HYDROXYMETHYLFURFURAL CONTENT OF HONEY AS AN INDICATOR OF ITS ADULTERATION WITH INVERT SUGARS

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Honey is principally a solution of glucose and fructose, with minor amounts of sucrose, reducing disaccharides, and higher sugars. The average composition of United States honey, and the ranges of components (Table 1) [18] shows that sucrose is not a potential adulterant, and that completely inverted sucrose (i.e. invert sugar) could be added to honey in considerable amounts without leading to common analytical values outside their natural ranges.

TABLE 1. Average percentage carbohydrate composition of 490 samples of honey, with the range of values[18].

Component	*Average*	*Standard deviation*	*Range*
Fructose	38·19	2·07	27·25-44·26
Glucose	31·28	3·03	22·03-40·75
Sucrose	1·31	0·95	0·25- 7·57
Maltose†	7·31	2·09	2·74-15·98
Higher sugars	1·50	1·03	0·13- 8·40

† i.e. reducing disaccharides

Several colorimetric tests were devised many years ago to indicate the addition of acid-inverted syrup to honey. These tests—the resorcinol (Fiehe) test and the aniline (Feder) test—have been studied intensively, and modified. Considerable early controversy was concerned with the interpretation of the colours produced. The reagents were known to react with the hydroxymethylfurfural (HMF) formed from fructose by action of acid and heat. Invert sugar prepared with acid contains variable amounts of HMF, depending on the conditions used. The minimum amount of added invert sugar detectable by these tests thus depends on their sensitivity, and on the characteristics of the invert syrup used.

It was recognized long ago[1] that if honey is heated sufficiently it gives a positive result in these tests. Such heating was said to destroy the flavour characteristics. More recently it became evident[4] that extended ordinary storage also leads to accumulation of enough HMF for a positive result, the period of storage being a function of the temperature. After quantitative methods for HMF in honey became available in the 1950s [13, 19], studies were made of the factors affecting the rate of HMF formation in honeys.

The detection of adulteration of honey by the addition of invert sugar will depend upon the difference between the HMF content of authentic honey in commerce and

* Agricultural Research, Science and Education Administration, U.S. Department of Agriculture. Retired 15 April, 1978.

that of honey to which economically attractive amounts of invert syrup have been added. It thus becomes necessary to establish an upper limit for the HMF content of honey which has been stored and processed under conditions of good manufacturing practice. To this end, the scientific literature has been examined for reports on the HMF content of commercial honey and the effect of heating and storage on the HMF content of honey.

The HMF content of commercial honey

The publication[18] of two quantitative methods for HMF in honey provided an opportunity in European countries for the extension of examinations of imported honey to include their HMF levels as well as diastase content. Europeans had for many years insisted that honey sold for direct consumption must meet minimum standards for diastase, to ensure that the honey had not been overheated to the point of obscuring its alleged health-giving properties. Enforcement of these rules has provided a considerable volume of analytical data on honey from the major exporting countries. When the quantitative HMF methods became available, limiting values for HMF content of honey were established, in addition to those for diastase values.

Data for non-European honeys entering the German Federal Republic and Switzerland in 55-gallon drums before 1960, and for Australian honeys reported in 1974, are summarized in Table 2. Much more extensive data were published in 1966[5], with HMF analyses of 1554 imported samples of commercial honeys received between 1960 and 1966 by laboratories in Switzerland and the German Federal Republic (Fig. 1). For the purpose at hand, the 39 Swiss honeys (in Fig. 1) are not included.

A few additional scattered values may be found in the literature, but they are insignificant in comparison with the above results. The average HMF content for all 1728 samples of honey reported in Table 2 and Fig. 1 is 1·24 mg/100 g.

TABLE 2. HMF content (mg/100g) of non-European honeys imported into the German Federal Republic and Switzerland[9], and of Australian honeys[2].

Source	*No. samples*	*Average*	*Standard deviation*	*Range*
German imports	38	4·0	3·1	0·2-11·7
Swiss imports	32	3·4	2·8	0·4-12·1
Australian :				
eucalyptus	60	0·31		
other sources	21	0·37		
commercial Australian blends	10	1·0		
commercial imports honey	13	2·7		

Effect of heat and storage on HMF in honey

As noted earlier, it has been known for many years that a positive colour test for HMF could result from overheating or long storage of honey. Much controversy in the literature revolved about this, because the colour tests yielded different results with different investigators, thus in effect creating differing standards for judging adulteration by invert sugar.

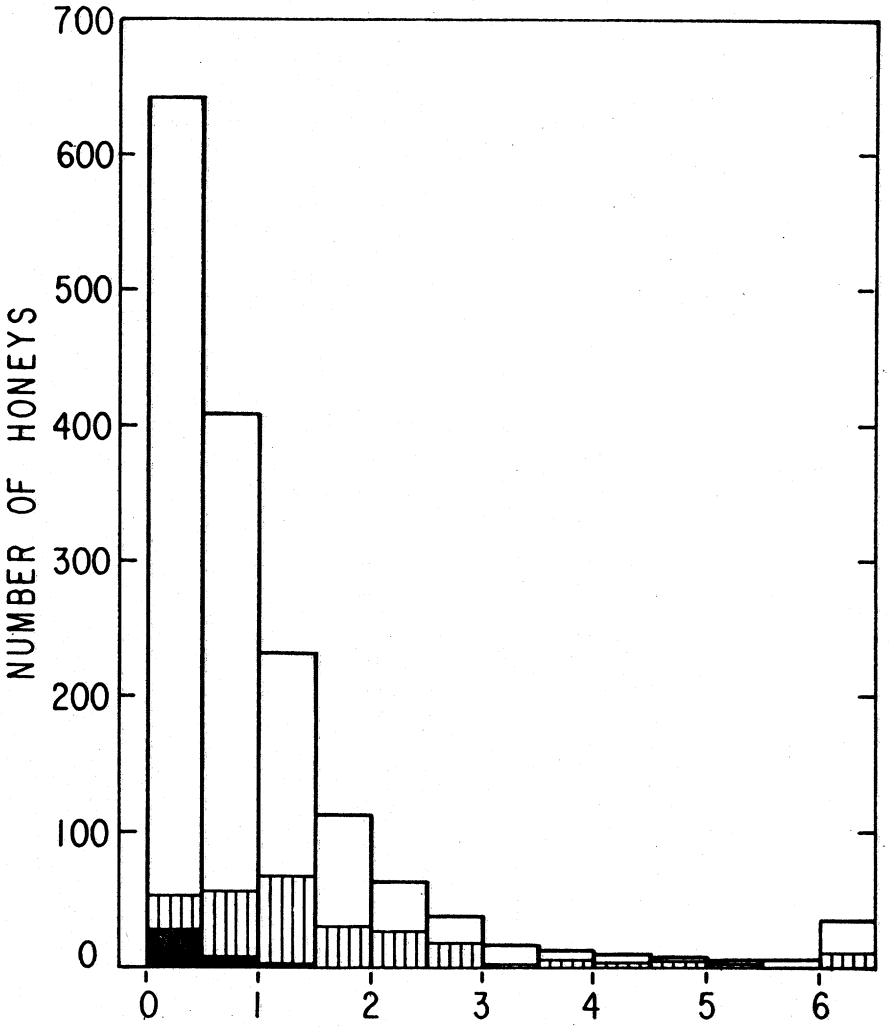

FIG. 1. Frequency distribution of the HMF content of 1596 samples of honey[5].

White bars 1307 imported honeys, Bremen laboratory
Hatched bars 247 imported honeys, Basel laboratory
Black bars 39 Swiss honeys, Basel laboratory

Nothing is to be gained by reviewing the large number of earlier studies which attempted to show that the Fiehe and Feder tests were, in fact, suitable for distinguishing between heated and adulterated honey. Authors in the USA[8, 12, 14, 16], some of whom used collaborative testing, all concluded that the colour tests were useful, as did French authors[6] in 1961. Earlier work reported the conditions of heating or storage that lead to a positive colour test; difficulty with interpreting the results arises

because the tests appear to have differing sensitivity in different hands. With the introduction of Winkler's methods[18] for quantification of HMF in honey, more nearly objective studies could be made of the effects both of storage and heating and of the compositional factors influencing HMF formation. Variability among honeys was demonstrated in this respect, and the rate of HMF formation shown to be correlated directly with moisture content and with initial HMF content of the honey[13]. Other unknown factors also affected rates: the acidity of honey had a positive effect on the formation of HMF[12], and the lower rate of HMF formation in heated Swiss honeys was ascribed to their higher pH (4·5-5·0) than most other honeys (pH 3·8)[11].

Several groups of workers have reported the effect on HMF content of various heat treatments of honey. The temperature exposures ranged from those normally used in honey processing, to those that were deliberately excessive in terms of deleterious effect on general organoleptic qualities.

The HMF content of four samples of honey increased during storage for 13-15 months at 20°C[13], that of one sample by 3·3 mg/100 g. In one California alfalfa honey HMF increased from about 0·3 to about 7 mg/100 g during 48 hours at 68°C; Fig. 2 shows increases in three other heated alfalfa honeys[13], and Table 3 shows the effect of a temperature of 50°C on several honeys imported into Switzerland. European (mostly French) honeys have been held for 10 h at 70°C[6]; 16 out of 25 were harvested just previously, and their HMF content (measured spectrophotometrically) was equal to or less than 13 mg/100 g, attainable by adding only 2% of an invert sugar with an HMF content of 650 mg/100 g.

The data of the investigations reported above were obtained from small-scale laboratory tests. An exception is the work of Hadorn and Zürcher[10], who followed the HMF content in 300-kg drums as they were taken through the procedure normally used in Switzerland to liquefy the contents. Drums were held in a room at 48°C, and all the honey reached this temperature within 24 h. The HMF content in three drums was 1·2, 1·3, 1·2 mg/100 g before liquefying, and 2·2, 2·7 and 2·4 respectively after 120 hours.

TABLE 3. HMF content (mg/100 g) of imported honeys before and after heating at 50°C[9].

Source of honey	*Heating period (h)*	*Initial HMF*	*Final HMF*
Guatemala	100	2·5	5
	300		16
Central America	100	0·6	2
	300		8
California	100	1·6	4·3
	200		8
Mexico	100	0·1	0·8
	200		2·6
Heating temperature 60°C			
Yucatan 1	45	5·6	14
2	45	0·6	3·5

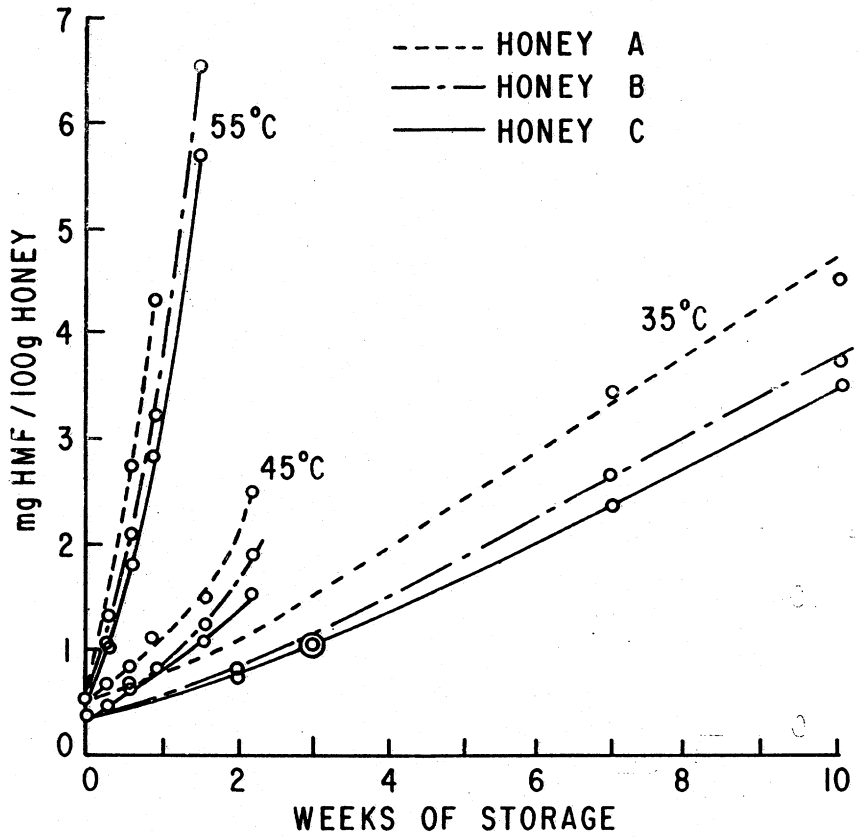

FIG. 2. Increase in the HMF content of three alfalfa honeys during storage at 35°, 45° and 55°C[13].

In the USA, White, Kushnir and Subers[16] subjected three honey samples to storage at seven temperatures ranging from −20°C to 60°C and analysed them for enzyme and HMF contents. In France Gonnet examined the accumulation of HMF in honey heated at 60, 70 and 80°C, and also the HMF content of a honey mixed with invert syrup (3% to 19%) which had been prepared with citric acid and contained 170 mg/100 g HMF (Fig. 3)[7].

Existing guidelines on HMF content of honey

As a result of researches described above, several proposals have been made to establish limits on the HMF content (among other criteria) of genuine honeys. All these proposals are of European origin, and they arise from the expressed desire of the countries concerned to provide to their consumers honey with minimal practicable heat exposure.

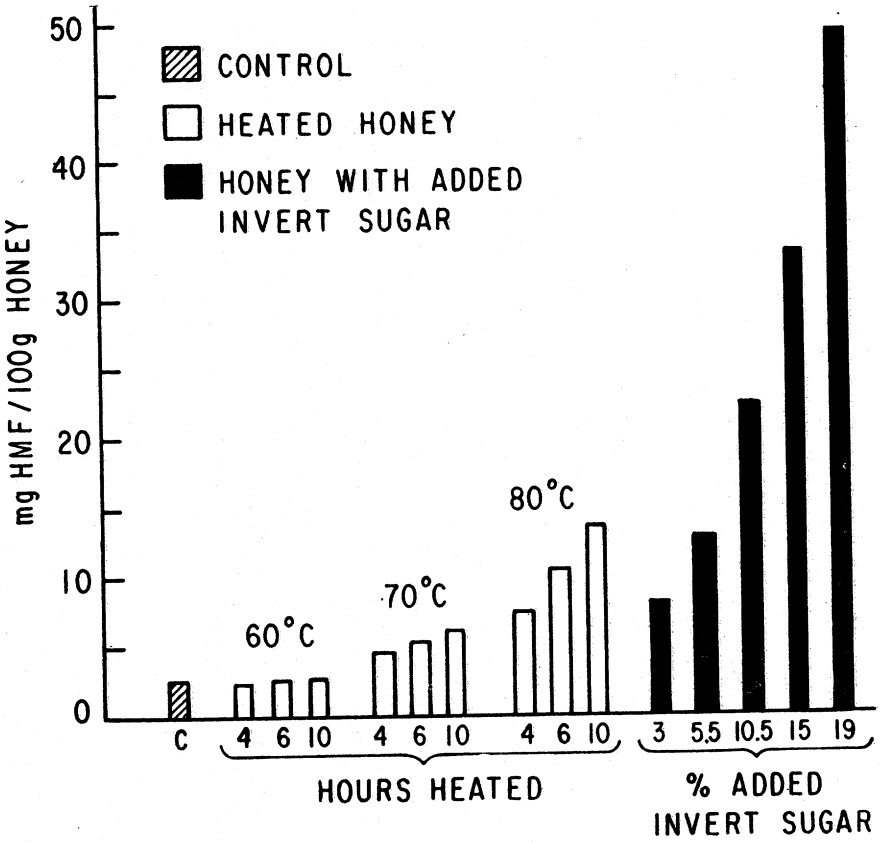

FIG. 3. Influence of heat, and of addition of invert sugar, on the HMF content of honeys[7].

Hadorn, Zürcher and Doevelaar[11] proposed a maximum of 3 mg/100 g for 'acceptable, old, or slightly heat-damaged honey' and listed values of 0·1-0·3 for 'acceptable, entirely fresh honey'. Duisberg and Hadorn[5] proposed a maximum for honey in bulk of 3 mg/100 g and, in consumer packages, 4 mg/100 g. The Codex Alimentarius in its *Recommended European regional standard for honey*[3] permits a maximum after blending and processing of 4 mg/100 g, except for honeys of naturally low enzyme content for which the maximum is 1·5 mg/100 g.

HMF content of invert sugar

The minimum percentage of added invert sugar syrup that is detectable in honey depends to a considerable extent upon the HMF content of the invert syrup, when HMF content is the test criterion. Five invert sugars, prepared on a laboratory scale by citric acid inversion, contained an average of 530 mg/100 g (range 450 to 650)[6]; a value of 170mg/100 g was obtained for a citric acid inverted syrup[7].

Current information on commercially available invert syrups, obtained from representatives of three major United States refiners, gave a general consensus that about 99% of the invert syrup sold is a mixture (about 1:1) of sucrose and invert sugar; this is of little value for adulterating honey because of its high sucrose content. Two of the commercial sources sell enzymatically converted, completely inverted products; they are produced without acid, so would not contain HMF as a result of the inversion process, although since they are made from molasses they do contain HMF. One refiner sells a clear 'full' invert which is produced by acid (not ion-exchange) inversion, but provides no information on its HMF content; however, the HMF content is monitored as a predictor of colour formation in storage.

It is likely that those who add invert syrup to honey will continue to carry out the inversion process themselves, because the process is relatively simple and inexpensive, and because no records are then available to document any purchase of invert syrup.

Suggested guidelines or action level for HMF in honey

In Fig. 4, two lines calculated from experimental data[16] show the number of days of exposure to the indicated temperatures that would bring about accumulation of HMF

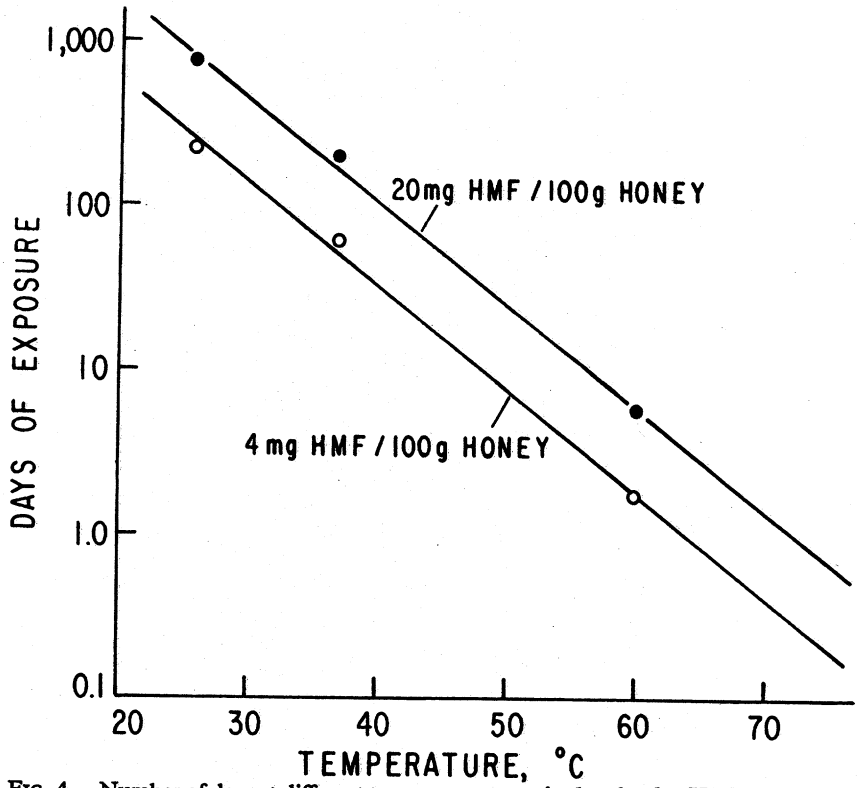

FIG. 4. Number of days at different temperatures required to develop HMF in honey at 4 and 20 mg/100 g[16].

at 4 and at 20 mg/100 g of an average honey. The lower level (4) is that given in the Codex Alimentarius Standard and is a conservative maximum value for authentic, normally processed and stored honey which is attained in actual practice in Europe. Holding the honey at a given temperature for about 3 times as long will increase the HMF content to 20, an excessive amount which is probably not encountered in normal processing and is accompanied by obvious deterioration of flavour quality.

It is therefore suggested that honey samples containing HMF at 20 mg/100 g or more should be regarded as possible mixtures of honey and invert syrup and, as such, candidates for further examination.

Summary

Recognition that hydroxymethylfurfural (HMF) in honey can arise from heating or storage has raised questions about the validity of using tests for HMF as an indicator of the addition of invert syrup to honey.

A review of the scientific literature on this subject permits the following conclusions:

1. The long-employed colour tests of Fiehe and Feder can successfully differentiate between heated/stored honey and honey mixed with invert sugar, provided the tests are carried out by recently established modifications which essentially quantify their results; a more direct and preferable approach is the simpler quantitative measurement of HMF.
2. Data from 1728 determinations of HMF in commercial honeys published from four laboratories between 1960 and 1974 show the average HMF content of honey to be 1·24 mg/100 g.
3. Existing European quality standards allow a maximum of 4 mg/100 g.
4. If the period of heat exposure (processing and/or storage) that would produce 4 mg/100 g HMF in honey is tripled, a level of 20 mg/100 g is attained.
5. The HMF content of citric acid inverted invert syrup is 170-650 mg/100 g.
6. A suggested action level of 20 mg/100 g would allow selection of honeys possibly adulterated with acid-converted invert syrup from normally stored and processed honeys, for further examination.

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Note added in proof:
A paper 'Hydroxymethylfurfural and honey adulteration' by White and Siciliano will appear early in 1980 in *J. Ass. Off. Anal. Chem.*, reporting analyses of 522 samples of U.S. honeys and tracing the HMF content of 24 lots through processing by 8 packers.