

Effect of Storage and Processing Temperatures 2046 on Honey Quality 75

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SUMMARY

Three honey samples were stored for periods ranging to 540 days at seven temperatures from -20 to 60°C. Loss of diastase and invertase and increase in hydroxymethylfurfural (HMF) content have been determined. Half-lives (in days) of the enzymes in honey are approximated as follows:

$$\text{Diastase, } \log t_{1/2} = \frac{\frac{1}{T} - 0.003000}{0.000130}$$

$$\text{Invertase, } \log t_{1/2} = \frac{\frac{1}{T} - 0.003083}{0.000113}$$

where T is between 283 and 353°K. A straight-line relationship is shown between storage temperature and the logarithm of the time required to reach an arbitrary level (3 mg/100 g) of HMF. The effect of commercial heating of honey on quality as indicated by enzyme and HMF content is discussed. A reduction of 10-15°F in storage temperature will reduce HMF production to 1/3 and enzyme loss to 1/5-1/6.

INTRODUCTION

Man's appropriation of the honey-bee's stored treasure gives him the responsibility of maintaining its original high quality and delectable fragrance. It is frequently necessary to heat honey to prevent fermentation by sugar-tolerant yeasts and to keep it liquid as long as possible. We have examined the effects of storage at several temperatures on some quality factors of honey to facilitate evaluation of commercial storage and processing conditions.

Diastatic activity is one of the oldest measures of honey quality. Europeans have for many years considered honey of low diastatic activity as overheated and hence unfit for table use, although it is becoming evident that a single criterion is not sufficient for this purpose (Duisberg and Gebelein, 1958; Hadorn and Kovacs, 1960). The presence in honey of excessive amounts of hydroxymethylfurfural (HMF) has been considered evidence of overheating and

implies loss of freshness. Because of the nature of HMF formation, it is likely that color and flavor deterioration will parallel it. Hadorn and Kovacs (1960) recently proposed that both HMF and diastase be considered when heating history is assessed. Hadorn *et al.* (1962) suggested several guidelines for evaluation of honey for use in Switzerland, as shown in Table 1.

Honey for import into West Germany is presently required to have appreciable levels of diastatic activity to qualify as first-grade honey (values of 5 and 8 units have been employed recently).

Although de Boer (1934) implied many years ago that extended storage (years) without heating can impair diastase levels and cause HMF accumulation, quantitative data have not been available until recently. In a study of the effect of room-temperature storage on honey composition, White *et al.* (1961) found diastase to have a half-life of about 17 months at 23-28°C. The monosaccharide content decreases about 9% per year, being converted to oligosaccharides. Evidence was given for an enzyme-increased acidity, which has since been shown (White *et al.*, 1963) to be due to glucose oxidase in honey. Schade *et al.* (1958) demonstrated that storage at 20°C would not prevent some HMF production. It was shown to

vary among honeys and depend on moisture content; storage at 35-55°C was examined.

Hadorn *et al.* (1962) recently analyzed a number of Swiss and imported honeys for HMF, invertase, and diastase, and noted the effects on these factors of storage at room temperature (20-23°C) and 50°C for some samples. They found Swiss honeys to be more stable than imported ones; this was ascribed to the lower acidity (pH 4.5-5.0) of the former than of the latter (more acid than pH 3.8). They also reported that HMF content of honey increases during storage at relatively low temperatures, implying that honey from subtropical regions could naturally have a high HMF content without being "overheated" or adulterated. Hadorn and Zürcher (1962) examined the effect of holding 300-kg drums of honey for 5 days in warm rooms at 48°C (118.4°F) and 43°C (109.4°F) to liquefy them, as commonly practiced there. The higher temperature caused a 50-65% loss of invertase and 20% loss of diastase, and doubled the HMF value. In a 43° room for five days, enzyme losses were about 5% and HMF increased about 25%. A considerable heat lag was noted in the drums.

Winkler (1955) originally proposed 4 mg per 100 g as an upper limit for HMF content for natural honey; according to Hadorn *et al.* (1962) the

Table 1. Swiss criteria for the evaluation of honey.^a

Classification	HMF (mg %)	Invertase ^b	Diastase ^c
Acceptable, entirely fresh honeys			
1) "Enzyme-rich"	0.1-0.3	16-25	18-40
2) "Enzyme-poor" because of heavy nectar flow	0.1-0.3	4-8	8-12
Acceptable, old or slightly heat-damaged honey	max. 3.0	min. 4	min. 8
Unacceptable, excessively long-stored honey (1-3 yrs. at room temperature)	4-8	mostly over 4	mostly over 8
Unacceptable, injured by short heating at high temperature	0.1-3.0	0-4	0-8
Unacceptable, injured by long heat treatment	4-15	0-4 occasionally higher	0-8 occasionally higher

^a Table 16 in the paper by Hadorn *et al.* (1962).^b g sucrose converted per hr per 100 g honey at 40°C.^c g starch converted per hr per 100 g honey at 40°C.

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upper limit for import into Germany is 3 mg per 100 g. The Fische test, a qualitative indication of HMF, is positive at about 0.9-1.4 mg per 100 g according to Schade *et al.* (1958); Hadorn and Kovacs (1960) reported a doubtful test at 3 and a positive test at 5 mg per 100 g.

When a honey type (for example, citrus or Southwestern alfalfa) is naturally low in enzyme activity, it is important to preserve such activity when the honey is intended for export. The quantitative effects of various processing and storage conditions have not been known. Data presented in this study on the effects of such conditions on diastase, invertase, and HMF content of honey should be useful in recommending storage and processing conditions for the best preservation of honey quality.

MATERIALS AND METHODS

Three samples of 1959 crop honey, of high, intermediate, and low diastase content were selected on the basis of their analyses from a group of twelve provided by a honey processor. The composition of these samples is shown in Table 2. Immediately upon receipt,

Table 2. Composition of honey samples.

Component	1	2	3
Moisture, %	17.5	18.2	18.2
Glucose, %	32.45	33.62	33.35
Fructose, %	38.69	38.29	38.57
Sucrose, %	1.99	0.46	0.07
Reducing disaccharides, %	5.42	5.20	4.81
Higher sugars, %	0.71	0.54	0.49
pH	3.84	3.85	4.22
Free acid, meq/kg	15.17	24.46	28.79
Lactons, meq/kg	8.54	10.82	6.56
Total acid, meq/kg	23.71	35.28	35.35
Ash, %	0.067	0.145	0.145
Nitrogen, %	0.034	0.092	0.170
Diastase ^a	15.1	30.6	45.8
Invertase ^a	4.06	15.92	17.39
HMF, mg/100 g	0.06	0.35	0.22
Inhibine	2	4	4
Color	White	Light amber	Dark amber

^a Grams substrate converted per 100 g honey per hr.

the contents of 60-lb cans of each sample were mixed thoroughly without heating. Sample 1 was liquid; 2 and 3 showed complete fine granulation. Approximately 80-g portions of each were placed in 2-ounce glass jars with rubber-sealed screw caps, and groups of samples were placed in storage at -20, 4, 21, 27, 37, 50, and 60°C. Jars were removed at appropriate intervals, and the contents of each were mixed without heating and analyzed

for diastase, invertase, and moisture. They were then placed at -20°C for later determination of HMF.

Diastase. The Schade *et al.* (1958) method as adopted by the AOAC (Horwitz, 1960) was used. (A modification introduced into the Schade method by Hadorn (1961)—the use of 2-cm cells for photometric measurement—results in lower values for diastase than by the AOAC version.) Units are equivalent to Gothe numbers, or grams starch hydrolyzed per hr at 40°C per 100 g of honey.

Invertase. The procedure described below was developed for this study.

Make 10.00 g honey to 100 ml with water in a volumetric flask. Pipette 10 ml into a 25-mm (flat width) cellulose dialysis tube and dialyze overnight against running tap water.

Transfer the contents of the sac to a 100-ml volumetric flask and make to volume. Pipette 20-ml portions into two 25-ml volumetric flasks, one for assay and one for a blank. Heat the latter in a boiling water bath for 3 min; cool. To each flask add 1.25 ml of 1M acetate buffer, pH 5.80, and immerse for 10 min in a water bath at 35°C. Then add 1.25 g dry sucrose, start timing, make to volume with 35° water, mix, and incubate at 35°. Before the hour has elapsed, pipette portions of 1-5 ml into prewarmed 25 x 250-mm test tubes held in the 35° bath. The size of the portions is chosen to fall within the 0.1-2.5-mg range of the Shaffer-Somogyi micro-method for reducing sugars (Horwitz, 1960, method 29.055, p. 430). After 1 hr of incubation, the inversion is

Table 3. Effect of storage at various temperatures on enzyme and hydroxymethylfurfural content of honey.

Time and temp.	Sample 1			Sample 2			Sample 3		
	Diastase ^a	Invertase ^b	HMF ^c	Diastase ^a	Invertase ^b	HMF ^c	Diastase ^a	Invertase ^b	HMF ^c
-20°C									
76 days	14.9	4.67	30.0	16.10	44.6	17.85
181	14.1	4.09	28.0	15.97	47.7	17.47
274	15.0	4.24	29.4	16.05	44.8	17.77
386	15.1	31.6	45.5
539	14.3	3.87	28.8	15.76	46.2	17.06
4°C									
79	15.0	4.02	29.8	16.16	47.6	17.85
188	15.1	4.07	0.06	30.0	15.69	0.33	44.2	17.07	0.20
276	15.1	3.91	31.0	15.60	45.5	17.21
371	14.4	3.50	0.08	30.6	15.85	0.43	46.9	17.22	0.22
538	15.1	4.04	0.06	30.6	15.36	0.35	45.4	17.06	0.22
21°C									
81	13.9	3.67	0.27	28.6	15.06	0.68	41.2	16.15	0.43
152	3.02	0.44	26.1	13.00	1.06	38.0	13.91	0.90
232	13.5	2.89	0.61	26.6	12.06	1.44	35.5	12.37	1.27
274	12.7	2.36	0.66	26.1	12.16	1.62	37.1	11.80	1.49
371	13.5	2.44	0.96	25.0	10.59	2.24	36.2	10.62	2.19
538	11.6	2.19	1.43	23.2	9.24	3.57	32.6	8.40	3.22
27°C									
63	13.4	2.51	0.62	28.2	11.63	1.30	41.7	11.86	1.23
131	12.5	1.67	1.55	23.2	7.95	3.20	33.0	7.78	3.33
248	9.6	1.20	2.62	19.2	4.92	5.22	26.4	4.69	6.66
350	7.9	4.36	15.9	8.14	21.3	10.5
540	7.05	8.06	13.9	14.5	11.3	20.6
37°C									
7	11.9	2.95	0.32	25.4	13.37	0.65	41.1	14.75	0.65
14	2.33	0.62	24.4	10.23	1.09	36.6	12.07	1.23
28	10.8	1.63	0.91	23.7	7.79	2.24	35.4	8.19	2.83
35	1.34	1.15	5.57	2.81	5.39	3.84
56	8.3	0.95	2.15	16.4	3.56	5.24	22.6	3.12	7.40
98	6.45	4.59	13.5	9.59	17.4	14.3
128	6.07	7.84	10.0	16.0	10.2	24.6
50°C									
5 hr.	3.61	0.30	14.40	0.60	16.29	0.54
8 hr.	13.1	3.22	0.30	28.8	11.83	0.57	46.2	14.91	0.56
1 day	13.2	2.31	0.42	26.3	9.41	0.72	42.9	11.05	0.86
2	11.1	1.40	0.60	22.8	5.82	1.22	36.6	8.67	1.32
3	11.3	0.80	23.0	3.99	3.24	34.9	6.35	1.81
5	6.55	1.52	19.5	5.85	29.4	3.50
9	3.05	11.7	8.07	18.3	8.71
60°C									
2 hr.	2.19	0.29	7.99	0.57	11.45	0.51
4	1.99	0.39	5.99	0.67	9.25	0.62
8	11.8	1.26	0.47	25.0	4.24	0.89	40.0	4.34	0.91
16	9.70	0.59	0.72	17.6	1.47	1.47	29.2	0.96	1.66
24	8.15	1.09	17.6	2.28	24.4	3.17
47	5.40	2.32	11.4	4.98	13.8	7.44
72	4.45	4.31	6.74	8.98	6.74	12.23

^a Grams starch converted per 100 g honey per hr under assay conditions.

^b Grams sucrose converted per 100 g honey per hr under assay conditions.

^c Milligrams per 100 g honey.

stopped by the addition of 5.00 ml Shaffer-Somogyi copper reagent to the tubes, water to make 10 ml total, and the reducing value is determined by the Shaffer-Somogyi method. Units are calculated from the degree of inversion of 5% sucrose solution by a dialyzed honey at 35°C, pH 5.0, and are here reported as grams sucrose hydrolyzed per 100 g honey under the test conditions, to give values comparable to the diastase values. Hadorn's units are similar, except that incubation is at 40°C rather than 35°C.

HMF. The Winkler (1955) method, using barbituric acid and *p*-toluidine, was found superior to several optical methods (Schade *et al.*, 1958; Winkler, 1955) for the determination of HMF in stored honey, and was used in this work. It was standardized against HMF solutions that were assayed spectrophotometrically, using 16,700 for molar absorptivity at 284 m μ (Singh *et al.*, 1948). Results are in mg HMF per 100 g honey.

RESULTS AND DISCUSSION

The results of these analyses are given in Tables 3 and 4. When the

Table 4. Rate constants^a for enzyme inactivation in honey.

	Temp. °C	Diastase k	Invertase k
Sample 1	21	0.0004214	0.001840
	27	0.001873	0.00462
	37	0.009810	0.03220
	50	0.1647	0.539
	60	0.6253	3.51
Sample 2	21	0.0005356	0.001039
	27	0.001933	0.00478
	37	0.01042	0.02736
	50	0.1049	0.490
	60	0.5533	4.218
Sample 3	21	0.0006489	0.001340
	27	0.002141	0.00523
	37	0.01189	0.03054
	50	0.0915	0.357
	60	0.6253	3.641

$$^a k = \frac{1}{t(\text{days})} \ln \frac{100}{\% \text{ activity remaining}}$$

logarithm of percent activity remaining is plotted against time, generally straight lines result, though in a few cases the data fit two intersecting lines, as diastase at 37° for sample 2, and at 37 and 60° for sample 1. In general, as expected, first-order kinetics are indicated for the heat inactivation of diastase and invertase in undiluted honey, over the temperature ranges studied that showed inactivation, 26–60°C. For either enzyme, the percentage inactivation for all three samples at a given time and temperature agreed within 15% or less; in some cases the corresponding

values were within 5%.

To obtain a relationship between temperature and enzyme destruction, rate constants for inactivation were calculated from the lines giving the best visual fit with the data in Table 3, using the first-order equation:

$$k = \frac{1}{t} \ln \frac{100}{x}$$

with t = time in days, x = percent activity remaining. The results are shown in Table 4 and plotted against the reciprocal of the absolute temperature in Fig. 1. From the equa-

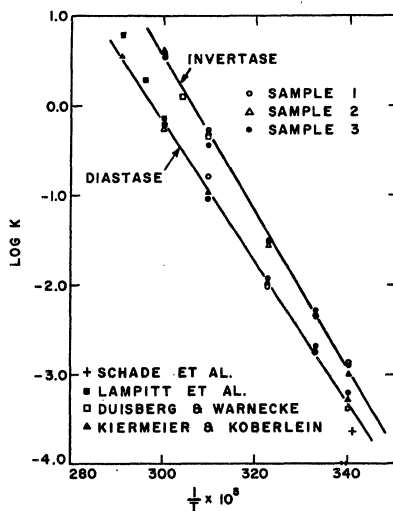


Fig. 1. Effect of temperature on rate of heat inactivation of diastase and invertase in honey. For diastase, $\log k = 22.764 - \frac{35010}{2.303 ET}$; for invertase, $\log k = 26.750 - \frac{39730}{2.303 ET}$

tions given in the legend, rate constants for destruction of invertase and diastase in honey (in fraction of original level per day) may be calculated for any temperature between about 20° and 70°C; extrapolation an additional ten degrees in each direction would seem to be reasonable.

Several points calculated from data in the literature are also shown in Fig. 1. Agreement is reasonably good considering the diversity of honey types and the varying assay methods.

Fig. 2 shows the half-lives of diastase and invertase in honey, calculated from the lines in Fig. 1. It also shows two lines for diastase in honey obtained from a plot of the data in Table 5 of Lampitt *et al.* (1929) for "commercial" honey and an English honey. Four points from the data of Hadorn *et al.* (1962) for a rather acid Yucatan honey are also shown.

Other half-life values (for Swiss honey of considerably less acidity) do not agree with our data; in fact, in storage at 20–23°C Hadorn *et al.* reported diastase to be lost slightly faster than invertase for these honeys, with the reverse true at 50°.

The equations for our half-life lines in Fig. 2 are as follows:

$$\text{Diastase: } \log t_{1/2} = \frac{1}{T - 0.003000} - 0.000130$$

$$\text{Invertase: } \log t_{1/2} = \frac{1}{T - 0.003083} - 0.000113$$

where $t_{1/2}$ is the half-life in days, and T is storage temperature in degrees Kelvin. These equations will provide estimates of the time required for half of the invertase or diastase in honey to disappear during storage (or processing) at any temperature between 10°C (283.1°K, 50°F) and 80°C

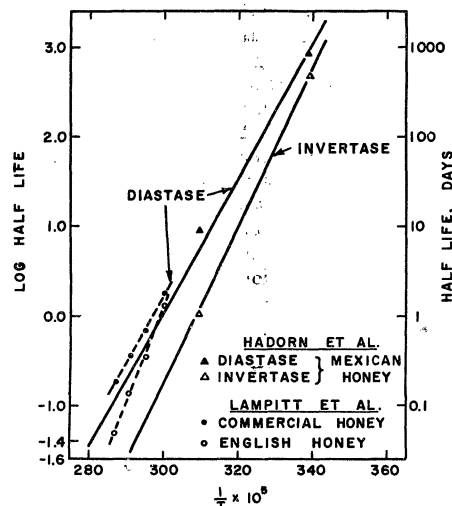


Fig. 2. Half-life of diastase and of invertase in honey. Solid lines—calculated from lines in Fig. 1. Broken lines—data of Lampitt *et al.* (1929) for diastase. Points—values from literature as indicated.

Table 5. Calculated half-lives of honey enzymes.

Temp.	Half-life	
	Diastase	Invertase
10°C	12,600 days	9,600 days
20	1,480	820
25	540	250
30	200	83
35	78	28
40	31	9.6
50	5.38	1.28
60	1.05	0.47 hr
70	5.3 hr	47 min
80	1.2	8.6
90°F	126 days	72
145°F	16.2 hr	48 days
160°F	4.5 hr	38.0 hr
		39 min

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(353.1°K, 176°F). Table 5 shows the results of such calculation for several temperatures in this range.

An invertase preparation was made from honey by a method described elsewhere (White *et al.*, 1963). Samples in 0.01M phosphate at pH 5.9 were heated at 50 and 60°C. Rate constants for the inactivation are shown in Table 6 and compared with

Table 6. Heat inactivation of invertase in honey and in buffer.^a

Temp.	Honey k ^b	Buffer k ^b	Ratio
50°C	0.0204	0.492	24
60	0.175	4.09	23

^a pH 5.9 phosphate, 0.01 M.
^b Per hour.

those for invertase in undiluted honey. The inactivation rate for the enzyme in buffer is about 24 times that of the enzyme in honey.

The effect of storage temperature upon the accumulation of HMF in the three honeys is shown in Table 3. Differences in rate among the honeys were much larger than found for enzyme loss. HMF accumulated about twice as fast in sample 3 as in sample 1, with sample 2 intermediate.

Without intending to recommend 3.0 mg per 100 g as a reasonable limit, the time of storage required at the various temperatures to attain this level of HMF in the three honeys was obtained by plotting data from Table 3. Fig. 3 shows the relationship be-

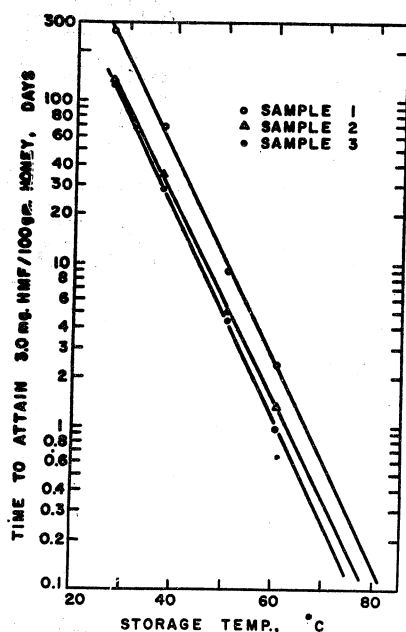


Fig. 3. Effect of temperature on rate of accumulation of HMF in honey.

tween temperature and rate of production of HMF in these honeys, expressed as days required to reach 3 mg per 100 g. The clover honey (sample 1) could be held twice as long as the darker fall-flower types before reaching the 3-mg level. The figure permits an approximation of the time required to reach the 3-mg level at any temperature over the range from 25 to 80°C.

The data in Table 5 indicate that climatic conditions can indeed have an important effect on honey quality. When stored at 90°F (which is quite possible in parts of the United States), four months will destroy half of the diastase; half of the invertase is lost in about 1½ months.

Frequently recommended conditions for pasteurizing honey (Fabian and Quinet, 1928; Milum, 1949) are 145°F for 30 minutes. At this temperature, half of the diastase is lost in 16 hr, and half of the invertase in 3 hr. In 18-36 hr, 3 mg HMF per 100 g honey will appear. Unless heating and subsequent cooling are rapid in such processing, many hours could be required for a batch of honey to reach safe temperatures from 145°F. Such treatment becomes highly important when processing honey to delay granulation [160°F for 30 min (Milum, 1949)], since 4.5 hr, and 40 minutes, respectively, will destroy half of the diastase and invertase. About 5-12 hr will produce 3 mg HMF per 100 g honey.

The effects of heat accumulate, so that the effects of processing and storage must be considered together. For optimum preservation of flavor, enzyme content, and color, and minimum HMF production is necessary. A reduction of 10-15°F in the temperature at which honey is stored for six months, which can be attained by air-conditioning, will substantially reduce the gradual but definite color and flavor deterioration that is characteristic of ordinary storage and provide a more nearly fresh honey flavor at the end of the period. Reducing storage temperatures by this amount will reduce HMF production to ½ and enzyme loss to about 1/6-1/8 of that at the higher temperature. The desirability of reducing storage temperature under commercial conditions can easily be ascertained by holding representative samples at freezer temperatures for the duration of the storage period and comparing them with the same honey held under the conditions in question.

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