Note in chemical analysis of feedstuffs

(Proximate analysis & Van Soest procedure)

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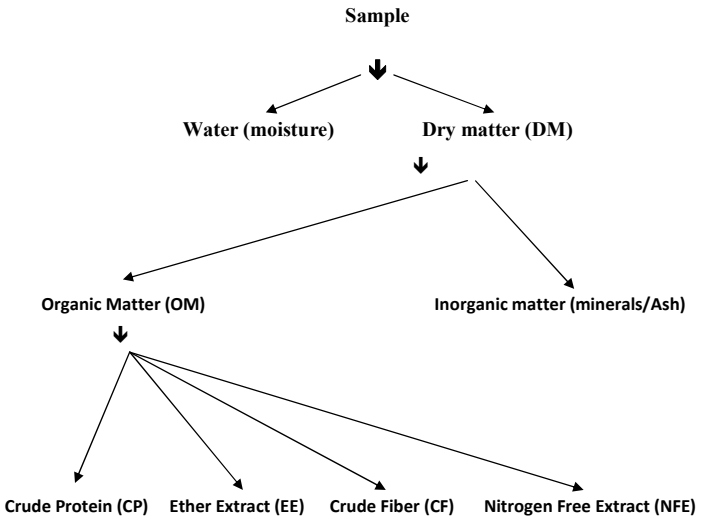
chemical analysis of feedstuffs

Introduction: The nutritional quality of a feed ingredient is dependent on its chemical constituents which carry a nutritional function. These constituents represent feed nutrients. When feeding animals, nutritionists select a combination of ingredients that supply the right amounts of a series of feed nutrients that fulfill the animal requirements. Therefore, when preparing rations, ingredients are treated as carriers of feed nutrients. Thus, the quality and value of a given ingredient will largely depend on the concentration of its nutrients. The most common system for estimating the nutritional value of a feed is so-called Weende system (developed in Germany), named also ***proximate analysis***. The system measures the critical nutrients in feeds (water, minerals/ash, crude protein, crude fat, crude fiber and nitrogen-free extract) by But as with any system; this system has a number of problem or shortcomings. The most important one refers to the crude fiber fraction and consequently the nitrogen-free extract (which is not directly determined but calculated by difference). Nowadays, there are improved methods to determine the fibrous fraction of feeds such as ***Van Soast procedure***.

Proximate analysis



Determination of Dry Matter (DM)

Feed sample is expressed on three DM basis:

As fed: refers to the sample of feed as it is consumed by the animal.

Partially dry: refers to a sample of as fed that has been dried in a forced-air oven at a temperature of 55°C and has been equilibrated with the air, the samples after these process would usually contain more than 85% DM (less than 15% moisture).

Dry: refers to a sample of feed that has been dried at 100°C (or 105°C) or 135°C until all the moisture has been removed.

Principle: Moisture is evaporated from the sample by oven drying either at 100°C (or 105°C) for determination of DM in forages and feeds (ground air-dry or partially-dried (more than 85% DM) samples); or at 135°C for forages and feeds with low volatile acid content. Dry matter is determined as the residue remaining after drying.

Equipment:

- Forced-air drying oven at 100°C (or 105°C) or 135°C, capable of maintaining temperature at ±2°C. Oven should be equipped with a wire rod shelf to allow the circulation of air. It should be vented and operated with vents open.
- Aluminium or Porcelain dish and Porcelain crucibles.
- Electronic analytical balance, sensitive to 0.1 mg

Procedure:

1. If only moisture is to be determined on the sample, use an aluminium or porcelain dish, but if ash determination is to follow on the dry matter residue, use a porcelain crucible.
2. Dry the appropriate container at 100°C (or 105°C) or 135°C for at least 2 hr.
3. Move containers rapidly to desiccators, and immediately cover desiccator and allow containers to cool to room temperature within approximately 1 hr after samples have been placed into it.
4. Weigh empty containers (W1) to nearest 0.1 mg, removing one at a time from desiccator and keeping desiccator closed between container removals.
5. Tare balance and weigh (W2) approximately 2 g ground sample into each container or weigh approximately 2 g ground sample into each container and record weight of container and sample (W3).
6. Shake dish or crucible gently to uniformly distribute the sample and expose the maximum area for drying, and Place containers and samples into preheated oven and leave it for 24 hr at 100°C or 16 hr (overnight) at 105°C or 2 hr at 135°C, after oven has returned to temperature.
7. Move samples to desiccator, seal desiccator and allow cooling to room temperature.
8. Weigh containers and dried sample (W4), recording weight.

Calculation:

If empty container is tared to zero in step 4

$$\text{DM \%} = \frac{W4 - W1}{W3} \times 100$$

If empty container is not tared to zero in step 4

$$\text{DM \%} = \frac{W4 - W1}{W2 - W1} \times 100$$

- Where W1 = weight of empty container (tare wt.) in grams
- W2= weight of sample and container in grams
- W3= weight of sample in grams
- W4 = weight of dry sample and container in grams

Moisture % = 100 - DM %

Two steps for total DM determination of wet samples

For wet samples (less than approximately 85% DM) it is necessary to partially dry the sample in forced-air oven before grinding. The goal is to dry the unground sample while keeping sample temperature below 55 to 60°C so that chemical composition is affected minimally. Drying at low temperature (less than 60°C) does not remove all water from the sample; therefore partial drying does not represent the DM of the sample. Following drying, the sample is ground and analyzed for final DM (the remaining 3 to 15% moisture) and other chemical constituents. Therefore, a two-step procedure for determining DM is recommended:

Step 1: Determine partial DM on unground sample (less than 85% DM).

Step 2: Determine final DM on ground sample and multiply partial DM times final DM to determine total DM, or see the calculation used the moisture content later.

Determination of partial DM

This procedure is applicable to all types of wet samples of forages and feeds (less than 85% dry matter, greater than 15% moisture), and has minimal effect on chemical composition.

Principle: Moisture is evaporated from sample and partial DM is determined as the residue remaining after oven drying at 55°C. Some moisture remains in the sample because drying at this temperature does not remove all water. Sample should be equilibrated at room temperature for 2 to 4 hrs before measuring partial DM to minimize the potential change in moisture that can occur during grinding and storage.

Equipment:

- Forced-air drying oven set at 55°C.
- Electronic analytical balance, sensitive to 0.01 g
- Pans, sufficient in size to hold 100 to 250 g coarse forage.

Procedure:

1. Dry empty pans at 55°C for 2 hr if non-absorbent (glass, metal) or 8 hr if absorbent (paper products).
2. Weigh empty pans and record weight (W1) to nearest 0.01 g.
3. Tare empty container to zero and weigh constant volume of coarse forage into pans and record weight (W2), filling pans to maximum sample depth of 1.5 inches. Use following approximate weights:
   - Low (less than 50%) moisture content 70 to 130 g
   - High moisture content 150 to 250 g
4. Dry in forced-air drying oven at 55°C for 16 to 24 hr.
5. Air-equilibrate samples for 2 to 4 hr and weigh the sample and pan and record the weight (W3).

Calculations:

$$\text{Partial DM \% (as fed basis)} = \frac{W3 - W1}{W2} \times 100$$

- Where W1 = weight of empty pan in grams (tare wt.).
- W2 = weight of sample in grams
- W3 = dry weight of sample and pan in grams

Total DM % = partial DM (at 55°C) X final DM (at 105°C)

Another calculation method of Total DM:

$$\text{Total moisture \%} = A + \frac{(100 - A) \times B}{100} \times 100$$

- Where A = initial moisture % (partially drying at 55°C).
- B= final moisture % (final drying at 105°C).

Total DM % = 100 % - Total moisture %

Determination of Ash

This procedure is applicable for the determination of ash in all types of dried, ground forages and feeds. It is not applicable for liquid feeds.

Principle: A dried, ground sample is ignited in a furnace at 550-600°C to oxidize (burn off) all organic material. Ash is determined by weighing the resulting inorganic residue (does not volatile at that temperature).

Equipment

- Porcelain crucibles, with numbered with furnace-proof ink.
- Muffle furnace.
- Electronic analytical balance, sensitive to 0.1 mg
- Desiccator, with vented lid.
- Drying oven.

Procedure:

1. Remove crucibles which have been dried for at least 2 hr at 100°C from oven, to desiccator. Cool and recording weight to the nearest 0.1 mg (W1).
2. Weigh 1.5 to 2.0 g of sample into the crucible, recording weight of crucible and sample (W2).
3. Ash in furnace at 550-600°C for 2-3 hr after the furnace reaches temperature.
4. Allow crucibles to cool in furnace to less than 200°C and place it in desiccator with vented top. Cool and weigh crucible and ash (W3).

Calculation:

$$\text{Ash \% (as fed basis)} = \frac{W3 - W1}{W2 - W1} \times 100$$

$$\text{Ash \% (as DM basis)} = \text{Ash \% (as fed basis)} \times \frac{100}{DM\ \%}$$

1. W1 = weight of crucible in grams (tare wt.).
2. W2 = weight of crucible and sample in grams
3. W3 = weight of crucible and ash in grams

Organic matter % (OM) (DM basis) = 100 % - Ash % (DM basis)

Determination of nitrogen and crude protein

(Block digestion)

Principle: The Kjeldahl method (macro) is the standard method of nitrogen determination. The original method in about 1810 was improved in 1970 by the introduction of aluminium block heaters which greatly increased efficiency of the digestion, and further improved in 1974 by the introduction of highly efficient steam distillation. This method is applicable for the determination of nitrogen (N) in all types of forages and feeds.

The "block" method consists of three basic steps as in original Kjeldahl method: 1) digestion (boiling) of the sample in concentrated sulfuric acid with a catalyst (copper or selenium), which results in remove all OM and conversion of nitrogen to ammonia sulfate; 2) NaOH is then added to convert the NH_4SO_4 to ammonia (NH_3)which is distillates into a trapping solution (weak acid, boric acid); and 3) quantification of the ammonia in boric acid by titration with a standard acid solution (HCl acid). The amount of acid neutralized by the ammonia is an estimate of the amount of nitrogen in the sample. Crude protein is estimated by multiply the nitrogen with a constant factor.

1) Digesion:

$$CHOSN + H_2SO_4 + \text{Catalyst} \longrightarrow (NH4)_2SO_4 + CO_2 + H_2O + SO_2$$

2) Distillation:

$$(NH4)_2SO_4 + 2NaOH \longrightarrow \underline{2NH3 + 2H2O} + Na_2SO_4$$

$$2NH_4OH$$

$$NH_4OH + H_3BO_3 \longrightarrow NH_4H_2BO_3 + H_2O$$

3) Titration:

$$NH_4H_2BO_3 + HCl \longrightarrow NH_4Cl + H_3BO_3$$

Equipment:

- Block digester, capable of maintaining 420°C and digesting 20 samples at a time of 20-45 min in 250 ml tubes constricted at the top.
- Fume hood.
- Weighing paper, nitrogen-free.
- Electronic analytical balance, sensitive to 0.1 mg.
- Steam distillation apparatus - digestion tube connected to distillation trap (by rubber stopper), which is connected to condenser.

Reagents:

- Digestion sulfuric acid 95-98%, reagent grade.
- Catalyst tablets (potassium sulfate + either copper sulfate or selenium).
- Sodium hydroxide, 40% w/w solution. Dissolve 2k g low N NaOH and dilute to 5 L
- Boric acid solution (**t**rapping sol.) 4%, dissolve 400 g boric acid (H_3BO_3) in distilled water containing 70 ml 0.1% alcoholic solution of methyl red and 100 ml 0.1% alcoholic solution of bromocresol green, dilute to 10 L with distilled water.
- Standard hydrochloric acid (HCl) solution (titrating sol.) 0.1-0.2 N, for boric acid trapping solution, prepare it by diluting 85-170 ml 36.5 to 38% HCl to 10 L with distilled water and then standardize it against standard alkali such as Na_2co_3 or NaOH.

Procedure:

Digestion

4) Weigh ground sample into digestion tube, recording weight (W) to nearest 0.1 mg. Weight range should depend on protein content of sample (0.8-1.5g) for (4-50% CP).
5) Add sufficient catalyst tablets, and then add 10-12 ml concentrated sulphuric acid.
6) Place tubes set on block digester preheated to 420°C (Digester must be equipped with an exhaust system and/or placed in an acid fume hood.), digest about 45 min.
7) Remove tubes and let cool about 10 min in a fume hood (time will depend upon airflow around tubes), then add directly about 25-30 ml of distilled water to the bottom of each tube to dissolve acid digest completely).

Distillation/Titration

8) Place NaOH in alkali tank of steam distillation unit. Make sure that sufficient NaOH is dispensed from unit to neutralize all acid in tube and excess (about 50-55 ml) before conducting distillation.
9) Place 250 ml titration flask containing trapping solution (about 25 ml 4% boric acid containing indicator) on the receiving platform, with tube from the condenser extending below the surface of the trapping solution.
10) Attach digestion tube containing diluted, cooled digest to distillation unit.
11) Dispense appropriate volume of distilled water (50 ml) and the base (NaOH) solution (50 ml).
12) Steam distillation until 100-125 ml distillate collects in titration flask.
13) Remove titrating flask from unit, rinsing condenser tip with water.
14) Titrate trapping solution containing ammonia (N) with 0.1 or 0.2 N HCl to neutral gray endpoint, record volume of acid (V_A) required to nearest 0.01 ml, and then titrate reagent blank (V_B) similarly. Colour change is from pink to green to gray to purple.

Calculation:

$$\text{N \% (as fed basis)} = \frac{(V_A - V_{BI}) \times N_{HCL} \times 0.014}{W} \times 100$$

$$\text{N \% (as DM basis)} = \text{N \% (as fed basis)} \times \frac{100}{\text{DM \%}}$$

- V_A = volume, in ml, of standard HCl required for sample
- V_{BI} = volume, in ml, of standard HCl required for blank
- N_{HCl} = normality of acid standard
- 0.014 = milliequivalent weight of N, in grams
- W = weight of sample in grams

CP % (DM basis) = N % (DM basis) X F

- F = 6.25 for all forages and feeds except :
- F = 5.70 for wheat grains and F= 6.38 for milk and milk products.

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Ether Extract (EE) (Crude Fat) Determination

This method is applicable for the determination of crude fat in dried forages and mixed feeds. It is not applicable for oilseeds, liquid feeds, and feeds containing dairy products.

Ether Extract - This fraction is also called crude fat, because ether dissolves lipids and lipid like compounds.

Principles: A dried, ground sample is extracted with ether which dissolves fats, oils, triglycerides, free fatty acids, pigments, waxes, sterols and other fat soluble substances. The analysis is conducted by continually washing a sample in ether. This is accomplished by placing the sample is an apparatus where ether can flow through the sample and collect in a lower spot (in a beaker). The ether is continually evaporated from this spot and condensed above the sample so it can flow back over the sample, extracting ether soluble materials. When process is completed, the ether is distilled (evaporated from the fat solution) and collected in another container and the remaining residue is dried and weighed, and referred to as ether extract or crude fat. Both the ether and the samples must be free of moisture to avoid co-extraction of water-soluble components in the sample such as carbohydrates, urea, glycerol, lactic acid etc. If water-soluble components are present in large amounts in the sample, they are washed out of the sample prior to drying. Low temperatures are used to evaporate the ether and remove residual moisture to prevent oxidation of the fat. Petroleum ether does not dissolve all of the plant lipid material, and therefore it cannot be substituted for diethyl ether.

Equipment:

- Goldfisch fat extraction apparatus, 6-flask unit, equipped with glass thimble holders and ether reclaiming tubes
- Extraction thimbles, 22 x 80 mm,
- Alundum (porous clay), coarse Fat beakers, pyrex, with ground lip, engraved with a number, 50 x 85 mm
- Drying oven
- Analytical balance, sensitive to 0.1 mg
- Desiccator and tongs
- Filter paper, Whatman #1, 11 cm, or equivalent
- Steam bath in a hood (optional)
- Gloves, white nylon, lintless

Reagents:

Anhydrous Diethyl Ether; purified for fat extraction. To prevent ether from absorbing water, purchase it in small containers and keep containers tightly closed.

Procedure: Sample drying

1. Weigh by difference a 1.5 to 2 g of ground sample into a clean thimble recording the weight to nearest 0.1 mg (W1), (if the sample contains large amounts of carbohydrates, urea, glycerol, lactic acid or water-soluble components, weigh 2 g sample into a small filter cone. Extract with five 20 ml portions of deionized water allowing each portion to drain, and then insert the paper and sample into thimble).
2. Dry for 5 hr at 100-105^{o}C.
3. Dry beakers to be used for fat determination for at least 1 hr at 100-105^{o}C. Cool the appropriate number of fat beakers in a desiccator. Weigh and record the weight (W2).
4. When the drying period is over, remove the samples from the oven to a desiccator. (This is a convenient stopping point. The samples should be stored in a desiccator if not immediately extracted.)

Extraction

5. Line the fat beakers up in front of the extractor and match the thimbles with their corresponding fat beakers.
6. Slip the thimble into a thimble holder and clip the holder into position on the extractor under the condenser of the Goldfisch fat extraction apparatus.
7. Add about 40 ml of diethyl ether (one glass reclaiming tube full) to each fat beaker.
8. Wearing white gloves, place the beaker onto the extractor with screw ring clamp, which is tightened as much as possible by hand. If the clamp is too loose, insert another gasket inside the ring.
9. Raise the heaters into position until they are in contact with the beakers. Leave about a 1/4 inch gap between the beaker and the heating element if necessary.
10. Turn on the main power switch, the heater switch, and the water that cools the condenser.
11. After the ether has begun to boil, check for ether leakage. This can be detected by sniffing around the ring clamp. If there is leakage, check the tightness of the clamp and if necessary replace the gasket(s).
12. Extract for minimum of 4 hr on a Hi setting (condensation rate of 5 to 6 drops per second), or for 16 hr on a Low setting (condensation rate of 2 to 3 drops per sec).
13. After the extraction is completed, lower the heaters, shut off the power and water, and allow the ether to drain out of the thimbles (about 30 min). This is a good stopping point.

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Ether distillation and weighing of fat residue

14. Remove the thimble from the holder, and rinse the holder with a small portion of diethyl ether from the washbottle. Clip an ether reclaiming tube in place and reattach the fat beaker.
15. Reposition the heaters and turn on the electricity and water. Proceed to distil the ether using a Hi setting. Watch Closely.
16. Distil until a thin layer of ether remains in the bottom of the beaker, and then lower the heater. Do not allow beakers to boil dry. Overheating will oxidize the fat. When the last beaker has finished, shut off the power and water.
17. Wipe the exterior of the beaker clean with a Kimwipe as it is being removed from the extractor.
18. Empty the reclaiming tubes into the "USED" diethyl ether container.
19. Complete evaporating ether in beakers in open air under a hood. If there is no hurry, air moving through the hood will be sufficient without heat. A steam bath may be used to speed up the evaporation. Beakers should remain in the hood until all traces of ether are gone. Carefully sniff each beaker to determine if any ether remains.
20. Place the beakers in a 102°C explosion proof oven. Warning: If a beaker containing ether is placed in the oven an explosion may occur.
21. Dry for 1/2 hr. No longer. Excessive drying may oxidize the fat and give high results.
22. Cool in a desiccator and weigh and record weight (W2).
23. The fat beakers are best cleaned by warming on a steam bath or on a hot plate on a low setting. Add some used ether to dissolve the fat. The use of a rubber policeman is helpful. After soaking the beakers in a detergent, wash them using hot water and vigorous brushing. The thimbles are best cleaned by blowing out with air.

Calculations:

$$\text{Ether Extract \% (as fed basis)} = \frac{W3 - W2}{W1} \times 100$$

$$\text{Ether Extract \% (as DM basis)} = \text{Ether Extract \% (as fed basis)} \times \frac{100}{DM\ \%}$$

$$\text{Or} = \frac{W3 - W2}{W1 \times DM\% / 100} \times 100$$

- Where W1 = weight of sample, in grams
- W2 = weight of empty dry beaker (tare weight), in grams
- W3 = weight of dry beaker and fat residue, in grams

Crude Fiber Determination (CF)

The method is based on the solubilization of non-cellulosic compounds by sulfuric acid and sodium hydroxide solutions. Thus, quantify the materials in the feed that form the cell wall (cellulose, certain hemicelluloses and lignin) and provide relatively low energy digestibility. However, later it was shown that crude fiber also included pectin, and the major disadvantage of this technique is that, hemicelluloses, pectin and part of lignin were not recovered in the crude fiber fraction.

Principle: A moisture- free and ether extracted sample is digested with a weak acid solution, then a weak base solution. The organic residue is collected in a filter crucible. The loss of weight on ignition is called crude fiber.

Reagents:

a) Sulfuric acid (H_2SO_4) solution 1.25% (0.255 ± 0.005 N). 12.5g (6.79 ml) 98% concentrated H_2SO_4 / 1000 ml distilled water, mix and control the concentration by titration.
b) Sodium hydroxide (NaOH) solution 1.25% (0.313 ± 0.005 N). 12.5 g / 1000 ml distilled water, mix and control the concentration by titration.
c) n-octanol as antifoam.
d) Anhydrous acetone or ethyl alcohol

Equipment:

- Digestion apparatus consisting of individually controlled heaters and water cooled condensers designed to maintain constant volume of solution throughout digestion.
- Digestion containers: a 600 ml tall form beaker is recommended
- Ashing dishes/crucibles.
- Desiccator.
- Filtering device (Buchner filter).
- Linen cloth with about 20 threads per cm.
- Suction filter: To accommodate filtering devices. Attach suction flask to trap in line with vacuum source.
- Vacuum source with valve to break or control vacuum.

Procedure:

The residue from the ether extract determination may be used (use the original weight of sample before drying and extracting with ether). If the residue is not used, proceed as follows to obtain a dry ether extract free sample.

1. Weigh by difference about 1.5-2.0 g of sample to the nearest 1mg (W1) into a clean thimble, dry in an oven at 105^{o}C overnight, then extract with ether (as in ether extract determination). Removing fat is not necessary if the sample has less than 1% ether extract).
2. Transfer the extracted residue to the digestion containers (weigh 6 samples set).
3. Add 200 ml boiling sulfuric acid solution (after preheating by the hot plate in order to reduce the time required for boiling). Put a permanent mark on a 200 ml level of the beaker to measure the acid and base solutions rapidly into the beaker.
4. Add 3-5 drops of n-octanol as antifoam agent.
5. Put the beaker on the preheated extraction heater and cover with condenser, boil exactly 30 minutes from the onset of boiling. Rotating the beaker periodically to prevent solids from adhering to sides.
6. When the time of the first acid treated sample has been finished, remove the sample and filter onto a linen cloth in a fluted funnel (Buchner filter) connected to vacuum for draining sulfuric acid.
7. Wash three times with 30 ml (crucible filled up to the top) of hot deionized water, or until washings are no longer acid.
8. After draining the last wash, wash the sample back into the container with 200 ml of preheated (boiling) sodium hydroxide (NaOH) solution and add 3-5 drops of antifoam.

   Note: Allow a total of five minutes from time of removal of acid treated sample from hot plate until alkali treated sample starts to boil; with this timing sequence, six determinations can be run at a time by one operator.
9. Place beaker back on hot plate and boil for exactly 30 minutes.
10. When the time of the first base treated sample has been finished, remove the sample, and filter onto a linen cloth as before, wash the sample with boiling water as point 7.

    Note: you can use filter crucible, sintered glass; coarse porosity or Gooch crucible in this step instead the linen cloth.
11. Perform a last washing with cold deionized water aimed to cool the residue and then wash the residue three times with 25 ml of acetone.
12. Quantitatively transfer the residue to a porcelain crucible.
13. Dry the residue in an oven at 105 °C for overnight or up to constant weight. Cool in desiccators to room temperature and weigh (W2). This weight represents the crude fiber plus ash weight.
14. Ignite the contents of crucible in a muffle furnace at 550-600°C for 2-3 hours and reweighed after cooling in a desiccators to room temperature (W3). Report the loss in weight as crude fiber.

Calculations:

$$\text{Crude Fiber \% (as fed basis)} = \frac{W3 - W2}{W1} \times 100$$

$$\text{Crude Fiber \% (as DM basis)} = \text{Crude Fiber \% (as fed basis)} \times \frac{100}{DM\ \%}$$

- Where W1 = weight of sample, in grams
- W2 = weight of crucible and residue after drying, in grams
- W3 = weight of dry crucible and residue after burning, in grams

Modification of Weende's method according to Wijkstrom for CF determination

Among different modifications proposed; that by T. Wijkstrom reduces boiling from 30 to 10 minutes by using more concentrated reagents. All the other steps of Weende's method remain unmodified.

Reagents:

a) Sulfuric acid (H_2S0_4) 0.640 N, 32.0 g of concentrated acid (reagent grade 98%, d.1.84) to 1000 ml with distilled water.

b) Potassium hydroxide (K0H) 0.556 N, 31.2 g (reagent grade free of carbonate) to 1000 ml with distilled water.

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Analytical procedure by Tecator automatic apparatus

A	Grind the sample
B	Weight the sample in the crucible (FO)
C	Insert 6 crucibles in the instrument
D	Add 1st preheated reagent in the columns
E	Add 3-4 drops of antifoam agent
F	Boil exactly for 30 minutes
G	Filter the remaining reagent
H	Wash 3 times with hot water
I	Add 2nd preheated reagent in the columns
L	Add 3-4 drops of antifoam agent
M	Boil exactly for 30 minutes
N	Filter the remaining reagent
O	Wash 3 times with hot water
P	Wash once with cold water
Q	Wash 3 times with cold solvent
R	Dry (105 °C) the residue in the crucibles
S	Weight the residue in the crucibles (F1)
T	Ash (550 °C) the residue in the crucibles
U	Weight the ash in the crucibles (F2)
V	$\%\ \text{crude fiber} = \frac{F1 - F2}{FO} \times 100$

Determination of Nitrogen Free Extract (NFE)

Principle: We have no direct measure of the most digestible part of feed, which is a soluble carbohydrate. These are estimated by adding up ash, crude fiber, crude protein, and ether extract after the analyses have been completed, and subtracting from 100. What ever is left is called the Nitrogen Free Extract (NFE).

Calculations:

NFE % (as DM basis) = 100 % - [ash% + CF% + EE% + Cp%; all as DM basis]

To adjusting to an as fed basis =

$$\frac{\text{Constituent on DM basis \%}}{100} \times \frac{\text{as fed DM \%}}{100} \times 100$$

What is problematic about this technique? What if you make a mistake in one of the other analyses, or what if one of those does not really measure what you assume it does? The mistake ends up affecting the NFE fraction.

The crude protein, ash, and ether extract, portion of proximate analysis are still widely used to analyze both plant and animal material. However, the crude fiber method has many problems and has been widely criticized. One of the big problems is that two important components of plant fiber, hemicellulose and lignin, are soluble in acid and/or alkali. Thus, they are not included in the crude fiber, but are counted in the NFE fraction.

While crude fiber is suppose to be the most indigestible fraction of a feed, its digestibility ranges from 0 to 90% in ruminants and in some situations, NFE appears less digestible than crude fiber.

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Van-Soest procedure for determination of Detergent fibrous fractions

In Germany, early chemists (Thaer, 1809; Henneberg and Stohmann, 1860) attempted to define the nutritive matter of livestock feeds with chemical analysis (proximate analysis including DM, Ash, CP, EE, CF and NFE). Their chemical methods (Weende system) were adapted to assay the TDN (Henery and Morrison, 1910). But, there are some problems (shortcomings) of the proximate analysis refer to carbohydrate (CF and NFE) determination.

Problems of the proximate analysis

Component	Supposed to content	Contains	Missing	Excess
CF	Fibrous matter	• Cellulose, • part of lignin	• Hemicellulose, • Part of lignin, • Insoluble ash	None
NFE	Soluble carbohydrate	• Soluble carbohydrate, • Hemicellulose, • Part of lignin, • Insoluble ash	None	• Hemicellulose, • Part of lignin, • Insoluble ash

In the 1960's a different method of fiber analysis was developed which has gained widespread acceptance for plant analysis. This is detergent fiber analysis, although it is referred to as Van Soest fiber analysis after the scientist who developed it. Van Soest (1964) was the first to use detergents to partition forage DM. His detergent designed to separate plant tissue into cell wall (NDF) constituents and cell contents (CC). Cell walls (structural carbohydrates) are further partitioned into hemicelluloses, cellulose, lignin and insoluble ash. While cell contents are partitioned into soluble carbohydrate, protein, solvent extract and soluble ash (also includes compounds like tannins and soluble fiber such as pectin, which is up to 100% digestible by microorganisms). This system allows the chemical partitioning of feeds into nutritive matter (non-structural carbohydrates (sugars, starches, organic acids ...), protein, fat and soluble ash) that is digestible by enzymes produced in the digestive system of animals, and partially nutritive matter that is digested by enzymes produced by microorganisms within the digestive tract (cellulose and hemicellulose), and non-nutritive matter that has no known nutritive value to animals (lignin, insoluble ash).

One of the important advantages of the detergent fiber analysis method is that the analysis divides the fiber into chemically meaningful classes. Crude fiber analysis does not give a consistent breakdown of fiber components. The problem with proximate analysis is the variety of compounds, both fiber and non-fiber that end up in the NFE portion and the hemicelluloses and part of lignin that is not represented in crude fiber. Thus, detergent fiber is a better technique for analyzing forages, especially for ruminants. This will be obvious when we compare the results of the two types of analyses.

Principle: The sample is first boiled in a neutral detergent solution, then filtered. The difference in weight is due to neutral detergent soluble or cell contents. The residue that remains is termed neutral detergent fiber (NDF). The sample is then boiled in an acid detergent solution which solubilizes hemicellulose. There are a couple of choices at this stage of the analysis. Treatment of the ADF with 72% sulfuric acid to remove cellulose, then ashing of the residue to estimate lignin. Another method, that is now standard for complete detergent fiber analyses, is to treat the ADF with $KMnO_4$ (potassium permanganate). This removes lignin, leaving cellulose and insoluble ash. This residue then ashed to estimate cellulose and also the isoluble ash.

Dietary fiber in humans diets is determined by procedures in which:

- fats are removed by treatment with ether.
- enzymes (amylase and proteases) are used to remove compounds that would be digestible by the animal (such as protein and starch).
- ethanol is mixed with the sample to precipitate soluble fiber.
- the sample is filtered and the residue is an estimate of dietary fiber.

Fiber (i.e. undigested residue) for one species in one situation may not be fiber for another species or another situation.

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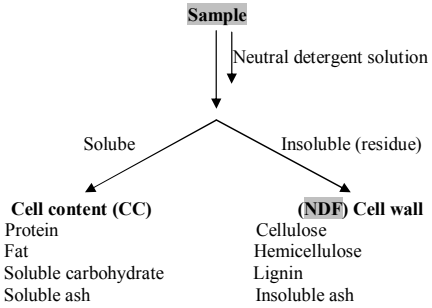
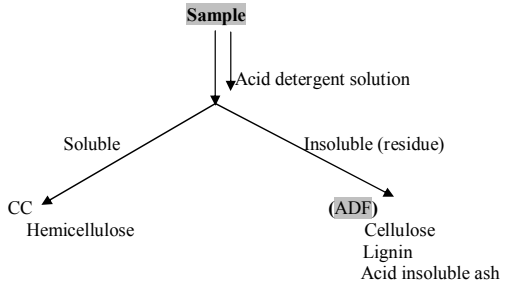
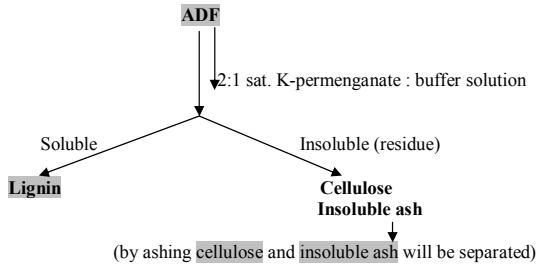
Van - Soest procedure



(CC % = 100 % - NDF %)



(Hemicellulose % = NDF % - ADF %)



Determination of Neutral Detergent Fiber (NDF) and cell contents (CC)

This method is applicable for the determination of NDF in all types of forages, but cannot be used with feeds that have a high protein and low fiber content.

Principle: The neutral detergent procedure for cell walls is a rapid method for the total fiber in fibrous plant feedstuffs. It divides the DM of feeds to nutritive available (soluble) constituents [cell contents] and incompletely available constituents or depends on microbial fermentation [cell walls; NDF].

Equipment:

- Refluxing apparatus.
- Berzelius beakers (600 ml).
- Fritted glass (Gooch) crucibles (coarse porosity, 50 ml).
- Analytical electronic balance, sensitive to 0.1 mg.
- Suction filtering device with trap in line and valve to break vacuum.
- Forced-air drying oven set at 100°C

Reagents:

- Neutral detergent solution: To make approximately 1 liter, mix: 30g Sodium lauryl sulfate, 18.61g Ethylenediaminetetraacetic acid (EDTA) disodium salt**,** 6.81g Sodium borate decahydrate ($Na_2B_4O_7.10H_2O$), 4.56g Sodium phosphate dibasic (Na_2HPO_4), anhydrous, 10 ml 2-ethoxy-ethanol (Triethylene glycol, reagent grade) in 1L distilled water. Chick solution pH ranging from 6.95 to 7.05 and adjust as necessary.

  Preparing a NDF solution. Pour one-half of distilled water into mixing container. Place on stir pl hood and begin stirring. Add remaining reagents except for triethylene glycol. (Caution: wear dust mask when weighing and transferring the sodium lauryl sulfate.) Slowly add remaining distilled water to container to limit foaming of the detergent. When approximately three-fourths of the distilled water has been added to the container, add the triethylene glycol. The triethylene glycol will reduce foaming of the detergent solution. Allow to stir overnight. Use heated stirrer if material fails to dissolve. Keep container at 20°C or higher to avoid solution precipitation. Verify pH of solution to be between 6.95 and 7.05. Adjust with HCl or NaOH as required if not within range.

- Sodium sulfite, anhydrous (Na_2SO_3),
- Acetone, reagent grade.

Procedure:

1. Dry 50 ml fritted glass crucibles overnight at 100°C and weigh (W1), recording weight to nearest 0.1 mg.
2. Wet samples should be oven dried at 55°C to greater than 85% dry matter, then ground to pass a 1 mm screen.
3. Thoroughly mix sample, then weigh 1 g into 600 ml beaker and recording weight (W2).
4. Preheat extraction heating (reflux) unit to a temperature that permits boiling of neutral detergent solution within 5 min.
5. Add 0.5 g of sodium sulfite using previously calibrated scoop.
6. Add 100 ml of neutral detergent solution (room temperature) and swirl beaker until the sample and sodium sulfite are completely suspended.
7. Put beaker on the heating unit under a cool water condenser. Samples should come to a boil in 5 to 10 min. Reduce heat as boiling begins, to avoid foaming. Swirl beaker to resuspend any particles that have crept up the sides of the beaker. Adjust boiling to an even level and reflux for 60 minutes, timed from onset of boiling.
8. Remove sample from heating unit and allow settling for 30-60 sec before filtering.
9. Preheat the fritted glass crucible for filtering by adding 40 ml of boiling water, and remove water with vacuum.
10. Carefully swirl beaker to suspend solids and decant the first 30-40 ml of solution from the beaker into the crucible. Rinse lip of beaker to prevent solution with particles from running down outside of beaker. Keep beaker in "decant" position while emptying. Remove the solution with a minimum amount of vacuum at first and increasing it only as more force is needed.
11. Close vacuum and rinse the remaining residue from the beaker into the crucible using a fine stream of boiling water. Be certain that no particles remain in the beaker or on the lip to run down the outside as the beaker is turned upright.
12. Remove vacuum, break up mat, and fill crucible with hot water. Filter and wash twice by adding 30 to 40 ml boiling water to residue.
13. Rinse sample twice with 30 ml of acetone, allowing at least 2 min soaking time between rinses, and suck sample dry, and remove sample from manifold.
14. Dry crucibles at 100°C for 8 hr or overnight and recording weight (W3).

Calculation:

$$\text{NDF \% (as fed basis)} = \frac{W3 - W1}{W2} \times 100$$

$$\text{NDF \% (as DM basis)} = \text{NDF \% (as fed basis)} \times \frac{100}{\text{DM \%}}$$

- Where: W1 = weight the empty crucible (tare weight) in grams
- W2 = weight of sample in grams
- W3 = weight of crucible and dry fiber in grams

Cell contents: It is the more readily nutrients are enclosed by the cell wall. It includes protein, soluble carbohydrate, soluble minerals and lipids. A high percentage of cell contents is a good index of high nutritive value of a feed. It is determined as follow:

- Cell contents % = 100 % - cell walls % (NDF %).

Calculate nonfibrous carbohydrates (NFC) by difference as follows:

NFC = 100 - (NDF% + CP% + Fat% + ash%)

NFE = 100 - (CF% + CP% + Fat% + ash%).

NSC = Total non-structural carbohydrates is measured by enzymatic methods (Smith, 1981).

Remark: There are three major modifications of the NDF method, each of which generates different values depending upon the feed that is analyzed. The former original NDF method (van Soest and Wine, 1967, Goering and Van Soest, 1970 and later modified by Van Soest et al., 1991) used sodium sulphite to remove contaminating proteins (nitrogenous) from NDF be cleaving disulfide bonds and dissolving many crosslinked proteins and it crucial for heated feeds (Hintz et al., 1996). It was discovered that the original method did not adequately remove starch grains and corn silage. The neutral detergent residue modification was developed that included a heat-stable amylase in the procedure to remove starch, however, sulphite was removed from the procedure because of concerns about the possible loss of lignin and phenolic compounds (Van Soest et al., 1991). The amylase-treated NDF modification (aNDF) was developed to measure NDF in all types of feeds and uses both heat-stable amylase and sodium sulfite to obtain NDF with minimum contamination by either starch or protein. Sodium sulfite improve the filtration of fiber residues during the NDF procedure and allows the methods to be used on all types of feeds and feed mixtures, including heated feeds and protein supplement.

Determination of Amylase Neutral Detergent Fiber (aNDF)

This method is applicable for the determination of NDF in all types of forages and feeds.

Principle: A neutral detergent solution is used to dissolve the easily digested pectin and plant cell contents (proteins, sugars and lipids), leaving a fibrous residue (aNDF) that is primarily cell wall components of plants (cellulose, hemicellulose and lignin). Detergent is used to solubilize the proteins while sodium sulfite helps to remove some nitrogenous matter. EDTA is used to chelate calcium and remove pectin at boiling temperatures, while triethylene glycol helps to remove some nonfibrous matter from concentrate feeds. Heat-stable amylase is used to remove starch. Two additions of amylase (one during refluxing and one during filtration) have been observed to aid aNDF analyses and minimize filtering difficulties. It used in hot solutions to inactivate potential contaminating enzymes that might degrade fibrous constituents.

Equipment:

- Same as used with cell wall (NDF) analysis procedure.

Reagents:

- Same as used with cell wall (NDF) analysis procedure.
- Heat-stable alpha-amylase solution, reagent grade.

Procedure:

1. Dry 50 ml fritted glass crucibles overnight at 100°C and hot weigh (W1), recording weight to nearest 0.1 mg.
2. Wet sample should be oven dried at 55°C to greater than 85% dry matter, then ground to pass a 1 mm screen.
3. Thoroughly mix sample, then weigh **0.5** g (0.45 to 0.55 g) (W2) into 600 ml beaker, and add 0.5 g of sodium sulfite using previously calibrated scoop.
4. Add **50** ml of neutral detergent solution and swirl beaker until the sample and sodium sulfite are completely suspended.
5. Preheat extraction heating (reflux) unit to a temperature that permits boiling of neutral detergent solution within 5 min.

6. Place beaker on the heating unit under a cool water condenser. Samples should come to a boil in 4 to 5 min. Samples normally foam vigorously for 1 to 2 min. Do not reduce temperature of heating unit during this time.
7. After 5 min, remove beaker from the reflux unit and add 2 ml of the standardized amylase solution.
8. Swirl beaker to thoroughly mix the amylase in the neutral detergent solution and resuspend any particles that have crept up the sides of the beaker. Detach any sample attached to the sides or bottom of the beaker using a rubber policeman. Rinse off policeman with aNDF solution.
9. Return beaker to the reflux unit and allow coming to a boil. Reflux for 60 min. Five to 10 min after adding amylase, rinse down the sides of the beaker with neutral detergent solution.
10. Remove sample from heating unit and allow settling for 30-60 sec before filtering.
11. Preheat the fritted glass crucible for filtering by adding 40 ml of boiling water. Remove water with vacuum.
12. Carefully decant the first 30-40 ml of solution from the beaker into the crucible. Rinse lip of beaker to prevent solution with particles from running down outside of beaker. Keep beaker in "decant" position while emptying. Remove the solution with a minimum amount of vacuum.
13. Close vacuum and rinse the remaining residue from the beaker into the crucible using a fine stream of boiling water. Be certain that no particles remain in the beaker or on the lip to run down the outside as the beaker is turned upright. Apply minimum vacuum to filter.
14. Immediately fill crucible half full of hot water and add 2 ml of standardized amylase solution. Allow to react for approximately 45 to 60 sec, while policing particles from sides of the beaker.
15. Rinse beaker with boiling water while inverted over the crucible until all residue is transferred.
16. Filter and wash twice by adding 30 to 40 ml boiling water to residue in fritted glass crucible and allowing it to soak for 2 min each time.
17. Rinse sample twice with 30 ml of acetone, allowing at least 2 min soaking time between rinses. And Rinse policeman, vacuum sample dry, and remove sample from manifold.
18. Dry crucibles at 100°C for 8 hr or overnight and hot weigh recording weight (W3).

Calculation: aNDF %

- Same as used with cell wall (NDF) analysis procedure.

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Determination of Acid Detergent Fiber (ADF)

Principle: The ADF fiber procedure provides a rapid method for ligno-cellulose in feedstuffs. An acidified detergent solution is used to dissolve cell contents and hemicellulose leaving a residue of cellulose, lignin, and heat damaged protein and insoluble minerals including silica (ash). ADF is determined as the residue remaining after extraction. The difference between the NDF and ADF is an estimate of hemicellulose.

Equipment:

- Refluxing apparatus.
- Berzelius beakers (600 ml).
- Fritted glass (Gooch) crucibles (coarse porosity, 50 ml).
- Analytical electronic balance, sensitive to 0.1 mg.
- Suction filtering device with trap in line and valve to break vacuum.
- Forced-air drying oven set at 100°C.

Reagents:

- Acid detergent solution: 1 liter of 1 N sulfuric acid ±0.005N. Normality must be verified by titration with a primary base standard before adding CTAB. A solution approximately 1.0 N sulfuric acid can be made by adding 51.04 g (27.7 ml) of concentrated sulfuric acid (95-98% purity) (AOAC) to 972.3 ml water (correct for assay). Add water (if normality too high) or sulfuric acid (if normality too low) to adjust normality to 1.00N ±0.005N. Then add 20 g Cetyl trimethylammonium bromide (CTAB), stir to facilitate solution.
- Acetone, reagent grade.

Procedure:

1. Dry 50 ml fritted glass crucibles overnight at 100°C and weigh (W1), recording weight to nearest 0.1 mg.
2. Samples should be oven dried at 55°C to more than 85% DM, then ground to pass a 1 mm screen.
3. Thoroughly mix and weigh sample (approximately 0.9 to 1.1 g) into beaker (W2).
4. Add 100 ml acid-detergent solution at room temperature. Place beaker on heater under the cold water condenser.
5. Heat to boiling in 5-10 min; reduce heat to avoid foaming as boiling begins. Reflux 60 min from onset of boil, adjusting boiling to slow, even level.
6. After about 30 min, wash down sides of beaker with minimal amount of acid detergent solution. A wash bottle is convenient for dispensing solution.

7. Remove beaker, swirl, and filter through tared (step 1) fritted glass crucible, using minimal vacuum. Rinse the beaker with boiling water while inverted over the crucible to insure quantitative transfer of all fiber particles into the crucible.
8. Soak twice with boiling (95-100°C) water by breaking up mat and filling crucible each time with vacuum off and allowing to soak a minimum of 15 to 30 sec (2 min recommended) after each wash. While filling the crucible with hot water or acetone, rinse the top edge and sides to remove residual acid detergent.
9. Rinse twice with 30-40 ml acetone by filling crucible each time with vacuum off, allowing a minimum of 15 to 30 sec (2 min recommended) before vacuuming dry.
10. Dry 8 hr or overnight in forced-air oven (100°C) and weigh, recording weight (W3).

Comments:

- Sulfuric acid for ADF solution must be standardized to be between 0.995 and 1.005 N. Variation in normality outside of this range can result in low or high ADF values.
- Timing of refluxing is critical and should not vary more than 5 min from the 60 min described by the method.
- Acid must be thoroughly washed from the sample because it will become concentrated when water is removed during drying. The combination of strong sulfuric acid and high temperature can char the sample and result in low ADF values. If black discoloration occurs during drying, repeat the analysis.
- The proper vacuum is critical to good filtering. It should be sufficient to remove the solutions rapidly but not so great that fiber particles plug the fritted disk.
- Rinse water must be in excess of 95°C. This is particularly true of samples containing pectic substances, mucilages or glycoproteins.

Calculation:

$$\text{ADF \% (as fed basis)} = \frac{W3 - W1}{W2} \times 100$$

$$\text{ADF \% (as DM basis)} = \text{ADF \% (as fed basis)} \times \frac{100}{DM\ \%}$$

- W1 = weight of empty crucible (tare weight) in grams
- W2 = weight of sample in grams
- W3 = weight of crucible and dry fiber in grams

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Determination of lignin, cellulose and insoluble ash by permanganate method

Principle: Interfering matter is removed by preparing ADF, which is chiefly composed of lignin, cellulose and insoluble minerals. Then, the permanganate method is aimed to free cellulose from encrusting lignin (contained in ADF) by the oxidation of lignin with an excess of acetic acid-buffered potassium permanganate solution, containing trivalent iron and monovalent silver as catalysts, leaving cellulose and insoluble minerals (ash). The insoluble ash (an estimate of silica content), which is a primary factor in reducing digestibility in many grasses. Lignin is measured as the weight lost by these treatments, while cellulose is determined as weight lost upon ashing.

Reagents:

Solvent for lignin

• Permanganate saturated solution: dissolve in 1L distilled water:

a) 50 g Potassium permanganate ($KMnO_4$), reagent grade.

b) 0.05 g Silver sulfate (Ag_2SO_4) as dealogenating agent

The salts are added to water and stirred for easier dissolution. Keep in dark bottle.

• Buffer solution: for 1L use the following amounts ofchemicals:

a) 6.0 g Ferric nitrate ($Fe(NO_3)_3$)

b) 0.15 g Silver nitrate ($AgNO_3$)

c) 500 ml Acetic acid glacial ($C_2H_4O_2$)

d) 5 g Potassium acetate ($C_2H_3KO_2$)

e) 400 ml tert-Butyl alcohol ($C_4H_{10}O$)

f) 100 ml Distilled water

Dissolve into distilled water iron and silver nitrate. Add, in the order, acetic acid, potassium acetate and butanol.

• Permanganate/buffer mixed solution

Mix 2 volumes of permanganate solution with 1 volume of buffer solution. The mixed solution must be clear and red. The mixed solution can be kept in a refrigerator at 4 °C up to a week. Concentrated solutions have a longer shelf life.

• Demineralizing solution: for 1L, dissolve:

50 g Oxalic acid dihydrate ($C_2H_2O_4$ 2 H_2O) in 700 ml Ethanol 95% (C_2H_6O), add 50 ml hydrochloric acid 37%, (HCl 12 N about) and 250 ml distilled water while stirring and mix.

• Ethanol 80%
• Acetone

Procedure:

1. Dry 50 ml fritted glass crucibles overnight at 100°C and weigh (W1), recording weight to nearest 0.1 mg.
2. Grind the air dried sample to pass 1 mm screen.
3. Weigh in a beaker 1 g of grinded sample to the nearest 1 mg (W2).
4. Add 100 ml of acid detergent solution at room temperature and some drops of n-octanol.
5. Heat to boiling and reflux 60 minutes from onset of boiling.
6. Remove beaker, swirl, and filter through tared (step 1) fritted glass crucible, using minimal vacuum. Rinse the beaker with boiling water while inverted over the crucible to insure quantitative transfer of all fiber particles into the crucible.
7. Rinse twice with 30-40 ml cold acetone by filling crucible each time with vacuum off, allowing a minimum of 15 to 30 sec (2 min recommended) before vacuuming dry.
8. Dry 8 hr or overnight in forced-air oven (100°C) and weigh, recording weight (W3).

Note: It is possible to start using the residue represented by ADF determination method.

9. Place crucibles containing the ADF in a shallow glass tray containing approximately 1 cm cold water (to reduce flow of solution out of crucibles). Fiber in crucibles should not be wet. Place a short glass rod in each crucibles to stir contents, and to break lumps.
10. Add approximately 25 ml of mixed permanganate/buffer solution (solvent for lignin) and stir. Do not overfill.
11. Allow crucibles to stand 90 ± 10 minutes at room temperature of 20-25°C. The red color of permanganate must be present during the whole period; if not add fresh reagent.
12. Remove crucibles and filter on filtering apparatus. Do not wash.
13. Place in a clean glass pan, and slowly fill crucibles no more than half full with demineralizing solution. Caution: foam can develop.
14. Leave it 5 minutes, then filter and repeat demineralizing treatment at least once. The residue must be white (20-30 minutes). Rinse down the sides of the crucibles with a fine stream of demineralising solution from a squeeze bottle. Treat until fiber is white.
15. Wash twice with 80% ethanol and twice with acetone.
16. Dry 8 hours or overnight at 105°C and let cool in a desiccators and weigh, recording weight (W4).
17. Ash for 3 hours at 500°C, cool in desiccators and weigh, recording weight (W5).

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Calculation:

$$\text{Lignin \% (as fed basis)} = \frac{W3 - W4}{W2} \times 100$$

$$\text{Lignin \% (as DM basis)} = \text{Lignin \% (as fed basis)} \times \frac{100}{\text{DM \%}}$$

$$\text{Cellulose \% (as fed basis)} = \frac{W5 - W4}{W2} \times 100$$

$$\text{Cellulose \% (as DM basis)} = \text{Cellulose \% (as fed basis)} \times \frac{100}{\text{DM \%}}$$

$$\text{Insoluble ash (silica) \% (as fed basis)} = \frac{W5 - W1}{W2} \times 100$$

$$\text{Insoluble ash (silica) \% (as DM basis)} = \text{Insoluble ash (silica) \% (as fed basis)} \times \frac{100}{\text{DM \%}}$$

Where W1 = weight of empty crucible (tare weight), in grams

W2 = weight of sample, in grams

W3 = weight of crucible and dry fiber (ADF), in grams

W4 = weight of crucible and dry residue (cellulose and insoluble ash) in grams

W5 = weight of crucible and insoluble ash, in grams

Remark:

To adjusting to dry basis:

$$\frac{\text{Analysis \% on as fed basis}}{\text{Dry matter \%}} \times 100$$