METHOD 10.1

Effective 1st January 2018

DETERMINATION OF SUGAR: LUFF-SCHOORL METHOD

1. Scope and Field of Application

This method is for the determination of glucose, reducing sugars expressed as glucose and total sugar expressed as sucrose in feeding stuffs. Where necessary, lactose shall be measured separately and taken into account when calculating the results.

2. Principle

The sugars are extracted in dilute ethanol; the solution is clarified with Carrez solutions I and II. After eliminating the ethanol, the sugars are determined before and after inversion by the Luff-Schoorl method.

3. Reagents

3.1 Ethanol solution: 40% (V/V) neutralised to phenolphthalein.

3.2 Carrez solution I: dissolve 21.9g zinc acetate dihydrate and 3g glacial acetic acid in water. Dilute to 100ml with water.

3.3 Carrez solution II: dissolve in water 10.6g potassium ferrocyanide. Dilute to 100ml.

3.4 Methyl orange solution, 0.1% (w/v).

3.5 Hydrochloric acid solution, 4mol/litre.

3.6 Hydrochloric acid solution, 0.1mol/litre.

3.7 Sodium hydroxide solution, 0.1mol/litre.

3.8 Luff-Schoorl reagent.

3.8.1 Copper sulphate solution: dissolve 25g copper sulphate pentahydrate, free from iron, in 100ml water.

3.8.2 Citric acid solution: dissolve 50g citric acid monohydrate in 50ml water.

3.8.3 Sodium carbonate solution: dissolve 143.8g anhydrous sodium carbonate in approximately 300ml of warm water. Leave to cool.

Stirring carefully, pour the citric solution (3.8.2) into the sodium carbonate solution (3.8.3). Add the copper sulphate solution (3.8.1) and make up to 1 litre with water. Leave to settle overnight and filter. Check the concentration of the reagent thus obtained (Cu 0.05mol/litre; Na_2 $CO_3$2N 1mol/litre). The solution's pH should be approximately 9.4.

3.9 Sodium thiosulphate solution, 0.1mol/litre.

3.10 Starch solution: dissolve 5g soluble starch in 30ml water and add this to 1 litre boiling water. Boil for 3 minutes, allow to cool and add 10mg mecuric iodide as preservative.

3.11 Sulphuric acid solution, 3mol/litre.

3.12 Potassium iodide solution, 30% (w/v).

3.13 Granulated pumice stone boiled in hydrochloric acid, washed in water and dried.

3.14 3-methylbutan-1-ol.

3.15 Ethanol solution, 80% (V/V).

4. Apparatus

Rotary shaker, 35-40 rpm.

5. Procedure

5.1 *Extraction of the sample*

5.1.1 *All straight and compound feeding stuffs except those listed under 5.1.2 and 5.1.3.*

Weigh 2.5g of the prepared sample to the nearest mg and transfer to a 250ml volumetric flask. Add 200ml 40% ethanol (3.1) and mix on the rotary shaker (4.) for 1 hour. Add 5ml Carrez solution I (3.2) and stir for approximately 30 seconds. Add 5ml of Carrez solution II (3.3) and stir for one minute. Make up to volume with 40% ethanol (3.1), mix and filter. Remove 200ml of the filtrate and evaporate to approximately half volume in order to eliminate most of the ethanol. Transfer the residue quantitatively to a 200ml volumetric flask using warm water, cool, make up to volume with water, mix and filter if necessary. This solution is used to determine the amount of reducing sugars and, after inversion, of total sugars.

5.1.2 *Feedingstuffs rich in molasses and other feeding stuffs which are not particularly homogeneous.*

Weigh 20g of the prepared sample and place with 500ml water in a 1 litre volumetric flask. Mix for 1 hour on the rotary shaker (4.). Clarify using Carrez I (3.2) and II (3.3) reagents as described in 5.1.1, this time using four times the quantity of each reagent. Make up to volume with 80% ethanol (3.15), mix and filter. Proceed as described in 5.1.1 from "Remove 200ml of the filtrate...".

5.1.3 *Molasses and straight feeding stuffs which are rich in sugar and almost starch free.*

Weigh 5g of the prepared sample and place with 200ml distilled water in a 250ml volumetric flask. Mix for 1 hour, or more if necessary, on the rotary shaker (4.). Clarify using Carrez I (3.2) and II (3.3) reagents as described in 5.1.1. Make up to volume with water, mix and filter.

5.2 *Determination of reducing sugars*

Using a pipette, remove not more than 25ml of the solution containing less than 60mg reducing sugars expressed as glucose. If necessary, dilute the solution to 25ml with distilled water and determine the content of reducing sugars by the Luff-Schoorl method as in 5.4 below.

5.3 *Determination of total sugars after inversion*

Using a pipette take 50ml of the solution and transfer to a 100ml graduated flask. Add a few drops of methyl orange solution (3.4). Carefully and stirring continuously, add hydrochloric acid (3.5) until the liquid turns a definite red. Add 15ml hydrochloric acid (3.6) and immerse the flask in a boiling water bath for 30 minutes. Cool rapidly to approximately 20^{o}C and add 15ml sodium hydroxide solution (3.7). Make up to 100ml with water and mix. Remove not more than 25ml of the solution containing less than 60mg reducing sugars expressed as glucose. If necessary, dilute the solution to 25ml with distilled water and determine the content of reducing sugars by the Luff-Schoorl method as in 5.4 below.

5.4 *Titration by the Luff-Schoorl method*

Using a pipette, take 25ml of Luff-Schoorl reagent (3.8) and transfer to a 300ml Erlenmeyer flask and add 25ml of the clarified sugar solution from 5.2 or 5.3. Add 2 granules of pumice stone (3.13) and 1ml 3-methylbutan-1-ol (3.14), heat, while swirling by hand, over a free flame of medium height so as to bring the liquid to the boil in approximately two minutes. Place the flask immediately on an asbestos-coated wire

gauze with a hole approximately 60mm in diameter, under which a flame has been lit. The flame shall be regulated in such a way that only the base of the flask is heated. Fit a reflux condenser to the Erlenmeyer flask and boil for exactly 10 minutes. Cool immediately in cold water and after approximately five minutes titrate as below.

Add 10ml of potassium iodide solution (3.12) and immediately add 25ml of sulphuric acid (3.11) added carefully in small increments to prevent excessive foaming. Titrate with sodium thiosulphate solution (3.9) until a dull yellow colour appears; add the starch indicator (3.10) and complete the titration.

5.5 *Blank titration*
Carry out the same titration (without boiling) on a mixture of 25ml of Luff-Schoorl reagent (3.8) and 25ml of water, after adding 10ml of potassium iodide solution (3.12) and 25ml of sulphuric acid (3.11).

6. Expression of Results
Calculate the difference between the sample titration (5.4) and the blank titration (5.5) expressed in mg sodium thiosulphate solution 0.1 mol/litre. Using the table (8.), establish the amount of glucose in mg which corresponds to the difference between the values of the two titrations.

Express the result as a percentage of the sample.

7. Observations

7.1 The difference between the content of total sugars after inversion, expressed as glucose, and the content of reducing sugars, expressed as glucose, multiplied by 0.95, gives the percentage content of sucrose.

7.2 In order to determine the content of reducing sugars, excluding lactose, two methods may be adopted:

7.2.1 For an approximate calculation, multiply by 0.675 the lactose content established by a different method of analysis and subtract the result obtained from the content of reducing sugars.

7.2.2 For an accurate calculation of reducing sugars, excluding lactose, the same sample must be used for the two final determinations. One of the analyses is carried out on part of the solution obtained under 5.1.1, the other on part of the solution obtained during the determination of lactose by the method laid down for that purpose (after fermenting the other types of sugar and clarifying).

In both cases the amount of sugar present is determined by the Luff-Schoorl method and calculated in mg of glucose. One of the values is subtracted from the other and the difference is expressed as a percentage of the sample.

Example:
The two volumes taken correspond, for each determination, to a sample of 250 mg.

In the first case 17 ml of sodium thiosulphate solution 0,1 mol/litre corresponding to 44.2 mg of glucose is consumed; in the second, 11 ml, corresponding to 27.6 mg of glucose.

The difference is 16.6 mg of glucose.

The content of reducing sugars (excluding lactose), calculated as glucose, is therefore:

$$\frac{4 \times 16.6}{10} = 6.64\%$$

8. Table of Values for 25ml of Luff-Schoorl Reagent

ml of $Na_2 S_2 O_3$ 0.1 mol/litre, two minutes' heating, 10 minutes' boiling

$Na_2 S_2 O_3$ 0.1 mol/litre	Glucose, fructose invert sugars $C_6 H_{12} O_6$		Lactose C12 H22 O11		Maltose C12 H22 O11	
ml	mg	difference	mg	difference	mg	difference
1	2.4	2.4	3.6	3.7	3.9	3.9
2	4.8	2.4	7.3	3.7	7.8	3.9
3	7.2	2.5	11.0	3.7	11.7	3.9
4	9.7	2.5	14.7	3.7	15.6	4.0
5	12.2	2.5	18.4	3.7	19.6	3.9
6	14.7	2.5	22.1	3.7	23.5	4.0
7	17.2	2.6	25.8	3.7	27.5	4.0
8	19.8	2.6	29.5	3.7	31.5	4.0
9	22.4	2.6	33.2	3.8	35.5	4.0
10	25.0	2.6	37.0	3.8	39.5	4.0
11	27.6	2.7	40.8	3.8	43.5	4.0
12	30.3	2.7	44.6	3.8	47.5	4.1
13	33.0	2.7	48.4	3.8	51.6	4.1
14	35.7	2.8	52.2	3.8	55.7	4.1
15	38.5	2.8	56.0	3.9	59.8	4.1
16	41.3	2.9	59.9	3.9	63.9	4.1
17	44.2	2.9	63.8	3.9	68.0	4.2
18	47.1	2.9	67.7	4.0	72.2	4.3
19	50.0	3.0	71.7	4.0	76.5	4.4
20	53.0	3.0	75.7	4.1	80.9	4.5
21	56.0	3.1	79.8	4.1	85.4	4.6
22	59.1	3.1	83.9	4.1	90.0	4.6
23	62.2		88.0		94.6	