TDF Method (AOAC 2009.01, AACC 32-45.01) 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. Oven—capable of maintaining a temperature of 105 ± 2°C.
3. Fiber Recovery instrument capable of separately recovering IDF, SDF, and 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-I, DF-S, DF-FT, ANKOM Technology).
5. Bag Weigh Holder—used for eliminating static during 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 to ensure complete closure (HS, ANKOM Technology).
8. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (X45, *Moisture Stop* weigh pouch, ANKOM Technology).
9. Marking pen—solvent and acid resistant (F06, ANKOM Technology).
10. Acetone rinse stand (TDF51 Rinse Stand, ANKOM Technology).

Reagents

Use Deionized (DI) or Distilled (DW) water throughout.

(a) *Ethanol* 95%.

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

(c) *Acetone*—reagent grade.

(d) *Sodium maleate buffer solution*—50 mM, pH 6.0 and 2 mM $CaCl_2$. Dissolve 11.6 g of maleic acid in 1600 ml of water and adjust the pH to 6.0 with 4 M (160 g/L) NaOH solution. Add 0.6 g of calcium chloride ($CaCl_2.2H_2O$) and adjust the volume to 2 L.
IMPORTANT: <u>Do not add sodium azide to the buffer solution when using the TDF instrument.</u> Although the methods call for sodium azide in the buffer as a preservative, it is not required for the method. The buffer solution should be made fresh each day. Strong reaction may occur if sodium azide is added to the buffer and used in the TDF instrument.

(e) *Enzyme solutions*— The AOAC and AACC methods require the following enzyme activity per sample:

- Pancreatic α-Amylase: 2,000 Units
- Amyloglucosidase (AMG): 136 Units
- Protease: 35 Tyrosine Units

Prepare enzyme solutions for use in the ANKOM^{TDF} Dietary Fiber Analyzer as follows:

AOAC 2009.01/2011.01	Enzyme	Enzyme Concentration
	α-Amylase (Pancreatic)	2000 Ceralpha U/g sample
	Amyloglucosidase	136 Glucose U/g sample
	Protease	35 Tyrosine U/g sample

ANKOM^{TDF} Enzyme Solutions	*Enzyme Concentration
<u>α-Amylase (Pancreatic)/AMG solution</u> 1. Measure 0.667 g α-Amylase Porcine Pancreatic (TDF130) into a 50 ml volumetric flask 2. Add 1.04 ml AMG concentrate (TDF84 or TDF85) to the same 50ml volumetric flask 3. Make up to mark with sodium maleate buffer	1000 Ceralpha U/ml 68 Glucose U/ml
<u>Protease solution</u> 1. Dilute 5 ml Protease concentrate (TDF82 or TDF83) to 25ml with DI or DW water	35 Tyrosine U/ml
*The ANKOM^{TDF} Dietary Fiber Analyzer delivers 2 ml of the amylase/AMG solution and 1ml of the protease solution to each of the six stations per run. If you are not using the ANKOM enzyme concentrates, you must prepare enzyme solutions to the recommended concentration, and it must include 0.02% w/v of Sodium Azide (to prevent microbial growth).	

(f) *Diatomaceous earth (DE)*—(ANKOM DE1/DE2, Celite 545 AW, No. C8656, Sigma Chemical Co. or equivalent).

(g) *Trizma Base, 0.75 M*—Add 90.8 g of Trizma (Sigma cat. No. T-1503) to ca 800 ml of DI or DW water and dissolve. Adjust volume to 1 L.

(h) *Acetic acid solution, 2 M*—Add 115 ml of glacial acetic acid (Fluka 45731) to a 1 L volumetric flask. Dilute to 1 L with DI or DW water.

(i) *D-Sorbitol (Internal standard solution)*—100 mg/ml. Weigh 100 g of dry D-Sorbitol into a beaker, dissolve in water and transfer to a 1 L volumetric flask with water and dilute to volume. Transfer to a polypropylene bottle and add 0.2 g of Sodium Azide as a preservative. (NOTE: Handle sodium azide with caution only after reviewing MSDS.)

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 samples as needed based on the AOAC 2009.01 method and adjust sample weights accordingly.

Calculations (all weights in grams)

$$\%\ TDF = \%\ WSWAIDF + \%\ WASDF$$

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

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

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)
WASDF	=	Soluble Dietary Fiber determined by HPLC

TDF Procedure *(see the TDF Analysis section of the Automated AOAC 2009.01/2011.25 and AACC 32-45.01 Method document for more details)*

1. Label the filter bags using a solvent resistant marker.
2. Fill chemical containers to the Min. Level line or above.
3. Fill the Protease enzyme container to the 15 ml line or above. Fill the Amylase/AMG mixture and Trizma Base containers to the 30 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 9-28 below).
9. Install SDF bags by gently pulling the black SDF Delivery Nozzle toward you and pulling each bag up so that the nozzle is inside the bag. 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 bags 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 pulling the black IDF Delivery Nozzle toward you and pulling each bag up underneath the nozzle. Pull the bag up so that the top of the filter part of the IDF bag is just below the bottom of Clamp Bar B 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 bags 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 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.

TDF Procedure (continued)

22. Check that the Nitrogen supply is connected to the instrument and turned on.
23. Set Filter Times.
24. Set the manual pH checks.
25. Add 1 ml of Internal Standard or instruct the instrument to prompt for a later addition.
26. Press the START button to begin the automated processes.
27. Check the pH after the Trizma Base has been added. (If configured, the instrument will stop so you can make this check.) Adjust to 7.9-8.4 as needed.
28. Check the pH after the Acetic Acid has been added. (If configured, the instrument will stop so you can make this check.) Adjust to approximately 4.3 as needed.
29. 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.
30. 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.
31. Place each bag in the Drying Rack and place the rack in an oven set to 105°C. Dry to constant weight (about 90 min).
32. Remove all of the bags from the oven and place them in desiccant pouches to cool.
33. Removing only one filter bag from the desiccant pouches at a time, place each filter bag in a tared Bag Weigh Holder and record the weights.
34. Determine the protein content within the SDF residue. See the “Protein Determination Procedure – SDF / TDF” for more information.
35. Determine the ash content within the SDF residue. See the “Ash Determination Procedure – IDF / SDF / TDF” for more information.
36. Using an HPLC, determine the WASDF from the filtrate in the instrument Filtrate Cups.
37. Calculate % TDF values.

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