

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V23

HYDROXYMETHYLFURFURAL (HMF) IN HONEY

Correspondence on this method may be sent to R. Wood, Statutory Methods (Chemistry and Microbiology) Department, Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Food Safety Directorate, Norwich Research Park, Colney, Norwich NR4 7UQ

1. Scope and Field of Application

The method allows the determination of the HMF content of honey. It is the same in principle as that described in CAC/12-1969, Codex Alimentarius Commission Recommended European Regional Standard for Honey, and is based on ref. 9.2.

2. Definition

The hydroxymethylfurfural content: the HMF content as determined by the method specified.

3. Principle

Spectrophotometric determination using barbituric acid and *p*-toluidine solutions.

4. Reagents

All reagents should be of recognised analytical grade unless specified otherwise.

4.1 Barbituric acid solution

4.1.1 Transfer 500 mg of barbituric acid to a 100 ml volumetric flask using 70 ml of water. Place the flask on a very hot water bath until the barbituric acid dissolves, allow to cool and make up to volume with water.

4.1.2 Barbituric acid

4.2 *para*-Toluidine solution

4.2.1 Weigh 10.0 g of *p*-toluidine and dissolve in approximately 50 ml of *iso*-propanol by heating gently on a water bath. Transfer the solution to a 100 ml volumetric flask with *iso*-propanol and add 10 ml of glacial acetic acid. Allow to cool and make up to the calibration mark with *iso*-propanol. Do not use for at least 24 hr. Store the solution in the dark. Care should be taken when handling *p*-toluidine.

4.2.2 *iso*-Propanol

4.2.3 *p*-Toluidine

4.2.4 Acetic acid, glacial

4.3 Water, oxygen-free.

Bubble oxygen-free nitrogen into boiling water for several minutes. Allow the water to cool before use.

4.4 Hydroxymethylfurfural, pure, for preparation of standard solution (6.2).

5. Apparatus

5.1 Spectrophotometer, calibrated to read at 550 nm.

5.2 Volumetric flasks, 50 ml and 100 ml capacity.

5.3 Pipettes, 1 ml, 2 ml and 5 ml.

6. Procedure

6.1 Preparation of the sample for analysis

The mass of the sample presented to the laboratory for analysis shall be at least 200 g. The prepared sample shall always be kept in an air-tight and moisture-tight container.

6.1.1 Liquid honey or pressed honey

Mix carefully by stirring or shaking without heating.

6.1.2 Comb honey

Remove the upper part of the combs, if they are sealed. Completely separate the honey from the combs without heating, if practicable by passing through a sieve; the mesh of the sieve is formed by wires woven to form square apertures of side 0.50 mm.

6.2 Preparation of the sample solution

Weigh 10 g sample of prepared honey (6.1) and dissolve it without heating in 20 ml of oxygen-free water (4.3). Wash the entire contents into a 50 ml volumetric flask (5.2) and make up to volume with water (4.3); designate "honey solution". The solution should be analysed as soon as it has been prepared.

6.3 Photometric determination

6.3.1 Sample determination

Take two test tubes and pipette into each of them 2.0 ml of honey solution (6.2); then add to each tube 5.0 ml of the *p*-toluidine solution (4.2). Pipette into one of the tubes (blank) 1.0 ml of water (4.3) and into the other (sample) 1.0 ml of barbituric acid solution (4.1). Agitate both tubes. Add the reagents quickly so as to complete the operation within a minute or two. Read off the extinction of the solution in the sample tube compared with the blank tube at 550 nm using a 1 cm cell, as soon as the maximum absorbance value is reached.

6.3.2 Standard determination

The method should be calibrated using a standard solution of HMF (4.4). Check the purity of the HMF standard by spectrophotometric assay at 284 nm, where the value of *E* (molar) is 16.830. Prepare a solution of HMF in water so that a 10 µg standard of HMF in 2 ml of water can be used in place of the honey solution in 6.3.1. Carry out the colour reaction as in 6.3.1 and measure the absorbance as in 6.3.1.

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

The level of HMF is calculated and expressed as mg of HMF per kg of prepared honey.

8.1 If the method is not standardised, an approximate value for HMF level (mg HMF per kg honey) is given by:

$$\text{HMF content (mg/kg)} = \frac{\text{Absorbance} \times 192}{\text{Cell path length (cm)}}$$

8.2 Using a standard determination, the HMF level (mg of HMF per kg of honey) is given by:

$$\text{HMF content (mg/kg)} = \frac{\text{Absorbance of sample solution} \times 25}{\text{Absorbance of standard solution}}$$

9. References

- 9.1 DW Lord, MJ Scotter, AD Whittaker and R Wood, J. Assoc. Publ. Analysts, 1989, **26**, 51-76.
- 9.2 JH Turner, PA Roberts, CL Barrich and RH Cotton, Anal. Chem., 1954, **26**, 898.
- 9.3 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16.

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V.0 of the series⁽³⁾.

A number of laboratories taking part in the collaborative trial of this method experienced some difficulty in obtaining the intralaboratory precision expected. Moreover, the substantial observed differences between repeatability and reproducibility values suggest that interlaboratory precision is not altogether satisfactory. Further investigation is required, and the possibility of an alternative method must be considered; meanwhile, the method may be used within the constraints of the observed precision (Table 1), but cannot be recommended for enforcement purposes.

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r ,

deduced from the collaborative trial data summarised below (Table 1). Overall, r may be taken as 9 mg/kg, though better precision might be expected. When analysing honey with HMF content above 20 mg/kg, this corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r , of less than 7%. However, at lower HMF contents the method becomes less precise (RSD_r up to 30%).

A2 Reproducibility

The absolute difference between two test results obtained under reproducibility conditions should not be greater than the reproducibility, R , deduced from the collaborative trial data summarised below (Table 1). Overall, R may be taken as 30 mg/kg, corresponding to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R , of up to 34%.

A3 Trueness (Bias)

Accuracy was not tested by spiking samples with known concentrations of HMF. However, there is no reason to suspect systematic bias.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggests an accuracy which, if maintained, corresponds to an extrapolated lower limit of acidity of roughly 9 mg of HMF per kg of honey for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed eight samples of honey once (four samples from different countries in blind duplicate). The samples did not require preparation (6.1) before analysis.

Table 1 summarises the statistical data; the HMF levels are expressed as mg of HMF per kg of honey.

TABLE 1
Statistical Analysis of Hydroxymethylfurfural (HMF) in Honey Samples

Sample	1/7	2/5	3/8	4/6
Number of Laboratories retained after eliminating outliers	18	14	17	17
Number of Laboratories eliminated as outliers	1	5	2	2
Number of results accepted after eliminating outliers	36	28	34	34
LEVEL OF ANALYTE				
Mean observed value $\bar{x}$	11.4	21.2	41.8	54.8
REPEATABILITY				
Standard Deviation S_r	3.4	0.89	2.9	1.6
Relative Standard Deviation $RSD_r(\%)$	29	4	7	3
Repeatability r [2.8 xh S_r]	9.4	2.5	8.2	4.6
REPRODUCIBILITY				
Standard Deviation S_R	3.9	2.5	10.0	7.2
Relative Standard Deviation $RSD_R(\%)$	34	12	24	13
Reproducibility R [2.8 xh S_R]	11.0	7.0	27.9	20.2

A6 Key to Table 1

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility