DRAFT TANZANIA STANDARD

Honey - Determination of reducing sugars

TANZANIA BUREAU OF STANDARDS

0. Foreword

This draft Tanzania standard prescribes the method for determination of reducing sugar in honey by Lane –Eynon method.

In the preparation of this draft Tanzania standard assistance was derived from AOAC Official Method 920.183 Sugars (reducing) 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 specifies the method for determination of reducing sugar in 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 of method

The method is a modification of the Lane and Eynon procedure involving the reduction of Soxhlet's modification of Fehling's solution by titration at boiling point against a solution of reducing sugars in honey using methylene blue as an internal indicator.

The maximum accuracy of this type of determination is attained by ensuring that the reduction of the Fehling's solution of the reducing sugars in the honey solution is carried out at constant volume. A preliminary titration is, therefore, essential to determine the volume of water to be added before the determinations are carried out to satisfy this requirement

4. Apparatus

4.1. Analytical balance

4.2. Volumetric flask

4.3. Erlenmeyer flask

4.4. Burette

4.5. Pipette

4.6. Hot plate

4.7. wire gauze

5. Reagents

5.1. Primary reagent

5.1.1. copper sulphate pentahydrate ($CuSO_4.5H_2O$; MW = 249.71)

5.1.2. sodium potassium tartrate (C_4H_4K; $NaO_6.4H_2O$; MW = 282.23)

5.1.3. Distilled water

5.1.4. sodium hydroxide (NaOH)

5.1.5. pure sucrose

5.1.6. hydrochloric acid

5.1.7. Methylene blue

5.1.8**.** alum ($K_2SO_4Al_2$ $(SO_4)_3.24H_2O$)

5.1.9. ammonium hydroxide

5.1.10. barium chloride solution

5.2 Preparation of reagent solution

5.2.1. *Soxhlet's modification of Fehling's solution*

Solution A: Dissolve 69.28g copper sulphate pentahydrate ($CuSO_4.5H_2O$; MW = 249.71) with distilled water to 1litre. Keep for one day before titration.

Solution B: Dissolve 346g sodium potassium tartrate (C_4H_4K; $NaO_6.4H_2O$; MW = 282.23) and 100g sodium hydroxide (NaOH) with distilled water to 1litre. Filter through prepared asbestos.

5.2.2. *Standard invert sugar solution (10 g/L)*

Weigh accurately 9.5g pure sucrose, add 5 ml hydrochloric acid (ca. 36.5 percent w/w pure HCl) and dilute with water to about 100ml, store this acidified solution for several days at room temperature (ca. 7 days at 12^{o}C to 15^{o}C, or 3 days at 20^{o}C to 25^{o}C), and then dilute to 1litre. (N.B. Acidified 1.0 percent invert sugar remains stable for several months). Neutralize a suitable volume of this solution with 1M sodium hydroxide solution (40g/L) immediately before use and dilute to the required concentration (2g/L) for the standardization.

5.2.3. *Methylene blue solution*

Dissolve 2g in distilled water and dilute to 1litre.

5.2.4. *Alumina cream*

Prepare cold saturated solution of alum ($K_2SO_4Al_2$ $(SO_4)_3.24H_2O$) in water. Add ammonium hydroxide with constant stirring until solution is alkaline to litmus, let precipitate settle and wash by decantation with water until wash-water gives only slight test for sulphate with barium chloride solution. Pour off excess water and store residual cream in stoppered bottle.

6. **Procedure**

6.1 *Preparation of test sample – First procedure* (applicable to honey which may contain sediment)

a) Transfer an accurately weighed sample of approximately 25g (W_1) from the homogenized honey to 100ml volumetric flask, add 5ml alumina cream (5.2.4)5.4); dilute to volume with water at 20^{o}C and filter.

b) Dilute 10ml of this solution to 500ml with distilled water (diluted honey solution).

Or

6.2. Preparation *for test sample – Second procedure*

a) Weigh accurately a representative quantity of about 2g (W_2) of the homogeneous honey sample, dissolve in distilled water and dilute to 200ml in a calibrated volumetric flask (honey solution).

b) Dilute 50ml of the honey solution to 100ml using distilled water (diluted honey solution).

6.3 *Standardization of the modified Fehling's solution*

Standardize the modified Fehling's solution A so that exactly 5ml (pipette), when mixed with approximately 5ml of Fehling's solution B, will react completely with 0.050g invert sugar added as 25ml dilute invert sugar solution (2g/L).

6.4 Alternatively, pipette 5ml Fehling's solution A and mix with approximately 5ml of Fehling's solution B. Then determine the amount (mg) of the invert sugar that will react with the mixture.

Percent reducing sugar = ((D X E)/V/M) x 100

Where,

M = mass of honey sample (mg)

D = volume of diluted honey solution which would contain all of the original honey sample on the basis of the solution

V = volume of diluted honey solution used up in the titration to reduce 5ml of Fehling's solution

E = the amount (mg) of standard invert sugar which reacts with 5ml of Fehling's solution

6.5 *Preliminary titration*

The total volume of the added reactants at the completion of the reduction titration must be 35ml. This is made up by the addition of a suitable volume of water before the titration commences. Since the compositional criteria of the honey standard specify that there should be more than 60 percent reducing sugars (calculated as invert sugar), a preliminary titration is necessary to establish the volume of water to be added to a given sample to ensure the reduction is carried out at constant volume. This volume of water to be added is calculated by subtracting the volume of diluted honey solution consumed in the preliminary titration (x ml) from 25ml.

Pipette 5ml Fehling's solution A into a 250ml Erlenmeyer flask and add approximately 5ml Fehling's solution B. Add 7ml distilled water, a little powdered pumice or other suitable antibumping agent, followed by about 15ml diluted honey solution from a burette. Heat the cold mixture to boiling over a wire gauze, and maintain moderate ebullition for 2 min. Add 1ml 0.2 percent aqueous methylene blue solution whilst still boiling and complete the titration within a total boiling time of 3 minutes, by repeated small additions of diluted honey solution until the indicator is decolorized. It is the colour of the supernatant liquid that must be observed. Note the total volume of diluted honey solution used (x ml).

7. *Determination*

Calculate the amount of added water necessary to bring the total reactants at the completion of the titration to 35ml by subtracting the preliminary titration (x ml) from 25ml.

Pipette 5ml Fehling's solution A into a 250ml Erlenmeyer flask and add approximately 5ml Fehling's solution B.

Add (25-x) ml distilled water, a little powdered pumice or other suitable antibumping agent and, from a burette, all but 1.5ml of the diluted honey solution volume determined in the preliminary titration. Heat the cold mixture to boiling over a wire gauze and maintain moderate ebullition for 2 min. Add 1.0ml 0.2 percent methylene blue solution whilst still boiling and complete the titration within a total boiling time of 3 min, by repeated small additions of diluted honey solution until the indicator is decolorized. Note the total volume of diluted honey solution (y ml). Duplicate titration should agree within 0.1ml.

8. **Calculation and expression of results**

Where the first procedure (6.1) has been used;

$$C = \frac{25 \times 1000}{W_1 Y_1}$$

Where the second procedure (6.2) has been used;

$$C = \frac{2 \times 1000}{W_2 Y_2}$$

Where C = g invert sugar per 100 g honey

W_1 = weight (g) of honey sample taken according to sub-section 6.1

W_2 = weight (g) of honey sample taken according to sub-section 6.2

Y_1 = volume (ml) of diluted honey solution consumed in the determination carried out according to the first procedure (6.1).

Y_2 = volume (ml) of diluted honey solution consumed in the determination carried out according to the second procedure (6.2).

8. Notes on the procedure

It is essential to the accuracy and repeatability of the determination that the volume of water necessary to bring the reactant mixture to a total volume of 35 ml be determined for each individual sample. The following table gives typical volumes which may be encountered at the preliminary titration stage for the incremental contents of invert sugar shown, assuming the test sample (6.1) weighs about 25 g or test sample (6.2) weighs about 2 g:

Invert sugar content	Volume of distilled water to be added
60	8.3
65	9.6
70	10.7
75	11.6

9. Standardization of Fehling's solution

Dry a quantity of standard dextrose in a vacuum oven for 2 hours at 100^{o}C and then dissolve exactly 5.0 gram in distilled water, dilute to 500 ml and mix thoroughly. Pipette 25.0 ml of the Fehling's solution into a boiling flask, and then bring to boil and titrate with standard dextrose solution as directed under "procedure". Adjust the concentration of the Fehling's solution by dilution or addition of copper sulphate so that titration requires 12.2 ml of standard dextrose solution.