Determination of hydroxymethylfurfural in honey by White spectrophotometric method

The method determines the concentration of 5-(hydroxymethyl-)furan-2-carbaldehyde. The result is usually expressed in milligrams per kilogram.

The determination of the hydroxymethylfurfural (HMF) content is based on the determination of UV absorbance of HMF at 284 nm. In order to avoid the interference of other components at this wavelength the difference between the absorbances of a clear aqueous honey solution and the same solution after addition of bisulphite is determined. The HMF content is calculated after subtraction of the background absorbance at 336 nm

REAGENTS

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: dilute 30 g of zinc acetate, $Zn(CH_3COO)_2 \cdot 2H_2O$ and make up to 100 ml.

Sodium bisulphite solution 0.20 g/100 ml: dissolve 0.20 g of solid sodium bisulphite $NaHSO_3$, in water and dilute to 100 ml. **Prepare fresh daily.**

EQUIPMENT

- Spectrophotometer operating in a wavelength range including 284 and 336 nm;
- 1 cm quartz cells;
- Magnetic stirrer;
- Filter paper (general purpose);
- 50 ml beaker
- 50, 100 ml and 10 ml volumetric flasks.

PROCEDURE

Sample preparation:

Accurately weigh approximately 5g of honey into a 50 ml beaker. Dissolve the sample in approximately 25 ml of water and transfer quantitatively into a 50 ml volumetric flask. Add 0.5 ml of Carrez solution I and mix. Add 0.5 ml of Carrez solution II, mix and make up to the

_____

mark with water. Filter through paper; rejecting the first 10 ml of the filtrate. Pipette 5.0 ml in each of two 2 volumetric flasks (10 ml). Add 5.0 ml of water to one of the volumetric flask and mix well (the sample solution). Add 5.0 ml of sodium bisulphite solution 0.2% to the second volumetric flask and mix well (the reference solution). Dilution of sample and reference solutions is carried out as follows:

Additions to volumetric flaks (10 ml)	Sample solution	Reference solution
Initial honey solution	5 ml	5 ml
Water	5 ml	-
0,2% sodium bisulfite solution	-	5 ml

Determination:

Determine the absorbance of the sample solution against the reference solution at 284 and 336 nm in 10 mm quartz cells within one hour. If the absorbance at 284 nm exceeds a value of about 0.6, dilute the sample solution with water and the reference solution with sodium bisulphite solution to the same extent in order to obtain a sample absorbance low enough for accuracy. If dilution is necessary.

CALCULATION

$$A_{HMF} \text{ [mg/kg]} = (A_{284} - A_{336}) \cdot 149.7 \cdot 5 \cdot D/W$$

Where:-

A_{284} = absorbance at 284 nm

A_{336} = absorbance at 336 nm

126 = molecular weight of HMF

16830 = molar absorptivity ε [L/ mol*cm] of HMF at λ= 284 nm

149 7 = (126 1000 1000)/16830 10 5 = constant

1000 = conversion g into mg

10 = conversion 5 into 50 ml

1000 = conversion g of honey into kg

5 = theoretical nominal sample weight

D = dilution factor, in case dilution is necessary

W = Weight in g of the honey sample

Express results in mg/kg to 1 decimal place.