

## Neutral Detergent Fiber in Feeds Filter Bag Technique (For A200, A200I)

### DEFINITION

This method determines Neutral Detergent Fiber, which is the residue remaining after digesting in a detergent solution. The fiber residues are predominantly hemicelluloses, cellulose, and lignin.

### SCOPE

This method is applicable to grains, feeds, forages and all fiber-bearing material.

### APPARATUS

1. Analytical Balance—capable of weighing down to 0.1 mg.
2. Oven—capable of maintaining a temperature of  $102\pm 2^\circ\text{C}$ .
3. Digestion instrument—capable of performing the digestion at  $100\pm 0.5^\circ\text{C}$  and maintaining a pressure of 10-25 psi. The instrument must also be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM<sup>200</sup>, 65 rpm agitation, ANKOM Technology).
4. Filter bags—constructed from chemically inert and heat resistant filter media, capable of being heat sealed closed and able to retain 25 micron particles while permitting rapid solution penetration (F57, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, ANKOM Technology).
6. Desiccator pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (MoistureStop Weigh Pouch, ANKOM Technology).
7. Marking pen—solvent and acid resistant (F08, ANKOM Technology).

### REAGENTS

1. Neutral Detergent Solution—Add 30.0 g Sodium dodecyl sulfate, USP; 18.61g Ethylenediamine-tetraacetic disodium salt, dihydrate; 6.81 g Sodium borate; 4.56 g Sodium phosphate dibasic, anhydrous; and 10.0 ml Triethylene glycol, in 1 L distilled  $\text{H}_2\text{O}$  (premixed chemical solution available from ANKOM Technology). Check pH range to 6.9 to 7.1. Agitate and heat to aid solution. (see Notes, *Caution*).
2. Alpha-amylase—Heat-stable bacterial alpha-amylase: activity = 17,400 Liquefon Units / ml (FAA, ANKOM Technology).
3. Sodium sulfite— $\text{Na}_2\text{SO}_3$ , anhydrous (FSS, ANKOM Technology).

### PREPARATION OF SAMPLE

Grind samples in a centrifugal mill with a 2 mm screen or cutter type (Wiley) mill with a 1 mm screen. Samples ground finer may have particle loss from the filter bags and result in low values.

### PROCEDURE

1. Use a solvent resistant marker to label the filter bags. Weigh filter bag ( $W_1$ ) and zero balance.  
*Note*—Do not pre-dry filter bags; any moisture will be accounted for by the blank bag correction.
2. Weigh 0.45-0.55 g of prepared sample ( $W_2$ ) directly in filter bag. Avoid placing the sample on the upper 4 mm of the bag. Spread the sample uniformly inside the filter bag by shaking and flicking the bag to eliminate clumping.
3. Using a heat sealer, completely seal the upper edge of the filter bag within 4 mm of the top.  
*Note*—Use sufficient heat to completely seal the filter bag and allow enough cool time (2 sec) before removing the bag from the heat sealer.
4. Weigh one blank bag and include in run to determine blank bag correction ( $C_1$ , see Number Note 1).
5. **Pre-extract only samples containing soybean products or >5% fat:** Extract samples by placing 24 bags with samples into a container with a top. Pour enough acetone into container to cover bags and secure top. Shake the container 10 times and allow bags to soak for 10 minutes. Repeat with fresh acetone. Pour out acetone and place bags on a wire screen to air-dry. **Exception - Roasted soybean:** Due to the processing of roasted soy a modification to the extraction is required. Place roasted soy samples into a container with a top. Pour enough acetone into container to cover bags and secure top. Shake the container 10 times and pour off acetone. Add fresh acetone and allow samples to soak for twelve hours. After soak time, pour out acetone and place bags on a wire screen to air-dry.
6. Place a maximum of 24 bags into the Bag Suspender. All nine trays should be used regardless

of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees. Insert the Bag Suspender with bags into the fiber analyzer vessel and place the weight on top to keep it submerged.

*Note*—Prior to inserting the Bag Suspender, if the vessel temperature is warm from a previous run, add cold water and exhaust.

7. When processing 24 sample bags, add 1900-2000mL of ambient ND solution to the fiber analyzer vessel. If processing less than 20 bags, add 100 ml/bag of ND solution (use minimum of 1500 mL to ensure Bag Suspender is covered). Add 20 g (0.5 g/50mL of ND solution) of sodium sulfite and 4.0 mL of alpha-amylase to the solution in the vessel.
8. Turn Agitate and Heat on and confirm agitation. Set timer for 75 min and close lid.
9. At end of extraction, turn Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening 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 lid.
10. After the solution has been exhausted, close the exhaust valve and open the lid. Add 1900mL of (70-90°C) rinse water and 4.0 mL of alpha-amylase to the first and second rinses. Turn Agitate on and rinse for 5 min. The lid may be sealed with the Heat on or left open with the Heat off. Repeat hot water rinses a total of three times.
11. When the rinsing process is complete remove the samples. Gently press out excess water from bags. Place bags in a 250 mL beaker and add enough acetone to cover bags and soak for 3-5 min.
12. Remove bags from acetone and place on a wire screen to air-dry. Completely dry in oven at 102±2°C (most ovens will complete drying within 2-4 hrs).

*Note*—Do not place bags in the oven until acetone has completely evaporated.

13. Remove bags from oven, place directly into a collapsible desiccant pouch and flatten to remove air. Cool to ambient temperature and weigh bags ( $W_3$ ).

*Note*—Do not use conventional desiccator container.

## CALCULATIONS

$$\% \text{ NDF (as-received basis)} = \frac{(W_3 - (W_1 \times C_1))}{W_2} \times 100$$

Where:  $W_1$  = Bag tare weight

$W_2$  = Sample weight

$W_3$  = Dried weight of bag with fiber after extraction process

$C_1$  = Blank bag correction (final oven-dried weight divided by the original blank bag weight)

## NOTES

### *Caution*

Powdered chemicals will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical.

Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling.

## NUMBERED NOTES

1. A running average blank bag correction factor ( $C_1$ ) should be used in the calculation of fiber. The inclusion of a blank bag in each run is mainly used as an indicator of particle loss. A  $C_1$  larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed then grinding method needs to be evaluated.