Method 11.3 – Miscellaneous: a-amylase activity

1. Rationale

This method is applicable to α-amylase, a starch hydrolysing enzyme, and determines the percentage of starch that is hydrolysed in 15 minutes under specified controlled conditions. The method refers to Method 7.8 (the SMRI starch method) to determine the starch content of the samples.

2. Principle

A solution of solubilized potato starch, sugar and calcium chloride is prepared. A specified volume of this solution is hydrolysed for 15 minutes at 70°C at a pH of 6.5. The starch content of this solution is determined and expressed as a percentage of the starch content of the original solution.

3. Apparatus

3.1 **Hot plate** with a magnetic stirrer and stirrer bar

3.2 **Water bath** operating at 70 or 90°C

3.3 **Conical flask**: 250 cm^3

3.4 **Beakers**: 250 and 1 000 cm^3

3.5 **Watch glass**: 100 mm ϕ

3.6 **Volumetric flasks**: 50, 100, 200 and 1 000 cm^3

3.7 **Pipettes**: 0.5, 1, 25, 100 cm^3

3.8 **Analytical balance** readable to 0.0001 g

3.9 **Thermometer**: 0 - 100°C

3.10 **Stop watch**

3.11 **pH meter** calibrated with pH 4 and 7 buffer solutions

3.12 **Buchner flask**: 500 cm^3

3.13 **Buchner funnel**: 60 mm ϕ

3.14 **Filter paper**: Whatman No. 91 or equivalent (185 mm ϕ)

4. Reagents

4.1 **Refined sugar** (first boiling)

4.2 Ethanol

Ethanol (CH_3CH_2OH, absolute alcohol) is a flammable liquid and is toxic when swallowed. It may cause damage to the eyes and safety glasses must be worn during use.

4.3 Potato starch, BDH

4.4 Silver nitrate (0.0171 M)

Silver nitrate is corrosive and should only be handled with gloves.

Dissolve 2.9049 g analytical grade silver nitrate ($AgNO_3$) in distilled water. Transfer to a 1 000 cm^3 volumetric flask, make to the mark and mix. The solution is light sensitive and should be stored in an amber container.

4.5 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.

4.6 Hydrochloric acid (1:1)

Hydrochloric acid is a corrosive acid and should only be handled with gloves while wearing safety glasses.

Carefully add 100 cm^3 of concentrated hydrochloric acid (HCl, 32%) 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 heat.

4.7 Kieselguhr (acid washed)

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

Non-metal containers and stirring rods must be used 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 one hour and filter under vacuum through the Watman No 91 filter paper. 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.

4.8 Calcium chloride solution (2.5%)

Weigh 2.5 g anhydrous calcium chloride ($CaCl_2$) and transfer to a 100 cm^3 volumetric flask. Make to the mark with distilled water.

4.9 Sodium hydroxide solution (0.05 M)

Sodium hydroxide (NaOH) is a corrosive base and should only be handled with gloves while wearing safety glasses.

Weigh 4.00 g sodium hydroxide pellets accurately and dissolve in 150 cm^3 distilled water. Cool the solution, transfer quantitatively to a 100 cm^3 volumetric flask and make to the mark with distilled water to prepare a 1 M sodium hydroxide stock solution.

Pipette 10 cm^3 of the stock solution into a 200 cm^3 volumetric flask and make to the mark with distilled water.

5. Procedure

Dissolve about 0.05 g (or a suitable quantity) of the enzyme in distilled water in a 50 cm^3 volumetric flask and make to the mark.

Dissolve 400 g refined sugar in 600 cm^3 distilled water in a 1 000 cm^3 beaker and add 1 cm^3 of the calcium chloride solution.

Weigh 1 g potato starch in a 250 cm^3 beaker and add 150 cm^3 distilled water. Cover the beaker with a watch glass and heat gradually to boiling on the hot plate magnetic stirrer with continuous stirring. Boil for 15 minutes to solubilise the starch. Cool the solution and add to the sugar solution in the 1 000 cm^3 beaker. Mix well and adjust the pH to 6.5 with the sodium hydroxide solution. Transfer to a 1 000 cm^3 volumetric flask and make to the mark with distilled water.

Pipette 100 cm^3 of this mixture into a conical flask, cover with a watch glass and place in a water bath at 90°C. If the enzyme is sensitive to high temperatures use 70°C. Pipette 0.5 cm^3 of the enzyme solution into the sugar solution, mix and allow the reaction to proceed for 15 minutes.

Immediately pipette 25 cm^3 of the reaction mixture into 100 cm^3 ethanol in a 250 cm^3 beaker to stop the reaction. Also pipette 25 cm^3 of the original sugar solution into another 250 cm^3 beaker as the control sample. Add 2 g acid washed Kieselguhr to each beaker, stir well and leave for one hour.

Determine the starch content of the two samples according to Method 7.8, the SMRI starch method.

6. Calculations

$$\text{Starch hydrolysed in 15 minutes (\%)} = \frac{S_c - S_s}{S_c} \times 100$$

where S_s ≡ Starch in the sample (mg/100 cm^3)
S_c ≡ Starch in the control sample (mg/100 cm^3)

Report as percentage to the nearest unit.

7. Example

Starch in the control sample	=	84 mg/100 cm^3
Starch in the reaction sample	=	18 mg/100 cm^3
Starch hydrolysed in 15 minutes	=	78.57%

Report as 79%

8. References

SASTA (1985). *Laboratory Manual for South African Sugar Factories.* 3rd Edition: 363 - 364.

SMRI (1997). Determination of the starch in raw sugar. *SMRI Test Methods*, TM054.