## TDF Method (AOAC 991.43, AACC 32-07.01, NMKL 129,2003) using the ANKOM$^{TDF}$ Dietary Fiber Analyzer

### Definition

Using Filter Bag Technology, this method determines the amount of TDF within a given sample using the weight of the recovered TDF residue corrected for ash and protein content.

### Scope

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

### Apparatus

1. Analytical Balance—capable of weighing 0.1 mg.
2. Drying Oven—capable of maintaining a temperature of 105 ± 2°C.
3. Fiber Recovery instrument capable of recovering TDF residue. The instrument must be capable of automatically adding all reagents, mixing the sample to ensure proper digestion, and controlling digestion and precipitation temperatures (ANKOM$^{TDF}$ Dietary Fiber Analyzer, ANKOM Technology).
4. Filter Bags (DF-S, DF-FT, ANKOM Technology).
5. Bag Weigh Holder—used for eliminating static during the bag weighing process (TDF52, ANKOM Technology).
6. Drying Rack—used for drying filter bags (TDF50, ANKOM Technology).
7. Heat sealer—sufficient for sealing the filter bags closed (1915, ANKOM Technology).
8. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (*MoistureStop* weigh pouch, ANKOM Technology).
9. Marking pen—solvent and acid resistant (F08, ANKOM Technology).
10. Acetone rinse stand (TDF51 Rinse Stand, ANKOM Technology).
11. Ashing Oven.
12. Protein Determination equipment—Kjeldahl recommended.

### Reagents

Use Distilled Water throughout.

(a) *Ethanol* 95%.

(b) *Ethanol 78%*—Place 821 ml 95% ethanol into 1 L volumetric flask, dilute to volume with $H_2O$.

(c) *Sodium Azide solution*—Add Sodium Azide (or equivalent) to $H_2O$ so the final solution has a 0.02% concentration of Sodium Azide.

(d) *Heat-stable α-amylase solution*—Dilute 5 ml of Megazyme amylase to 100 ml with the Sodium Azide solution.

(e) *Protease*—Dilute 10 ml of Megazyme protease to 100 ml with the Sodium Azide solution.

(f) *Amyloglucosidase solution*—Dilute 20 ml of Megazyme AMG to 100 ml with the Sodium Azide solution.

(g) *Diatomaceous earth (DE)*—(ANKOM XTC, Celite 545 AW, No. C8656, Sigma Chemical Co. or equivalent).

(h) *MES*—2-(*N*-Morpholino)ethanesulfonic acid (No. M-8250, Sigma Chemical Co., or equivalent).

(i) *TRIS*—Tris(hydroxymethyl)aminomethane (No. T-1503, Sigma Chemical Co., or equivalent).

(j) *MES-TRIS buffer solution*—0.05M MES, 0.05M TRIS, pH 8.2 at 24°C. Dissolve 19.52 g MES and 12.2 g TRIS in 1.7 L $H_2O$. Adjust pH to 8.2 with 6*N* NaOH, and dilute to 2 L with $H_2O$. (*Note:* It is important to adjust pH to 8.2 at 24°C. However, if buffer temperature is 20°C, adjust pH to 8.3; if temperature is 28°C, adjust pH to 8.1. For deviations between 20 and 28°C, adjust by interpolation.)

(k) *Hydrochloric acid solution*—0.561*N*. Add 93.5 ml 6*N* HCl to ca 700 ml $H_2O$ in 1 L volumetric flask. Dilute to 1 L with $H_2O$.

(l) *Acetone*—reagent grade.

(m) *Sodium Azide*—reagent grade.

### Sample Preparation

1. Grind samples in a centrifugal mill with a 0.5 mm screen. Samples ground finer may have particle loss from the filter bags and result in low values.
2. De-fat and de-sugar samples as needed based on the AOAC 991.43, AACC 32-07.01, or NMKL 129,2003 methods. Adjust sample weights accordingly.

### TDF Procedure *(see the TDF Analysis section of the Operator's Manual for more detail)*

1. Label the filter bags using a solvent resistant marker.
2. Fill all chemical containers to the Min. Level line or above.
3. Fill all enzyme containers to the 10 ml line or above.
4. Place each filter bag in a tared Bag Weigh Holder and record the weight.
5. Place ca 1 g of DE in each of six tared and numbered tins and record the weights.
6. Place 0.5±0.05 g of sample in each of six tared and numbered tins and record the weights.
7. Remove all Clamp Bars from the instrument.
8. Follow the instructions on the Touch Screen Display.
9. Install SDF bags by gently pulling the black SDF Delivery Nozzle toward you and pulling the bag up underneath the nozzle. Pull the bag up so that the top of the bag is about 35 mm (1.375 inches) above the top of Clamp Bar C and return the nozzle to its original position to hold the bag in place. Center each bag within the black lines located on the back of Clamp Bar C.
10. Re-install Clamp Bar D.
11. Flatten the bag to remove any wrinkles.
12. Press the check mark button (☑) on the "SDF Bags (and clamp bar D) installed?" screen on the Touch Screen Display to pinch the bags just above the filter.
13. Add DE to each SDF bag, rinsing with no more than 3 ml of Distilled Water to ensure complete transfer.
14. Install IDF *Flow-Thru* bags by gently pulling the black IDF Delivery Nozzle toward you and pulling the bag up underneath the nozzle. Pull the bag up so that the top of the bag is about 35 mm (1.375 inches) above the top of Clamp Bar A and return the nozzle to its original position to hold the bag in place. Center each bag within the black lines located on the back of Clamp Bar A.
15. Place at least 20 mm (0.75 inches) of the bottom of each IDF *Flow-Thru* bag inside the top of each corresponding SDF bag.
16. Re-install Clamp Bar B.
17. Flatten the bag to remove any wrinkles.
18. Press the check mark button (☑) on the "IDF *Flow-thru* Bags (and clamp bar B) installed?" screen on the Touch Screen Display to pinch the bags.
19. Re-install Clamp Bar C.
20. Secure the front of each SDF filter bag in place with the hook located on the front part of Clamp Bar C.
21. Transfer the prepared sample into each of the IDF *Flow-Thru* bags, keeping it below the IDF Delivery Nozzle.

## Calculations (all weights in grams)

$$\%\ TDF = \left[ \frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} \right] X\ 100$$

$$= \left[ \frac{[((f_{F1} - f_{S1} - D_1) + (f_{F2} - f_{S2} - D_2))/2] - P - (A_2 - D_2) - B}{(M_1 + M_2)/2} \right] X\ 100$$

Where:

| | | |
|---|---|---|
| $M_1, M_2$ | = | Original wt for duplicate samples adjusted for pre-treatment fat and sugar losses (g) |
| $R_1, R_2$ | = | Residue for duplicate samples (g) |
| $f_F$ | = | Final Filter Bag (g) |
| $f_S$ | = | Initial Filter Bag (g) |
| D | = | Original wt of Diatomaceous Earth (g) |
| P | = | Protein of residue and bag (g) |
| A | = | Ash of residue and bag (g) |
| B | = | Blank (g) |
| | = | $[(BR_1 + BR_2)/2] - P_B - (A_B - D_B)$ |
| | = | $[((f_{BF1} - f_{BS1} - D_{B1}) + (f_{BF2} - f_{BS2} - D_{B2}))/2] - P_{B1} - (A_{B2} - D_{B2})$ |
| $BR_1, BR_2$ | = | Residue for duplicate blanks (g) |
| $f_{BF}$ | = | Final Blank Filter Bag (g) |
| $f_{BS}$ | = | Initial Blank Filter Bag (g) |
| $P_B$ | = | Protein of Blank Filter Bag (g) |
| $A_B$ | = | Ash of Blank Filter Bag (g) |
| $D_B$ | = | Original wt of Diatomaceous Earth in Blank Filter Bag (g) |

## TDF Procedure (continued)

22. After the automated recoveries are complete, rinse the SDF bags twice with acetone. ANKOM recommends the use of the ANKOM TDF51 Rinse Stand for the acetone rinses.
23. After the acetone has evaporated, using a setting of 3 to 4, press the Heat Sealer arm down for 3 to 4 seconds to seal each SDF bag just above the filter. This keeps all residue contained to the filter area.
24. Place each filter bag in the Drying Rack and place the rack in an oven set to 105°C. Dry to constant weight (about 90 minutes).
25. Remove all of the bags from the oven and place them in a desiccant pouch to cool.
26. Remove each bag one at a time and record their weights.
27. Determine the protein content within the TDF residue. See the "Protein Determination Procedure – SDF / TDF" for more information.
28. Determine the ash content within the TDF residue. See the "Ash Determination Procedure – IDF / SDF / TDF" for more information.
29. Calculate the % TDF value.