Introducing the Automated Fiber Analyzer

- Performs Crude Fiber, ADF and NDF determinations
- Automatically adds required solutions and hot water rinses
- Batch Process up to 24 samples at one time
- Accurate and Precise results, time after time
- Computerized controls eliminate technician variability

The ANKOM\textsuperscript{2000} Fiber Analyzer automates fiber determinations. Capable of Acid Detergent, Neutral Detergent and Crude Fiber analysis, the ANKOM\textsuperscript{2000} eliminates chemical and hot water handling. The samples are prepared using ANKOM Filter Bag Technology and placed in a bag suspender. At that point the technician simply selects the analytical method on the computer controller and pushes a button. The system automatically adds the appropriate chemicals and performs the necessary rinses. The solution is heated and solubilization takes place without manual intervention. When the process is complete, the technician simply removes the bags and completes a final drying before weighing the samples.

Also Available

Used in over 85 countries around the world, the ANKOM\textsuperscript{2000} Fiber Analyzer provides a low cost alternative for the laboratory seeking increased efficiency. Technicians simply add the required solutions and hot water rinses according to the method and the ANKOM\textsuperscript{2000} handles the rest.

HELPING TO FEED THE WORLD!
## Specifications

### Sample Specifications

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td><strong>Sample Size</strong></td>
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<tr>
<td><strong>Fiber Range:</strong></td>
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<tr>
<td><strong>Samples per batch:</strong></td>
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### Samples per day

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<td><strong>Acid Detergent Fiber</strong></td>
<td>144</td>
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<tr>
<td><strong>Neutral Detergent Fiber</strong></td>
<td>120</td>
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<tr>
<td><strong>Crude Fiber</strong></td>
<td>96</td>
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### Instrument Specifications:

<table>
<thead>
<tr>
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<th><strong>ANKOM 2000</strong></th>
<th><strong>ANKOM 200</strong></th>
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<tr>
<td><strong>Dimensions:</strong></td>
<td>18”w x 14.5”d x 23”h</td>
<td>16”w x 9”d x 20”h</td>
</tr>
<tr>
<td><strong>Operating Temperature</strong></td>
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<td>100°C</td>
</tr>
<tr>
<td><strong>Power Requirements</strong></td>
<td>120 – 240V, 50/60 Hz</td>
<td>120-240V, 50/60 Hz</td>
</tr>
<tr>
<td><strong>Weight</strong></td>
<td>46 kg.</td>
<td>20 kg</td>
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</table>

### Helping To Feed The World!

ANKOM Technology is the developer of Fiber Bag Technology (FBT) used around the world for fiber and fat analysis. With customers in over 85 countries, ANKOM has a reputation for quality and innovation. Constantly seeking to develop better methods for time consuming analytical methods, ANKOM Technology focuses on customers needs. We offer instruments, chemicals and other ancillary products to support fiber studies, crude fat extraction, total fat extraction, in vitro and in situ research and more. We work hard to keep costs low with quality and service high.
Introducing the Automated Fiber Analyzer

• Performs Crude Fiber, ADF and NDF determinations
• Automatically adds required solutions and hot water rinses
• Batch Process up to 24 samples at one time
• Accurate and Precise results, time after time
• Computerized controls eliminate technician variability

The ANKOM 2000 Fiber Analyzer automates fiber determinations. Capable of Acid Detergent, Neutral Detergent and Crude Fiber analysis, the ANKOM 2000 eliminates chemical and hot water handling. The samples are prepared using ANKOM Filter Bag Technology and placed in a bag suspender. At that point the technician simply selects the analytical method on the computer controller and pushes a button. The system automatically adds the appropriate chemicals and performs the necessary rinses. The solution is heated and solubilization takes place without manual intervention. When the process is complete, the technician simply removes the bags and completes a final drying before weighing the samples.

Also Available

Used in over 85 countries around the world, the ANKOM Fiber Analyzer provides a low cost alternative for the laboratory seeking increased efficiency. Technicians simply add the required solutions and hot water rinses according to the method and the ANKOM handles the rest.
Specifications

Sample Specifications
Sample Size: 0.5 – 1.0 gram
Fiber Range: 0% - 100%
Samples per batch: up to 24

Samples per day
Acid Detergent Fiber = 144
Neutral Detergent Fiber = 120
Crude Fiber = 96

Instrument Specifications:
ANKOM®
Dimensions: 18"w x 14.5"d x 23"h
Operating Temperature: 100°C
Power Requirements: 120 – 240V, 50/60 Hz
Weight: 46 kg.

ANKOM®
Dimensions: 16"w x 9"d x 20"h
Operating Temperature: 100°C
Power Requirements: 120–240V, 50/60 Hz
Weight: 20 kg

Helping To Feed The World!

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Acid Detergent Fiber in Feeds - Filter Bag Technique (for A2000 and A2000I)

Definition
This method determines Acid Detergent Fiber, which is the residue remaining after digesting with H₂SO₄ and CTAB. The fiber residues are predominantly cellulose and lignin.

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. Oven—capable of maintaining a temperature of 102 ± 2°C.
3. Digestion instrument—capable of performing the digestion at 100 ± 0.5°C and maintaining a pressure of 10-25 psi. The instrument must be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM²⁰⁰₀ with 65 rpm agitation, ANKOM Technology).
4. 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 and F₅₈, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, ANKOM Technology).
6. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (MoistureStop weigh pouch, ANKOM Technology).
7. Marking pen—solvent and acid resistant (F₀₈, ANKOM Technology).

Reagents
1. Acid Detergent Solution—Add 20 g cetyl trimethylammonium bromide (CTAB) to 1 L 1.00N H₂SO₄ previously standardized (premixed chemical solution available from ANKOM). Agitate and heat to aid solution. **CAUTION 1**: Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. Always combine acid to water and not the reverse. **CAUTION 2**: CTAB will irritate mucous membranes. A dust mask and gloves should be worn when handling this chemical.

Sample Preparation
Grind samples in a centrifugal mill with a 2 mm screen or cutter type (Wiley) mill with a 1 mm screen. Samples ground finer may have particle loss from the filter bags and result in low values.

ADF Procedure (see the ADF 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 (W₁) and zero the balance. **NOTE**: Do not pre-dry filter bags. Any moisture will be accounted for by the blank bag correction.
3. Place 0.45 – 0.50 g of prepared sample in up to 23 of the bags and record the weight (W₂) of each. Avoid placing the sample in the upper 4 mm of the bag.
4. Include at least one empty bag in the run to determine the blank bag correction (C₁).
   **NOTE**: A running average blank bag correction factor (C₁) 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 C₁ larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag during the extraction. 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. Place up to 3 bags on each of eight Bag Suspender Trays and flicking the bags to eliminate clumping.
6. Spread the sample uniformly inside the filter bags by shaking.

Exception – Roasted soybean: Due to the processing of roasted soy a modification to the extraction is required. Place roasted soy samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top. Shake the container 10 times and allow bags to soak for 10 minutes. Repeat with fresh acetone. Pour out acetone and place bags on a wire screen to air-dry.

Exception – Roasted soybean: Due to the processing of roasted soy a modification to the extraction is required. Place roasted soy samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top. Shake the container 10 times and pour off the acetone. Add fresh acetone and allow samples to soak for twelve hours. After the soak time, pour out the acetone and place the bags on a wire screen to air-dry.
7. Using a heat sealer, completely seal each filter bag closed within 4 mm 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.
8. Pre-extract only samples containing >5% fat: Extract samples by placing bags with samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top. **CAUTION 3**: Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling.

CAUTION 3: Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling.

9. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.
10. Read the Temperature Controller on the right side of the instrument. If the temperature is higher than 20°C, cool the Vessel as follows:
   a. Fill the Vessel with cold water.
   b. When the Temperature Controller reads 20°C, return the sample to the Vessel and cool further by lowering the Temperature Controller to 15°C.
   c. Repeat steps a and b if necessary.

(Procedure continued on next page.)
Calculations

\[
\% \text{ ADF (as-received basis)} = \frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}
\]

Where:

- \( W_1 \) = Bag tare weight
- \( W_2 \) = Sample weight
- \( W_3 \) = Dried weight of bag with fiber after extraction process
- \( C_1 \) = Blank bag correction (running average of final oven-dried weight divided by original blank bag weight)

ADF Procedure (continued)

11. If you are using Cubetainers for your chemicals, attach the AD solution hose to the Cubetainer and then to Port B on the instrument.

12. 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.

13. Follow the instructions on the ANKOM2000 display:
   a. Select ADF.
   b. Close the Vessel Lid.
   c. Confirm hot water is on (>70°C).
   d. Press START.

14. When the ADF extraction and rinsing procedures are complete, open the Vessel Lid and remove the filter bags. Gently press out excess water from the bags. Place bags in a 250ml beaker and add enough acetone to cover bags and soak for 3-5 minutes.

15. 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 has completely evaporated.

16. 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 (\( W_3 \)). NOTE: Do not use a conventional desiccator container.
Neutral Detergent Fiber in Feeds - Filter Bag Technique (for A2000 and A2000I)

**Definition**
This method determines Neutral Detergent Fiber, which is the residue remaining after digesting in a detergent solution. The fiber residues are predominantly hemicellulose, cellulose, and lignin.

**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. Oven—capable of maintaining a temperature of 102 ± 2°C.
3. Digestion instrument—capable of performing the digestion at 100 ± 0.5°C and maintaining a pressure of 10-25 psi. The instrument must be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM2000 with 65rpm agitation, ANKOM Technology).
4. 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 and F58, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, ANKOM Technology).
6. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (MoistureStop weigh pouch, ANKOM Technology).
7. Marking pen—solvent and acid resistant (F08, ANKOM Technology).

**Reagents**
1. Neutral Detergent Solution—Add 30g Sodium dodecyl sulfate (USP), 18.61g Ethylenediaminetetraacetic disodium salt (dehydrate), 6.81g Sodium borate, 4.56g Sodium phosphate dibasic (anhydrous), and 10.0ml Triethylene glycol to 1L distilled H₂O (premixed chemical solution available from ANKOM Technology). Check that pH is from 6.9 to 7.1. Agitate and heat to aid solution. **CAUTION:** Powdered chemicals will irritate mucous membranes. A dust mask and gloves should be worn when handling these chemicals.
3. Sodium sulfite—Na₂SO₃, anhydrous (FSS, ANKOM Technology)

**Sample Preparation**
Grind samples in a centrifugal mill with a 2mm screen or cutter type (Wiley) mill with a 1mm screen. Samples ground finer may have particle loss from the filter bags and result in low values.

**NDF Procedure** *(see the NDF 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 (Wₑ) and zero the balance. **NOTE:** Do not pre-dry filter bags. Any moisture will be accounted for by the blank bag correction.
3. Place 0.45 – 0.50g of prepared sample in up to 23 of the bags and record the weight (Wₛ) 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 (Cₑ).
   **NOTE:** A running average blank bag correction factor (Cₑ) 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 Cₑ larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag during the extraction. 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. **Pre-extract only samples containing >5% fat:** Extract samples by placing bags with samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top. **CAUTION:**: Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling. Shake the container 10 times and allow bags to soak for 10 minutes. Repeat with fresh acetone. Pour out acetone and place bags on a wire screen to air-dry.
   **Exception** – **Roasted soybean:** Due to the processing of roasted soy a modification to the extraction is required. Place roasted soy samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top. Shake the container 10 times and pour off the acetone. Add fresh acetone and allow samples to soak for twelve hours. After the soak time, pour out the acetone and place the bags on a wire screen to dry.
7. Spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
8. 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.
9. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.
10. If you are using Cubetainers for your chemicals, attach the ND solution hose to the Cubetainer and then to Port A on the instrument.
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.
   *(Procedure continued on next page.)*
% NDF (as-received basis) = \[ \frac{100 \times (W_3 - (W_1 \times C_1))}{W_2} \]

Where:
- \( W_1 \) = Bag tare weight
- \( W_2 \) = Sample weight
- \( W_3 \) = Dried weight of bag with fiber after extraction process
- \( C_1 \) = Blank bag correction (running average of final oven-dried weight divided by original blank bag weight)

**NDF Procedure (continued)**

12. Follow the instructions on the ANKOM\textsuperscript{2000} display:
   a. Select NDF. (Wait to close the Vessel Lid.)
   b. Confirm hot water is on (>70°C).
   c. Press START.
   d. After the ND solution has been automatically inserted and agitation begins, manually add 20g of Na\textsubscript{2}SO\textsubscript{3} and 4.0ml of alpha-amylase.
   e. Close the Vessel Lid.

13. Attach the Amylase Dispenser Assembly to Port B on the instrument. Add 8.0ml of alpha-amylase and enough water to fill the dispenser. The ANKOM\textsuperscript{2000} will automatically add the amylase solution to the first and second rinse.

14. When the NDF extraction and rinsing procedures are complete, open the Vessel Lid and remove the filter bags. Gently press out excess water from the bags. Place bags in a 250ml beaker and add enough acetone to cover bags and soak for 3-5 minutes.

15. 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 has completely evaporated.

16. 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 (\( W_3 \)). NOTE: Do not use a conventional countertop or cabinet desiccator.
Crude Fiber Method
Method 1

Crude Fiber Analysis in Feeds - Filter Bag Technique (for A2000 and A2000I)
AOAC Approved Procedure Ba 6a-05

Definition
This method determines Crude Fiber which is the organic residue remaining after digesting with 0.255N H₂SO₄ 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® 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). See Numbered Notes 1.
6. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, 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 H₂SO₄/100ml. 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 ± 0.005N. 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 (Sₚ, RSDₚ, 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 (Wₑ) 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 (Wₛ) 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 (Cₑ).

NOTE: A running average blank bag correction factor (Cₑ) 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 Cₑ 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. Spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
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. Use sufficient heat to completely seal each filter bag closed within 4mm of the top to encapsulate the sample. NOTE: Do not pre-dry filter bags. Any moisture will be accounted for by the blank bag correction.
9. Include at least one empty bag in the run to determine the blank bag correction (Cₑ).

NOTE: A running average blank bag correction factor (Cₑ) 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 Cₑ 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.
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## Crude Fiber Method
### Method 1

<table>
<thead>
<tr>
<th>Calculations</th>
</tr>
</thead>
</table>
| \[
\% \text{ Crude Fiber} = \frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}
\] |

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)

9. Read the Temperature Controller on the right side of the instrument. If the temperature is higher than 20°C, cool the Vessel as follows:
   a. Fill the Vessel with cold water.
   b. When the Temperature Controller reads 20°C, run the Flush Procedure to drain the water.

10. Attach each Cubetainer hose to the Cubetainer and to its specific port. Port A is used for Crude Fiber Acid solution. Port B is used for Crude Fiber Base solution.

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.

12. Follow the instructions on the ANKOM2000 display:
   a. Select Crude Fiber.
   b. Close Vessel Lid.
   c. Confirm hot water is on (>50°C).
   d. Press START.

13. When the Crude Fiber extraction and 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.

14. 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 has completely evaporated.

15. 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.

16. 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.

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<th>Collaborative Laboratory No.</th>
<th>Whole Corn</th>
<th>Cattle Feed</th>
<th>Alfalfa</th>
<th>Whole Soy</th>
<th>Poultry Starter</th>
<th>Calf Starter</th>
<th>Swine Feed</th>
<th>Horse Feed</th>
<th>Soy Meal</th>
<th>Pig Starter</th>
<th>Dog Food</th>
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<td>22.6</td>
<td>9.8</td>
<td>4.7</td>
<td>11.0</td>
<td>17.5</td>
<td>6.4</td>
<td>3.7</td>
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<td>2</td>
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<td>10.7</td>
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<td>1</td>
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Mean 1.69 14.44 22.62 9.60 4.65 10.73 17.27 6.21 3.70 2.83 1.25

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

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Outliers: C-Chocho, G-Grubbs, DG-Dougle Grubbs

Official Method Laboratories

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

HORRAT VALUE 3.07 1.14 1.18 1.75 1.82 1.11 0.62 1.42 1.83 1.75 6.34

*Official Method AOCS Ba 6-84/AOAC 962.09
Method 8 – determining Acid Detergent Lignin in beakers

Reagents

Sulfuric acid (72% by weight) – ANKOM Technology FSA72 or dilute reagent grade \( \text{H}_2\text{SO}_4 \) to a specific gravity of 1634 g/L at 20°C (24.00 N) by adding 1200 g \( \text{H}_2\text{SO}_4 \) to 350 ml \( \text{H}_2\text{O} \) in a 1 L MCA volumetric flask with cooling. Standardize this solution to 1634 g/L at 20°C specific gravity by removing solution and adding \( \text{H}_2\text{O} \) or \( \text{H}_2\text{SO}_4 \) as required.

Safety Precautions (see attached MSDS)

WARNINGS:

Acetone is highly flammable. Use fume hood when handling acetone and avoid inhaling or contact with skin. Ensure that all the acetone has evaporated before placing in the oven.

Wear rubber gloves and face shield when handling sulfuric acid. Always add sulfuric acid to water. If acid contacts skin, wash with copious amounts of water.

Apparatus

a) Filtration device – ANKOM Technology – F57 Filter Bags
b) Impulse bag sealer – ANKOM Technology – 1915 Heat Sealer
c) Desiccator – ANKOM Technology – Desiccant/MoistureStop pouch – X45
d) 2L & 3L Beaker

Procedure

1) Grind the sample to pass through a 1 mm screen (2mm screen when using a cyclone mill).
2) Weigh each Filter Bag (\( W_1 \)), record the weight, and tare the balance.
3) Add 0.5 g (±0.05 g) of air-dried sample (\( W_2 \)) directly into each Filter Bag.
4) Weigh and seal one (1) blank bag and include it in the digestion to determine the blank bag correction (\( C_1 \)).
5) Seal the bags closed 4 mm from the open edge using the heat sealer.
6) Spread the sample uniformly inside each filter bag by flicking the bag to eliminate clumping.
7) Perform ADF determinations using Fiber Analyzer (See ADF Procedure).
8) After performing ADF determinations, place dried bags with samples into a 3 L beaker and completely cover the bags with 72% \( \text{H}_2\text{SO}_4 \) (approximately 250 ml).

**IMPORTANT:** Bags must be completely dry and at ambient temperature before adding concentrate acid. If moisture (even ambient moisture) is present in the bags, heat generated by the \( \text{H}_2\text{SO}_4 \) and \( \text{H}_2\text{O} \) reaction will adversely affect the results.

9) Place a 2 L beaker inside the 3 L beaker to keep bags submerged. Agitate the bags at the start and at 30-minute intervals by gently pushing and lifting the 2 L beaker up and down approximately 30 times.
10) After 3 hours pour off the \( \text{H}_2\text{SO}_4 \) and rinse with tap water to remove all acid.

**IMPORTANT:** If acid remains in the bags when they go into the oven, the samples will burn, resulting in values that are higher than they should be.

11) Repeat rinses until pH paper shows neutral color when touching the bags. Rinse with approximately 250 ml of acetone for 3 minutes to remove the water. Handle the bags gently during rinsing. Fine lignin particles can exit the filter if not handled carefully.
12) Dry the bags in an oven at 105°C for 2-4 hours.

**WARNING** – Do NOT place bags in an oven until all acetone has evaporated.

13) Remove the bags from the oven and place them directly into Desiccant/MoistureStop pouches and flatten to remove air. Cool to ambient temperature and weigh the bags (W₃).

14) Prepare each bag for the ash procedure.

   14.1) Fold each bag from bottom to top. Because the bags are wider at the top than at the bottom, there will be a little extra material on each side after the first fold.

   14.2) Fold each bag from right to left. The extra material now lines up on left side.

   14.3) Heat seal the bag at the location of the extra material.

15) Ash the bags in pre-weighed crucibles (30 or 50 ml) at 525°C for 3 hours or until C-free. Cool and calculate weight loss (W₄).

16) Calculate blank bag ash correction (C₂) using weight loss upon ignition of a blank bag sequentially run through ADF and lignin steps.

17) Calculate percent ADL.

    \[
    \begin{align*}
    \text{ADL (as-received basis)} & = \frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2} \\
    \text{ADL}_\text{DM (DM basis)} & = \frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2 \times DM} \\
    \text{ADL}_\text{OM (DM basis)} & = \frac{(W_4 - (W_1 \times C_2)) \times 100}{W_2 \times DM}
    \end{align*}
    \]

    Where:
    
    \begin{align*}
    W_1 & = \text{Bag tare weight} \\
    W_2 & = \text{Sample weight} \\
    W_3 & = \text{Weight after extraction process} \\
    W_4 & = \text{Weight of Organic Matter (OM) (weight loss on ignition of bag and fiber residue)} \\
    C_1 & = \text{Blank bag correction (final oven-dried weight/original blank bag weight)} \\
    C_2 & = \text{Ash corrected blank bag (Loss of weight on ignition of bag/original blank bag)} \\
    DM & = \text{Dry Matter}
    \end{align*}