DRAFT TANZANIA STANDARD

Honey- Determination of Sucrose

TANZANIA BUREAU OF STANDARDS

0. Foreword

This draft Tanzania standard prescribes the method for determination of sucrose in Honey.

In the preparation of this draft Tanzania standard assistance was derived from AOAC Official method 920.184 Sucrose 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 standard prescribes the method for determination of sucrose 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. Apparatus

3.1. Analytical balance

3.2. Volumetric flask

3.3. Erlenmeyer flask

3.4. Burette

3.5. Pipette

3.6. Hot plate

3.7. Wire gauze

4. Reagents

4.1. Primary reagent

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

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

4.1.3. Distilled water

4.1.4. sodium hydroxide (NaOH)

4.1.5. pure sucrose

4.1.6. hydrochloric acid

4.1.7. Methylene blue

4.1.8. alum ($K_2SO_4Al_2$ $(SO_4)_3.24H_2O$)

4.1.9. ammonium hydroxide

4.1.10. barium chloride solution

4.2 Preparation of reagent solution

4.2.1 Soxhlet 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.

4.2.2 Standard invert sugar solution

Standard invert sugar solution (10 g/L)

Weigh accurately 9.5g pure sucrose, add 5ml hydrochloric acid (ca. 36.5 percent w/w pure HCl) and dilute with water to about 100 ml, 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.

4.2.3 Hydrochloric acid (6.34M aqueous)

4.2.4 Sodium hydroxide solution (5M aqueous)

4.2.5 Methylene blue solution 2g/l
Dissolve 2g in distilled water and dilute to 1litre.

4.2.6. *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.

5. Procedure

5.1 *Preparation of test sample*

5.1.1. 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 (3.6) 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

5.1.2. 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).

5.2 *Hydrolysis of the test sample*

The honey solution (50ml) is placed in a 100ml graduated flask, together with 25ml distilled water; heat the test sample to 65^{o}C over a boiling water-flask. The flask is then removed from the water-bath and 10 ml of 6.34M hydrochloric acid added. The solution is allowed to cool naturally for 15 minutes, and then brought to 20^{o}C and neutralized with 5M sodium hydroxide, using litmus paper as indicator, cooled again, and the volume adjusted to 100ml (diluted honey solution).

5.3 *Titration*

Preliminary titration

The total volume of the added reactants at the completion of the reduction titration must be 35 ml. 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 25 ml.

Pipette 5 ml 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 (xml).

6. *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 (xml) from 25ml.

Pipette 5 ml 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.

7. Calculation and expression of results

Calculate percent invert sugar (g invert sugar per 100 g honey) after inversion using the appropriate formula as for percent invert sugar before inversion in.

6.1. Where the first procedure (4.1.1) has been used;

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

6.2. Where the second procedure (4.1.2) has been used;

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

Where

C = g invert sugar per 100g honey

W_1 = weight (g) of honey sample taken according to sub-section 4.1.1

W_2 = weight (g) of honey sample taken according to sub-section 4.1.2

Y_1 = volume (ml) of diluted honey solution consumed in the determination carried out according to the first procedure 4.1.1.

Y_2 = volume (ml) of diluted honey solution consumed in the determination carried out according to the second procedure 4.1.2.

Apparent sucrose content = (invert sugar content after inversion minus invert sugar content before inversion) X 0.95

The result is expressed as g apparent sucrose /100 g honey.