DRAFT TANZANIA STANDARD

Honey – Determination of hydroxymethylfurfural (HMF) content

TANZANIA BUREAU OF STANDARDS

0. Foreword

This draft Tanzania standard prescribes the Spectrophotometric method for the determination of hydroxymethylfurfural (HMF) content of honey.

In the preparation of this draft Tanzania standard assistance was derived from AOAC Official Method 980.23 Hydroxymethylfurfural in Honey

In reporting the result of a test or analysis made in accordance with this standard, if the final value observed or calculated, is to be rounded off, it shall be done in accordance with TZS 4

1. Scope

This draft standard prescribes the method for determination of hydroxymethylfurfural (HMF) content of honey.

2. Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

TZS 4, rounding off numerical values

TZS 59, Water - Distilled quality – Specification

3. Principle

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

The determination of 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 bisulphate is determined. The HMF content is calculated after subtraction of the background absorbance at 336 nm.

4. Apparatus

4.1 Spectrophotometer, operating in a wavelength range including 284 nm and 336 nm.

4.2 Quartz cell, 1cm

4.3 Vortex mixer

4.4 Filter paper, general purpose

4.5 Beaker, 50ml

4.6 Volumetric flask, 50mL.

4.7 Test tube, 18 mm x 150 mm

4.8. Pipette 5ml

4.9. Analytical balance

5. Reagents

5.1. Primary reagent

5.1.1. potassium hexacyanoferrate (ll)

5.1.2. zinc acetate

5.1.3. solid sodium hydrogen sulfite

5.2. Preparation of reagent solution

5.2.1. *Carrez solution I***,** dissolve 15g of potassium hexacyanoferrate (ll), $K_4Fe(CN)_6.3H_2O$ in water and make up to 100mL.

5.2.2 *Carrez solution II***,** dilute 30g of zinc acetate, $Zn(CH_3.COO)_2.2H_2O$ and make up to 100 mL.

5.2.3 *Sodium bisulphite solution***,** 0.20g/100 g, dissolve 0.20g of solid sodium hydrogen sulfite, $NaHSO_3$, (metabisulphite, $Na_2S_2O_5$), in water and dilute to 100mL. The solution should be prepared on the day of use.

6 Procedure

Accurately weigh approximately 5g of sample into a 50mL beaker. Dissolve the sample in approximately 25 mL of water and transfer quantitatively into a 50mL volumetric flask.
Add 0.5mL of Carrez solution I and mix. Add 0.5mL of Carrez solution II, mix and make up to the mark with water (a drop of ethanol may be added to suppress foam). Filter through paper, rejecting the first 10mL of the filtrate. Pipette 5.0mL in each of two test tubes (18 mm x 150 mm). Add 5.0mL of water to one of the test tubes and mix well (the sample solution). Add 5.0 mL of sodium bisulphite solution 0.2% to the second test tube and mix well (the reference solution).

Dilution of sample and reference solutions is carried out as follows:

Additions to test tube	**Sample solution**	**Reference solution**
Initial honey solution	5.0 mL	5.0 mL
Water	5.0 mL	-

0.2% sodium bisulphite solution - 5.0 mL

Determine the absorbance of the sample solution against the reference solution at 284nm and 336 nm in 1cm quartz cell within 1 h. If the absorbance at 284nm exceeds a value of about 0.6, dilute the sample solution with water and 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;

$$\text{The dilution, D} = \frac{\text{Final volume of sample solution}}{10}$$

7. Calculation and expression of results

The HMF content, expressed in mg/kg, of the sample is calculated using the following formula:

$$\frac{(A_{284} - A_{336}) \times 149.7 \times 5 \times D}{W}$$

Where,

A_{284} is the absorbance at 284 nm;

A_{336} is the absorbance at 336 nm;

149.7 is the factor $= \frac{126 \times 1000 \times 1000}{16830 \times 10 \times 5}$;

126 is the molecular weight of HMF;

16830 is the molar absorptivity of HMF at λ = 284 nm;

1000 is the conversion g into mg;

10 is the conversion 5 into 50 mL;

1000 is the conversion g of honey into kg;

5 is the theoretical nominal sample weight;

D is the dilution factor, in case dilution is necessary; and

W is the weight of honey taken.

Results are expressed in mg/kg, to one decimal place.

NOTE:-

1. There is alternative method using HPLC.
2. The values with the two methods (HPLC and spectrophotometric) are not significantly different from each other.