

SUGARS AND SUGAR PRODUCTS

Hydroxymethylfurfural and Honey Adulteration

JONATHAN W. WHITE, JR and JAMES SICILIANO

U.S. Department of Agriculture, Agricultural Research, Science and Education Administration, Eastern Regional Research Center, Philadelphia, PA 19118

The value of the determination of hydroxymethylfurfural (HMF) in the detection of invert sirup adulteration of honey is examined. Analysis of 481 samples of extracted honey and 41 comb honeys from producers, and samples of honey before and after processing from 8 packers provided basic data for establishing guidelines for HMF content of honey. A sample containing 20 mg/100 g or more should be considered as possibly adulterated and subjected to additional analysis for confirmation of the presence or absence of adulteration. Extremely high (about 50 mg/100 g) values are conclusive, however.

Detection of invert sirup added to honey has been a problem for nearly a century. Addition of moderate amounts of invert sirup does not cause glucose and fructose levels to fall outside of the normal range for honey. Qualitative color tests used in years past depended on the detection of hydroxymethylfurfural (HMF), which was produced during the acid-catalyzed inversion of sucrose. Indeed, the 12th edition of *Official Methods of Analysis* of the AOAC includes a resorcinol test for commercial invert sugar (31.138). The general unreliability of such tests is indicated by a note: "Resorcinol test, when neg., may not be regarded as conclusive evidence of absence of com. invert sugar sirup in honey."

Problems and evaluations of these tests are discussed elsewhere (1). The description in 1955 (2) of 2 quantitative methods for HMF in honey stimulated interest in their use for evaluation of honey quality. Based essentially on data from over 1700 samples of honey imported into Germany and Switzerland, the honey standards of the Codex Alimentarius (3) included a maximum value for HMF in table honey of 4 mg/100 g. This value was selected to assure that table honey available in the participating countries is not denatured by heat, thus destroying health-giving properties they believed to be present. Honey with higher HMF content is relegated to the manufacturing trades at lower prices.

For many years honey has been known to contain HMF arising from action of normal honey acidity (av. pH 3.9) on fructose at ambient temperatures and at an accelerated rate during heat processing or storage at elevated temperatures. This caused early difficulties with qualitative tests for invert sirup adulteration and must be recognized in differentiating between normally processed honey and that containing added invert sirup.

Several of the important honey adulterants do not contain significant amounts of HMF. High fructose corn sirup (HFCS) and many conventional (non-fructose) corn sirups (CCS) are lower than processed honey in HMF. Other tests are required to detect their addition to honey.

From the studies of Schade *et al.* (4), Hadorn and Kovacs (5), Gautier *et al.* (6), Hadorn and Zürcher (7), White *et al.* (8), and Gonnet (9) on the production of HMF in honey by heat and storage, it is apparent that, as with all other aspects of honey chemistry, this area is characterized by extreme variability. No fixed formula can be devised to predict exactly the effect of storage and heating on HMF content of honey. White *et al.* (8) found a linear relationship between storage temperature of honey and the logarithm of the time required to accumulate a given amount of HMF. This finding was based on extensive storage studies of 3 honey samples and was used to estimate that about 3 times the heat exposure required to produce an HMF concentration of 4 mg/100 g honey is needed to produce an HMF level of 20 mg/100 g honey (1). Any guideline established for a permissible HMF level in honey cannot be the sole basis for condemnation as adulterated. Additional compositional evidence is required. The measurement of HMF is a relatively easy procedure with the new bisulfite method (10) which is intended as a screening procedure for questioned samples to reduce the need for complete carbohydrate analysis for demonstrating the presence of added invert sirup (11).

A study was therefore conducted to determine the HMF levels of United States honey as produced by beekeepers and to estimate the effect of United States commercial processing on HMF content. The objective was to provide a practical maximum level of HMF beyond which a sample would be suspected of being adulterated with invert sirup, without discriminating against genuine honey abused by heat or storage. A collection of 480 samples of United States honey, certified as genuine by their producers, was obtained for another purpose (12), and we determined the HMF content of these samples to obtain baseline values for such honey. We also analyzed honey in the comb to establish baseline values for the HMF content of unprocessed honey. By enlisting the cooperation of a number of honey packers, we also analyzed samples of honey before and after normal processing to obtain information on the increase of HMF caused by thermal processing.

Experimental

Producer Samples

Samples were from the 1974 and 1975 crop years, and information on floral type, heating, and storage history was provided for most of the 480 samples that were voluntarily submitted. Samples were refrigerated immediately when received and were not heated before HMF analysis.

Processing Samples

Packers, whose joint output represents at least 70% of United States commercial honey, were requested to provide samples of honey before and after their customary processing for both retail and bulk pack. This was done because circumstances prevented visits to the processing plants for the direct collection of samples. Usable samples were received from 7 honey packers and extensive data, which are included here with permission, from another.

Comb Honey Samples

A total of 41 samples of honey in the comb were received. These were crushed and separated from the wax residues by gravity straining through four layers of cheesecloth, without heat.

Table 1. Hydroxymethylfurfural (HMF) content of United States honey as received from producers

Type	No. of samples	HMF, mg/100 g		
		Mean	s	Range
Liquid	481	0.62	0.99	0.00-13.6
Comb	41	0.27	0.26	0.03- 0.92

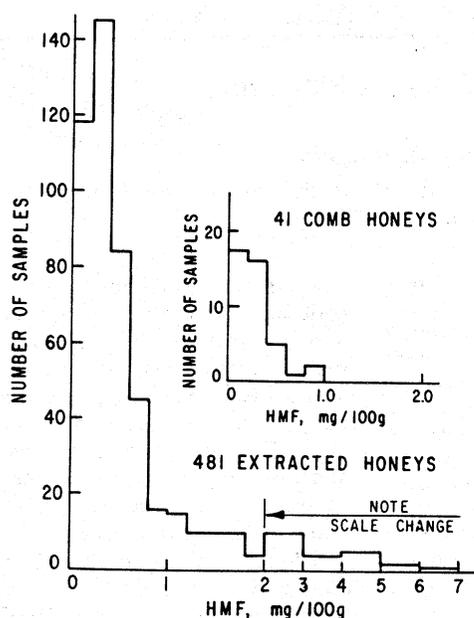


FIG. 1.—Distribution of hydroxymethylfurfural content among 481 samples of extracted honey direct from producers and among 41 comb honeys.

They were prepared within 24 hr of receipt and were stored below 5°C until analyzed for HMF.

Determination of HMF

The barbituric acid-toluidine method of Winkler (2) was used, with a Stasar II spectrophotometer equipped with a flow-through cell. In this method, 5 mL 10% *p*-toluidine in 50% isopropanol containing 10% acetic acid is added to 2 mL of a 20% honey solution. Then 1 mL 0.5% barbituric acid is added, and the absorbance is read against a blank containing water instead of barbituric acid. The time-maximum absorbance occurs at 550 nm. The reaction mixture was retained in the cell until the maximum value for absorbance had been observed, the time depending on ambient temperature. The instrument was calibrated with HMF (Sigma Chemical Co., St. Louis, Missouri) which was assayed by the strength of the UV absorption maximum at 280 nm, with $\epsilon = 16830$ as standard (13).

Results and Discussion

The HMF contents of the producer and comb honey samples are shown in Table 1. Figure 1 shows the distribution of values for both sets of samples. The comb honey samples had been exposed only to ambient temperatures. Two samples with HMF about 0.9 mg/100 g originated from warm climates (Florida and south-

Table 2. Effect of commercial processing upon hydroxymethylfurfural content of honey

Packer	Pack	Hydroxymethylfurfural, mg/100 g			Mean diff.
		Before	After	Diff.	
A	retail	1.71	2.79	1.08	1.11
	bulk (drum)	1.69	2.91	1.22	
	bulk (cans)	1.95	3.00	1.05	
B	retail	1.48	1.36	-0.12	1.56
	bulk	2.32	2.20	-0.12	
	bulk	5.51	7.40	1.90	
	retail	1.90	4.01	2.11	
	retail	5.58	7.60	2.02	
	bulk	5.09	8.65	3.56	
C	bulk	16.7	16.7	0	3.47
	bulk	8.72	18.5	9.78	
	bulk	2.93	6.19	3.26	
	bulk	3.85	6.21	2.36	
	bulk	2.74	4.69	1.95	
D	retail	0.0	1.69	1.69	2.04
	retail	0.0	1.64	1.64	
	retail	1.11	3.89	2.78	
E	retail	1.12	1.27	0.13	0.11
	retail	1.38	1.58	0.20	
	retail	1.12	1.12	0.00	
F	retail	0.28	1.75	1.47	1.47
G	retail	0.73	2.31	1.58	1.28
	retail	0.87	2.71	1.84	
	bulk	0.61	1.03	0.42	
Average increase, all data					1.58

ern California). The extracted honey collection represents the varied heating and straining practices of the producers. Records of heating were provided; 31 producers heated their honey to 150°F or more, some holding it at high temperatures for several hours. Many heated to 120–140°F; many did not heat the honey at all. The wider range of values and the higher mean and deviation for extracted honey compared with comb honey reflects the treatment of the honey after extraction. The sample with the highest HMF content had been heated excessively (10 hr at 155°F) and stored 10 months in Florida temperatures. This degree of heat treatment is totally unnecessary and is destructive to honey flavor and aroma.

Honey is processed by heat and straining or pressure filtration to delay granulation and to eliminate yeast spores. The exact procedures used differ among packers and would be ex-

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Table 3. Hydroxymethylfurfural content of honey during processing and packing^a

Process	HMF, mg/100 g		
	1	2	3
Sampled from 55 gal. drum	0.42	0.35	0.45
After melting in hot oven	0.47	0.63	0.54
After 15 hr in settling tank	0.60	0.91	0.70
Immediately after bottling	0.58	0.94	0.84
Cased, stacked, stored 9 days	1.18	1.30	1.28
After 1 year storage	2.77	3.41	3.43
Increase from processing	0.76	0.95	0.83
Mean	0.85		

^a Data provided by R. W. Meloy, Sioux Honey Association.

pected to have variable effects on HMF content. The results of analyses of before and after processing samples sent by cooperating packers are shown in Table 2. Retail pack is table honey in small glass containers; bulk pack usually refers to darker, lower-grade honey sold in 60 lb tins or 55 gal. steel drums for food manufacturing, but high quality, light-colored honey also is sold in bulk for this purpose. Data from 4 additional packers were not used because analytical results indicated that the same lots of honey were not followed through the processing, since after values were appreciably lower than before values. The spread in HMF increase reflects differences in individual processing practices. It seems reasonable to estimate that commercial processing increases HMF content of honey by about 2 mg/100 g honey.

In a large, modern plant, 3 lots of honey were sampled at various stages of processing, and data for HMF content at each stage are presented in Table 3. The effectiveness of the procedure for bulk melting is indicated by the small increase in HMF content from the raw honey to post-settling sample; the increase averaged only 0.33 mg/100 g. Bottling, casing, and stack heat caused another 0.46 mg/100 g increase. The effect of one year's storage was to increase HMF levels further by an average of 255%. The average increase in HMF content (0.85 mg/100 g) resulting from the processing procedures used in this plant was smaller than any of those in Table 2, except for that of packer E, a very small operation.

Guidelines for HMF Content of Commercial Honey

The establishment of a value for the HMF content of honey beyond which a sample must

be considered possibly adulterated with invert sirup is difficult. Processing and storage of honey, even at relatively low temperatures, can add significantly to HMF concentration. Honey may be exposed to tropical ambient temperatures for months before shipment. A review of the literature (1) for this purpose indicated that a value of 20 mg HMF/100 g honey is reasonable for a guideline, based on the effect of tripling the heat exposure (processing or storage) needed to produce an HMF level of 4 mg/100 g. Data presented here are consistent with this guideline.

No sample should be condemned solely on the basis of containing 20 mg (or more) HMF/100 g honey. Other evidence of abnormal composition must also be present. For example, only 6 of 15 samples cited as adulterated (11) had an HMF content >20 mg/100 g, and 3 samples that appeared to contain no honey and consisted only of invert sirup had low values for HMF content (1.4, 1.9, 2.8 mg/100 g). In the latter 3 samples, the fructose/glucose ratio was below 1.0, and total monosaccharides were above and total disaccharides were below the honey compositional limits given in that paper. Low levels of HMF in these 3 samples indicated that the product was not an acid-invert sirup.

We concluded that knowledge of HMF content of a honey sample is informative but not conclusive of adulteration with invert sirup unless extremely high (>50 mg/100 g) values are obtained. Decisions regarding adulteration with invert sirup must be based on deviation of several compositional parameters from honey norms (11).

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