TDF Method (AOAC 991.43/985.29/2001.03, AACC 32-07.01) 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- Total, Soluble, and Insoluble Dietary Fiber in Foods and Feeds

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 (HS, 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 DI or DW Water throughout.

Solutions common to AOAC 991.43, 985.29, and 2001.03 methods

(a) *Ethanol* 95%.

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

(c) *Enzyme solutions*—Make enzyme solutions according to the Enzyme Dilution Table below.

Enzyme	Dilute ANKOM concentrates (AOAC 991.43)
α-Amylase	TDF80 or TDF81: Dilute 5 ml to 25 ml with DI/DW water
Protease	TDF82 or TDF83: Dilute 5 ml to 25 ml with DI/DW water
Amyloglucosidase	TDF84 or TDF85: Dilute 5 ml to 25 ml with DI/DW water

Enzyme	Dilute ANKOM concentrates (AOAC 985.29)
α-Amylase	TDF80 or TDF81: Dilute 10ml to 25 ml with DI water
Protease	TDF82 or TDF83: Dilute 5 ml to 25 ml with DI water
Amyloglucosidase	TDF84 or TDF85: Dilute 5 ml to 25 ml with DI water

Enzyme	Make up Enzyme Solutions (AOAC 2001.03)
α-Amylase	300 ± 30 Ceralpha U/ml (in DI water)
Protease	600-990 Glycose U/ml (in DI water)
Amyloglucosidase	35-75 Tyrosine U/ml (in DI water)

(d) *Diatomaceous earth (DE)*—(ANKOM DE1, DE2, or equivalent).

(e) *Acetone*—reagent grade.

Solutions unique to the AOAC 991.43 method

(f) *MES*—2-(*N*-Morpholino) ethanesulfonic acid (MES, ANKOM Technology, or equivalent).

(g) *TRIS*—Tris(hydroxymethyl)aminomethane (TRIS, ANKOM Technology, or equivalent).

(h) *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.)

(i) *Hydrochloric acid solution*—0.561*N*. Dilute 93.5 ml of 6*N* HCl to 1 L with H_2O.

Solutions unique to the AOAC 985.29/2001.03 methods

(j) *Hydrochloric acid solution (AOAC 985.29/2001.03)*—0.65*N*. Dilute 325 ml of 1*N* HCl to 500 ml with H_2O.

(k) *Phosphate buffer solution*—0.08M, pH 6.0. Dissolve 1.40 g Sodium Phosphate dibasic, anhydrous (Na_2HPO_4) (or 1.75 g dihydrate) and 9.68 g Sodium Phosphate monobasic monohydrate (NaH_2PO_4) (or 10.94 g dihydrate) in a 1 L volumetric flask. Dilute to 1 L with H_2O. Adjust pH to 6.0.

(l) *Sodium Hydroxide Solution*—0.55*N*. Dissolve 11.00 g Sodium Hydroxide (NaOH ACS) in a 500 ml volumetric flask. Dilute to 500 ml with H_2O.

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/or de-sugar samples as needed based on the AOAC 991.43/985.29/2001.03 and AACC 32-07.01 methods. Adjust sample weights accordingly.

TDF Procedure *(see the TDF Analysis sections 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 15 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 (as detailed in steps 9-25 below).
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. This will close bar D which will pinch the bags just above the filter.
13. Add DE to each SDF bag, rinsing the tin with no more than 3 ml of 78% to ensure complete transfer and that all the DE is below the SDF Delivery Nozzle.
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 Bags (and clamp bar B) installed?" screen on the Touch Screen Display. This will close bar B which will pinch the bags just above the filter.
19. Re-install Clamp Bar C.
20. Add sample into each of the IDF *Flow-Thru* bags. Rinse the tin with no more than 3 ml of DI or DW water to ensure complete transfer and that all the sample is below the IDF Delivery Nozzle.
21. Secure the front of each SDF filter bag in place with the hook located on the front part of Clamp Bar C.
22. Check that the Nitrogen supply is connected to the instrument and turned on.
23. Set Filter Times.
24. Set the manual pH check.
25. Press the START button to begin the automated processes.
26. **AOAC 985.29/2001.03 ONLY:** Check the pH during the IDF process before the Protease digestion. The Touch Screen Display will remind you to do this. Adjust to 7.3-7.7.
27. Check the pH during the IDF process, after the HCl has been added. (If configured, the instrument will stop so you can make this check.) Adjust to 4.0-4.7 (AOAC 991.43) or 4.0-4.6 (AOAC 985.29/2001.03) as needed.
28. After the automated processes are complete, rinse the SDF bags twice with acetone. ANKOM recommends the use of the ANKOM TDF51 Rinse Stand for the acetone rinses.
29. After the acetone has evaporated, with your Heat Sealer set between 3 and 4 (settings may vary depending on the heat sealer and the power source), press the Heat Sealer arm down for 3 to 4 seconds to seal each bag just above the filter. This keeps all residue contained to the filter area while handling the bags.
30. 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).
31. Remove all of the bags from the oven and place them in a desiccant pouch to cool.
32. Remove each bag one at a time and record their weights.
33. Determine the protein content within the TDF residue. See the "Protein Determination Procedure – SDF / TDF" for more information.
34. Determine the ash content within the TDF residue. See the "Ash Determination Procedure – IDF / SDF / TDF" for more information.
35. Calculate the % TDF value.

Calculations (all weights in grams)

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

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

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)