APPLICATION NOTE - DETERMINING SUCROSE CONTENT IN MILK BY POLARIMETRY

Application Need: The dairy industry needs to be able to accurately determine the sucrose content of milk and milk products. The **sucrose content** is the total amount of unaltered sucrose in the milk product, and is determined by the procedure specified in the International Standard ISO 2911. It is expressed as a percentage by mass.

Solution: Use Reichert's Polar1 polarimeter to perform the ISO 2911 procedure to ascertain the sucrose content.

Overview

To determine sucrose content using polarimetry, a beam of polarized sodium light is rotated when falling through a sugar solution. The angle of rotation is specific for each type of sugar, and is in direct proportion with the sugar concentration and the distance the light has traveled (the standard tube length in polarimeters is 20 cm). The milk solution is treated with ammonia to bring mutarotation of lactose to final equilibrium, and it is neutralized by acetic acid. The neutralized milk solution is clarified by successive addition of zinc acetate and potassium hexacyanoferrate (II) to precipitate protein and fat in the milk. The next step is filtration. Using one part of the filtrate, the rotation angle of sucrose is measured. Then mild hydrochloric acid is added to invert the sucrose into fructose and glucose, and the filtrate is measured again. Glucose and fructose both have different specific rotations than sucrose. The difference in the two measurements is used to calculate the sucrose percentage.

Procedure

Preparation of sample

- Shake and invert the container of the sample of milk several times. Open the container and stir the sample carefully using a glass rod.
- Transfer the sample to a glass bottle and close it.

If the sample is too old or too thick:

- Transfer the sample to another glass bottle. The sample must be warmed up to 40°C in a water bath (6.5)[1] for about 2 hours. Every 15 minutes during the warming time, take the sample out and shake it well. Cool it down to room temperature. **Determination**
- Weigh exactly 40 g of sample milk into a beaker (6.8).
- Add 50 ml of hot water (80-90°C) and mix well.
  Note: All mixing should be done by *rotating the flask*, rather than by shaking it, unless noted, to avoid the formation of air bubbles.
- Transfer the mixture to a 200 ml volumetric flask (6.2), rinsing the beaker with successive quantities of water at 60°C until the total volume is between 120 and 150 ml. Mix by rotating the flask and cool to about 20°C.
- Add 5 ml NH_3 solution (7.3), mix by rotating the flask, and allow to stand for 15 minutes.
- Add a sufficient quantity of the CH_3COOH solution (7.4) to neutralize the solution and mix by rotating the flask.
  Note: The exact amount of CH_3COOH required is determined by titration of the NH_3 solution, using bromothymol blue as the indicator.
- Add 12.5 ml of $Zn(CH3COO)_2$ (7.1) solution and then 12.5 ml of $K_4Fe(CN)_6$ (7.2) solution and mix gently by rotating the flask.
- Bring the content of the flask to 20°C and dilute to the 200 ml mark with water at 20°C.
- Close the flask with a dry stopper and mix thoroughly by shaking vigorously.

[1] The numbers in parentheses refer to sections of the ISO 2911 standard.

- Allow the flask to stand for 15 minutes, then filter through a dry filter paper (6.13), rejecting the first 25 milliliters of filtrate. Collect the remaining clear filtrate in a clean and dry Erlenmeyer (6.8).
- Close the Erlenmeyer and cool to 20°C in a water bath (6.6), making sure that the contents are below the level of the water in the water bath **(this is Solution A).**
- Pipette (6.11) 40 ml of the filtrate (Solution A) into a 50 ml volumetric flask (6.3), and add 6 ml HCl (7.5).
- Place the flask in the water bath at 60°C (6.4) for 15 minutes, taking care that the entire bulb of the flask is immersed. Mix by rotating the flask during the first 5 minutes.
- Cool the flask to 20°C in water bath (6.6), dilute the 50 ml mark with water at 20°C, and mix. Allow to stand for 1 hour at this temperature **(this is Solution B).**

Measurement

To measure the samples, use direct polarization.

- Fill a clean 200 mm-long polarimeter tube with Solution A at 20°C. Place the filled sample tube in the polarimeter and record the optical rotation (A).
- Do the same for Solution B and record the optical rotation (B).

Calculation

To calculate the sucrose content, use the following formula:

$$S = \frac{A-1.25B}{Q} * \frac{V-\Delta V}{V} * \frac{V}{L*m}$$

S: % sucrose m: weight of the sample in grams
A: the reading of solution A
B: the reading of solution B

Note: if the invert polarization is measured at a temperature (t) other than 20 □ 0.2°C, the value of B should be multiplied by:
(1+0.0037[t-20])

L: the length, in decimeters, of the polarimeter tube
Q: the inversion division factor (see Additional Calculation below) V: the volume, in milliliters, of diluted sample before filtration
ΔV: the correlation, in milliliters, for the volume of the precipitate formed during the clarification, as shown below:

$$\Delta V = \frac{m}{100}(1.08F + 1.55P)$$

Where *F* is the percentage of fat in the sample and *P* is the percentage of protein in the sample.

For example, using the 200 mm tube, the formula above for % sucrose simplified to:

$$S = \frac{A-1.25B}{Q} * \frac{200-\Delta V}{2*m}$$

Additional Calculation

When calculating the sucrose value as shown above, you must determine the following value:

- **Value of the inversion division factor Q**
  The following formula gives an accurate value for *Q*, when the light source is sodium light and the rotation is measured in angular degrees:

Q = 0.8825 + 0.0006 (C-9) – 0.0033 (t-30) Where:
C is the percentage of total sugars in the inverted solution according to the polarimetric reading *t* is the temperature, in degrees Celsius, of the inverted solution during the polarimetric reading.

Remarks:

1. The percentage of total sugars C in the inverted solution may be calculated from the direct reading and the change of inversion per standard procedure, using the standard values for the specific rotations of sucrose, lactose, and inverted sugar. For normal condensed milk, the correction 0.0006 (C-9) can be ignored, because C is very close to 9.
2. Variation in temperature from 20°C makes little difference in the direct reading, but variation of more than 0.2°C during the invert reading requires a correction. The correction factor given to B is only accurate between 18-22°C.

Product Recommendations:

Polar 1 Polarimeter – Reichert Cat #14001000