Determination of hydroxymethylfurfural in honey by Winkler spectrophotometric method

The method determines the concentration of 5-(hydroxymethyl-)furan-2-carbaldehyde, defined as the constituents of honey which are capable of combining with barbituric acid and p-toluidine under the conditions of the test.

To aliquot parts of a honey solution, solutions of p-toluidine and barbituric acid are added and the resultant colour is measured against a blank in 1-cm cuvettes at 550 nm.

REAGENTS

p-toluidine-solution:

NOTE: p-toluidine is carcinogenic and presents a risk to health. Contact with the reagent should be avoided. If possible, use one of the other two methods for HMF determination.

Dissolve 10.0 g p-toluidine in 50 ml 2-propanol by gently warming on a water bath. Transfer with a few ml of 2-propanol to a 100 ml volumetric flask and mix with 10.0 ml glacial acetic acid. After cooling to ambient temperature, fill to volume with 2-propanol.

Store in the dark for at least 24 hours before use. Discard after three days or if there is undue coloration.

Barbituric acid solution: Transfer 500 mg barbituric acid as quickly as possible to a 100 ml volumetric flask with about 70 ml water. Dissolve by warming the stoppered flask gently on a water bath. Cool to ambient temperature and dilute to volume.

Carrez solution I: dissolve 15 g of potassium hexacyanoferrate(II), $K_4Fe(CN)_6 \bullet 3H_2O$ in water and make up to 100 ml.

Carrez solution II: dissolve 30 g of zinc acetate, $Zn(CH_3COO)_2 \cdot 2H_2O$ in water and dilute to 100 ml with water.

EQUIPMENT

- Spectrophotometer for measuring absorbance at 550 nm;

- 1 cm cells;
- Volumetric flasks, 10, 50 and 100 ml;
- Beaker;
- Filter paper, analytical grade;
- Magnetic stirrer;

PROCEDURE

Preparation of the sample solution

Weigh about 10 g of honey to the nearest mg. Dissolve in about 20 ml water and quantitatively transfer to a 50 ml volumetric flask. Add 1 ml of Carrez I, shake well, add 1 ml of Carrez II, shake once more, dilute to volume with water and mix once more. Filter the solution through filter paper. Discard the first 10 ml of the filtrate. Complete the rest of the analysis immediately.

In the case of very clear samples, clarification with Carrez' reagents is not necessary.

Determination

Pipette 2.0 ml of the sample solution to each of two volumetric flasks (10 ml) and add 5.0 ml p-toluidine solution to both. Add 1.0 ml of water to one flask (blank value) and 1.0 ml of barbituric acid solution to the other with gentle shaking. Carry out without delay and complete in 1 - 2 minutes. Measure the absorbance of the sample against the blank as soon as the colour intensity has reached a maximum (3 – 4 minutes after adding the barbituric acid solution), using 1cm cells at 550 nm.

CALCULATION

The content of HMF is calculated as follows:

$$\textbf{HMF} = (192 \cdot A \cdot 10)/\text{weight of honey in grams}$$

where:

A = Absorbance,

192 = Factor for dilution and extinction coefficient,

Express results in mg/kg to 1 decimal place.