EXPERIMENT 4 DETERMINATION OF REDUCING SUGARS, TOTAL REDUCING SUGARS, SUCROSE AND STARCH

Structure

4.1 INTRODUCTION

Several methods are available for estimation of reducing sugars. They include chemical, polarimetric and chromatographic methods. However, for routine analysis of food products, Lane and Eynon chemical method is most widely used. Non-reducing sugars and starch are first converted into reducing sugars for estimation.

Objectives

After studying and performing this experiment, you should be able to:

- prepare food products for estimation of reducing sugars, total sugars and starch; and
- determine reducing sugars, total sugars and starch in food products by Lane and Eynon method.

4.2 EXPERIMENT 4a: REDUCING SUGARS

4.2.1 Principle

Lane and Eynon method is based on the principle of reduction of Fehling's solution by reducing sugars. Fehling's solution is a mixture of copper sulphate and alkaline Rochelle salt (sodium potassium tartarate). Rochelle salt complexes with the cupric hydroxide formed in alkaline solution and prevent it from precipitation. Reducing sugars reduces the complexed cupric hydroxide to red, insoluble cuprous oxide under the experimental conditions. An oxidation-reduction indicator, usually methylene blue, detects the end point of the reaction.

The first step in the estimation of reducing sugars by Lane and Eynon method is the determination of Factor for Fehling's solution. Fehling factor is the quantity of invert sugar in grams required to completely reduce the Fehling's solution (usually 5 ml each of Fehling's A and B solutions).

Total sugars include reducing sugars and non-reducing di - and oligo-saccharides like sucrose, which on mild acid hydrolysis are converted into reducing sugars. Starch is hydrolysed by strong acids into glucose.

4.2.2 Requirements

Equipment and Apparatus

Chemical balance, 1mg sensitivity

Hot plate

Burette (50 ml cap.) with an off-set tip

Volumetric flask, 250 ml

Pipette, 5 ml and 25 ml

Conical flask, 250 ml

Weighing bottle

Funnel (small)

Whatman No. 1 filter circles

Chemicals and Reagents

Fehling's solution A: Dissolve 69.28 g copper sulphate ($CuSO_4.5H_2O$) in distilled water and dilute to 1000 ml. Filter and store in amber colour bottle.

Fehling's solution B: Dissolve 346 g Rochelle salt (Potassium sodium tartrate: $KNa\ C_4H_4O_6.\ 4H_2O$) and 100 g NaOH in distilled water. Dilute to 1000 ml. Filter and store in amber colour bottle.

Neutral lead acetate solution: Prepare 20% neutral lead acetate solution.

Potassium oxalate solution: Prepare 10% potassium oxalate ($K_2C_2O_4.\ H_2O$) solution.

Methylene blue indicator: Prepare 1% methylene blue solution in distilled water.

4.2.3 Procedure

i) Standardization of the Fehling's Solution for Invert Sugar

Accurately weigh 4.75g of AR grade sucrose. Transfer to 500 ml volumetric flask with 50 ml distilled water. Add 5 ml conc. HCl and allow to stand for 24 hr. Neutralise the solution with NaOH using phenolphthalein as end point indicator and make up to volume. Mix well and transfer 25 ml to a 100 ml volmetric flask and make up to volume (1 ml = 2.5 mg of invert sugar). Transfer to a burette having an off-set tip and titrate against Fehling's solution as described below for sample.

Observations

Titre $= V_1$ = ------ ml

Calculations

$$\text{Factor for Fehling's solution (g of invert sugar)} = \frac{\text{Titre} \times 2.5}{1000}$$

$$= 0.0025 \times V_1 = \text{------- g}$$

ii) Determination of Reducing Sugars

Preparation of sample

Weigh accurately 10-50 g sample as such (juices, beverages etc.) or homogenized sample (jams, preserves etc.) and transfer to 500 ml volumetric flask. Add about 100 ml water and neutralize with NaOH solution to phenolphthalein end point. Add 10 ml neutral lead acetate solution, shake and let stand for 10 min. Add potassium oxalate solution in small amounts until there is no further precipitation. Make up to volume, mix the solution well and filter through Whatman No. 1 filter circle. Transfer the filtrate to a 50 ml burette having an off-set tip.

Preliminary titration: Pipette out 5 ml each of Fehling A and B solutions into 250 ml conical flask. Mix and add about 10 ml water and a few pumice stone or glass beads. Dispense the sugar solution from the burette. Heat the solution to boiling. Add 3 drops of methylene blue indicator. Continue the addition of the sugar solution drop wise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 to 50 ml). Maintain a total boiling peiod of 3 min. Note down the titre value.

Final titration: Pipette out 5 ml each of Fehling A and B solutions into a 250 ml conical flask. Add sample solution about 0.05 to 1.0 ml less than titre value of the preliminary titration. Heat the flask to boiling. Add 3 drops of methylene blue indicator. Complete the titration within 1 min by adding 2 to 3 drops of sugar solution at a time, until the indicator is decolourized. At the end point, the boiling liquid assumes the brick red colour. Note down the titre value. Perform the titration in duplicate and take the average.

Note: i) Preliminary titration must be finished within 3 min.
ii) Conical flask should not be disturbed or removed from the burner before the titration is finished.

4.2.4 Observations

Weight of the sample = W = ------- g

Dilution volume for the sample = V_2 = ------ ml

Volume of clarified sample solution required for Fehling's reaction (titre) = V_3 = ------- ml

4.2.5 Calculations

Based on the factor for Fehling's solution, V_3 ml sample solution contains:

0.0025 V_1 g reducing sugar (as invert sugar)

$$\text{Therefore, \% Reducing Sugars in the sample} = \frac{0.0025 \times V_1 \times V_2 \times 100}{V_3 \times W}$$

$$= \frac{0.25 \times V_1 \times V_2}{V_3 \times W} = X\ \%$$

4.2.6 Results

Reducing sugars (as invert sugar) = % by wt.

4.3 EXPERIMENT 4b: TOTAL REDUCING SUGARS

4.3.1 Principle

Total reducing sugars represent reducing sugars and non-reducing di and oligo saccharides, which can be hydrolysed into reducing sugars with dilute acids.

4.3.2 Requirements

Same as for experiment 4a.

4.3.3 Procedure

Pipette an aliquot of 50 ml of the clarified, de-leaded filtrate to a 100 ml volumetric flask. Add 5 ml of conc. HCl and allow to stand at room temperature for 24 hours. Neutralise with conc. NaOH solution followed by 0.1N NaOH using phenolphthalein as end point indicator. Make up to volume and transfer to 50 ml burette having an off-set tip. Perform the titration against Fehling's solution similar to the procedure described for reducing sugars, and determine the total sugars as invert sugars.

4.3.4 Observations

Volume of the acid hydrolysed sample solution required for Fehling solution (titre) = V_4 = ------ ml

4.3.5 Calculations

Based on the factor for Fehling's solution, total reducing sugars in

V_4 ml = 0.0025 × V_1 g

As 50 ml of the clarified and de-leaded solution is diluted twice (50 ml to 100 ml) after hydrolysis, dilution volume of the sample = ($2 \times V_2$).

$$\text{Therefore, \% Total reducing sugars (as invert sugars)} = \frac{0.0025 \times V_1 \times 2 \times V_2 \times 100}{V_4 \times W}$$

$$= \frac{0.5 \times V_1 \times V_2}{V_4 \times W} = Y\ \%$$

Total reducing sugars comprises of reducing sugars and non-reducing sugars, which can be hydrolysed into reducing sugars under the experimental conditions. This non-reducing sugar is usually expressed in terms of sucrose.

As 0.95 g sucrose on hydrolysis yields 1 g invert sugar (glucose + fructose):

% Sucrose in the sample = (Total reducing sugars – % Reducing sugars originally present) × 0.95

$$= (Y - X) \times 0.95$$

[% Total sugars = (% Reducing sugars + % Sucrose)]

4.3.6 Results

Sucrose content in the sample = % by weight

4.4 EXPERIMENT 4c: STARCH

4.4.1 Principle

Starch is hydrolysed to glucose with strong acid and the reducing sugar formed is estimated by Lane and Eynon method. Sample containing sugars are washed to free it from them before hydrolysing the starch. Traces of lipids present are also washed off with petroleum ether before hydrolysing the starch.

4.4.2 Requirements

500 ml conical flask with std. joint to fix Liebig condenser
Petroleum ether
Alcohol – 95%and 50%
10% Alpha naphthol solution in alcohol
Sulphuric acid- conc.
Stainless steel vessel (5 lit. cap.), 10" dia.
Test tube – 10 ml
Centrifuge

4.4.3 Procedure

For samples containing sugars and less starch, to weighed quantity (50-100 g), add a little of water and heat to 60°C. Allow to stand for some time to obtain a solution of starch. Add about 100 ml 95% alcohol and centrifuge (2000 rpm, 10 min.) to settle the precipitate. Filter and wash the residue with about 50% alcohol until the filtrate gives no positive test for sugars. To test for sugars, to a few ml of the filtrate in a test tube, add a drop of 10% alpha naphthol reagent. Allow 1 ml of pure conc. H_2SO_4 to flow slowly down the side of the test tube

so as to form a layer beneath the aqueous solution. If sugars are present, a red ring will appear within a few seconds at the junction of the two layers. Use the 50% alcohol-washed and dried precipitate for starch hydrolysis as below.

For starchy materials, weigh accurately about 2-5 g sample in a beaker. Add ether and stir well. Allow to settle and decant the ether portion and discard. Repeat the step 3-4 times and dry the sample.

Transfer the prepared sample to a conical flask and add 200 ml water and 20 ml conc. HCl. Place the flask in a steel vessel filled with 2/3rds volume of water. Connect the flask to a water-circulating condenser. Heat the vessel and allow the water to boil for exactly 2 hr. Shake the digestion flask intermittently during boiling. Remove the flask and cool the contents. Transfer the contents to a 500 ml volumetric flask and neutralise with sodium hydroxide. Filter through Whatman No. 1 filter circle. From the filtrate determine the reducing sugar content using Lane and Eynon titrimetric procedure.

4.4.4 Observations

Weight of sample	= W	= ------ g
Hydrolysed sample volume made up to:	= V_5	= ----- ml
Titre	= V_6	= ----- ml

4.4.5 Calculations

Based on the factor for Fehling's solution, reducing sugar content of V_6 ml of the hydrolysed starch solution = $0.0025 \times V_1$ g

The theoretical yield of reducing sugars (glucose) on complete hydrolysis of starch is: 0.90 g starch = 1 g glucose. However, for practical purposes, the currently accepted factor is: 0.925 g starch = 1 g glucose.

$$\text{Therefore, \% Starch content of the sample} = \frac{0.0025 \times V_1 \times V_5 \times 100 \times 0.925}{V_6 \times W}$$

4.4.6 Results

Starch content of the sample = per cent by weight.

4.5 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.