DRAFT TANZANIA STANDARD

Determination of Starch in Raw Sugar

TANZANIA BUREAU OF STANDARDS

0. Foreword

This draft Tanzania standard prescribes the method for determination of Starch in Raw Sugar.

In the preparation of this draft Tanzania standard assistance was derived from ICUMSA Method GS1-16 (2013) The Determination of Starch in Raw Sugar by a Modified BSES Method - Official.

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 Tanzania standard prescribes the method for determination of Starch in Raw Sugar

2. Field of application

This method has been adopted by the Australian sugar industry for the determination of starch and is applicable on raw cane sugar The upper concentration of applicability of the method is 300 mg/kg

3. Principle

Cane starch is composed of amylose and amylopectin with amylose being the fraction that forms a blue colour with iodine. Cane starch can be occluded in the raw sugar crystal and affects the filtration rate of liquor from the refinery carbonation process. The amylose fraction is responsible for this effect.

The sugar is dissolved in water and the solution is digested with hot calcium chloride-acetic acid solution to solubilize any starch present. Potassium iodide iodate solution is added to form the blue starch-iodine complex. The absorbance of this complex is read in a spectrophotometer at 700 nm. At this wavelength, the effect on the absorbance of the impurities in raw sugar is minimal.

4. Reagents

WARNING AND SAFETY PRECAUTIONS

USERS OF THIS METHOD ARE ADVISED TO CONSULT THEIR NATIONAL HEALTH AND SAFETY LEGISLATION BEFORE HANDLING THESE REAGENTS.

Use only reagents of recognized analytical grade and distilled water or water of equivalent purity.

4.1 Calcium chloride solution - 40% (m/m). Dissolve 53.0 ± 0.1 g of calcium chloride dihydrate in distilled water and dilute to 100.0 ± 0.1 g with distilled water. As calcium

chloride is highly deliquescent, stocks should not be exposed to air. The strength of the calcium chloride solution must be checked and, if necessary,

adjusted to 40.0 ± 0.3% (m/m) with distilled water or with calcium chloride dihydrate (approximately 75.5% $CaCl_2$,).

Check the solution strength by measuring its specific gravity. The specific gravity of 40% (m/m) calcium chloride solution is 1.3942 at 24 °C. Within the temperature range 20-30 °C, it decreases on an average by 0.0007 per degree above 24 °C and increases by the same amount per degree below 24 °C. In the region of 40% (m/m), a change of 0.1 % in calcium chloride concentration produces a change of 0.001 in specific gravity. Measure the specific gravity by pycnometer, hydrometer or other instrument capable of the accuracy required. A convenient method is as follows:

• Allow stoppered containers of calcium chloride solution and distilled water to stand for a number of hours (e.g. overnight) so that both reagents come to ambient temperature.

• Weigh a clean, dry 100 mL volumetric flask to 0.02 g. Fill to the mark with calcium chloride solution and weigh again to 0.02 g. Note the temperature of the solution to 0.1 °C.

• Rinse out the flask and fill to the mark with distilled water at the same temperature as the calcium chloride solution, and weigh to 0.02 g. From the masses of the solution and water, and the temperature, calculate the specific gravity to 0.001.

4.2 Acetic acid - 1 mol/L approx. Dilute 57 ± 1 mL of glacial acetic acid (ρ_{20}= 1.049 g/mL) to one litre with distilled water.

4.3 Acetic acid - 0.033 mol/L approx. Dilute 3.3 ± 0.03 mL of 1 mol/L acetic acid (3.2) to 100 mL with distilled water.

4.4 Calcium chloride-acetic acid reagent. Using a pH meter and stirrer, adjust the pH of a 100 mL aliquot of calcium chloride solution (3.1) to pH 3.0 ± 0.1 with 0.033 mol/L acetic acid. Because of the large salt concentration, allow sufficient time for the pH meter to equilibrate.

Adjust the pH of the bulk of the calcium chloride solution in the same proportion. Although the pH of a calcium chloride-acetic acid solution tends to alter on standing, do not readjust to pH 3.0 before using.

4.5 Potassium iodate solution - 0.0017 mol/L. Dry about 0.5 g potassium iodate at 105-110 °C for 1 hour. Dissolve 0.3566 ± 0.0002 g of the dried reagent in distilled water and make to 1 litre. Store in the dark in a brown, glass-stoppered bottle.

4.6 Potassium iodide solution - 10 g/100 mL. Dissolve 10.0 ± 0.1 g of potassium iodide in distilled water and dilute to 100 mL. Store in the dark in a brown, glass-stoppered bottle. Discard the solution if it becomes yellow.

4.7 Potassium iodide-potassium iodate reagent. This reagent must be prepared on the day it is to be used. Mix 10.0 ± 0.5 mL of potassium iodide solution (3.6) with 90.0 ± 0.5 mL of distilled water. To this solution add 100.0 ± 0.5 mL of potassium iodate solution (3.5).

Mix the reagents and keep in a brown, glass-stoppered bottle. This reagent must be discarded after one day.

4.8 Standard starch solution - 900 mg/L. Use an analytical grade potato starch with low ash (<0.3 g/100 g). Determine in duplicate its moisture content, correct to 3 decimal places, by drying about 2g (weighed to 0.000 1g) at 105 -110 °C for 2 hours.

4.8.1. Weigh into a 25 mL beaker $\frac{(0.9000.100)}{(100- W_{H2O})}$

± 0.0001 g of fresh starch, i.e. equivalent to 0.9000 ± 0.0002 g of anhydrous starch, where W_{H2O}, represents the water content of the starch.

4.8.2. To the weighed quantity of starch, add 5 mL of cold distilled water and mix with a glass rod. Before the starch settles, transfer the mixture quantitatively to 500 mL of boiling water in a litre conical flask so that no slurry touches the wall of the flask.

4.8.3. With at least three additional 5 mL portions of distilled water, transfer all the starch to the flask. This operation should be completed within one minute.

4.8.4. Boil the starch for 3 minutes ± 10 seconds, timed from the moment the first 5 mL of starch slurry enters the boiling water.

4.8.5. Rinse a one litre volumetric flask with hot distilled water. Quantitatively transfer the hot solution through a glass funnel to the one litre volumetric flask.

Wash the conical flask at least twice with hot distilled water, by adding the water to the 25 mL beaker and then transferring it to the conical flask. Continue washing the conical flask with hot distilled water and transferring to the volumetric flask until the latter is filled to approximately 900 mL.

4.8.6. Swirl to mix the flask contents and cool under running water to room temperature. Make the solution to one litre, stopper and mix well. Store in a refrigerator.

NOTE - The solution will keep for one week, but if possible, the standardization should be carried out on the same day as the solution is prepared, to eliminate all possibility of its deterioration.

4.9 Standard starch solution - 180 mg/L. Using a Class, A bulb pipette, pipette 20.00 mL of the 900 mg/L standard starch solution (3.8) into a 100 mL volumetric flask. Dilute to 100 mL with distilled water. Mix well by inverting and shaking. This reagent will not keep and must be prepared on the day the standardization is carried out.

4.10 Starch-free sucrose. Use only sugar, tested to be free of starch, e.g. first strike refined white sugar.

5 Apparatus

Ordinary laboratory apparatus and glassware. All apparatus should be visually checked for damage prior to use.

5.1 Spectrophotometer - suitable for the measurement of absorbance at 700 nm with a set of matched 2 cm cells.

5.2 Water bath - controlled at 95-100 °C.

5.3 Flask shaker.

5.4 Volumetric flask - with wide neck (Kohlrausch), 100 mL capacity. A minimum of 6 flasks are needed for preparation of the calibration standards.

5.5 Bulb pipettes - Class A (AS 2166 or equivalent), 2 mL, 4 mL, 6 mL, 8 mL, 10 mL, 20 mL, 30 mL.

5.6 Automatic dispenser - 30 mL.

5.7 Balance - to weigh up to 100.00 ± 0.01 g.

6 Procedure

Including Standardization of Method

6.1 Preparation of standards

6.1.1. Weigh 7.20 ± 0.02 g of starch-free sucrose (3.10) into each of six 100 mL Kohlrausch volumetric flasks (4.4).

6.1.2. Pipette, using Class A bulb pipettes 0, 2.00, 4.00, 6.00, 8.00 and 10.00 mL aliquots of the 180 mg/L standard starch solution (3.9) respectively into each of the six flasks. The final solutions in the flasks correspond to 0, 50, 100, 150, 200 and 250 mg/kg of starch in sugar.

6.1.3. Pipette, using a 25 mL graduated pipette, respectively into each of the six flasks 14.0 ± 0.1, 12.0 ± 0.1, 10.0 ± 0.1, 8.0 ± 0.1, 6.0 ± 0.1, and 4.0 ± 0.1 mL of distilled water to make the liquid volume in each flask to 14.0 mL. Swirl each flask to completely dissolve the sugar.

6.1.4. Using an automatic dispenser or a 30 mL bulb pipette add 30.0 ± 0.1 mL of the calcium chloride acetic acid reagent (3.4) into each flask. Mix well.

6.1.5. Stopper each flask loosely and place in the boiling water bath (4.2) for 15 ± 1 minutes. Commence timing from the moment the flasks are placed in the bath. Swirl each flask about 5 and 10 minutes after placing in the bath to aid dissolution of the starch.

NOTE - The flasks must be placed in the heating bath within 30 minutes after adding water to dissolve the sugar.

6.1.6. After 15 minutes, remove the flasks from the heating bath and cool to room temperature in a running water bath.

6.1.7. To each flask, add 30.00 ± 0.05 mL of 0.033 mol/L acetic acid (3.3). Use a 30 mL automatic pipette or a bulb pipette. Mix well.

6.2 Measurement of absorbance

6.2.1. Determine the cell corrections for a pair of matched 2 cm cells using distilled water.

6.2.2. Add to the 0 mg/kg test solution 20.00 ± 0.05 mL of the potassium iodide-potassium iodate reagent (3.7) using a 20 mL bulb pipette or automatic dispenser. Mix well and make to the mark with distilled water. Stopper the flask and mix well.

6.2.3. Immediately rinse twice and fill a 2 cm cell with the 0 mg/kg test solution. Read the absorbance of the solution in a spectrophotometer at 700 nm against distilled water as the reference.
Correct the absorbance reading for any cell correction (see above). Record the absorbance to 0.001 units.
NOTE- The absorbance of the test solution must be read between two and five minutes after adding the iodide-iodate reagent to the test solution.

6.2.4. Repeat the two steps above for each concentration of starch involved in the standardization.

NOTE - The reading of the 0 mg/kg starch standard should not exceed 0.0 10 absorbance for a 2 cm cell. As a guide the reading for the 200 mg/kg starch standard should be about 0.290 absorbance for a 2 cm cell at 700 nm

6.3 Preparation of graph

6.3.1. Plot "starch content of sugar in mg/kg" against "absorbance".

6.3.2. A straight line must be obtained for the graph. If three or more points lie off the line of best fit by more than 5% of the line's absorbance value at that particular starch concentration, the standardization must be repeated. A Microsoft Excel spreadsheet or similar procedure is recommended.

6.3.3. The graph used by any laboratory for routine analysis should be constructed using the mean of independent standardizations by at least two analysts. Each set of results and the mean must comply with the 5% limits above. In addition, the slopes of the individual lines must not differ by more than 5%.

6.4 Preparation of test solution

6.4.1 Weigh 7.20 ± 0.02 g of raw sugar into each of two 100 mL Kohlrausch volumetric flasks:

(a) sample blank solution

(b) sample test solution

6.4.2. Using an automatic dispenser or 25 mL graduated pipette add 14.0 ± 0.1 mL, of distilled water to each flask. Swirl the flasks to completely dissolve the sugar.

6.4.3. Add to each flask 30.00 ± 0.05 mL of calcium chloride-acetic acid reagent (3.4) using an automatic dispenser or a 30 mL bulb pipette. Mix well.

6.4.4. Stopper each flask loosely and place the boiling water bath (4.2) at 95-100 °C for 15 ± 1 minutes. Commence timing from the moment the flask is placed in the bath. Swirl each flask after 5 and 10 minutes, to aid dissolution of starch.

NOTE -- The flasks must be placed in the boiling water bath within 30 minutes of adding water to dissolve the sugar.

6.4.5 Remove the flasks from the bath and cool in a running water bath to room temperature

6.4.6. To each flask, add 30.00 ± 0.05 mL of 0.033 mol/L acetic acid (3.3). Use a 30 mL bulb pipette or automatic dispenser. Mix well.

6.4.7. Make the sample blank solution (a) above, to the mark with distilled water and mix well.

6.4.8. Using a bulb pipette or automatic dispenser add 20.00 ± 0.04 mL of potassium iodide-iodate reagent (3.7) to the sample test solution (b) above, and make to the mark with distilled water. Stopper the flask and mix well.

6.4.9. Read the absorbance of the sample test solution in a 2cm cell in a spectrophotometer at 700 nm, against the sample blank as the reference solution. Record the absorbance to 0.001 units.

NOTE - The readings must be made between two and five minutes after the addition of the potassium iodide-iodate reagent to the sample test solution.

6 Expression of results

6.1 Calculation.

Read the starch content of sample in mg/kg directly from the calibration graph (6.3). Record the result to 1 mg/kg starch in raw sugar.

6.2 Precision

Sample	ΔW starch	s_r	r	S_R	R
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Raw	0-300	4.5	12.6	9.4	26.3

ΔW starch Range of starch content in raw sugar,

s_r, Repeatability standard deviation in mg/kg,

r Repeatability (probability P = 95%),

S_R Reproducibility standard deviation in mg/kg,

R Reproducibility (probability P = 95%).