

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V 22

WATER-INSOLUBLE SOLIDS 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 water-insoluble solids 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.

2. Definition

Water-insoluble solids content: the content of water-insoluble solids as determined by the method specified.

3. Principle

The water-insoluble solids content is determined gravimetrically, after filtration, of the honey in solution, and drying the residue for 1 hr. at 135°C.

4. Reagents

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

4.1 Phloroglucinol solution, 1% *m/V* in ethanol

4.2 Sulphuric acid, concentrated

5. Apparatus

5.1 Analytical balance, capable of weighing to 0.1 mg.

5.2 Sintered glass crucible, pore size 15-40 µm.

5.3 Drying oven, electrically heated, thermostatically controlled at a temperature of $135 \pm 1^\circ\text{C}$.

5.4 Desiccator, containing an efficient desiccant, e.g. dried silica gel.

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

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. If necessary, further heat at 65°C until liquefaction 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 Dry a sintered glass crucible (5.2) for 1 hr. at 135°C in the oven (5.3), allow to cool in the desiccator (5.4) and weigh to an accuracy of 0.1 mg (m_1).

6.2.2 Accurately weigh about 20 g of honey (m_0) and dissolve it in a suitable volume (ca 200 ml) of water at 80°C; mix well.

6.2.3 Filter through the previously dried and weighed sintered glass crucible (6.2.1).

6.2.4 Wash through the crucible carefully with water at 80°C until free from sugars. Thorough washing with warm water is essential; use Mohr's test (6.2.5) to check that washing is complete.

6.2.5 Mohr's test

Add phloroglucinol solution (4.1) to the filtrate in a test tube. Mix. Run a few drops of concentrated sulphuric acid (4.2) down the side of the tube; colour is produced at the interface if sugars are present.

6.2.6 Dry the crucible for 1 hr. at 135°C in the oven (5.3), allow to cool in the desiccator (5.4) and weigh to an accuracy of 0.1 mg.

6.2.7 Repeat the drying until constant weight (m_2) is obtained.

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 water-insoluble solids content, calculated as a percentage by mass of the prepared sample, is given by:

$$\% \text{ water-insoluble solids content} = 100 \times (m_2 - m_1)/m_0$$

where:

m_0 is the mass of the test sample, in g (6.2.2);

m_1 is the mass of the dried sintered glass crucible in g (6.2.1);

m_2 is the mass of the dried crucible and filtered solids, in g (6.2.7).

9. References

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

9.2 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⁾.

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). Within the range 0.01- 0.03% water-insoluble solids, r may be taken as 0.02% solids. This corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r of 24- 71%. Similar or better precision may be expected at levels up to 0.5% solids and beyond.

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 0.026% water-insoluble solids, corresponding to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R , of 31-93%.

A3 Trueness (Bias)

Accuracy was not tested by spiking samples with known concentrations of water-insoluble solids. 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 water-insoluble solids content of roughly 0.02% solids 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 levels of water-insoluble solids are expressed as a percentage by mass of the sample.

TABLE 1
Statistical Analysis of the % Water-insoluble Solids (*m/m* %) in Honey Samples

Sample	1/7	2/5	3/8	4/6
Number of Laboratories retained after eliminating outliers	15	16	17	16
Number of Laboratories eliminated as outliers	4	3	2	3
Number of results accepted after eliminating outliers	30	32	34	32
LEVEL OF ANALYTE				
Mean observed value $\bar{x}$	0.02	0.01	0.03	0.01
REPEATABILITY				
Standard Deviation S_r	0.01	0.01	0.01	0
Relative Standard Deviation $RSD_r(\%)$	27	63	26	32
Repeatability r [2.8 xh S_r]	0.02	0.02	0.02	0.01
REPRODUCIBILITY				
Standard Deviation S_R	0.01	0.01	0.01	0.01
Relative Standard Deviation $RSD_R(\%)$	36	63	26	84
Reproducibility R [2.8 xh S_R]	0.02	0.02	0.02	0.03

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