Practice: **DETERMINATION OF SACCHARIDES (Schoorl method)**

**PRINCIPLE**

Purified aqueous extract of a test sample is filtered and sucrose is hydrolyzed (inverted) to reducing sugars, which reduce cupric (Cu$^{ll}$) ions in alkaline boiling solution to cuprous (Cu$I$) oxide. The surplus of Cu$^{ll}$ is determined by iodometry using sodium thiosulfate titration.

**SCOPE**

This adaptation of the Schoorl method was designed for the determination of total sugar (i.e., reducing sugars + easily hydrolyzed sucrose) content in baked products analysis. The method is based on the Czech Standard ČSN 56 0116-7, Český normalizační institut, 1994.

**REAGENTS**

- Carrez solution I
- Carrez solution II
- Hydrochloric acid, 20 % solution
- Sodium hydroxide, 30 % solution
- Methyl orange, ethanolic solution (indicator)
- Fehling’s solution I
- Fehling’s solution II
- Starch colloidal solution (indicator)
- Sulfuric acid, 25 % solution
- Potassium iodide, crystalline
- Hydrochloric acid, diluted (1:5, v/v)
- Sodium thiosulfate, titrimetric solution, c = 0.1 mol L$^{-1}$ (approximate concentration)
- Potassium dichromate – TOXIC SUBSTANCE! Work only under direct supervision of an assistant!

**PROCEDURE**

**Aqueous extract preparation**

Weigh exactly about 2.5 g of tested sample from homogenized granulated analytical sample and transfer to a 200 mL volumetric flask. Add about 100 mL tempered water (about 30 °C) and mix the content of flask by swirling. Leave the dispersion for 30 min with occasional gentle agitation. After 30 min, rinse parts of the sample on the walls and pipet 5 mL zinc sulphate solution (Carrez I) when swirling the flask to mix them properly. Then add 5 mL potassium hexacyanoferrate solution (Carrez II) when swirling the flask. At 20 °C, fill the flask with water to bring total volume of the mixture to 200 mL. After proper mixing the flask content, filter through paper filter No. 388 and collect filtrate in a clean and dry flask.
Sucrose hydrolysis (inversion)

Pipet 50 mL of the filtrate to 100 mL volumetric flask, add 5 mL 20 % hydrochloric acid (use a cylinder). Prepare a water bath (≈ 70 °C), put the flask in, and insert thermometer in the flask. The temperature should increase to 67 °C within 3 min. When is this temperature reached, let the solution invert exactly 5 min at 67-70 °C. After that, cool the flask quickly to room temperature in a cold running water bath. Add a drop of methyl orange solution and neutralize it with 30 % sodium hydroxide (transition from red to yellow). At 20 °C, fill the flask with water to bring total volume of the mixture to 100 mL and mix it properly.

Reduction of cupric ions

Pipet 10 mL Fehling’s solution I and 10 mL Fehling’s solution II to 300 mL conical flask. Immediately, pipet exactly 20 mL inverted sample and 10 mL water. Add several small glass beads (or porous ceramics) to prevent bumping while boiling and cover the mouth of the flask with a small glass funnel. Place the flask on a hot plate adjusted to bring the solution to boil in 3 min and continue boiling for exactly 2 minutes (total heating time of 5 min). Then, cool the flask quickly to room temperature in a cold running water bath. The surplus of Cu^{II} is determined by iodometry using sodium thiosulfate titration.

Note 1. If the concentration of sugars is above 20 %, it is better to pipet 10 mL sample and 20 mL water for Cu^{II} reduction.
Note 2. After reduction, blue solution must persist (evidence of the presence of Cu^{II} ions) above the red precipitate of Cu_{2}O.

Titration

Add about 3 g of crystalline potassium iodide to the flask. After iodide dissolution, add 10 mL 25 % sulfuric acid. Titrature immediately the released iodine with approximately 0.1 M sodium thiosulfate solution. Near the end point add 3 mL of starch indicator solution, and continue titrating carefully while agitating the solution continuously until the blue starch – iodine color is discharged (creamy white color should last for about 3 min).

Blank determination

Conduct two blank determinations in identical fashion substituting purified water for the inverted sample.

Standardization of sodium thiosulfate titrimetric solution with potassium dichromate

Standardize 0.1 M sodium thiosulfate solution with calculated amount of potassium dichromate using the following procedure. Weigh out about 2 g potassium iodide crystals in Erlenmayer flask and dissolve them in several drops of water. Add 5 mL diluted hydrochloric acid (1:5), 25 mL of water and transfer accurately weighted amount of potassium dichromate from small beaker (is safer than weighing boat). Allow the mixture in stoppered flask to stand 5-6 minutes in the dark. Then open the flask and rinse the stopper with water and dilute the solution with 100–200 mL water. Titrate the
released iodine with 0.1 M sodium thiosulfate until the yellow color almost disappears. Add several drops (0.5 mL) of the starch indicator. A blue color will appear. Titrate with the 0.1 M sodium thiosulfate until the blue color just disappears (slight green color may appear). Record the volume of thiosulfate.

Note 3. Standardize prior to use.

**Number of determinations**

Conduct two determinations of a tested sample prepared from an analytical sample.

**Calculations**

Subtract the volume of sodium thiosulfate solution (sample titration) from the volume of sodium thiosulfate solution (blank sample titration – usually amounts 27-28 mL). Result of the subtraction (in mL) corresponds to an amount of sucrose (see Table I). The amount of sucrose is for 20 mL of sample pipetted for reduction and titration, and for accurate 0.1 M sodium thiosulfate solution. Calculations considering dilutions during sample treatment and actual sodium thiosulfate concentration must be done. The sucrose concentration should be expressed in g per 100 g of the sample matrix.

**REFERENCES**

Test A: DETERMINATION OF SACCHARIDES (Schoorl method)

1. Summarize concisely the principles of titrimetric determination of reducing saccharides with Fehling’s reagent.
2. What happens with sucrose during inversion?
3. Write down and enumerate equations used at iodometric determination of exact concentration of Na₂S₂O₃ (help: reduction of K₂Cr₂O₇, formation of I₂, titration of I₂ with Na₂S₂O₃ solution).
4. Calculate the amount of K₂Cr₂O₇ required for the determination of exact concentration of 0.1 M Na₂S₂O₃, when the consumption of 0.1 M Na₂S₂O₃ for titration is 20 mL. M.w. (K₂Cr₂O₇) = 294.18 g mol⁻¹.