Method 7.10 – Raw sugar: dextran by the haze method

1. Rationale

This method is applicable to cane raw and refined sugar. Dextran is defined as a predominantly straight-chain glucose polymer with mainly α-1,6-glucosidic linkages formed by the action of certain species of bacteria (particularly *Leuconostoc mesenteroides*) on sucrose during cane and juice storage. High dextran levels are therefore associated with sucrose loss and cause processing problems in factories and refineries. This method is used to rate sugars in terms of quality and is sensitive to high molecular weight (HMW) dextrans.

2. Principle

The haze formed by dextran-like polysaccharides in the presence of alcohol is measured. Soluble starch is hydrolysed by incubation with an α-amylase enzyme. Protein is removed by precipitation with trichloroacetic acid (TCA) followed by filtration with acid-washed kieselguhr. The dextran haze is produced by addition of ethanol to the filtrate. The turbidity of the dextran haze is measured spectrophotometrically at a wavelength of 720 nm. The method is standardised against a commercially available dextran.

3. Apparatus

3.1 **Spectrophotometer** operating at 720 nm

3.2 **Optical glass** cells: 2 × 20 mm

3.3 **Water baths**

Boiling water bath operating at 50 - 60°C
Cooling water bath using cold tap water

3.4 **Flask shaker**

3.5 **Analytical balance** readable to 0.0001 g

3.6 **Top pan balance** readable to 0.01 g

3.7 **Stopwatch**

3.8 **Volumetric flasks**: 25, 100, 200 and 500 cm^3

3.9 **Bulb pipettes**: 1, 2, 3, 4 and 10 cm^3

3.10 **Graduated pipettes**: 5 and 10 cm^3

3.11 **Funnel** to fit into a 100 cm^3 volumetric flask

3.12 **Automatic pipettor** or safety pipette: 10 cm^3

3.13 **Buchner funnel**: 55 mm ϕ

3.14 Buchner flask: 250 cm^3

3.15 Burettes: 25 and 50 cm^3

3.16 Beaker: 150 cm^3

3.17 Filter paper: Whatman No. 5, 55 mm ϕ

3.18 Oven operating at 105°C

3.19 Moisture dish

4. Reagents

4.1 Dextran, Pharmacia (Dextran T500, 500 000 MW)

4.2 Dextran solution (0.8 mg/cm^3)

Prepare the standard dextran solution daily. Do not store overnight.

Determine the moisture content of the dextran in duplicate to 2 decimal places by drying approximately 2 g of the solid in an oven at 105°C for 3 hours. Record the mass to 0.0001 g and calculate the moisture content from the loss in mass as indicated in 6.1. Duplicate values must be within 1% of the mean of the duplicates.

All subsequent masses must be adjusted accordingly to give a known mass of dried dextran. The dried sample must be discarded as it will not have the same solubility as fresh dextran.

Weigh a quantity of the undried dextran that contains 0.16 g of dry dextran into a 100 cm^3 beaker and record the actual mass to 0.0001 g. Determine the amount of dextran to be weighed using the following formula:

$$\text{mass dextran (g)} = 0.16 \text{ g dry dextran} \times \frac{100}{100 - \text{moisture}(\%)}$$

Dextran is not readily soluble in water. Firstly add 1 to 2 cm^3 of distilled water to the dextran to form a slurry. Allow the particles to become uniformly hydrated by standing for about 10 minutes with occasional stirring. Add more water in small aliquots while a gel mass remains. When about 25 cm^3 have been added and no gel is present, wash the slurry into a 200 cm^3 volumetric flask with water to a volume of about 80 cm^3. Place the flask in a boiling water bath for 30 minutes. Cool to room temperature in a cold water bath and make to the mark with distilled water.

4.3 Dextran solution (0.08 mg/cm^3)

Pipette 10 cm^3 of the 0.8 mg/cm^3 dextran solution into a 100 cm^3 volumetric flask, make to the mark and mix well. Prepare this solution freshly.

4.4 TCA solution (100 g/litre)

TCA (trichloroacetic acid) is a corrosive acid and will cause respiratory tract burns. Work in a fume cupboard while wearing gloves and safety glasses.

Dissolve 10.0 ± 0.1 g of TCA in distilled water, transfer to a 100 cm^3 volumetric flask and make to the mark. Always add the acid to the water and never the other way around. This reagent will keep for two weeks, stored at 5°C in a dark brown bottle.

4.5 Ethanol

Ethanol (EtOH) is a flammable liquid and is toxic. Avoid contact with the eyes by wearing safety glasses.

Denatured absolute alcohol (DAA) which is a commercially available ethanol that contains 2.0 ± 0.2 % (m/m) methanol and less than 0.5 % (m/m) water may be used. If particulate matter is visible, filter through Whatman No. 5 filter paper. Store in an airtight container.

4.6 **Refined sugar**: first boiling

Use first boiling refined sugar with a starch content of less than 2 mg/litre. To this end the absorbance that develops when 8.0 cm^3 of sucrose/TCA solution plus 4.5 cm^3 distilled water are diluted to 25 cm^3 with denatured alcohol should not exceed 0.003 AU in a 2 mm cell at 720 nm when read against a blank of 8.0 cm^3 of sucrose/TCA solution diluted to 25 cm^3 with distilled water.

4.7 **Sucrose/TCA solution** (500 g/litre sucrose)

This solution should be made up freshly as required.

Dissolve 250.0 ± 0.1 g refined sugar in distilled water in a 500 cm^3 volumetric flask. Add 78 cm^3 of the TCA solution, make to the mark and mix.

4.8 α-Amylase enzyme

Any α-amylase is suitable as long as the α-1,6-glucoside link in dextran is not attacked. To this end use one of the standard dextran solutions prepared in 5.1 as a sample and analyse according to the procedure in 5.2. The absorbance should be within 5% of the reading obtained for the same dextran standard when the procedure is followed without the enzyme treatment.

The enzyme activity of the α-amylase determined according to Method 11.3 should be at least 85%.

4.9 **Hydrochloric acid** (concentrated, 32%)

Hydrochloric acid (HCl) is a corrosive acid and should only be handled in a fume cupboard with gloves while wearing safety glasses.

4.10 **Silver nitrate** (0.0171 M)

Silver nitrate ($AgNO_3$) is corrosive and should only be handled with gloves while wearing safety glasses.

Dissolve 2.9049 g silver nitrate in distilled water. Transfer to a 1 000 cm^3 volumetric flask, make to the mark and mix. The solution is sensitive to light and should be stored in an amber container.

4.11 **Nitric acid** (concentrated or fuming)

Nitric acid (HNO_3) in its concentrated form (also called fuming nitric acid) is a corrosive acid and the fumes may cause severe damage to the lungs and respiratory tract. Always open in a fume cupboard using gloves and safety glasses. Decant carefully into a clean and dry smaller container for regular use.

4.12 Acid-washed kieselguhr

Add 50 ± 5 g of kieselguhr to 1 litre of distilled water. Add 50 ± 5 cm^3 of concentrated hydrochloric acid (HCl) to the mixture and stir for 5 minutes. Filter the kieselguhr through any fast-flowing filter paper in a large Buchner funnel and wash with distilled water until the filtrate is free of chloride.

To test for chloride add some of the silver nitrate solution and a few drops of concentrated nitric acid to the filtrate. The solution will turn milky white in the presence of chloride.

Dry the washed kieselguhr for 6 hours at 96 - 100°C and store in a closed container.

5. Procedure

5.1 Preparation of the standard graph

Prepare the standard solutions according to the amounts in Table 1 in 25 cm^3 volumetric flasks. It is recommended that only 4 or 6 standards and the blank be prepared per batch because of the time constraints in the method.

Use 5 cm^3 graduated pipettes or a series of bulb pipettes to add the aliquots of the standard dextran solutions and water to a total volume of 12.5 cm^3. Add 8.0 cm^3 of the sucrose/TCA solution to each of the volumetric flasks using the 10 cm^3 graduated pipette. Do not pipette by mouth but use a safety bulb or automatic pipette.

Table 1: Standard dextran solutions for standard graph

Std no	0.08 mg/cm^3 dextran solution (cm^3)	0.8 mg/cm^3 dextran solution (cm^3)	Distilled water (cm^3)	Dextran concentration (mg/kg sugar)
1	0.0	-	4.5	0
2	1.0	-	3.5	20
3	2.0	-	2.5	40
4	3.0	-	1.5	60
5	4.0	-	0.5	80
6	-	1.0	3.5	200
7	-	1.5	3.0	300
8	-	2.0	2.5	400
9	-	2.5	2.0	500
10	-	3.0	1.5	600
11	-	3.5	1.0	700
12	-	4.0	0.5	800
13	-	-	17.0	Blank

Make flask number 13 up to the mark using distilled water and mix by shaking. This is the blank solution.

The dextran concentration is calculated as follows:

$$\text{Dextran (mg/kg sugar)} = \text{standard concentration} \times \frac{1000}{\text{sugar concentration}} \times 1000$$

$$= \frac{\text{aliquot} \times \text{dextran solution}}{25\ cm^3} \times \frac{25\ cm^3 \times 1000}{8\ cm^3 \times 500\ mg/cm^3} \times 1000$$

Within 20 minutes of addition of the TCA solution slowly add denatured alcohol to the 25 cm^3 mark of the volumetric flask from a 50 cm^3 burette while gently swirling. The time for the alcohol addition should be between 30 and 60 seconds. Mix the contents of the flask gently by inverting three times. Avoid vigorous shaking of the flask as it may cause coagulation of the dextran haze. Start the stopwatch immediately after mixing. As the absorbance must be read at a precise time after the mixing step it is recommended that alcohol be added to the dextran standards at uniform time intervals (3 minutes).

Determine the cell correction at 720 nm of a pair of matched 20 mm cells containing distilled water, *i.e.* the difference in absorbance values due to the cells. This value is expected to be 0.000 AU.

Approximately 17-18 minutes after the completion of the mixing step rinse the reference cell three times and fill with the blank solution. In a similar way, rinse and fill the second cell with the standard solution. Clean the optical faces of the cells with a tissue, check that striations are absent.

At 20 minutes ± 10 seconds after the completion of the mixing step record the absorbance of the sample solution against that of the blank solution at 720 nm to 0.001 AU.

Read all of the dextran standards using the above procedure. It is necessary to refill the blank solution cell for each determination.

5.2 Dextran determination in raw sugars

Weigh 32.0 ± 0.1 g of the raw sugar sample in a 100 cm^3 volumetric flask. Add approximately 60 cm^3 distilled water and dissolve. Add 0.1 cm^3 of the α-amylase enzyme. Mix the contents well and stopper the flask. Place the flask in a shaking water bath at 55 ± 5°C for 15 ± 2 minutes. Cool the flask to room temperature in a cold water bath.

Measure 10.0 cm^3 of the TCA solution in a glass volumetric flask and transfer to the volumetric flask. Make to the mark and mix well. Pour this solution into a 150 cm^3 beaker already containing two heaped teaspoons (about 6 - 8 g) of acid-washed kieselguhr and mix well. Filter the mixture through a 5.5 cm Buchner funnel with a Whatman No. 5 filter paper under vacuum, using the first 10 to 15 cm^3 of filtrate to rinse the funnel and flask.

Within 20 minutes of addition of the TCA solution add 12.5 cm^3 of the filtrate to each of two clean and dry 25 cm^3 volumetric flasks. Slowly add denatured alcohol to the 25 cm^3 mark of one volumetric flask from a 50 cm^3 burette while gently swirling. The time for the alcohol addition should be between 30 and 60 seconds. Mix the contents of the flask gently by inverting three times. Avoid vigorous shaking of the flask as it may cause coagulation of the dextran haze. Start the stopwatch immediately after mixing.

Add distilled water to the 25 cm^3 mark of the second volumetric flask and mix. This is the sample blank. Determine the cell correction at 720 nm of a pair of matched 20 mm cells containing distilled water, *i.e.* the difference in absorbance values due to the cells. This value is expected to be 0.000 AU.

Approximately 17-18 minutes after the completion of the mixing step rinse the reference cell three times and fill with the blank solution. In a similar way, rinse and fill the second cell with the test solution. Clean the optical faces of the cells with a tissue, check that striations are absent.

At 20 minutes ± 10 seconds after the completion of the mixing step record the absorbance of the sample solution against that of the blank solution at 720 nm to 0.001 AU.

Immediately after reading, visually inspect the contents of the sample solution cell to check for flocculation. If the haze has flocculated the analysis is invalid and must be repeated. If the dextran haze absorbance is higher than the upper limit of the calibration graph, repeat the analysis but mix the sample with sucrose (refined sugar with less than 2 mg/litre starch), *e.g.* use 16.0 ± 0.1 g of raw sugar sample and 16.0 ± 0.1 g of sucrose.

6. Calculations

6.1 Dextran moisture

$$\text{Dextran moisture (\%)} = \frac{M_1 - M_2}{M_1} \times 100$$

where M_1 ≡ mass of dextran before drying (g)
M_2 ≡ mass of dextran after drying (g)

6.2 Standard graph

Calculate the actual concentration of dextran in each standard using the moisture in the dextran (4.1) and the actual mass that was used to make up the first standard.

$$\text{dextran solution (mg/cm}^3\text{)} = \text{dry mass (mg)} \div \text{volume (cm}^3\text{)}$$
$$= \left(\text{mass (mg)} \times \frac{100 - \text{moisture (\%)}}{100} \right) \div 200 \text{ cm}^3$$

The dextran concentrations of the standards are calculated as follows:

$$\text{dextran (mg/kg sugar)} = \text{standard concentration} \times \frac{1000}{\text{sugar concentration}} \times 1000$$

$$= \frac{\text{aliquot} \times \text{dextran solution}}{25 \text{ cm}^3} \times \frac{25 \text{ cm}^3 \times 1000}{8 \text{ cm}^3 \times 500 \text{ mg/cm}^3} \times 1000$$

$$= \text{aliquot} \times \text{dextran solution} \times 250$$

Apply the cell correction to the absorbance readings of all the standards. Plot the actual dextran concentration (mg/kg sugar) against the corrected absorbance for each standard and draw the graph of best fit. The calibration graph should be gradual at low dextran concentrations and become almost linear at high dextran concentrations. It is advisable to express the graph in terms of a regression equation for input into a spreadsheet or Laboratory Information Management System (LIMS). Individual points should lie within 5% of the absorbance value of the graph of best fit at low concentrations and within 3% of the absorbance value of the graph of best fit at high concentrations.

6.3 Samples

Apply the cell correction to the absorbance value of the raw sugar solution. Obtain the dextran concentration in the raw sugar directly from the standard graph using the absorbance of the sample solution. If the sample was diluted with standard sugar, multiply the result by the dilution factor. Express results to the nearest mg/kg sugar. Values lower than 25 mg/kg are simply reported as < 25 mg/kg.

7. Example

7.1 Dextran standard (0.8 mg/cm³)

Dextran moisture = 15.415%

mass dextran = $0.16 \text{ g dry dextran} \times \frac{100}{100 - \text{moisture}(\%)}$

= 0.1892 g

Actual mass of dextran = 0.1895 g
Actual dry mass of dextran = 0.1603 g
Concentration in 200 cm³ = 0.8015 mg/cm³

7.2 Standard graph

Dextran in each standard solution (mg/litre) = aliquot × dextran solution × 250

Subtract the cell correction from all of the absorbances.

Table 2: Actual dextran standard concentrations and absorbances

Std no	0.08 mg/cm³ dextran solution (cm³)	0.8 mg/cm³ dextran solution (cm³)	Dextran concentration (mg/kg sugar)	Absorbance (AU)
1	0.0	-	0	0.000
2	1.0	-	20	0.004
3	2.0	-	40	0.011
4	3.0	-	60	0.017
5	4.0	-	80	0.024
6	-	1.0	200	0.074
7	-	1.5	301	0.127
8	-	2.0	401	0.183
9	-	2.5	501	0.234
10	-	3.0	601	0.297
11	-	3.5	701	0.358
12	-	4.0	801	0.424
13	-	-	Blank	0.000

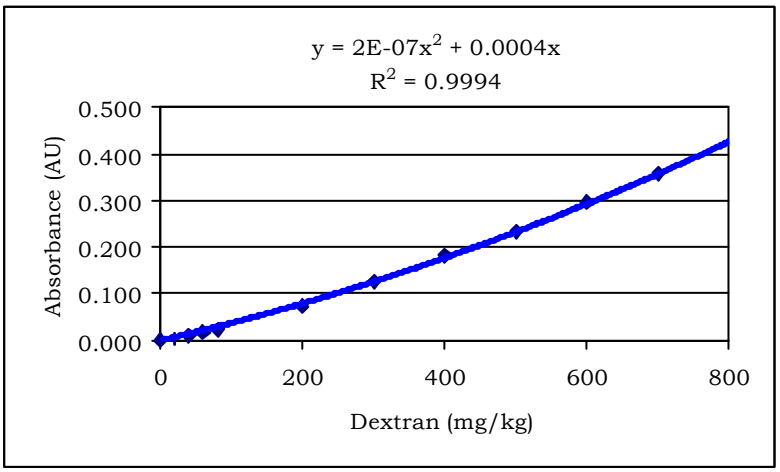

Figure 1: Standard dextran graph

7.3 Sample

Cell correction	=	0.000 AU
Absorbance of blank	=	0.001 AU
Absorbance reading	=	0.051 AU
Absorbance of sample	=	0.050 AU
From the graph, dextran of sample	=	118 mg/kg

8. Precision

The tolerance associated with the analysis is ± 25 mg/kg.

9. References

ICUMSA (1994). The determination of dextran in raw sugar by a modified alcohol haze method. *ICUMSA Methods Book,* Method GS1-15.

McCowage, RJ (1994). Referee General Subject 1. *Proc ICUMSA*, 21: 30 pp.