MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

MAFF VALIDATED METHOD V19 **APRIL 1992**

METHOD FOR ACIDITY IN HONEY

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COSHH AND SAFETY CONSIDERATIONS

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulation, 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.

1. SCOPE AND FIELD OF APPLICATION

The method allows the determination of the acidity of honey. It is the same as that described in CAC/12-1969, Codex Alimentarius Commission Recommended European Regional Standard for Honey.

2. DEFINITION

Acidity: the content of acid (expressed in milli-equivalents of acid per kg) as determined by the method specified.

3. PRINCIPLE

A plot of the neutralisation curve of honey is obtained by titration of a sample with sodium hydroxide. The acidity is calculated from the total titrant used at the equivalence point.

4. REAGENTS

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

4.1 Standard sodium hydroxide solution, 0.05 mol/l (carbonate-free).
4.2 Water, carbon dioxide-free, prepared by boiling and cooling distilled water immediately prior to use.

5. APPARATUS

5.1 pH meter
5.2 Magnetic stirrer
5.3 Analytical balance
5.4 Volumetric flask, 50 ml
5.5 Beaker, 50 ml
5.6 Pipettes, 25 ml
5.7 Burette, capable of being read to 0.05 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 200g. The prepared sample shall always be kept in an air-tight and moisture-tight container.

6.1.1 Liquid honey or pressed honey

If the sample is free from granulation, mix carefully by stirring or shaking. If the honey is granular, place in a closed container on a water bath, taking care not to immerse it, and heat for 30 min. at 60°C; further heat, if necessary, at 65°C until liquefication is complete, occasionally shaking the container. Mix carefully and allow to cool rapidly as soon as the sample liquefies. Do not heat honey which has to be used for the determination of hydroxymethylfurfural content or diastase activity. If the honey contains foreign matter (eg wax, twigs, bees or particles of honeycombs), heat the sample to 40°C on a waterbath and strain the honey through cheesecloth in a hot-water jacketed funnel before sample preparation.

6.1.2 Comb honey

Remove the upper part of the combs, if they are sealed. Completely separate the honey from the combs by passing through a sieve; the mesh of the sieve is formed by wires woven to form square apertures of side 0. 50 mm. If part of the wax or comb passes through the sieve, heat the sample as described under 6.1.1 and strain the honey through a filter. If the honey is granular, heat it until the wax liquefies, stir, allow to cool and remove the wax.

6.2 Analysis of the prepared sample

6.2.1 Accurately weigh approximately 5 g of honey. Dissolve in a few ml of water, transfer quantitatively into a 50 ml volumetric flask (5.4), and make up to volume with water. Pipette 25 ml from the flask into a beaker (5.5).

6.2.2 Place a magnetic stirrer (5.2) in the beaker, stir the liquid gently and titrate potentiometrically with sodium hydroxide solution (4.1). Add the sodium hydroxide in increments of 0.05 ml only. Note the pH immediately after every addition of alkali solution.

6.2.3 Plot the neutralisation curve of change of pH (on ordinate axis) against the volume of sodium hydroxide solution (on the abscissa). Determine from the graph the pH of neutralisation, ie at the inflection. Note that the volume of alkali solution plotted on the abscissa for a given change of pH should be the mean of the two volumes over which the pH change occurs.

7. EXPRESSION OF RESULTS

The acidity, expressed as milli-equivalents of sodium hydroxide necessary to raise the pH of 1000 g of prepared honey to the neutralisation point, is given by:

$$\text{Acidity (meq/kg)} = \frac{1000 \times V \times M}{m}$$

where;

m is the mass in g of the test sample, ie 0.5 x weight of sample taken (6.1);

M is the molarity in mol/1 of the sodium hydroxide solution;

V is the volume in ml of the sodium hydroxide added to obtain the pH at the equivalence point.

8. REFERENCE

8.1 DW Lord, MJ Scotter, AD Whittaker and R Wood, J. Assoc. Publ. Analysts, 1989, **26**, 51-76.

8.2 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 [2].

Al. 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). At acidities above 12 meq/kg, r may be taken as 3 meq/kg. This corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of less than 9%. At lower acidities, the method appears less precise (r up to 5 meq/kg), with a RSD_r of up to 25%.

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 to be 6-9 meq/kg, corresponding to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of 20-50%. In particular, at higher acidities (above 12 meq/kg), the better precision (R = 7 meq/kg, CV = 20%) can be expected.

A3. TRUENESS (BIAS)

Accuracy was not tested by spiking samples with known concentrations of acid. 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 5 meq/kg 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 acidities are calculated from the titre and expressed as meq/kg.

TABLE 1
STATISTICAL ANALYSIS OF ACIDITY (meq/kg) IN HONEY SAMPLES

Sample	1/7	2/5	3/8	4/6
Number of laboratories retained after eliminating outliers	18	19	17	17
Number of laboratories eliminated as outliers	1	0	2	2
Number of accepted results after eliminating outliers	36	38	34	34
LEVEL OF, ANALYTE -				
Mean observed value x	7.0	6.05	13.5	13.5
REPEATABILITY				
Standard deviation S_r	1.68	1.04	0.71	0.36
Relative standard deviation RSD_r (%)	24	16	5.3	2.6
Repeatability r [2.8xS_r]	4.7	2.9	2.0	2.6
REPRODUCIBILITY				
Standard deviation S_R	3.0	2.2	2.5	2.5
Relative standard deviation RSD_R (%)	43	34	19	19
Reproducibility R [2.8xS_R]	8.5	6.2	7.1	7.1

A6. KEY TO TABLE 1

SYMBOL	DEFINITION
-	
x	Overall observed mean value
Sr	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