Crude Fiber Analysis in Feeds - Filter Bag Technique (for A200 and A200I)

AOCS Approved Procedure Ba 6a-05

Definition

This method determines Crude Fiber which is the organic residue remaining after digesting with 0.255N H_2SO_4 and 0.313N NaOH. The compounds removed are predominantly protein, sugar, starch, lipids and portions of both the structural carbohydrates and lignin.

Scope

This method is applicable for all feed materials such as grains, meals, pet foods, mixed feeds, forages, and the following oilseeds: corn and soybeans.

Apparatus

1. Analytical Balance—capable of weighing 0.1 mg.
2. Oven—capable of maintaining a temperature of 102 ± 2°C.
3. Electric muffle furnace—with rheostat control and pyrometer that will maintain a temperature of 600 ± 15°C.
4. Digestion instrument—capable of performing the digestion at 100 ± 0.5°C and maintaining a pressure of 10-25psi. The instrument must be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM^{2000} with 65rpm agitation, ANKOM Technology).
5. Filter Bags—constructed from chemically inert and heat resistant filter media, capable of being heat sealed closed and able to retain 25 micron particles while permitting solution penetration (F57 or F58, ANKOM Technology).
6. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (HS or HSi, ANKOM Technology).
7. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (*MoistureStop* weigh pouch, ANKOM Technology).
8. Marking pen—solvent and acid resistant (F08, ANKOM Technology).

Reagents

1. Sulfuric acid solution—0.255 ± 0.005N. 1.25g H_2SO_4/100ml. Concentration must be checked by titration.
   CAUTION1: Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. Always add acid to water and not the reverse.
2. Sodium hydroxide solution—0.3130 ± 005N. 1.25g NaOH/100ml. Concentration must be checked by titration.
   CAUTION2: Sodium hydroxide can severely burn the skin, eyes, and respiratory tract. Protective clothing should be worn when working with this acid. Always add caustic material to water and not the reverse.

Sample Preparation

Grind samples in a centrifugal mill with a 2mm screen or cutter type (Wiley) mill with a 1mm screen. Samples ground finer (fiber particles less than 25 microns) may have particle loss through the filter bags that result in lower fiber values (up to 0.5% units).

Precision

Results of the collaborative study (see Tables 1&2) indicate the precision (S_r, RSD_r, r) that the analyst should use as a benchmark for evaluating replication in the same laboratory.

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

1. Use a solvent resistant marker to label the filter bags to be used in the analysis.
2. Weigh and record the weight of each empty filter bag (W_1) and zero the balance. NOTE: Do not pre-dry filter bags. Any moisture will be accounted for by the blank bag correction.
3. Place 0.95 – 1.00g of prepared sample in up to 23 of the bags and record the weight (W_2) of each. Avoid placing the sample in the upper 4mm of the bag.
4. Include at least one empty bag in the run to determine the blank bag correction (C_1).
   NOTE: A running average blank bag correction factor (C_1) should be used in the calculation of fiber. The inclusion of at least one blank bag in each run is mainly used as an indicator of particle loss. A C_1 larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed then the grinding method needs to be evaluated.
5. Using a heat sealer, completely seal each filter bag closed within 4mm of the top to encapsulate the sample. NOTE: Use sufficient heat to completely seal the filter bags and allow enough cool time (2 sec) before removing each bag from the heat sealer.
6. Extract fat from samples by placing all bags into a 250ml container. Add enough petroleum ether to cover bags and soak for 10 minutes.
   CAUTION3: Petroleum ether is extremely flammable. Avoid static electricity. A fume hood should be used at all times when using petroleum ether.
   Pour off the solvent and allow the bags to air-dry. To eliminate sample clumping, spread the sample uniformly inside the filter bags by shaking and flicking the bags.
7. Place up to 3 bags on each of eight Bag Suspender Trays (maximum of 24 bags). Stack the trays on the center post of the Bag Suspender with each level rotated 120 degrees in relation to the tray below it. Place the empty 9th tray on top. NOTE: All nine trays must be used regardless of the number of bags being processed.
8. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.
9. Turn the instrument Power Switch to the ON position.
10. Before inserting the Bag Suspender into the Vessel, read the Temperature Controller on the instrument. If the temperature is higher than room temperature, fill the Vessel with cold tap water. The temperature on the Controller will decrease. When the value on the Controller reaches its lowest number and starts to increase, open the Exhaust Valve and exhaust the water. Repeat this process until the number on the Temperature Controller equilibrates to room temperature.
11. Open the Vessel Lid and insert the Bag Suspender with bags into the Vessel and place the Bag Suspender Weight on top of the empty 9th tray to keep the Bag Suspender submerged.

Calculations

$$\% \text{ Crude Fiber} = \frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}$$

Where:

- W_1 = Bag tare weight
- W_2 = Sample weight
- W_3 = Weight of Organic Matter (loss of weight on ignition of bag and fiber)
- C_1 = Ash corrected blank bag factor (running average of loss of weight on ignition of blank bag/original blank bag)

Crude Fiber Procedure (continued)

12. When processing 24 sample bags, add 1900-2000 mL of ambient temperature acid solution to the fiber analyzer vessel. If processing less than 20 bags, add 100 mL/bag of acid (0.255*N* H_2SO_4) solution (use minimum of 1500 mL to ensure Bag Suspender is covered).
13. Turn Agitate and Heat ON and confirm agitation.
14. Set the timer for 40 minutes and close the lid so that the vessel is sealed.
15. When the extraction is complete, turn Agitate and Heat OFF.
16. Open the drain valve (slowly at first) and exhaust the hot solution before opening the Vessel Lid. NOTE: The solution in the Vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the Vessel Lid.
17. After the solution has been exhausted, close the exhaust valve and open the Vessel Lid. Add 1900-2000 mL of 50-90°C rinse water. Turn Agitate on and rinse for 5 minutes. If the Heat is ON, the Vessel Lid should be closed. If the Heat is OFF, the Vessel Lid can be open. Repeat 5 minute hot water rinse 1 more time for a total of 2 rinses.
18. When processing 24 sample bags, pour 1900 - 2000 ml of ambient temperature base (0.313*N* NaOH) solution over the Bag Suspender in the Vessel. If processing less than 20 bags, add 100 ml/bag of the base solution (minimum of 1500 ml).
19. Turn Agitate and Heat ON and confirm agitation.
20. Set the timer for 40 minutes and close the lid so that the vessel is sealed.
21. When the extraction is complete, turn Agitate and Heat OFF.
22. Open the drain valve (slowly at first) and exhaust the hot solution before opening the Vessel Lid. NOTE: The solution in the Vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the Vessel Lid.
23. After the solution has been exhausted, close the exhaust valve and open the Vessel Lid. Add 1900 mL of 50-90°C rinse water. Turn Agitate on and rinse for 5 minutes. If the Heat is ON, the Vessel Lid should be closed. If the Heat is OFF, the Vessel Lid can be open. Repeat 5 minute hot water rinse 2 more time for a total of 3 rinses.
24. When the Crude Fiber rinsing processes are complete, open the Vessel Lid and remove the samples. Gently press out excess water from the bags. Place the bags in a 250ml beaker and add enough acetone to cover the bags and soak for 3-5 minutes.

    CAUTION4: Acetone is extremely flammable. Avoid static electricity. A fume hood should be used at all times when using acetone.
25. Remove the filter bags from the acetone and place them on a wire screen to air-dry. Completely dry in an oven at 102 ± 2°C. (In most ovens the filter bags will be completely dry within 2-4 hours.) NOTE: Do not place bags in the oven until the acetone in the bags has completely evaporated.
26. Remove the filter bags from the oven and immediately place them directly into a collapsible desiccant pouch and flatten to remove any air. Cool to ambient temperature and weigh the filter bags. NOTE: Do not use a conventional desiccator container for this step.
27. Ash the entire filter bag/sample in a pre-weighed crucible for 2 hours at 600 ± 15°C, cool in a conventional desiccator and weigh to calculate loss of weight of organic matter (W_3).

Table 1. Results of the international collaborative study of the Filter Bag Technique for crude fiber compared with three laboratories using an Official Crude Fiber Method.

Collaborative Laboratory No.	Rep	Whole Corn	Cattle Feed	Alfalfa	Whole Soy	Poultry Starter	Calf Starter	Swine Feed	Horse Feed	Soy Meal	Pig Starter	Dog Food
						% Crude Fiber						
1	1	2.1	14.5	22.6	9.8	4.7	11.0	17.5	6.4	3.7	2.8	1.3
	2	1.8	14.2	22.4	9.9	4.9	10.7	17.2	6.5	4.0	2.9	1.3
2	1	1.7	14.8 C	22.5	7.2 C	4.4	10.4	17.4	5.8	3.4	2.6	7.1 C
	2	2.0	20.2 C	23.0	10.1 C	4.7	11.1	17.4	6.0	3.5	2.8	1.0 C
3	1	1.6	14.1	22.5	10.1	4.6	10.8	17.6	6.6	3.9	3.1	2.0
	2	1.9	14.6	22.5	10.3	4.7	10.9	17.6	6.8	4.0	3.2	1.6
4	1	1.6	14.2	22.2	9.5	4.4	10.6	17.1	6.2	3.4	3.0	1.3
	2	1.7	14.7	22.2	9.9	4.7	10.5	16.9	6.4	3.7	2.9	1.3
5	1	1.5	13.9	22.7	9.5	4.8	10.5	17.3	5.9	3.6	2.8	1.3
	2	1.8	14.5	22.4	10.1	4.7	10.5	17.6	6.0	3.5	2.7	1.4
6	1	1.8	14.1	22.6	9.3	4.7	10.9	17.2	6.3	3.7	2.8	1.2
	2	2.0	14.3	21.9	9.4	4.5	10.4	17.2	6.1	3.8	3.0	1.3
7	1	1.7	14.5	24.0	10.0	4.8	10.7	17.4	6.1	3.7	3.0	1.2
	2	1.5	14.8	23.6	10.0	4.3	10.4	17.4	6.2	4.0	2.9	1.1
8	1	1.6	15.0	22.3	9.3	4.6	10.7	17.4 C	6.0 C	3.7	2.5	0.5
	2	1.6	14.4	22.9	10.0	4.3	10.8	2.4 C	5.2 C	3.4	2.6	1.1
9	1	1.4	14.4	21.9	8.9	4.6	10.4	17.0	5.9	3.4	2.7	1.3
	2	1.8	14.3	22.6	9.6	4.2	10.4	16.6	5.9	3.7	2.7	1.2
10	1	1.7	14.1	21.4	9.3	4.5	10.8	17.0	6.3	3.8	2.9	1.4
	2	1.7	14.2	22.1	9.8	4.8	10.9	17.3	6.3	3.6	2.8	1.4
11	1	1.4	14.3	23.3	8.5	4.7	10.9	17.7 C	6.1	3.6	2.8	1.3
	2	1.5	15.9	24.1	8.9	5.5	11.9	19.1 C	6.2	4.2	2.9	0.6
Mean		1.69	14.44	22.62	9.60	4.65	10.73	17.27	6.21	3.70	2.83	1.25

Official Method Laboratories[a]											
				% Crude Fiber							
Central Analytical	1.8	14.5	23.0	10.2	4.4	9.3 G	14.7 G	6.8	2.9	1.9 G	3.4 G
Hahn Laboratories, Inc.	2.0	14.0	21.2	8.4	4.2	10.6	17.4	5.7	4.2	2.9	1.6
SDSU Olson Bio. Lab	2.4	14.2	23.8	10.1	4.6	10.8	17.4	6.8	4.1	2.8	1.3
Mean	2.05	14.23	22.67	9.57	4.40	10.70	17.40	6.43	3.73	2.85	1.45

Outliers: C-Chochran, G-Grubbs, DG-Double Grubbs

[a] AOCS Official Method Ba 6-84, AOAC 962.09

Table 2. Summary of the statistical analysis of the Filter Bag Technique crude fiber collaborative study, including comparison with the Official Method.

Sample type	Whole Corn	Cattle Feed	Alfalfa	Whole Soy	Poultry Starter	Calf Starter	Swine Feed	Horse Feed	Soy Meal	Pig Starter	Dog Food
Number of laboratories	11	10	11	10	11	11	9	10	11	11	10
Number of replicates	22	20	22	20	22	22	18	20	22	22	20
Overall FBT mean	1.69	14.44	22.62	9.60	4.65	10.73	17.27	6.21	3.70	2.83	1.25
Official Method mean[a]	2.05	14.23	22.67	9.57	4.40	10.70	17.40	6.43	3.73	2.85	1.45
S_r	0.16	0.44	0.36	0.32	0.26	0.28	0.18	0.10	0.20	0.09	0.23
S_R	0.19	0.44	0.67	0.48	0.27	0.33	0.28	0.27	0.22	0.17	0.31
RSD_r, %	9.6	3.1	1.6	3.3	5.5	2.6	1.1	1.6	5.3	3.3	18.1
RSD_R,%	11.4	3.1	2.9	5.0	5.8	3.1	1.6	4.3	6.0	6.0	24.5
r	0.46	1.23	1.00	0.88	0.72	0.80	0.51	0.27	0.55	0.26	0.64
R	0.54	1.23	1.86	1.34	0.75	0.94	0.78	0.75	0.62	0.48	0.86
HORRAT VALUE	3.07	1.14	1.18	1.75	1.82	1.11	0.62	1.42	1.83	1.75	6.34

[a] Official Method AOCS Ba 6-84/AOAC 962.09