DRAFT TANZANIA STANDARD

Honey – Determination of diastase activity

TANZANIA BUREAU OF STANDARDS

0. Foreword

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

In the preparation of this draft Tanzania standard assistance was derived from AOAC Official Method 958.09 Diastastic Activity of 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 diastase activity 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. Apparatus

3.1. stop-watch

3.2. Volumetric flask

3.3. pH meter

3.4. Volumetric flask

3.5. Analytical balance

3.6. Conical flask

3.7. Filtering crucible (pore size 90-150)

3.8. Water bath

3.9. desiccator

3.10. thick wire gauze

3.11. reflux condenser

3.12. Spectrophotometer to read at 660 nm

3.13. Pipette 5ml, 10ml

4. Reagents

4.1. Primary reagent

4.1.1. Iodine analytical grade

4.1.2. potassium iodide, analytical grade

4.1.3. distilled water

4.1.4. sodium acetate.$3H_20$

4.1.5. glacial acetic acid

4.1.6. sodium chloride, analytical grade

4.1.7. potato starch

4.1.8. 95% ethanol

4.1.9. 1 M hydrochloric acid

4.2. Preparation of solution

4.2.1. *Iodine stock solution*

Dissolve 8.8g of iodine analytical grade, in 30-40ml water containing 22g potassium iodide, analytical grade, and dilute to 1litre with water.

4.2.2 *Iodine solution 0.0007 N*

Dissolve 20g potassium iodine, analytical grade, in 30-40ml water in a 500ml volumetric flask. Add 5.0ml iodine stock solution and make up to volume. Make up a fresh solution every second day.

4.2.3 *Acetate buffer – pH 5.3 (1.59 M)*

Dissolve 87g sodium acetate.$3H_20$ in 400 ml water; add about 10.5ml glacial acetic acid in a little water and make up to 500ml. Adjust the pH to 5.3 with sodium acetate or acetic acid as necessary, using a pH meter.

4.2.4 *Sodium chloride solution 0.5M*

Dissolve 14.5g sodium chloride, analytical grade, in boiled-out water and make up to 500ml. The keeping time is limited by mould growth.

4.2.5 *Starch solution*

(a) Preparation of soluble starch

In a conical flask immersed in a water-bath and fitted with a reflux condenser, boil 20g of potato starch for one hour in the presence of a mixture of 100ml of 95% ethanol and 7ml of 1 M hydrochloric acid. Cool, filter through a filtering crucible (pore size 90-150) and wash with water until the wash/water ceases to give any chloride reaction. Drain thoroughly and dry the starch in air at 35^{o}C. The soluble starch must be stored in a well stoppered flask.

(b) Determination of moisture content of soluble starch

Accurately weigh a quantity of approximately 2g of soluble starch and spread in a thin layer over the bottom of a weighing bottle (diameter 5 cm). Dry for one and a half hours at 130^{o}C. Allow to cool in a desiccator and re-weigh. The weight loss with respect to 100g represents the moisture content. The moisture content of such starch should be 7-8% m/m depending on the humidity of the air in which the sample has been dried.

(c) Preparation of starch solution

Use a starch with a blue value between 0.5 – 0.55 using a 1cm cell, as determined by the method below. Weigh out that amount of starch which is equivalent to 2.0g anhydrous starch. Mix with 90ml of water in a 250ml conical flask. Bring rapidly to the boil, swirling the solution as much as possible, heating over thick wire gauze preferably with an asbestos centre. Boil gently for 3 min., cover and allow spontaneously to cool to room

temperature. Transfer to a 100ml volumetric flask, place in a water bath at 40^{o}C to attain this temperature and make up to volume at 40^{o}C.

5. Method for determining blue value of starch

The amount of starch equivalent to 1g anhydrous starch is dissolved by the above method, cooled and 2.5ml acetate buffer added before making up to 100ml in a volumetric flask. To a 100ml volumetric flask add 75ml water, 1ml M hydrochloric acid and 1.5ml of 0.02N iodine solutions. Then add 0.5ml of the starch solution and make up to volume with water. Allow to stand for one hour in the dark and read in 1cm cell using a spectrophotometer at 660nm against a blank containing all the ingredients except the starch solution. Reading on the absorbance scale = Blue value.

5.1. Procedure

5.1.1. Preparation of test samples

Honey solution

10.0g honey is weighed into a 50ml beaker and 5.0ml acetate buffer solution is added, together with 20ml water to dissolve the sample. The sample is completely dissolved by stirring the cold solution. 3.0ml sodium chloride solution is added to a 50ml volumetric flask and the dissolved honey sample is transferred to this and the volume adjusted to 50ml.

N.B. It is essential that the honey should be buffered before coming into contact with sodium chloride.

Standardization of the starch solution

The starch solution is warmed to 40^{o}C and 5ml pipetted into 10ml of water at 40^{o}C and mixed well. 1ml of this solution is pipetted into 10ml 0.0007 N iodine solution, diluted with 35ml of water and mixed well. The colour is read at 660nm against a water blank using a 1cm cell.

The absorbance should be 0.760 $\pm$ 0.020. If necessary the volume of added water is adjusted to obtain the correct absorbance.

5.1.2. Absorbance determination

Pipette 10 ml honey solution into 50ml graduated cylinder and place in 40^{o}C $\pm$ 2^{o}C water-bath with flask containing starch solution. After 15 minutes, pipette 5ml starch solution into the honey solution, mix and start stop-watch. At 5

minutes internals remove 1ml aliquot and add to 10.00ml 0.00007 N iodine solution. Mix and dilute to standard volume Determine absorbance at 660nm in spectrophotometer immediately using 1cm cell. Continue taking 1 ml aliquot at internals until absorbance of less than 0.235 is reached.

6. Calculation and expression of the results

The absorbance is plotted against time (min) on a rectilinear paper. A straight line is drawn through at least the last three points on the graph to determine the time when the reaction mixture reaches an absorbance of 0.235. Divide 300 by the time in minutes to obtain the diastase number (DN). This number expresses the diastase activity as ml 1 percent starch solution hydrolyzed by the enzyme in 1g of honey in 1 h at 40^{o}C. This diastase number corresponds with the Goethe scale number.

Diastase activity = DN = ml starch solution (1 percent/g honey/h at 40^{o}C).