STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

Method name: Starch
BIOVIT no.: 1159

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1. Introduction

This method is intended for analyzing starch in grain products / fertilizers / rumen and intestinal contents, m / mer. Most of the carbohydrates in grain products are made up by starch. Starch is a polysaccharide that consists of a high number of glucose units, which are linked together by α-glycosidic bonds.
The sample is dissolved in a buffer which has a pH around the optimum for α-amylase activity and contains calcium, which is important for the enzymes to work. When boiling the sample with heat-stable α-amylase present, the three-dimensional structure of the starch is broken down. Thus, starch becomes available for α-amylase which splits the long starch chains into shorter chains which will dissolve in the liquid phase. Then a buffer that has a pH around the optimum for amyloglucosidase activity is added, and the added amyloglucosidase cleaves the shorter chains down to glucose. The concentration of glucose is finally determined spectrophotometrically as a color reaction (see **"Arb 1013 glucose"** for details).

2. Reagents

- MOPS buffer (Sodium Salt)
- Sodium acetate buffer
- Calcium chloride dihydrate
- Heat stable α-amylase
- Amyloglucosidase
- Randox Glucose Assay (GLUC-HK GL8319)
- Clinical Chemistry Calibrator Level 2 CAL2350
- Clinical Chemistry Calibrator Level 3 CAL2351
- Quality control (Assayed Chemistry Premium Plus Level 2, Cat No-HN1530)
- Quality control (Assayed Chemistry Premium Plus Level 3, Cat No-HE1532)
- Acetone (for fat extraction, fat > 8 %)
- 80% ethanol (for sugar extraction, sugar > 4%)

MOPS buffer (50 mM, pH 7.0):
11.55 g Sodium salt of MOPS (Sodium Salt) is added to 900 mL distillated water in a beaker with the stirring magnet. During stirring, the pH is adjusted to 7.0 with 1 M (10%) hydrochloric acid (ca. 17 mL). Then 0.74 g of calcium chloride is added, and the volume is adjusted to 1 L in a volumetric bottle. The buffer is stored in the refrigerator.

Sodium acetate buffer (200mM, pH 4.5)
11.8 mL of Glacial acetic acid (1.05 g / l) is added to 900 mL of distillated water in a beaker with the stirring magnet.
While stirring, adjust the pH to 4.5 with 1M (4 g / 100 mL) NaOH. (ca. 60 mL). The volume is adjusted to 1 L in a volumetric bottle. The buffer is stored in the refrigerator.

3. Risk assessment
Glacial acetic acid: Flammable and highly corrosive.

NaOH: Highly corrosive. Wear gloves and eye protection.

HCl: Corrosive and irritates the respiratory tract.

R1 and R2 in the kit contain less than 0.1% sodium azide.

Wear heat resistant gloves when handling boiling water. If burning, rinse well under running cold water.

4. Recommended equipment

- 10 mL test tube (TT) with screw cap that can withstand boiling and centrifugation.
- TT tube w / cap.
- Pipettes and pipette tips 1-5 mL.
- Multi-pipette to 0.1, 0.2 and 4mL.
- Bench centrifuge with a swing-out rotor.
- Weigh out accurately to the nearest 0.1 mg.
- Vortex mixer.
- Thermostatic water bath, 50 °C.
- Boiling water bath, with tube rack.
- RX Daytona + instrument.

5. Sample material

For the analysis, a 100 mg ± 5 mg sample is needed. Degree of grinding: 0.5 mm.
If the sample is extracted by using acetone/ ethanol, a 120 mg ± 5 mg sample is needed.

6. Special remarks

Acetone treatment

If the samples contain more than 8% fat, they must be extracted with acetone in order to remove fat from the sample, before the samples degrade into glucose. See point 4a in the job description.

Sugar extraction

If the samples contain more than 4% sugar, they must be extracted with 80% ethanol in order to remove free sugar from the sample, before the samples degrade into glucose. See point 4b in the job description.

For grain products, is not necessary to extract free sugar, because the concentration of glucose is very low. (0.1-0.5%).

RX Daytona + Instrument:

Limit of detection: 0,049 mmol/L
Limit of quantification: 0,35 mol/L
Linearity: 0,35 – 61.9 mmol/L

7. Work procedure

1) Turn on thermostatic water bath and adjust to the desired temperature.

2) Weigh 100 mg sample (± 5 mg) into a 10 mL glass tube w / screw cap

- **120mg if the samples are to be extracted by acetone or ethanol!!**

3) Make a note of the exact sample weight.

4) *Acetone extraction: (only applies to samples with more than 8% fat)*

a) *Add 7 mL of acetone to each sample, mix it well (vortex), and leave on the bench for 5 min, then mix it again. Leave it for another 5 min. and centrifuge for 10min. v / 3000 rpm. Discard the supernatant. Repeat the previous step one more time.*

(7 mL acetone, 3x mix, stand in 5 min, centrifuges.) The samples must be left overnight in a fume hood to evaporate acetone.

<u>Sugar extraction:</u> (only applies to samples with more than 4% sugar).

b) *Add 10 mL of 80% ethanol, mix (vortex) the content and incubate the tube at 80°C for 5 min. Centrifuge the tube for 10 min at 3000 rpm on a bench centrifuge. Discard the supernatant.*
Resuspend the sample in 10 mL of 80% ethanol and incubate the tube at 80°C for 5 min. Mix it well (vortex) then centrifuge the tube for 10 min at 3000 rpm on a bench centrifuge. Carefully pour off the supernatant.

5) One chicken control sample should be included in parallel on each round of analysis. The control sample is in the closet above the weight in the lab on the 1st floor and is marked Chicken feed control. (0.5 mm).

6) Add 0.2 mL of 80% ethanol to each tube and mix in a vortex to completely wet the sample (important in aiding the complete dissolution of sample with a high starch content).

7) Add 3 mL of α-amylase in MOPS buffer (100µl amylase and 2.9 mL MOPS buffer) to each sample and mix well.

8) Incubate the samples in the boiling water bath for 6 min, with mixing after 2 and 4 min.

9) Add 4 mL of sodium acetate buffer and 0.1 mL of amyloglucosidase to each sample, mix the contents on a vortex mixer.

10) Incubate the tubes in a water bath at 50 °C for 30 min.

11) Centrifuge the samples for 10 min. at 3000 *rpm.*

12) Pipette a few mL of the supernatant into TT tubes, in order to store the sample.

13) The samples are analyzed on a RX Daytona + fully automatic analyzer.

- See separate manual for the use of the instrument

8. Calculation

$$\% \text{ starch} = \frac{\text{abs.sample} * 180 * 0{,}0073 * 162 * 100}{\text{mg sample} * 180}$$

abs. sample = glucose absorbance read on spectrophotometer (mmol / l)

180 = molecular weight of glucose (mg / mmol)

0.0073 = dilution factor (buffer + enzyme)

162/180 = glucose factor (conversion from glucose unit to starch)

mg sample = weighed sample

100 = the calculation in %