analysis of variance for the free acidity, lactone and total acidity values. The average changes in each of these categories are seen to be highly significant. It has been proposed (4, 19, 24) that an enzyme producing acidity occurs in honey. If this is the case, honey samples showing high diastase number might be expected to show a correspondingly high rate of acid production. Table 7 shows these values for 10 honey samples. Also shown in the table is an analysis of variance for regression. The F value obtained, 11.5, shows a highly significant re-

gression between the two sets of values. This is not meant to imply that the amylase enzyme system is responsible for acid production, but rather that the factors affecting amylase activity also influence the activity of the acid-producing enzyme.

DIASTASE

The amylase content of honey has long been used by Europeans as a measure of the heat treatment to which a honey has been exposed. The voluminous literature will not be reviewed here (10). Recently (6, 8) it has been proposed that diastase content alone is not a suitable criterion for the detection of overheated honey.

There appears to be relatively little information in the literature on the effect of storage of honey on its diastase content. De Boer (5), using the Gothe procedure, reported that diastase decreased gradually with age of honey, about 3 Gothe "steps" in 10 years. Schade, Marsh and Eckert (14),

TABLE 6
ANALYSIS OF VARIANCE

Source of Variability	D.F.	Free Acidity				Lactone				Total Acidity			
		S.S.	M.S.	F	S	S.S.	M.S.	F	S	S.S.	M.S.	F	S
Total.....	19	207.6				175.0				582.6			
Materials.....	9	190.6	21.17	31.0 ¹		159.2	17.69	31.1 ¹		523.9	58.2	35.9 ¹	
Storage.....	1	10.9	10.93	16.0 ¹		10.7	10.68	18.8 ¹		44.1	44.1	27.2 ¹	
Error.....	9	6.14	0.68		0.83	5.11	0.57		0.75	14.6	1.6		1.27

¹ Exceeds .01 probability level.

TABLE 7
REGRESSION OF ACID PRODUCTION BY HONEY ON
DIASTASE NUMBER

Sample	Diastase No	Change in Total Acidity per Year
91	38.0	3.16
258	35.3	2.74
92	33.3	1.50
94	19.1	0.46
96	27.8	2.66
107	18.5	0.59
97	8.0	0.18
108	20.0	1.84
109	10.7	1.59
98	21.7	1.58

Analysis of Variance for Regression				
Source	S.S.	D.F.	M.S.	F
Total	927.28	9		
Linear Regression	546.99	1	547	11.5 ¹
Deviations	380.29	8	47.5	

¹ Significant at .01 probability level.

using their improved procedure, reported diastase values for honey samples before and after storage for 13 to 15 months at 20° C. They reported that the diastase activity had "decreased slightly but not significantly in most cases." We have sub-

jected their data (the 7 samples in their Table 3) to the analysis of variance, and the changes were found significant at the 1% probability level (F = 11.7). Their data showed an average decrease for 7 samples of 10.1% in diastase number for the approximately 14 months storage at 20° C, or 0.72% per month.

We have determined diastase number for aliquots of 20 samples of honey after storage times of 4 to 21 months at -20° C and also at laboratory room temperature. Samples were from the 1956 and 1957 crops and were frozen on receipt at the laboratory at varying times (½ to 14 months) after their extraction. Full data on samples will be included in a later publication.

The data given in Table 8 show the effect on diastase number of room temperature, dark storage for varying times, based on the reasonable assumption that no change takes place on samples stored at -20° C. This table shows an average loss of diastase value for honey stored unheated at temperatures ranging from about 23° to 28° C of 2.95% per month, which is equivalent to a half-life of 17 months.

TABLE 8
EFFECT OF STORAGE ON DIASTASE CONTENT OF HONEY

No.	Storage Time	Diastase Values		Loss	-% Loss	Loss per Month
		Frozen	Room Temp			
234	21 mo.	61.2	30.9	30.3	49.5	2.36
430	20	32.6	18.6	14.0	42.9	2.16
361	20	14.6	8.11	6.5	44.5	2.23
326	19	17.6	7.23	10.4	59.1	3.11
238	17	10.6	7.59	3.01	28.3	1.66
403	13	6.74	3.97	2.77	41.1	3.16
91	13	38.0	21.8	16.2	42.6	3.28
258	13	35.3	20.8	14.5	41.1	3.16
92	13	33.3	19.0	14.3	42.9	3.30
94	13	19.1	12.9	6.2	32.5	2.50
96	13	27.8	18.4	9.4	33.8	2.60
97	13	8.00	4.42	3.58	44.7	3.44
98	13	21.7	15.8	5.9	27.2	2.09
261	13	10.3	8.40	1.90	18.4	1.41
142	13	22.4	13.2	9.2	41.1	3.16
104	9	10.8	8.15	2.65	24.5	2.72
121	8	22.6	15.9	6.7	29.6	3.70
179	8	16.7	11.4	5.3	31.7	3.96
333	8	15.2	9.38	5.8	38.1	4.76
214	4	15.2	12.8	2.4	15.8	3.95
Av	13.2	22.0	13.4		38.9	2.95

This may be compared to the 0.72% per month shown by the data of Schade *et al* (14) for a temperature probably 5–6° C lower. This at once shows the importance of low-temperature storage for honey in which diastase content must be maintained. Our data show a considerable variation in the rate of loss of diastase among the honey samples. Kiermeier and Koberlein (8) reported that the heat-sensitivity of honey diastase is related to the pH of the sample; Schade *et al* (14) are in agreement. We made an effort to relate several compositional factors to the rate of loss of diastase in storage but, as shown in Table 9, no relationship was obtained for ash, total acidity, hydrogen ion concentration, original diastase value, and moisture content. An analysis of variance for regression on the values for diastase loss vs original diastase number,

TABLE 9
CORRELATION OF DIASTASE LOSS RATE
WITH OTHER FACTORS

Factor	F Value ¹
Time of Storage	12.4 ⁴
Original Diastase No	2.7 ²
Moisture Content	0.1
Total Acidity	0.5
Hydrogen Ion Conc	0.07
Ash	1.9
Total Age	7.6 ³

¹ Calculated by analysis of variance for regression.

² Significant at .10 level.

³ Exceeds .05 probability level.

⁴ Exceeds .01 probability level.

for example, gave an F value of 2.66, significant at the 10% probability level. However, rate of loss was found to be correlated with storage time, with the rate for samples stored short times being significantly greater than the over-all rate for samples with longer storage periods. Analysis of variance of these data yields an F value for linear regression of 12.4, significant at the 1% probability level. A less significant relation was found between total age and rate of diastase loss. This does not provide information on the composition factors controlling rate of loss.

It may be seen from these data and also those of Schade and co-workers that storage temperature is a most important factor affecting retention of diastase in honey. Many studies relating diastase loss with degree of heating have been reported (6, 8, 10) investigating the thesis that diastatic activity is an indication of heating of honey. De Boer in his study of aging of honey did note that in general the changes occurring as honey ages are the same as those brought about by heating; he had particular reference to increase in hydroxymethylfurfural content. We have now, for the first time, evidence that over a storage period of 12–18 months, without heating, a honey may lose enough diastase to fall below the minimum values required for European acceptance as table honey.

With export practices, including storage by the producer, packer, shipper, time in transit through the Panama Canal to Europe, plus a possible 6 months in free-port storage before sampling and testing by the importer, a total elapsed time of 12–18 months from extraction to the European consumer may occur (17). Any storage temperature exceeding the average 26° C (79° F) in this study will appreciably increase diastase destruction beyond that found here. A few weeks or months of warehouse storage at 85 or 90° F or higher is commonly encountered in southwestern honey handling.

Although further studies of the effect of these relatively low temperatures on stability of honey are planned, the following practices for honey intended for export to Europe may be suggested:

1. Expeditious handling to shorten time from extractor to European customer.
2. Prompt removal from storage areas where honey is exposed to heat.
3. If long-term storage is necessary, it should be in cool or cold storage.
4. Sampling by importers for testing should be at arrival instead of after prolonged warehouse storage.

Complete analytical data on all of the honey samples used in this work will be included in a forthcoming publication on the composition of American honey.

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LITERATURE CITED

1. Auerbach, F. and E. Bodlander. 1924. Über ein neues Verfahren zur Unterscheidung von Honig und Kunsthonig. *Z. Nahr. Genussm.*, **47**, 233.
2. Austin, G. H. 1956. Maltose content of Canadian honeys and its probable effects on crystallization. *Proc. X Int. Cong. Entomol.*, Montreal, **4**, 1001.
3. Browne, C. A. 1908. Chemical analysis and composition of American honeys. *Bull. 110, Bur. Chem., U. S. Dept. Agr.*; Kardos, R. F. 1938. Der Rohrzuckergehalt im natürlichen Honig. *Z. Untersuch. Lebensm.*, **76**, 354.
4. Cocker, L. 1951. The enzymic production of acid in honey. *J. Sci. Food Agr.*, **2**, 411.
5. de Boer, H. W. 1934. De invloed van den ouderdom op de samenstelling van honig. *Chem. Weekblad*, **31**, 482.
6. Duisberg, H. and H. Gebelein. 1958. Über die Kontrolle von Erhitzungsschaden bei Honigen. *Z. Lebensm.-Untersuch. u.-Forsch.*, **107**, 489.
7. Edelman, J. 1954. Transfer reactions catalyzed by some sucrase preparations. *Biochem. J.*, **57**, 22.
8. Kiermeier, F., and W. Köberlein. 1954. Über die Hitzeinaktivierung von Enzymen in Honig. *Z. Lebensm.-Untersuch. u.-Forsch.*, **98**, 329.
9. Lochhead, A. G. 1933. Factors concerned with the fermentation of honey. *Zentr. Bakt., Parasitenk.* 2d Abt., **88**, 296; Stephen, W. A. 1946. The relationship of moisture content and yeast count in honey fermentation. *Sci. Agr.*, **26**, 258.
10. Lothrop, R. E., and H. S. Paine. 1931. Diastatic activity of some American honeys. *Ind. Eng. Chem.*, **23**, 71; Lampitt, L. H., E. B. Hughes, and H. S. Rooke. 1929. Furfural and diastase in heated honey. *The Analyst*, **54**, 381; 1930. The diastatic activity of honey. *The Analyst*, **55**, 666; Vansell, G. H. 1929. Diastase in honey. *J. Econ. Entomol.*, **22**, 926; de Boer, H. W. 1931. The behavior of diastatic ferments in honey when heated. *Bee World*, **12** (2), 13; Bartels, W. and A. Fauth. 1933. Beobachtungen bei der Untersuchung californischer Honige. *Z. Untersuch. Lebensm.*, **66**, 396.
11. *Official Methods of Analysis*. Association of Official Agricultural Chemists. 8th ed., 1955. Washington, D.C.
12. Milum, V. G. 1948. Some factors affecting the color of honey. *J. Econ. Entomol.*, **41**, 495.
13. Pigman, W. W. and R. M. Goepf, Jr. 1948. *Chemistry of the Carbohydrates*. Academic Press, New York, pp. 434, 515, 605.
14. Schade, J., G. E. Marsh and J. E. Eckert. 1958. Diastase activity and hydroxymethyl-furfural in honey and their usefulness in detecting heat alteration. *Food Research*, **23**, 446.
15. Thompson, A., K. Anno, M. L. Wolfrom and M. Inatome. 1954. Acid reversion products from D-glucose. *J. Am. Chem. Soc.*, **76**, 1309; Sato, A. and K. Aso. 1957. Kojibiose (2-O- α -D-glucopyranosyl-D-glucose): isolation and structure. *Nature*, **180**, 984.
16. Watanabe, T. and K. Aso. 1959. Isolation of kojibiose from honey. *Nature*, **183**, 1740.
17. Webster, Marvin, Agricultural Marketing Service, USDA. (Private communication).
18. White, J. W., Jr. (Unpublished data).
19. White, J. W., Jr. Enzymic production of acid in honey. Paper presented at first annual meeting of American Committee, Bee Research Association, Tampa, Fla., Jan. 1959.
20. White, J. W., Jr. 1959. Report on the analysis of honey. *J. Assoc. Offic. Agr. Chemists*, **42**, 341.
21. White, J. W., Jr., and N. Hoban. 1959. Composition of honey. IV. Identification of the disaccharides. *Arch. Biochem. Biophys.*, **80**, 386.
22. White, J. W., Jr., and J. Maher. 1953. Transglucosidation by honey invertase. *Arch. Biochem. Biophys.*, **42**, 360.
23. White, J. W., Jr., and J. Maher. 1954. Selective absorption method for determination of the sugars of honey. *J. Assoc. Offic. Agr. Chemists*, **37**, 466.
24. White, J. W., Jr., J. Petty, and R. B. Hager. 1958. The composition of honey. II. Lactone content. *J. Assoc. Offic. Agr. Chemists*, **41**, 194.