**Job Description**
**Department of Animal and Aquaculture Sciences, NMBU**

---

**Method name: ADFom (ash corrected)**
BIOVIT No .: Arb1037

---

## 1. Introduction / purpose

Modern methods in food and feed analyzes divide the chemical content into two main fractions

- cell walls
- cell contents

**A**cid **d**etergent **f**iber (ADF) is part of the cell wall and is defined as cellulose and equation. Cellulose and equation can be separated from the rest of the cellular material by washing the sample in question with an acidic soap solution. The material that is not washed away will then be defined as ADF and determined gravimetrically.

Unfortunately, the soap solution fails to dissolve all of the inorganic material in the sample. A small amount of insoluble silicates will be present in the finished analyzed sample and will therefore be part of the calculated ADF value. To correct for this inorganic part, the sample can be incinerated at 550 °C. The residues after incineration are a measure of the inorganic part of the sample and one can then decide what is called ash-corrected ADF, *ADF on organic matter basis*, (ADFom). Uded et *al*. recommends that this form be used when ADF values are to be published in peer-reviewed journals

## 2. Reagents and control sample

- Acetone
- "Acid Detergent" AD - solution
  - 20 g cetyltrimethylammonium bromide (CTAB)
  - 1 L sulfuric acid (1.00 N) = 565ml 95% sulfuric acid
- Control sample: The LabTek control (mix for NDF / ADF / threads / kjeldahl-N)

## 3. Risk assessment

After boiling and rinsing, the drain tap on the left side of the instrument **MUST** be opened (vertically = open) before the lid of the chamber is opened. If this is not done, the hot contents of the chamber will splash on the people standing around the instrument. This is due to the overpressure that occurs in the chamber during cooking and rinsing.

**Important:** Do not open the incinerator at 550 $^{o}$C. If there is still organic material left, a flame will go out when the door is opened! Use pliers and possibly gloves when taking the samples from the ashtray.
Should you burn yourself; use running cold water for the first few minutes. Then use lukewarm running water so that frost damage does not occur.

## 4. Equipment

- Arrived$^{200}$ Fiber Analyzer
- Heat Sealer
- Filter bags (F57 or F58 from Arrived)
- Assay weight (accuracy: 0.1 mg)
- Drying cabinet (103 ± 2 °C)
- Desiccators
- Marker (permanent marker)
- Hot Plate
- Water Boiler
- Measuring cup
- Glass w / lid
- Preheating oven (550 °C)
- Tulle glass (which can withstand over 550 °C)
- Steel board (to put the samples in)

## 5. Sample material

The method can be used on most types of samples, but the particle size should not be less than 1 mm in diameter for cutting mills or 2 mm for grinding mills. Smaller particles will increase the probability of errors in the analysis results.

## 6. Job description

Weighing of samples:

1. Label the filter bags (F57) with the sample number. (Permanently highlight black).
2. Weigh the filter bags and register the weight ($W_0$).
3. Tare the bag and weigh 0.45-0.5g sample in the filter bag. (Make sure there are no sample particles in the sealing area).

4. Register the test weight ($W_1$).
5. Seal the filter bag approx. 4mm from the opening. Hold the seal arm down for 2-3 seconds after the red light has gone out to cool the seal.
6. Shake and distribute the sample in the bag to prevent the sample from clumping.

Degreasing:

If the samples contain soybean products or exceed 5% fat, the samples must be degreased as follows:

1. Place the samples in a glass with a lid.
2. Fill the vial with acetone to cover the samples.
3. Put on the lid.
4. Shake the glass 10 times and leave for 10 minutes.
5. Drain the acetone.
6. Repeat steps 1-5 one more time.
7. Allow the samples to air dry.

If the samples contain *roasted* soybeans, the samples must be degreased as follows:
Sections 1-3 are followed equally from the previous degreasing procedure.

1. Shake the glass 10 times.
2. Drain the acetone.
3. Fill the glass with new acetone and leave the samples for 12 hours.
4. Drain the acetone.
5. Allow the samples to air dry.

Fill the bag holder:
The bag holder consists of 9 trays with space for 3 bags per. tray. Place the air-dried bags in the trays. The first 3 are placed in the recesses on the first board. The next 3 are placed in tray no. 2 etc. The trays are rotated 120o relative to each other. Tray No. 9, the top tray, should be empty. This acts as a lid for the other 8 trays.

**PROCEDURE ON ARRIVAL**

NB! The chamber must be at room temperature before the analysis can be started.

1. Turn on Arrived.
2. Place the bag holder in the chamber.
3. Put the soldering iron in place.
4. Add a maximum of 2.0 liters of ADF solution.
5. Press the HEAT and AGITATE buttons. See in the chamber that the sample holder is in motion.
6. Close the lid again.
7. Leave it for 60 minutes.
8. Turn off HEAT and AGITATE.
9. **NB !!! OPEN THE DRAIN AND EMPTY THE CHAMBER FOR SOLUTION AND TO REMOVE THE OVERPRESSURE.**
10. Open the lid of the chamber.
11. Close the drain tap.

Rinsing:

1. Add 1.9-2.0 liters of 70-90°C water.

   **NOTE:** If the HEAT button is not switched on, the lid can be left open during cleaning. If you turn it on, the lid **must** be closed again. (Optional method).
2. Press the AGITATE button and leave for 5 minutes.
3. Open the drain tap slowly and let the cleaning water drain.
4. Repeat steps 1-3 for 2nd wash.
5. Add 1.9-2.0 liters of 70-90°C water.

6. **DO NOT** press the HEAT button. Press the AGITATE button and leave the 3rd wash for 5 minutes. NB! Before emptying the chamber the 3rd time, the pH must be checked. If the pH is not neutral, continue washing until a neutral pH has been reached.

Further procedure:

1. Open the lid and remove the bag holder.
2. Place the samples in a 250ml beaker.
3. Gently squeeze out excess water with your hand and empty the beaker of water.
4. Add enough acetone to cover the samples and leave the samples for 3-5 minutes.
5. Pour out the acetone.
6. Gently squeeze excess acetone by hand and empty the beaker.
7. Let the bags air dry until they are completely free of acetone.
8. Place the bags in the drying cabinet at 102ºC (± 2ºC) for 2-4 hours.
9. Place the bags directly in the Desiccant Pouch zip bags.
10. Flatten the zip bag as much as possible to remove as much air as possible.
11. Allow the samples to cool to room temperature. (Ca.10-15min).
12. Weigh the samples and record the weight ($W_2$).

Ashing:

1. Mark the counting glass with the sample number
2. Weigh the counting glass and note the weight ($W_3$)
3. Place the bag on the counting glass and place the sample(s) in the incinerator

***NB! The numbering disappears during the incineration.***
***Therefore, note how you place the samples.***

4. Check that the incinerator is set to the correct temperature (550 °C) and start heating. Leave the samples for incineration for min. 4 hours, maximum 20 hours
5. Let them cool slightly at room temperature.
6. Pre-ashed samples are placed in a desiccator (with active desiccant) to cool.
7. When the temperature of the samples has become stable (room temperature), the samples are weighed ($W_4$).

**7. Calculation of the analysis result**

$$\left(\frac{(W_2 - W_0) \times F - (W_4 - W_3)}{W_1}\right) \times 1000\ g/kg = amount\ ADF\ in\ the\ sample\ (\frac{g}{kg})$$

**$W_0$** = bag weight (g) - bag weight

**$W_1$** = weight of weighted sample (g)

**$W_2$** = weight of sample + bag after drying (g) - dry weight

**$W_3$** = counting glass (g)

**$W_4$** = weight of ashed sample + counting glass (g)

**F** = pose correction factor = 0.9987