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Starch Digestibility; Degree of Starch Access

1. Application

This procedure covers the determination of starch digestibility in biomass samples.

2. Summary of Methods

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Sample Collection, Preservation, and Handling

Half of each sample is dried at 55^{o}C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm forage mill. The other half of each wet sample is retained for starch recovery (DSA).

6. Apparatus and Materials

6.1 Analytical balance, accurate to 0.1 mg.
6.2 LAB-Line Multi-Unit heaters or similar hot plates.
6.3 Graduate cylinders of appropriate sizes.
6.4 100 ml, 500 ml, and 1000 ml volumetric flasks, class A.
6.5 Timer.
6.6 1000 ml beakers.
6.7 Thermometer.

7. Reagents

Prepare all reagents as per normal starch assay, as listed below; noting larger volumes of reagents will be used.

7.1 Glucose calibration verification standards, such as YSI 2.0 and 9.0 mg/ml glucose standards.
7.2 Amyloglucosidase (suggested source, Sigma A-3042).
7.3 Corn Starch (Spectrum S1552).
7.4 Methanol, ACS reagent grade.
7.5 Phosphate buffer (pH 6.5, for solvent)

7.5.1 Dissolve 14 g $NaH_2PO_4*H_2O$ in reagent grade water and bring to volume in a 1000 ml volumetric flask.
7.5.2 Determine pH and add 15M NaOH drop wise until pH reaches 6.5.
7.6 Acetate buffer (pH 4.2)
7.6.1 Weigh 9.1 g of sodium acetate into 500 ml volumetric flask.
7.6.2 Add about 300 ml of reagent grade distilled water and mix until all solid is dissolved.
7.6.3 Add 22.3 ml (23.4 g) of glacial acetic acid.
7.6.4 Dilute to volume with distilled water and mix.
7.7 Amyloglucosidase working solution
7.7.1 Use the Sigma A-3042 amyloglucosidase containing 11,500 units of activity per milliliter.
7.7.2 Mix 2.5 ml of A-3042 amyloglucosidase with 475 ml of reagent grade distilled water in a 500 ml beaker to achieve 60 units of activity per milliliter of enzyme solution.
7.7.3 Prepare daily and store in the refrigerator.
7.8 25% TCA – dissolve 50.0 g trichloracetic acid in 200 ml reagent grade water.
7.9 Phosphate buffer
7.9.1 Dissolve 40 g NaH_2PO_4 and 10 g Na_2HPO_4 in reagent grade water
7.9.2 Bring to volume in a 1000 ml volumetric flask
7.10 α-Amylase, heat stable – use Sigma A-3306. In a 50 ml beaker mix 2 ml of A-3306 with 18 ml of reagent grade water to obtain 20 ml of working solution.

8. Methods

8.1 Determine the lab dry matter and total dry matter content of each sample using the "Sample Preparation & Lab Dry Matter" and "Total Dry Matter" procedures.
8.2 Determine the total starch content using the "Total Starch in Forages" procedure.
8.3 Weigh out approximately 20.0 g of wet corn silage, TMR, or small grain silage sample to the nearest 0.001 g and transfer to a 1000 ml beaker. Alternatively, weigh out approximately 4.0 g of dry corn, HM corn, grains, or other byproducts to the nearest 0.001 g and transfer to a 1000 ml beaker. Reocrd as W_{sample}, the initial sample weight.
8.4 A standard reference corn starch is run in parallel with each batch of samples and to monitor starch recovery of the assay. Weight 2.5 g of corn starch to the nearest 0.001 g and transfer to a 1000 ml beaker. Record the weight as $W_{standard}$, the initial standard reference material weight. As with unknown samples, absolute DM content of the corn starch must also be determined (105°C for 3 hours).
8.5 Add 150 ml of reagent grade water to each beaker. Swirl vigorously to ensure the sample is wetted and evenly dispersed. Note: A few drops of methanol may be used to pre-wet the sample, which will aid in its dispersion once the water is added.
8.6 Add 200 ml phosphate buffer (pH 6.5) to each beaker. Loosely cover and place beaker on a heating unit on setting 70.
8.7 After 5 minutes increase setting to 90. Heat for approximately 25 minutes, swirling frequently to wet any sample that may be clinging to the side of the beaker and to avoid clumping of sample in the middle/bottom of the beaker. Monitor the

temperatures of beaker solutions with a thermometer being careful not to allow samples to reach temperatures > 95°C. Samples will start boiling at this point.

8.8 Remove from heat.

8.9 Add 100 ml of reagent grade water to each beaker and swirl to mix.

8.10 Add 3 ml of heat-stable amylase working solution to each beaker when temperatures are near 80°C. Stir gently on stir plate while allowing beaker solutions to cool to below 50°C (approximately 25 minutes.) Use thermometer to monitor beaker solution temperatures.

8.11 Add 100 ml of acetate buffer to each beaker and continue stirring on stir plate to mix.

8.12 Add 50 ml amyloglucosidase working solution to each beaker when temperatures are < 50°C. Mix well and continue stirring at approximately 40°C for 60 minutes.

8.13 While samples are incubating at 40°C, set up a 10 ml test tube for each sample. Pipette 0.5 ml of TCA into each tube.

8.14 After 60 minutes of mixing, beaker solutions should be near room temperature.

8.15 Pipette 6 ml of beaker solution hydrolyzate into 10 ml test tubes containing TCA.

8.16 Pipette 3.5 ml of phosphate buffer into each test tube and mix well.

8.17 Set up and calibrate the YSI as described in the manufacturer's manual using the dextrose membrane, YSI 2357 system buffer, and YSI 2776 2.5 g/L calibrator solution. Program the instrument to auto calibrate every fourth sample or every fifteen minutes, set the sample size to 25µL, and use the following probe parameters:

- 8.17.1 Chemistry – dextrose
- 8.17.2 Units – g/L
- 8.17.3 Calibrator – 2.50 g/L
- 8.17.4 End point – 30 seconds
- 8.17.5 Cal Station # - 1

8.18 Verify the calibration of the YSI using the glucose calibration verification standards before starting the run. Re-verify the calibration periodically during the analysis and at the end of the run.

8.19 Measure the glucose levels in the enzyme blanks and in all the samples. The validated linear range of the instrument is 0 – 9.0 g/L dextrose. If the value reported exceeds the validated range, the hydrolyzate must be diluted appropriately and re-run.

9. Calculations

9.1 Calculate the amount of starch recovered from analysis of the reference corn starch as follows, 105°C dry weight basis:

9.1.1 % Standard recovered = [{($YSI_{standard}$, g/L * total volume, L} / {standard weight, g, $W_{standard}$ * (% total solids, T_{final} / 100)}] * 0.9 * 100%

9.1.2 Note: Corn starch recoveries of 93 to 95% have routinely been achieved with this protocol. Recoveries less than 90% indicate the data generated for the batch of samples should be rejected and the analysis repeated.

9.2 Calculate the amount of starch recovered in un-dried, un-ground silages, grains, TMR's, etc., on a 105°C dry weight basis:

9.2.1 % Starch = [(YSI_{sample}, g/L * total volume, L) / {standard weight, g, $W_{standard}$ * (% dry matter / 100)}] * 0.9 * 100%

9.2.2 Note 1: The factor 0.9 converts grams of glucose to grams of the anhydrosuger (starch, in this case). The factor can be calculated by dividing the molecular weight of glucose less one molecule of water (180-18) by the molecular weight of glucose.

9.2.3 Note 2: Because the sample is un-dried and un-ground, % dry matter = (Lab dry matter * Total dry matter)

9.3 Calculate the starch recovery percent ($Starch_{rp}$, % of starch) present in the sample, to two decimal places, on a 105°C dry weight basis by the following formula:

9.3.1 $Starch_{rp}$, % Starch = (% starch recovered, % of dry matter / total starch, % of dry matter) * 100%

10. Quality Control

A standard reference corn starch is run in parallel with each batch of samples and to monitor starch recovery of the assay. Starch recovery of corn starch should be > 93.0 percent. The recovery of reference corn starch is not used to adjust DSA starch determinations and is only used to monitor assay recover. When reference starch recoveries are low entire starch procedures should be evaluated and re-run.

11. Reporting

Report as Starch Digestibility (DSA), percent of starch present in the sample, to two decimal places, on a 105°C dry weight basis. If duplicate samples are run, report the average.

Starch Digestibility (DSA), % of Starch	Reference
> 96.0	Very High
96.0-93.0	High
93.0-90.0	Medium
< 90.0	Low

12. References

12.1 Blasel, H., P.C. Hoffman, and R.D. Shaver. 2005. "Degree of starch access: An enzymatic method to determine starch degradation potential of corn grain and corn silage." Anim. Feed Sci. and Technol.

12.2 NREL Ethanol Project Laboratory Analytical Procedure #001, "Standard Method for the Determination of Total Solids in Biomass."

12.3 Solvay Enzymes. 1996. "Fungal glucoamylase for Starch Hydrolysis." Diazyme L-200 Technical Notes.
12.4 YSI Incorporated. 12994. "Determination of Cook in Extruded Cereal Products." Application Note #319.