

## Crude Fiber Analysis in Feeds By Filter Bag Technique (For A200, A200I)

### 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 down to 0.1 mg.
2. Oven—capable of maintaining a temperature of  $102\pm 2^\circ C$ .
3. Electric muffle furnace—with rheostat control and pyrometer that will maintain a temperature of  $600\pm 15^\circ C$ .
4. Digestion instrument—capable of performing the digestion at  $100\pm 0.5^\circ C$  and maintaining a pressure of 10-25 psi. The instrument must also be capable of creating a similar flow around each sample to ensure uniformity of extraction. (ANKOM<sup>200</sup> w/65 rpm 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 rapid solution penetration (F57 or F58, ANKOM Technology, see Numbered Notes 1).
6. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, ANKOM Technology).
7. Desiccator 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— $0.255\pm 0.005N$ . 1.25 g  $H_2SO_4/100$  mL. Concentration must be checked by titration (see Notes, *Caution*).
2. Sodium hydroxide solution— $0.313\pm 0.005N$ . 1.25 g NaOH/100 mL. Concentration must be checked by titration (see Notes, *Caution*).

### PREPARATION OF SAMPLE

Grind samples through a centrifugal mill with a 2 mm screen or cutter type (Wiley) mill with a 1 mm screen.

Samples ground finer may show particle loss from the filter bags and result in low values.

### PROCEDURE

1. Use a solvent and acid resistant marker to label the filter bags. Weigh filter bag ( $W_1$ ) and zero balance.

*Note*—Do not pre-dry filter bags; any moisture will be accounted for by the blank bag correction.

2. Weigh 0.95-1.00 g of prepared sample ( $W_2$ ) directly in filter bag. Avoid placing the sample on the upper 4 mm of the bag.
3. Using a heat sealer, completely seal the upper edge of the filter bag within 4 mm of the top.

*Note*—Use sufficient heat to completely seal the filter bag and allow enough cool time (2 sec) before removing the bag from the heat sealer.

4. Weigh one blank bag and include in run to determine blank bag correction ( $C_1$ , see Number Note 2).
5. Extract fat from samples by placing all bags into a 250 mL container. Add enough petroleum ether to cover bags and soak for 10 min. Pour off solvent and allow bags to air-dry. Spread sample uniformly inside the filter bag by shaking and flicking the bag to eliminate clumping.

6. Place a maximum of 24 bags into the Bag Suspender. All nine trays are used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees. Insert the Bag Suspender with bags into the fiber analyzer vessel and place the Bag Suspender weight on top of the empty 9th tray to keep it submerged.

*Note*—Prior to inserting the Bag Suspender, if the vessel temperature is warm from a previous run, add cool water and exhaust.

7. When processing 24 sample bags, pour 1900-2000 mL of ambient temperature acid ( $0.255N H_2SO_4$ ) solution into the fiber analyzer vessel. If

processing less than 20 bags, add 100 ml/bag of acid solution (use minimum of 1500 mL to ensure Bag Suspender is covered).

8. Turn Agitate and Heat on and confirm that Bag Suspender is agitating properly. Close and completely seal the lid of vessel. Extract samples for a total of 40 min.
9. At end of extraction, turn Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening 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 lid.

10. After the solution has been exhausted, close the exhaust valve and open the lid. Add 1900 mL of (50-90°C) rinse water and agitate for 5 min. The lid may be sealed with the Heat on or left open with the Heat off. Repeat hot water rinse (total of two rinses).
  11. When processing 24 sample bags, pour 1900-2000 mL of ambient temperature base (0.313N 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).
  12. Turn Agitate and Heat on and confirm that Bag Suspender is agitating properly. Close and completely seal the lid of vessel. Extract samples for a total of 40 min.
  13. At end of extraction, turn Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening 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 lid.
14. After the solution has been exhausted, close the exhaust valve and open the lid. Add 1900 mL of (50-90°C) rinse water and agitate for 5 min. The lid may be sealed with the Heat on or left open with the Heat off. Repeat hot water rinse (total of three rinses).
  15. When the rinsing process is complete remove the samples. Gently press out excess water from bags. Place bags in a 250 mL beaker and add enough acetone to cover bags and soak for 3-5 min.
  16. Remove bags from acetone and place on a wire screen to air-dry. Completely dry in oven at 102±2°C (most ovens will complete drying within 2-4 hrs).

*Note*—Do not place bags in the oven until acetone has completely evaporated.

17. Remove bags from oven, place directly into a collapsible desiccant pouch and flatten to remove air. Cool to ambient temperature and weigh bags.

*Note*—Do not use conventional desiccator container.

18. Ash the entire bag/sample in pre-weighed crucible for 2 hrs at 600±15°C, cool in desiccator and weigh to calculate loss of weight of organic matter ( $W_3$ ).

## CALCULATIONS

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

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 (loss of weight on ignition of blank bag/original blank bag)

## PRECISION

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

## NOTES

### *Caution*

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.

Sodium hydroxide can severely burn 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.

Petroleum ether and acetone are extremely flammable. Avoid static electricity. A fume hood should be used at all times when using petroleum ether or acetone.

## NUMBERED NOTES

1. F57 filter bags may produce up to 0.5% units lower values on finely ground feed samples. Finely ground samples are samples with fiber particles less than 25 microns.

2. A running average blank bag correction factor ( $C_1$ ) should be used in the calculation of fiber. The inclusion of a 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 grinding method needs to be evaluated.

**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 <sup>a</sup>												
% 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

<sup>a</sup> 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 <sup>a</sup>	2.05	14.23	22.67	9.57	4.40	10.70	17.40	6.43	3.73	2.85	1.45
S <sub>r</sub>	0.16	0.44	0.36	0.32	0.26	0.28	0.18	0.10	0.20	0.09	0.23
S <sub>R</sub>	0.19	0.44	0.67	0.48	0.27	0.33	0.28	0.27	0.22	0.17	0.31
RSD <sub>r</sub> , %	9.6	3.1	1.6	3.3	5.5	2.6	1.1	1.6	5.3	3.3	18.1
RSD <sub>R</sub> , %	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

<sup>a</sup> Official Method AOCS Ba 6-84/AOAC 962.09