Method 6.8 – C-molasses: starch by the SMRI method

1. Rationale

This method is applicable to C-molasses and is based on the colorimetric determination of the starch/iodine complex in the absence of sugar.

2. Principle

The starch is separated from the sample by precipitation with alcohol and dissolved in a calcium chloride solution. The starch in solution is reacted with iodine and the colour of the starch/iodine complex is measured spectrophotometrically at a wavelength of 600 nm. A standard graph prepared using a standard potato starch solution is used.

3. Apparatus

3.1 **Spectrophotometer** operating at 600 nm

3.2 **Optical glass cell**: 10 mm

3.3 **Oven** operating at 105 ± 5°C

3.4 **Desiccator** with self-indicating silica gel

3.5 **Analytical balance** readable to 0.0001 g

3.6 **Beakers**: 100 and 12 × 250 cm^3

3.7 **Volumetric flasks**: 6 × 50, 100, 500 and 1 000 cm^3

3.8 **Pipettes**: 5, 10, 15, 20 and 25 cm^3

3.9 **Buchner flasks**: 6 × 250 and 500 cm^3

3.10 **Buchner funnels**: 6 × 60 and 190 mm ϕ

3.11 **Measuring cylinders**: 50 and 100 cm^3

3.12 **Hot plate**

A thermostatically controlled hotplate tray covered with a layer of sand to ensure even boiling must be used.

3.13 **Watch glass**: 100 mm ϕ

3.14 **Moisture dish** with lid: 80 mm ϕ, 10 mm height

3.15 **Stemless filter funnel**: 100 mm ϕ

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

3.17 Glass beads

3.18 Filter paper

Whatman No. 91, S&S 3000 or equivalent: 185 mm ϕ
Whatman No. 5 or equivalent: 55 mm ϕ
Whatman No. 6, Postslip medium white w/s or equivalent (for Brix): 185 mm ϕ

3.19 pH meter and buffer solutions 4 and 7

3.20 Conical flasks: 250, 500 cm^3

3.21 Refractometer readable to 0.01°Bx

3.22 Schott bottle: 250 cm^3

3.23 Filtration apparatus

funnel: 100 mm ϕ stemless
beakers: 2 × 150 cm^3
watch/cover glass: 100 mm ϕ

4. Reagents

4.1 Ethanol

Ethanol (CH_3CH_2OH, absolute alcohol) is a flammable liquid and is toxic when swallowed. Avoid contact with eyes by wearing safety glasses during use.

4.2 Ethanol (80%)

Add 80 cm^3 ethanol to a 100 cm^3 measuring cylinder and fill to the 100 cm^3 mark with distilled water.

4.3 Silver nitrate (0.0171 M)

Silver nitrate ($AgNO_3$) is corrosive. Wear gloves and safety glasses during use.

Dissolve 2.9049 g silver nitrate in distilled water. Transfer to a 1000 cm^3 volumetric flask, make to the mark and mix. The solution is sensitive to light and should be stored in an amber container.

4.4 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.

4.5 Hydrochloric acid (1:1)

Hydrochloric acid (HCl, 32%) is a corrosive acid and should only be handled with gloves while wearing safety glasses.

Carefully add 100 cm^3 of concentrated hydrochloric acid to 100 cm^3 of distilled water. Always add the acid to water and not the other way around. The dilution is exothermic and the solution will therefore heat.

4.6 Kieselguhr (acid washed)

Kieselguhr is an inert powder and should not be inhaled. Use a dust mask during handling.

Use non-metal containers and stirring rods to avoid acid corrosion. Add sufficient hydrochloric acid (1:1) to the Kieselguhr to form a loose slurry. Stir the slurry for a minimum of 1 hour and filter under vacuum through Whatman No. 91 paper in a large Buchner funnel. Wash the Kieselguhr with distilled water until the washings are chloride free.

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 Kieselguhr overnight at 105°C.

4.7 Acetic acid (2 M)

Acetic acid (CH_3COOH) in its concentrated form (also called glacial acetic acid) is corrosive and flammable and should not be inhaled. Always open in a fume cupboard using gloves and safety glasses. Decant carefully into a clean and dry smaller container for regular use.

Measure 114.5 cm^3 (120 g) of glacial acetic acid in a measuring cylinder and add to a 1 000 cm^3 volumetric flask containing about 500 cm^3 distilled water. The dilution is exothermic and the solution will heat. Cool under running water and make to the mark.

4.8 Calcium chloride solution (40% m/V or 25% m/m)

Weigh 800.0 g analytical grade calcium chloride dihydrate ($CaCl_2.2H_2O$) and dissolve in 1646 g of distilled water. Adjust the pH to 3.0 ± 0.2 with 2 M acetic acid using a pH meter. This gives a 25% (m/m) or 40% (m/V) solution of dry calcium chloride ($CaCl_2$) with a solution density of 1.6 g/cm^3. Store the solution in a closed container ensuring no contact with the atmosphere to prevent evaporation and absorption of CO_2 from air which would change the solution pH. If crystallization is observed, discard and prepare a new batch.

4.9 Potasium iodide solution (10%)

Potassium iodide (KI) is an irritant. Wear gloves and safety glasses during use.

The reagent is unstable and must be prepared immediately prior to use.

Weigh 10.0 g potassium iodide and dissolve in distilled water. Transfer to a 100 cm^3 volumetric flask and make to the mark.

4.10 Potassium iodate solution (0.0017 M)

Potassium iodate (KIO_3) is explosive and should be kept away from other chemicals. Handled with care in a fume cupboard while wearing gloves and safety glasses. Toxic fumes may form above 100°C.

Dry the potassium iodate powder overnight in a desiccator before use. Weigh 0.3567 g and dissolve in distilled water. Dilute to 1000 cm^3 in a volumetric flask.

4.11 Potato starch, BDH

4.12 Refined sugar: first boiling sugar

4.13 Celite 577

Celite is an inert powder and inhalation may cause asbestosis of the lungs. Wear a dust mask during use.

5. Procedure

5.1 Starch moisture content

Weigh accurately about 1 g potato starch in a moisture dish and dry in the oven at 105 ± 5°C for 1½ hours. Cool in a desiccator and reweigh. From the loss in mass, calculate the moisture content of the starch as indicated in 6.1. All subsequent masses must be adjusted according to the moisture content to give a known mass of dried starch. The dried sample must be discarded as it will not have the same solubility as fresh starch (retrogradation).

5.2 Preparation of the standard graph

It is necessary to prepare a new standard graph for every new calcium chloride solution.

Prepare a stock solution of starch by adding 500 mg fresh potato starch to 10 cm^3 of distilled water in a 100 cm^3 beaker to make a slurry. Pour the slurry into 300 cm^3 boiling distilled water in a 250 cm^3 conical flask being careful to rinse the beaker well with water. Continue boiling for 1 minute. Cool and transfer the solution quantitatively into a 500 cm^3 volumetric flask with distilled water. Make to the mark with distilled water. This is a 1 000 mg/litre solution and each 1 cm^3 contains 1 mg starch.

Prepare the standard starch solutions exactly according to the amounts indicated in Table 1 in 250 cm^3 beakers. Use appropriate pipettes for the liquids. Dissolve the sugar by swirling.

Table 1: Standard starch solutions

Standard	Refined sugar (g)	Water (cm^3)	Stock starch aliquot (cm^3)	Starch concentration (mg/litre)
1	25	30	0	0
2	25	25	5	10
3	25	20	10	20
4	25	15	15	30
5	25	10	20	40
6	25	5	25	50

Add 100 cm^3 alcohol and 2 g Kieselguhr to each beaker and mix well. Cover with a watch glass and stand for 1 hour.

Prepare a filter cake pad for each standard in the Buchner funnels using Whatman No. 5 filter paper and a 2 g Kieselguhr slurry by applying vacuum until the Kieselguhr is dry. Filter each solution under vacuum. Wash the cake with 3 × 5 cm^3 80% ethanol followed by 3 × 5 cm^3 100% ethanol. Allow the cake to dry partially and transfer the paper and cake to a new 250 cm^3 beaker.

Measure 40 cm^3 of the calcium chloride solution into a measuring cylinder. Scrape the Kieselguhr from the filter paper into the beaker and remove the paper by washing with some of the calcium chloride solution. Add the remainder of the calcium chloride solution and a few glass beads to the beaker. Cover the beaker with a watch glass, place on the hot plate and bring to the boil. Boil gently for 15 minutes then cool under running water.

Transfer the cooled solution to a 100 cm^3 volumetric flask using a small Buchner funnel to trap the glass beads and make to the mark. Add 1.7 cm^3 water to correct for the volume of Kieselguhr present and shake. Filter through a fluted No. 91 filter paper, discarding the first 40 cm^3 of filtrate.

Pipette 10 cm^3 of the clear filtrate into a 50 cm^3 volumetric flask and add 15 cm^3 distilled water. Add 2.5 cm^3 of the 2 M acetic acid solution, 0.5 cm^3 of the potassium iodide (KI) solution and 5 cm^3 of the potassium iodate (KIO_3) solution. Mix thoroughly, make to the mark and measure the absorbance at 600 nm in a 10 mm cell within 2 minutes of adding the potassium iodate solution, using water as the reference.

5.3 Sample Preparation

Weigh 5.0 ± 0.1 g well mixed molasses in a 250 cm^3 beaker. Dissolve in 30 cm^3 of hot distilled water. Add 110 cm^3 absolute alcohol and 2 g Kieselguhr, stir and cover with a watch glass. Stand for 1 hour. Prepare a filter pad in a Buchner funnel using Whatman No. 5 filter paper and a 2 g Kieselguhr slurry by applying vacuum until the Kieselguhr is dry. Filter the molasses solution under vacuum. Wash the cake with 3 × 5 cm^3 80% ethanol followed by 3 × 5 cm^3 100% ethanol. Allow the cake to dry partially.

Measure 40 cm^3 of the calcium chloride solution into a measuring cylinder. Scrape the Kieselguhr from the filter paper into a 250 cm^3 beaker and remove the paper from the beaker by washing with some of the calcium chloride solution. Add the remainder of the calcium chloride solution and a few glass beads to the beaker. Cover the beaker with a watch glass, place on the hot plate and bring to the boil. Boil gently for 15 minutes and cool under running water.

Transfer the cooled solution to a 100 cm^3 volumetric flask using a small Buchner funnel to trap the glass beads and make to the mark. Add 1.7 cm^3 water to correct for the volume of Kieselguhr present and shake. Filter through a fluted No. 91 filter paper, discarding the first 40 cm^3 of filtrate.

Pipette 20 cm^3 clear filtrate into a 50 cm^3 volumetric flask. Prepare a reagent blank in a separate volumetric flask using 20 cm^3 distilled water. Add 15 cm^3 distilled water, 2.5 cm^3 of the 2 M acetic acid solution, 0.5 cm^3 of the potassium iodide (KI) solution and 5 cm^3 of the potassium iodate (KIO_3) solution. Mix thoroughly, make to the mark and measure the absorbance at 600 nm in a 10 mm cell within 2 minutes of adding the potassium iodate solution, using water as the reference.

5.4 Brix determination

Weigh 50.00 ± 0.05 g of molasses into a 500 cm^3 conical flask. Weigh 200.00 ± 0.05 g distilled water into the same flask to bring the total mass to 250.00 ± 0.10 g. Record these masses to calculate the dilution factor. Stopper the flask and mix thoroughly on the sample shaker.

Pipette 50 cm^3 of the well-mixed stock solution and transfer to the 250 cm^3 Schott bottle. Weigh 1 g Celite 577 powder while wearing a dust mask and add to the Schott bottle. Mix and filter the solution through fluted Whatman No. 6 filter paper supported in a funnel which rests directly in a beaker. Seal the funnel with a watch glass to minimise evaporation. Discard the first 10 cm^3 of filtrate and collect about 20 cm^3 of the filtrate in another clean, dry beaker. Do not allow the filtrate to touch the bottom of the funnel or filter paper. Do not replenish the solution in the filter funnel.

5.4.1 Reading of the sample (filtrate)

Zero the refractometer using distilled water. If the reading is not 0.00°Bx at 20.0°C, record this value as the water blank.

Pour the filtrate into the refractometer cell compartment using three portions to ensure complete displacement of the previous solution. Record the reading once it stabilizes at 20.0°C.

6. Calculations

6.1 Starch moisture

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

where M_1 ≡ mass of starch before drying
M_2 ≡ mass of starch after drying

6.2 Standard graph

Subtract the absorbance of the blank (standard 1) from the absorbances of the other five solutions. Plot these absorbance values against the starch concentrations, taking the moisture content of the starch into account when calculating the starch concentrations of the standard solutions (mg/litre). This graph should be a straight line passing through the origin. The slope is calculated (absorbance over concentration) and used in the calculation of starch in the samples. The slope multiplied by 20 (to convert concentration to mg/50 cm^3) gives the calcium chloride factor (*i.e.* absorbance at 1.0 mg/50 cm^3 starch) which should be approximately 0.3.

6.3 Samples

Subtract the absorbance of the sample blank from the absorbance of the sample solution to get the absorbance of the sample. Calculate the amount of starch in the sample in mg/litre according to the equation below.

$$\text{Starch in sample} = \frac{\text{absorbance}}{\text{slope}} \times \frac{100\ cm^3}{1000} \times \frac{50\ cm^3}{5\ cm^3} \div 0.005\ kg$$

$$= \frac{\text{absorbance}}{\text{slope}} \times 200$$

$$\text{Starch on Brix} = \frac{\text{starch in sample}}{\text{Brix}} \times 100$$

Report to the nearest 5 mg/kg.

7. Example

7.1 Standard graph

Starch moisture = 16.95%

500 mg of starch therefore contains 415.3 mg dry starch

Calculate the starch concentration of each standard in terms of the dry starch according to the following formula (results are indicated in Table 2):

$$\text{Starch concentration (mg/litre)} = \frac{\text{dry mass}}{500\ \text{cm}^3} \times \frac{\text{aliquot}}{100\ \text{cm}^3} \times \frac{10\ \text{cm}^3}{50\ \text{cm}^3} \times 1000$$

Table 2: Calculated standard starch solutions

Standard	Absorbance (AU)	Absorbance (Solution - Blank)	Starch concentration (mg/litre)
1	0.048	-	-
2	0.171	0.123	8.3
3	0.293	0.245	16.6
4	0.416	0.368	24.9
5	0.538	0.49	33.2
6	0.661	0.613	41.5

From the calibration graph, the slope is 0.0147 AU litre/mg and the calcium chloride factor is 20 × 0.0147 = 0.294.

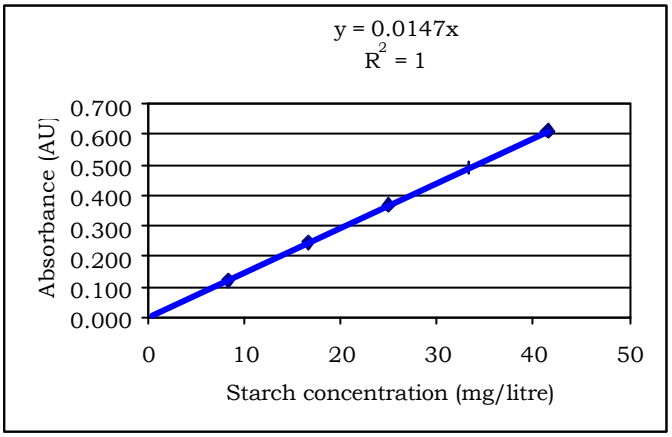

Figure 1: Standard starch graph

7.2 Samples

Brix of solution = 16.88°Bx
Brix of molasses = 16.88°Bx × 5
= 84.40°Bx

absorbance of solution = 0.184 AU
absorbance of blank = 0.020 AU
absorbance of sample = (0.184 – 0.020) AU
= 0.164 AU

$$\text{starch in sample} = \frac{0.164\ \text{AU}}{0.0147\ \text{AU litre/mg}} \times 200$$
= 2 231 mg/kg

$$\text{starch on Brix} = \frac{2231\ \text{mg/kg} \times 100}{84.40^{\circ}\text{Bx}}$$
= 2 643 mg/kg on Brix

Report as 2 643 mg/kg

8. References

SASTA (1985). *Laboratory manual for South African sugar factories.* 3rd Edition: 194 - 197, 313 - 314.

SMRI (1997). Determination of the starch in molasses. *SMRI Test Methods*, TM053.