METHOD 10.2

Effective 1st January 2018

LACTOSE IN FEEDINGSTUFFS

1. Scope and Field of Application

This method is for the determination of lactose in feedingstuffs containing more than 0.5% lactose.

2. Principle

The sugars are extracted from the sample with water and subjected to fermentation by the yeast *Saccharomyces cerevisiae.* The solution is clarified and filtered and the lactose content of the filtrate is determined by the Luff-Schoorl method.

3. Reagents

3.1 *Saccharomyces cerevisiae* suspension: suspend 25g of fresh yeast in 100ml water. This suspension will keep for 1 week if stored in a refrigerator.

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

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

3.4 Luff-Schoorl reagent.

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

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

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

Stirring carefully, pour the citric solution (3.4.2) into the sodium carbonate solution (3.4.3). Add the copper sulphate solution (3.4.1) 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.

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

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

3.7 Sulphuric acid solution, 3mol/litre.

3.8 Sodium thiosulphate solution, 0.1 mol/litre.

3.9 Starch solution: dissolve 5g soluble starch in 30ml water and add this to 1,000ml boiling water. Boil for 3 minutes, allow to cool and add 10mg mercuric iodide as preservative.

4. Apparatus

Water bath with thermostat set at 38-40°C.

5. Procedure

5.1 *Preparation of the solution*

Weigh 1g of the sample and transfer to 100ml graduated flask. Add 25-30ml water. Place the flask in a boiling water bath (4.) for 30 minutes and then cool to approximately 35°C. Add 5ml yeast suspension (3.1) (see 7.), mix and maintain the flask at 38-40°C for 2 hours. Cool to approximately 20°C.

Add 2.5ml Carrez solution I (3.2), stir for 30 seconds, then add 2.5ml Carrez solution II (3.3) and again stir for 30 seconds. Make up to 100ml with water, mix and filter. Transfer by pipette into a 300ml Erlenmeyer flask a suitable volume of filtrate not exceeding 25ml and containing from 40 to 80mg lactose. If necessary make up to 25ml with water.

5.2 *Reagent blank*

Carry out a blank test on the reagents following the procedure in 5.1, but omitting the sample.

5.3 *Determination*

Determine the lactose content of the prepared solution, using the Luff-Schoorl method as follows:

Transfer 25ml of Luff-Schoorl reagent (3.4) to the flask containing the sample solution and add 2 granules of pumice stone (3.5). Heat, while swirling by hand, over a free flame of medium height so as to bring the liquid to the boil in approximately 2 minutes. Place the flask immediately on a ceramic-coated wire gauze with a hole approximately 60mm in diameter, under which a flame has been lit. The flame should be regulated in such a way as to heat only the base of the flask. Fit a reflux condenser to the Erlenmeyer flask and boil for exactly 10 minutes. Cool immediately in cold water and after approximately 5 minutes titrate as follows:

Add 10ml potassium iodide solution (3.6) and immediately add 25ml of sulphuric acid (3.7) (added carefully in stages to prevent excessive foaming). Titrate with sodium thiosulphate solution (3.8) until a dull yellow colour appears; then add the starch indicator (3.9) and complete the titration.

5.4 *Blank titration*

To a flask containing 10ml potassium iodide solution (3.6) and 25ml sulphuric acid (3.7) transfer 25ml of Luff-Schoorl reagent (3.4) and 25ml water. Titrate this solution as directed in 5.3, but without boiling.

6. Expression of Results

Calculate the difference between the sample titration (5.3) and the blank titration (5.4) 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 the percentage of lactose in the sample.

7. Observations

For products containing more than 40% of fermentable sugar, use more than 5ml of yeast suspension (3.1).

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	