# ANKOM Method for Determining NDF

## Materials:

Digestion Apparatus – ANKOM Fiber Analyzer
ANKOM F57 Filter bags
Impulse bag sealer
Desiccator
Moisture stop weigh pouch

## Reagents:

**Neutral Detergent Solution:**

1. To 500 ml dd water, add 8 g Sodium hydroxide (Mallinckrodt Cat # SX059-1, FW 40.00, CAS # 1310-73-2 or Macron Cat # 770810, FW 40.00, CAS # 1310-73-2), 29.22 g ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich Cat # ED-1KG, FW 292.24, CAS # 60-00-4) and 13.62 g Sodium borate (Sigma Cat #S-9640, FW 381.37, CAS # 1303-96-4).
2. With heat, dissolve 9.12 g Sodium phosphate dibasic (anhydrous) J.T. Baker Cat #3828-01, FW 141.90, CAS # 7558-79-4) in 50 ml dd water.
3. Dissolve 63.33 g Sodium dodecyl sulfate (Sodium laurel sulfate) (MP Cat # 102918, FW 288.4, CAS # 151-21-3) in 1,000 ml dd water.
4. Pour 1, 2, and 3 above into 2,000 ml volumetric.
5. Bring up to volume with dd water and allow to stand overnight.
6. Check pH. It should be 6.8-7.2. Adjust with NaOH.

**Amylase solution:**

We use a heat stable alpha-amylase (DSM Maxamyl™ HT). Brand name: Validase HT340L, Valley Enzymes, South Bend, IN.

**Acetone** (Fisher Cat # A18-20, FW 58.08, CAS # 67-64-1)

## Procedure:

1. Weigh filter bag, record weight, and tare balance.
2. Weight 0.5 g air-dried sample, ground to pass through a 1 mm screen, directly into filter bag. Weigh two blank bags and include in digestion to determine bag correction.
3. Seal the bags closed within 0.5 cm from the open edge using the heat sealer.
4. Spread sample uniformly inside the filter bag by shaking and lightly flicking the bag to eliminate clumping.
5. A maximum of 24 bags may be placed inside the bag suspender. All nine trays are used regardless of the number of bags being processed. Place three bags per trays and stack trays on center post with each level rotated 120 degrees. The weight is placed on top of the empty ninth tray to keep the bag suspender submerged.

**Note – Samples containing soy or 0.5% fat:** Extract fat from sample by placing 24 bags with samples into a 500 ml bottle with a top. Pour enough acetone into bottle to cover bags and secure top. Shake the container 10 times and allow bags to soak 10 minutes. Repeat with fresh acetone. Pour out acetone and place bags on a wire screen to air dry.

An exception to this procedure is roasted soy. Roasted soy contains special properties, which require a modification to this procedure. Place roasted soy samples into a 500 ml bottle with a top. Pour enough acetone into bottle to cover bags and secure top. Shake the container 10 times and pour off acetone. Add fresh acetone and allow bags to soak for 12 hours. After soak time, drain off acetone and air dry.

6. When processing 24 bags add 1,900-2,000 ml of NDF solution into ANKOM Fiber Analyzer vessel. If processing less than 20 bags, add 100 ml/bag of NDF solution (minimum of 1,500 ml). Add 4 ml of alpha-amylase into the solution in the vessel.
7. Place bag suspender into the solution in the vessel. Turn Agitate and Heat ON, and confirm that the bag suspender is correctly agitating. Set the timer for 75 minutes and push Start. Close and seal the lid of vessel.
8. After 75 minutes, turn Agitate and Heat OFF, open the drain valve and exhaust hot solution before opening lid.
9. After solution is exhausted, close valve and open lid. Add approximately 2,000 ml hot water and 4.0 ml 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 for a total of four rinses.
10. Remove filter bags from suspender and gently press out water. Place bags into a beaker and soak in acetone for 3 minutes. Remove bags and lightly press out acetone.
11. Spread bags out and allow acetone to evaporate. Complete drying in a 100 °C oven for at least 2 hours. Remove bags from oven, place in a moisture stop pouch. Cool in desiccator and weigh bags.

**Calculations:**

Calculate percent NDF (DM basis):

$$[W3 - (W1 \times C1)] \times 100 \div W2 \times DM$$

Key: W1 = Bag tare weight
W2 = Sample weight
W3 = Weight after extraction process
C1 = Blank bag correction (final oven dried weight/original blank bag weight)