WBL-M-028: Acid Detergent Fiber and Lignin Analysis

Scope:

This method determines Acid Detergent Fiber (ADF), which is the fiber cellulose and lignin residue (cellulose and lignin) remaining after processing with acid detergent. Acid Detergent Lignin (ADL) can then be quantified using sulfuric acid extraction and ashing.

Equipment:

Grinding mill

Micro-Balance (Sartorius M500P)

Drying oven (Fisher Scientific Isotemp)

Muffle Furnace (Thermo Fisher Scientific BF51828C-1)

Ankom Model 200

Reagents:

1.) Acid detergent solution: Add 20 g cetyl trimethylammonium bromide (CTAB) to 1 L of 1N H_2SO_4. Agitate and heat to aid solution. OR, premixed solution (ANKOM FAD20CB).

2.) 1 N Sulfuric Acid (H_2SO_4): Prepare in a fume hood. Add ~500 mL of DDI water to a 1 L volumetric flask followed by 28 mL concentrated H_2SO_4. Allow solution to cool in a water bath before bringing to volume with DDI water (Concentrated H_2SO_4 Fisher Cat. # A300-212).

3.) 72% Sulfuric Acid (H_2SO_4) (ANKOM Cat. # FSA72)

4.) Acetone, purified (ANKOM Cat. # FACE)

Supplies:

Fiber filter bags (ANKOM Cat. # F57)

Heat sealer

Desiccator (ANKOM Cat. # X45)

50 mL beakers

3 L beaker

2 L beaker

Chemical resistant marker (ANKOM Cat. # F08)

QA/QC Requirements:

1 digestion duplicate, 1 digestion blank, and 2 QC Standards (1 cladium & 1 pine needle) for each set of 20 samples.

Procedure:

Sample Preparation

1.) If samples required grinding, grind samples in a centrifugal mill with a 2mm screen or cutter type (Wiley) mill with a 1mm screen. Samples ground finer may have particle loss from the filter bags and result in low values. If the sample size is too small for the mill, use a hand-grinding method with screens instead.

2.) After each sample, use the vacuum to clean the Wiley mill and all grinding components. If there are still sample remnants after vacuuming, clean the mill with ethanol

ADF Procedure

1.) Use a solvent resistant marker to label the filter bags to be used in the analysis.

2.) Weigh and record the weight of each empty filter bag and zero the balance **(W1)**. NOTE: Do not pre-dry filter bags. Any moisture will be accounted for by the blank bag correction.

3.) Place 0.45 – 0.50g of prepared sample in up to 24 of the bags and record the weight **(W2)** of each. Avoid placing the sample in the upper 4mm of the bag.

4.) Include at least one empty bag in the run to determine the blank bag correction **(C)**. NOTE: A running average blank bag correction factor should be used in the calculation of fiber. The inclusion of at least one blank bag in each run is mainly used as an indicator of particle loss. A **C** larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag during the extraction. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed, then the grinding method needs to be evaluated.

5.) Using a heat sealer, completely seal each filter bag closed within 4mm of the top to encapsulate the sample. NOTE: Use sufficient heat to completely seal the filter bags and allow enough cool time (2 sec) before raising the heat sealer arm to remove each bag from the heat sealer.

6.) Pre-extract only samples containing >5% fat (e.g. seeds, nuts, etc.): Extract samples by placing bags with samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top. Shake the container 10 times and allow the bags to soak for 10 minutes. Repeat with fresh acetone. Pour out acetone and place bags on a wire screen to air-dry.

7.) To eliminate sample clumping, spread the sample uniformly inside the filter bags by shaking and flicking the bags.

8.) Place up to 3 bags on each of eight Bag Suspender Trays (maximum of 24 bags). Stack the trays on the center post of the Bag Suspender with each level rotated 120 degrees in relation to the tray below it. Place the empty 9th tray on top. NOTE: All nine trays must be used regardless of the number of bags being processed.

9.) Verify that the Exhaust Hose is connected to the instrument and securely positioned in the drain.

10.) Turn the instrument Power Switch to the ON position.

11.) Before inserting the Bag Suspender into the Vessel, read the Temperature Controller on the instrument. If the temperature is higher than room temperature, fill the Vessel with cold tap water. The temperature on the Controller will decrease. When the value on the Controller reaches its lowest number and starts to increase, open the Exhaust Valve and exhaust the water. Repeat this process until the number on the Temperature Controller equilibrates to room temperature.

12.) Open the Vessel Lid and insert the Bag Suspender with bags into the Vessel and place the Bag Suspender Weight on top of the empty 9th tray to keep the Bag Suspender submerged.

13.) When processing 24 sample bags, add 1900-2000 mL of ambient temperature AD solution to the fiber analyzer vessel. If processing less than 20 bags, add 100 mL/bag of AD solution (use minimum of 1500 mL to ensure Bag Suspender is covered).

14.) Turn Agitate and Heat ON and confirm agitation. Set the timer for 60 minutes and close the lid.

15.) When the ADF extraction is complete, turn Agitate and Heat OFF.

16.) Open the drain valve (slowly at first) and exhaust the hot solution before opening the Vessel Lid. NOTE: The solution in the Vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the Vessel Lid.

17.) After the solution has been exhausted, close the exhaust valve and open the Vessel Lid. Add 1900-2000 mL of 70-90°C rinse water. Turn Agitate on and rinse for 5 minutes. If the Heat is ON, the Vessel Lid should be closed. If the Heat is OFF, the Vessel Lid can be open. Repeat 5 minute hot water rinses 2 more times. Just before draining the 3rd rinse, test the water with pH paper. If acid is present repeat rinses until neutral.

18.) After the rinsing procedures are complete, open the Vessel Lid and remove the filter bags. Gently press out excess water from the bags. Place bags in a 250ml beaker and add enough acetone to cover the bags and soak for 3-5 minutes.

19.) Remove the filter bags from the acetone and place them on a wire screen to air-dry. Completely dry in an oven at 102 ± 2°C. (In most ovens the filter bags will be completely dry within 2-4 hours.) Do not place bags in the oven until the acetone in the bags has completely evaporated. NOTE: When running a lignin procedure or a sequential (NDF/ADF or NDF/ADF/Lignin) with the F57 Filter Bag it is important not to dry the bags overnight after the NDF or ADF procedure. A drying timeframe of 2-4 hours at 100°C to 105°C is sufficient to thoroughly dry the bags after each procedure. Extended drying times or too high a temperature can compromise the bag's filtration media. In addition, be sure to check the water of the fourth rinse during the ADF procedure to ensure all the sulfuric acid has been removed from the bags. If litmus paper shows the presence of acid during the fourth hot water rinse, repeat until neutral.

20.) Remove the filter bags from the oven and immediately place them directly into a collapsible desiccant pouch and flatten to remove any air. Cool to ambient temperature and weigh the filter bags **(W3)**. NOTE: Do not use a conventional desiccator container.

ADL in Beakers Procedure

1.) After performing ADF determinations, place dried bags/samples into 3 L beaker and add sufficient quantity (approximately 250 ml) of 72% H2SO4 to cover bags. IMPORTANT: Bags must be completely dry and at ambient temperature before adding concentrate acid. If moisture is present in the bags, heat generated by the H2SO4 and H20 reaction will affect the results (sample inside bag will char).

2.) Place 2 L beaker inside 3 L beaker to keep bags submerged. Agitate bags at start and at 30-minute intervals by pushing and lifting 2L beaker up and down approximately 30 times.

3.) After 3 hours pour off H2SO4 and rinse with warm H2O to remove all acid. Repeat rinses until pH is neutral. Rinse with approximately 250 ml of acetone for 3 minutes to remove water.
WARNING: Do not place bags in the oven until acetone is completely evaporated.

4.) Complete drying in oven at 105° C for 2-4 hours. Remove bags from oven and place directly into MoistureStop weigh pouch and flatten to remove air. Cool to ambient temperature and weigh **(W4)**. Calculate blank bag correction using weight loss of a blank bag upon sulfuric acid extraction **(C1)**.

5.) Place each bag in a pre-weighed 50 mL beaker and ash at 525°C for 3 hours. Cool the contents of the beakers post-ashing and weigh **(W5)**. Calculate blank bag ash correction using weight loss upon ignition of a blank bag sequentially run through ADF and sulfuric acid extraction **(C2)**.

Calculations:

1.) **Cellulose (g)** = $W3 - W4 - (W1 \times C1)$

2.) **% Cellulose** = $\left(\frac{Cellulose\ (g)}{W2-W1}\right) \times 100$

3.) **Lignin (LOI) (g)** = $W4 - W5 - (W1 \times C2)$

4.) **% Lignin** = $\left(\frac{Lignin\ (g)}{W2-W1}\right) \times 100$

W1 = Empty bag weight

W2 = Initial dry sample weight prior to processing

W3 = Dried sample weight after ADF extraction process

W4 = Dried sample weight after sulfuric acid extraction process

W5 = Sample weight after ashing process

C1 = Sulfuric acid extraction blank bag correction (average of oven-dried weight after sulfuric acid extraction divided by original blank bag weight)

C2 = Ash blank bag correction (average of loss on ignition weight divided by original blank bag weight)

Quality Assurance:

Acceptance criteria:

Accuracy 85%-115%

Cladium acceptance: lignin concentration between 16%-22%

Pine needles acceptance: lignin concentration between 16%-22%

If mean calculated lignin concentrations for QA materials fall within the acceptance ranges, report sample data with no quality. If mean calculated lignin concentrations for QA materials are outside of the acceptance ranges, sample data will not be reported and samples in that batch will be re-run. If insufficient mass is available, sample data will be reported with the appropriate flag.

References:

1.) ANKOM Technology. Acid Detergent Fiber in Feeds – Filter Bag Technique (for A200 and A200I). ADF Method, Method 5. 2017.

2.) ANKOM Technology. Method 8 - Determining Acid Detergent Lignin in Beakers. 2016.