Determination of Starch

Materials:

50 ml glass screw cap tubes with Teflon lined screw caps
Re-pipette, capable of dispensing 25 ml
Pipettes calibrated to dispense 50 µl, 1 ml, and 9 ml
Water baths, 60 °C and 95 °C
Centrifuge (2,000 x g)
100 ml volumetric flasks
Spectrometer

Reagents:

A. 0.1 M Acetate Buffer, pH 5.0

1. Sodium Acetate (13.61 g) (Mallinckrodt Cat # 7364, FW 136.08, CAS # 6131-90-4 or Fisher Cat # S-220, FW 136.08, CAS # 6131-90-4)
2. Glacial Acetic Acid (2.22 ml) (Fisher Cat # S25118, FW 60.05, CAS # 64-19-7)

   Dissolve sodium acetate and acetic acid in 600 ml H_2O, adjust pH to 5.0 with HCl or NaOH and bring volume to 1 liter.

B. **Alpha-amylase** (Genencor International Inc., Rochester, NY 14608 (800-847-5311))

C. **Distillase L-400 Enzyme** (Genecor International Inc., Rochester, NY 14608 (800-847-5311)) (Genencor Cat # 101-00307-915)

D. **1% Sodium Azide,** dissolve 2 g Sodium azide (Sigma Cat # S-8032, FW 65.01, CAS # 26628-22-8) in 150 ml H_2O, dilute to 200 ml.

Procedure:

1. Dry samples and grind through a 1 mm screen.
2. Weigh 100 mg samples into 35 ml screw cap tubes with Teflon lined caps.

Note: This assay is capable of handling up to 150 mg starch per tube.

Feed samples are weighed out in quadruplicate. One set of duplicates for enzyme addition and the other as glucose blanks for determination of free glucose in the sample. Corn starch and corn controls should also be run with each set of samples analyzed (any sample which could potentially contain free glucose should be handled in this manner).

Fecal samples are weighed out in duplicate. Free glucose is usually minimal, so no blanks are needed, but a few samples should be checked to be sure.

An **enzyme blank** (containing only reagents) should also be included to determine free glucose in the enzymes.

3. Using a calibrated repipet, add 25 ml of the acetate buffer to all tubes. This volume must be accurate as the final calculations are based on this volume.

4. Add 50 µ of alpha-amylase to all tubes except the glucose blanks for the feed samples. Cap tubes and mix gently.
5. Place tubes in a 95 °C water bath for 30 minutes. Tighten caps after 3-5 minutes and gently shake tubes 3 times during incubation.
6. Move tubes to a 60 °C water bath and allow them to cool (about 10-15 minutes). One tube at a time, remove cap and add 50 µl Distallase L-400, replace cap tightly, mix, and return to the water bath. Do not add enzyme to the glucose blanks.
7. Incubate tube at 60 °C overnight. Mix gently at least 3 times during incubation.
8. Remove tubes from the water bath and centrifuge at 2000 x g for 10 minutes. Pipette exactly 9 ml supernatant into vials containing 1 ml of 1% sodium azide. Cap vails, mix, and freeze or refrigerate until glucose analysis.

Glucose Analysis:

Use the following working standards based on the sample type.

Stock Standard (1,000 mg/dl)

Add 1.0000 g dextrose, 10 ml of 1% sodium azide, and 50 ml H_2O to a 100 ml volumetric flask. Dissolve and bring to volume with H_2O.

Note: A commercial Glucose Standard Solution (Sigma Diagnostics) may also be used.

Working Standards

Add appropriate amounts of stock solution to 100 ml volumetric flasks with 10 ml 1% sodium azide. Bring to volume with H_2O.

Working Standards for forage, blanks, and low fecal samples:

ml Stock/ 100 ml	mg/dl glucose
0.5	5
1	10
2	20
3	30
4	40

Working standards for grains and high fecal samples:

ml Stock/ 100 ml	mg/dl glucose
5	50
10	100
15	150
20	200
25	250

Starch Analysis: Glucose Analysis with Microplate Reader

Materials:

Synergy H1 Microplate Reader

Procedure:

1. Dilute standards and samples.
    a. Vortex samples and pipette 500 ul samples and standards into 12 x 75 ml glass tubes. Dilute with 500 ul 18 Mohm water. Be sure to include a blank tube with 18 Mohm water only. **Do not dilute samples without enzyme added.** Use Eppendorf repeater pipette to add 18 Mohm water to tubes. For low starch samples, no dilution is needed. Run enzyme without dilution.
2. Micro-titre Reading (Molecular Devices Spectrophotometer)
    a. Warm up Synergy H1 spec to 37 °C. Do not read plates until temperature is reached.
    b. Pre-read blank plate.
    c. Pipette 20 µl blank, standards, and samples into wells of microplate.
    d. Set up program to read each well (label each well for respective contents).
    e. Add 200 µl glucose reagent to each well.
    f. Incubate plate for 3 minutes at 37 °C before reading.
    g. Read plate at wavelength of 340 nm.

Calculations:

$$mg\ glucose = \left(Glucose\ concentration\ of\ sample\ in \left(\frac{mg}{dl}\right) \times 0.25\ dl\right) - blank\ 1 - (blank\ 2 \times mg\ sample \times DM)$$

$$Blank\ 1 = Glucose\ conc. of\ enzyme\ blank \left(\frac{mg}{dl}\right) \times 0.25\ dl = mg\ free\ glucose\ from\ enzyme$$

$$Blank\ 2 = \frac{Glucose\ conc. of\ sample\ blank \left(\frac{mg}{dl}\right) \times 0.25\ dl}{mg\ sample\ in\ blank\ \times DM} = mg\ free\ glucose\ per\ mg\ sample$$

1.110 = correction factor for glucose recovery from starch based on:

Chain Length	Glucose, g/g glucan
2	1.052
5	1.085
10	1.099
20	1.105
50	1.109
100	1.110
500	1.111

1.111 = correction for the dilution of 9 ml supernatant with 1 ml of 1% sodium azide

$$\%\, Starch = \frac{\left(\frac{mg\ glucose}{1.110}\right) \times 1.111}{mg\ sample\ \times DM} \times 100$$

$$\%\, Starch = \frac{mg\ glucse}{mg\ sample\ \times DM} \times 100$$

Reference

Starch analysis: Herrera-Saldana, R. and J. T. Huber. 1989. Influence of varying protein and starch degradabilities on performing of lactating cows. J. Dairy Sci. 72:1477-1483.