**Method for Determining Neutral Detergent Fiber (aNDF)**

**ANKOM Technology - 05/03**

A. **Reagents**

(a) *Neutral Detergent Solution (ND)* - Add 30.0 g sodium lauryl sulfate, USP; 18.61 g Ethylenediaminetetraacetic Disodium Salt, Dihydrate; 6.81 g sodium tetraborate decahydrate; 4.56 g sodium phosphate dibasic, anhydrous; and 10.0 ml triethylene glycol, in 1 L distilled H₂O (premixed chemical solution Catalog #FND20 or FND20C). Agitate and heat to facilitate solubility. Check pH range to 6.9 to 7.1.

(b) *Alpha-amylase* - Heat-stable bacterial alpha-amylase: activity = 17,400 Liquefon Units / ml (catalog #FAA). One Liquefon Unit is the measure of digestion time required to produce a color change with iodine solution indicating a definite stage of dextrinization of starch substrate under specified conditions.

(c) *Sodium sulfite* - Na₂SO₃, anhydrous (catalog #FSS).

(d) *Acetone* - Use grade that is free from color and leaves no residue upon evaporation (catalog #FACE).

B. **Safety Precautions** - see MSDS provided with chemicals

(a) Acetone is highly flammable. Use fume hood when handling acetone and avoid inhaling or contact with skin. Make sure bags are completely dry and that all the acetone has evaporated before placing in oven.

(b) Sodium lauryl sulfate will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical.

C. **Apparatus**

(a) Digestion apparatus - ANKOM²⁰⁰/²²⁰ Fiber Analyzer

(b) Filter Bags – (Catalog #F57)

(c) Impulse bag sealer - Requires high enough temperature to melt and seal polymer in filter bags (Catalog #1915 or 1920)

(d) Desiccator - ANKOM MoistureStop weigh pouch( large zip-lock bag / desiccant inside)(Catalog#X45)

D. **Procedure**

(a) Prepare Filter Bags/Sample

1) Use a solvent resistant marker to label the filter bags with test and bag number

2) Weigh Filter Bag (W₁) record weight and tare balance.

3) Weigh 0.5g (±0.05 g) of air-dried sample (W₂) directly into filter bag, Sample should be ground to pass through a 1mm screen (2mm screen when using a cyclone mill), Weigh one blank bag and include in digestion to determine blank bag correction (C₁). This will account for any moisture or weight loss in the bag.

4) Seal the bags closed within 0.5cm from the open edge using the heat sealer.

5) Spread sample uniformly inside the filter bag by shaking and lightly flicking the bag to eliminate clumping.

6) A maximum of 24 bags may be placed in the bag suspender. All nine trays are 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. The weight is placed on top of the empty 9th tray to keep the bag suspender submerged. Insert the bag suspender with bags into the fiber analyzer vessel.
D. Procedure (con't)

7) NOTE: SAMPLES CONTAINING SOY PRODUCT OR >5% FAT - Extract fat from 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 Soy - Due to special properties of Roasted Soy a modification to the fat 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, drain off acetone as stated above and allow samples to air-dry before next step.

(b) Add 20 g (0.5 g/50 ml of ND solution) of sodium sulfite and 4.0 ml of heat stable alpha-amylase to 2000 ml of ND solution when processing 24 sample bags. Allow sodium sulfite to dissolve in the solution. Pour solution over the bag suspender in the vessel. If processing less than 20 bags add 100 ml/bag of detergent solution and reduce sodium sulfite as indicated above. NOTE: A minimum of 1500 ml is required. Confirm that all nine levels of Bag Suspender are covered.

(c) Turn Agitate and Heat ON and confirm that Bag Suspender is agitating properly. Set timer for 75 minutes and push Start. Close and seal lid of vessel.

(d) After 75 minutes turn Agitate and Heat OFF, open the drain valve (slowly at first) and exhaust hot solution before opening lid. WARNING: The solution in vessel is under pressure. The valve should be opened first to remove pressure before lid can be opened. Ensure exhaust hose is securely positioned for safe disposal of effluent.

(e) After the solution has been exhausted close valve and open the lid. Add approximately 2000 ml of hot (85° - 90°C) H2O and 4.0 ml of alpha-amylase to the first and second rinses. Lower lid but do not tighten. Turn Agitate ON and leave Heat OFF. Each rinse should last 3-5 minutes. Exhaust water and repeat rinse two more times (total of three rinses).

(f) After final rinse add “cold” tap water to assist handling and cool the vessel for the next run. Drain the water and remove bag suspender from vessel. Remove filter bags from bag suspender and gently press out excess water. Place in 250 mL beaker and cover bags with acetone. Allow bags to soak 3 minutes then remove and lightly press out excess acetone.

(g) Spread bags out and let air-dry. Completely dry in oven at 105°C (most ovens provide complete drying within 2 - 4 hours). WARNING: Do not place bags in the oven until acetone has completely evaporated. Remove bags from oven, place directly into MoistureStop weigh pouch and flatten to remove air. Cool to ambient temperature and weigh bags (W3).

E. Calculate percent aNDF (as-is basis) = \( \frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2} \)

aNDF (DM basis): = \( \frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2 \times DM} \)

aNDFOM (DM basis): = \( \frac{(W_4 - (W_1 \times C_2)) \times 100}{W_2 \times DM} \)

Where:  
W_1 = Bag tare weight  
W_2 = Sample weight  
W_3 = Weight after extraction process  
W_4 = Weight of Organic Matter (OM) (loss of weight on ignition of bag and fiber residue)  
C_1 = Blank bag correction (final oven-dried weight/original blank bag weight)  
C_2 = Ash corrected blank bag (loss of weight on ignition of blank bag/original blank bag weight)