Method 5.4 – Syrup: 
reducing sugars and sucrose by the 
Lane and Eynon method

1. Rationale

The method is applicable to factory syrups and relies upon the property of reducing sugars to reduce and precipitate copper from a known amount of Fehlings solution under standard conditions according to the Lane and Eynon method.

2. Principle

A sample of syrup is diluted so that the final mixture contains about 0.15 to 0.30 g reducing sugars per 100 cm$^3$. This solution is titrated against mixed Fehlings reagents that contain a strongly alkaline cupric complex salt solution using methylene blue as an indicator. As a guide, clear juice in a South African sugar factory usually requires to be diluted in a 1:3 ratio. It has been found that calcium interferes with the determination and it is recommended that EDTA be used as a sequestrant. In most factory products EDTA will also reduce the colour significantly which aids in the detection of the end point.

3. Definitions

3.1 Reducing sugars

Reducing sugars consists primarily, but not exclusively, of glucose and fructose and are obtained through the hydrolysis of sucrose.

3.2 Invert: an equimolar mixture of glucose and fructose.

4. Apparatus

4.1 Light duty balance readable to 0.01 g

4.2 Pipette: 5 cm$^3$

4.3 Volumetric flasks: 100, 200 and 1000 cm$^3$

4.4 Burette: 50 cm$^3$

4.5 Erlenmeyer flask: 500 cm$^3$

4.6 Hot plate

4.7 Gauze or screen: 0.15 mm pore opening

4.8 Stop watch

4.9 Glass beads

4.10 Filter paper: Whatman No. 91
5. **Reagents**

5.1 **Pumice powder** to prevent over-boiling

5.2 **Liquid paraffin**

5.3 **Methylene blue solution (1%)**

Dissolve 1.0 g methylene blue in distilled water and dilute to 100 cm$^3$ in a volumetric flask.

5.4 **Ethylene diamine tetra acetic acid, disodium salt dihydrate (4%)**

Ethylene diamine tetra acetic acid (EDTA), disodium salt dihydrate is mildly irritating to the skin, eyes and respiratory tract. Work in a fume cupboard while wearing gloves and safety glasses.

Weigh 4.00 g Ethylene diamine tetra acetic acid, disodium salt dihydrate, dissolve in distilled water and dilute to 100 cm$^3$ in a volumetric flask.

5.5 **Copper sulphate pentahydrate**

Copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) is highly irritating to the eyes, skin and respiratory tract. Work in a fume cupboard while wearing gloves and safety glasses.

5.6 **Sodium potassium tartrate tetrahydrate** ($\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$)

5.7 **Sodium hydroxide pellets**

Sodium hydroxide (NaOH, also called caustic) is a corrosive base and should be handled with care. Wear gloves and safety glasses during use.

5.8 **Fehlings Solutions** (A and B)

**Solution A**: Dissolve 69.278 g copper sulphate pentahydrate in distilled water and dilute to 1 000 cm$^3$ in a volumetric flask.

**Solution B**: Dissolve 346 g sodium potassium tartrate tetrahydrate in 500 cm$^3$ distilled water. Dissolve 100 g sodium hydroxide in 200 cm$^3$ of distilled water and cool to room temperature. Transfer both these solutions quantitatively to a 1 000 cm$^3$ volumetric flask. Mix thoroughly and make to the mark with distilled water. Filter the solution through a Whatman No. 91 filter paper. (It has been found that some suspended matter develops in this solution within two weeks. It is therefore advised to let this solution stand for 2 weeks before filtering).

**Note**: It is necessary to keep solutions A and B separate.

6. **Procedure**

6.1 **Standardisation of the Fehlings solutions**

Pipette 50 cm$^3$ standard invert solution into a 250 cm$^3$ volumetric flask and dilute to volume with distilled water. Fill the burette with the dilute standard invert solution. Pipette 5 cm$^3$ Fehlings A solution and 5 cm$^3$ Fehlings B solution into a 500 cm$^3$ Erlenmeyer flask. Add a little pumice powder, three glass beads and four drops of liquid paraffin.
Titrate this solution against the dilute standard invert solution in the burette according to the titration procedure in 6.3.

6.2 Sample preparation

Dilute the syrup so that the final mixture contains about 0.15 to 0.30 g reducing sugars per 100 cm$^3$. For example, dilute 23 g of syrup to 100 g with water, mix well and do the analysis. If the titre is too high or too low, adjust the dilution and repeat the analysis.

Weigh $50.00 \pm 0.01$ g of the diluted sample accurately into a 200 cm$^3$ volumetric flask, add 10 cm$^3$ EDTA (4%) and make to the mark with distilled water. Rinse and fill a 50 cm$^3$ burette with the diluted juice and adjust the level to zero.

Pipette 5 cm$^3$ of Fehlings A and 5 cm$^3$ of Fehlings B solution into the Erlenmeyer flask. Add a little pumice powder, 3 glass beads and four drops of liquid paraffin to prevent foaming.

Titrate this solution against the sample solution in the burette according to the titration procedure in 6.3.

6.3 Titration procedure

Add 15 cm$^3$ of the solution from the burette and note the colour of the mixture. Place the flask on a hot plate and bring the mixture to a boil in not more than 2¼ minutes. From this point on the flask may not be swirled or disturbed in any way. If the original colour persists after boiling for 10-15 seconds it indicates that most of the Fehlings solution has not yet been reduced. If this is the case hold the burette by hand and make further additions of 5 cm$^3$ at a time with a few seconds of boiling after each addition, until the original colour of the reagent fades to bright orange.

Add three or four drops of methylene blue and continue the addition of solution from the burette until the indicator is completely decolourised. The boiling liquid resumes the bright orange appearance which it had before the addition of the indicator. If less than 10 cm$^3$ of the burette volume is used, dilute the juice solution 1:1 with water and repeat the titration.

Take a reading from the burette as an approximate titration. Do a second titration by adding all but 1 cm$^3$ of the approximate titre of the sample solution at once. Heat the liquid and start the stopwatch as soon as the boiling point is reached. Boil the liquid for 2 minutes. Add three or four drops of the methylene blue indicator and complete the titration drop wise until the indicator becomes colourless. The titration should be completed within 3 minutes and during this time the mixture must be kept boiling continuously to exclude air.

Repeat the last step of the titration. Duplicate titres should agree to within 0.1 cm$^3$.

7. Calculations

7.1 Standardisation of the Fehlings solution

5 cm$^3$ of Fehlings A solution and 5 cm$^3$ Fehlings B solution should result in a standard invert solution titre of exactly 25.64 cm$^3$. If the titre is higher than 25.64 cm$^3$ Fehlings solution A should be diluted until the desired titre is obtained. If the titre is lower than 25.64 cm$^3$ copper sulphate should be added to Fehlings solution A until the desired titre is obtained.
7.2 Reducing sugars

The amount of reducing sugars in the sample solution is calculated using the equation below. Sucrose affects the titration in a predictable way. The equation assumes a sucrose content of 14% or 3.5 g/100 cm\(^3\) after dilution.

\[
\text{mass reducing sugars (mg/100 cm}^3) = 4748.9939 \times \text{titre}^{-0.9904}
\]

The percentage reducing sugars in the sample is calculated as indicated below.

\[
\text{reducing sugars (%) } = \frac{M_{RS}}{1000} \times \frac{1}{M_{DS}} \times \frac{M_{S}}{M_{T}} \times 100
\]

where

- \(M_{RS}\) = mass reducing sugars (mg) in 100 cm\(^3\)
- \(M_{DS}\) = mass diluted syrup (g) in 100 cm\(^3\)
- \(M_{S}\) = mass syrup (g) used for dilution
- \(M_{T}\) = total mass (g) of dilution

8. Example

Mass syrup in dilution = 23.00 g
Total mass of dilution = 100.00 g
Titre = 18.5 cm\(^3\)
Mass diluted sample in 200 cm\(^3\) = 50.00 g
Mass juice in 100 cm\(^3\) = 25.00 g
Reducing sugars in solution = 263.99 mg/100 cm\(^3\)

Reducing sugars in sample = \[
\frac{264 \, \text{mg/100 cm}^3}{1000} \times \frac{1}{25.00 \, \text{g}} \times \frac{23.00 \, \text{g}}{100.00 \, \text{g}} \times 100
\]

= 0.243%

Report as 0.25%

9. References


SMRI (1997). Determination of the reducing sugars in juice by the Lane and Eynon method. SMRI Test Methods, TM047.