Critical Factors in Determining Fiber in Feeds and Forages

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Introduction

From the nutritional perspective, fiber is defined as the hydrolytically indigestible partially fermentable components of feed. Chemically, these components are a variable mixture of cellulose, hemicelluloses, lignin, and soluble dietary fibers (e.g., pectins). Nutritionists need a practical and routine means of measuring fiber and must compromise between the theoretical concept of fiber and the utility of using chemical solubility to isolate and measure fractions that closely resemble the nutritionally defined fraction called fiber. Because there is no guarantee of direct correspondence between chemical solubility and nutritional availability, in reality, **fiber is defined by the method used to isolate it.** The actual definition of fiber becomes method-dependent, which explains why there are so many different fiber analyses (crude fiber, acid detergent fiber, neutral detergent fiber, amylase-neutral detergent fiber, total dietary fiber, etc.).

The abundance of fiber methods is complicated further by the modifications of each method that are commonly used. Sometimes these modifications are developed to meet the specific needs of a particular application or research project. Other times modifications are made for convenience or to increase the speed of fiber analysis. **Because fiber is defined by the method used to isolate it, it should be clear that method modifications have the potential to result in a fiber value that is not comparable with the parent method and cannot be reported using the same test name. The sensitivity of fiber values to a method suggests that fiber methods must be followed exactly to be reproducible.** To be acceptable, any modification of fiber methods must be evaluated thoroughly with several feed products representing various types of feed ingredients. The objective of this paper is to discuss some of the critical steps and conditions in fiber analyses and indicate the potential problems inherent in the methods themselves.

Acronyms

CF – crude fiber
ADF – acid detergent fiber
NDF – neutral detergent fiber
TDF – total dietary fiber
IDF – insoluble dietary fiber
SDF – soluble dietary fiber
SDFP – soluble dietary fiber precipitate
SDFS – soluble dietary fiber solubles
DP – degree of polymerization
AAFCO – Association of American Feed Control Officials

Fiber Definitions

Crude Fiber (CF)

This method was designed to divide carbohydrates into digestible and indigestible fractions. When CF content is high, the energy content of the feed is low because crude fiber is considered indigestible. Measuring CF was one part of the original system of analyzing the "digestible" fraction of feedstuffs. The CF method uses sequential acid and alkali extraction. It was developed by Henneberg and Stohmann during the 1860s at the Weende Experiment Station in Germany, and is part of the Weende system of proximate analysis. The CF extract was once used as a standard for fibrous components or the indigestible portion of carbohydrates in feed. However, some of these components are partially fermentable by microorganisms in the animal digestive system fermentation compartments (e.g., rumen, cecum, large bowel). Since CF accounts for most of the cellulose but only a portion of the hemicellulose and lignin and no ash, it underestimates true fiber. CF values are less than ADF values. Thus, CF is not a good indicator of digestibility by ruminant animals, and the use of this assay in feeds for ruminants has significantly declined. Crude fiber still is used today as the legal measure of fiber in grains and finished feeds.

Detergent Fibers

Animal nutritionists have replaced the CF assay with the Van Soest detergent fiber analysis system. The technique of using detergents to separate digestible and indigestible parts of plant tissues was originally proposed by Van Soest in 1963. The concept behind detergent fiber analysis is that plant cell substances can be divided into less digestible cell walls (made up of hemicelluloses, cellulose, and lignin) and the highly digestible cell contents (containing starch and sugars). The highly digestible cell contents are successfully separated from the cell walls by using two different detergent systems:

NDF = Hemicelluloses + Cellulose + Lignin + Ash

ADF = Cellulose + Lignin + Ash

Acid Detergent Fiber (ADF)

This fibrous component represents the least digestible fiber portion of forage or other roughage. This highly indigestible part of forage includes lignin, cellulose, silica, and insoluble forms of nitrogen, but not hemicelluloses. Forages with higher ADF values are lower in digestible energy than forages with lower ADF values, which means that as the ADF concentration increases, digestible energy concentration decreases. During laboratory analysis, ADF is the residue remaining after boiling a test material in acid detergent solution. ADF often is used in nutritional equations to calculate digestibility, total digestible nutrients (TDN) and/or net energy for lactation (NEL).

Neutral Detergent Fiber (NDF)

NDF is the residue or insoluble fraction left after boiling a feed material in neutral detergent solution. The NDF contains insoluble plant cell wall components that include cellulose, hemicelluloses, lignin, silica, and cutins. The hemicelluloses, cellulose, and lignin represent the fibrous content of the forage. Because they give the plant rigidity and enable it to support itself as it grows, these three components are classified as structural carbohydrates.

Although lignin is indigestible, hemicelluloses and cellulose can be (to varying degrees) fermented by microorganisms in animals with either a rumen (e.g., cattle, goats, sheep), a cecum (e.g., horses, rabbits, guinea pigs) or a large bowel (most species). NDF often is used in nutritional equations to calculate digestibility, total digestible nutrients (TDN) and/or net energy for lactation (NEL).

Total Dietary Fiber (TDF)

In 2001, the American Institute of Chemists defined dietary fiber as "those compositions that are resistant to digestion and absorption in the small intestine but can be fermented in the big intestine. It includes polysaccharides, oligosaccharides, etc., like cellulose, hemicellulose, gum, beta-glucans, pectin, lignin, polydextrose, fructo-oligosaccardides, resistant starch and dextrin."

According to the American Association of Cereal Chemists (AACC) "dietary fiber is the edible portions of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine and are either completely or partially fermented in the large intestine." Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances.

Codex Alimentarius further defines dietary fiber as carbohydrate polymers with three or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories:

- Edible carbohydrate polymers naturally occurring in the food consumed.
- Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological benefit to health, as demonstrated by generally accepted scientific evidence.
- Synthetic carbohydrate polymers that have been shown to have the physiological benefits to health as demonstrated by generally accepted scientific evidence to competent authorities.

As a result of these definitions, there are a number of analytical methods which can be used to estimate dietary fiber content. The method chosen is determined by the type of material and the specific dietary fiber fractions. The most common method used is AOAC 991.43 and the earlier 985.29.

Factors Resulting in Variation in CF, ADF, and NDF Analyses

The chemical principles in the analysis of CF have not changed since its introduction in the 1860s. Likewise, the chemical principles for the analysis of detergent fibers have stayed the same since Van Soest introduced them in the 1960s. While the principles have remained the same, the methods and equipment have been improved for greater throughput and reduction in tedious steps. Fiber was first determined by boiling a test material in a beaker and filtering through a Gooch crucible. Many labs still use this method. Tecator introduced the Fibertec® extraction system in 1976 that allows the simultaneous digestion and sequential filtration of six test portions thereby eliminating the need to transfer the solution to a filtering crucible. ANKOM introduced its system in 1992 that allows the determination of up to 24 test portions placed in filter bags in a pressurized kettle. Gerhardt followed this with its Fibretherm system

that allows simultaneous determination of 12 test portions placed in filter bags in a reflux kettle. **While there are different extraction systems and methods, they are all required to follow the critical conditions discussed below.**

It is noted that fibers in many forages are now being determined by NIRS (near-infrared reflectance spectroscopy). NIR is a secondary method that relies on internal calibrations based on chemical analysis. NIR involves a different set of critical conditions that are not discussed in this paper.

Subsampling and Segregation. In general, the fiber content of large particles is greater than that of small particles for most feed ingredients. Thus, any process that segregates a test material by particle size (such as shaking during shipment, grinding, or grab sampling) will produce a subsample that differs from the true average of the test material. One of the most problematic segregation processes is comminution (e.g., grinding). The tough, large particles that are retained in the grinding mill and finally pulverized to the extent they pass through the screen are high in fiber. If these particles are brushed or vacuumed from the mill and not included in the analytical sample, there is a bias error. If the analytical sample is not mixed thoroughly after grinding, a bias error occurs because the last material to exit the mill (which is higher in fiber) is not randomly incorporated leading to a biased test portion, especially if the test portion is improperly selected (e.g, as a single increment). The ground analytical samples need to be properly mixed before selecting a test portion. The mixing container must not be more than two-thirds full to achieve effective mixing. *Mixing in a container that is full or almost full is always ineffective.* One type of mixing technique that will work for many materials is the Paul Schatz motion which is a three dimensional motion combining a figure eight movement with rotation (see web site at http://www.wab.ch/e/produkte/turbula/turbula.html). The act of "mixing" should never assume to produce a material of uniform composition. In fact, mixing is often a second insidious segregation process (especially the common habit of "stirring"), leading to a false confidence and taking of short cuts in selection of a test portion.
Refer to AAFCO's *Guidelines for Preparing Laboratory Samples* for recommendations on subsampling, comminuting and mixing. http://www.aafco.org/Publications/Guidelines-for-Preparing-Laboratory-Samples

Drying of High Moisture Materials Before Comminution and Analysis. Proteins and carbohydrates can form insoluble compounds (Maillard or browning products) when exposed to high temperatures in the presence of moisture. These Maillard products are measured as artifact fiber and lignin. Thus, high moisture materials never should be exposed to temperatures above 60°C during drying, and a maximum of 50°C is preferred to avoid a high bias in fiber results.

Particle Size Reduction. Fiber methods function by extracting and solubilizing non-fibrous compounds from feed particles. It is expected that extraction efficiency should increase as the size of particles decreases because reagents and washing solvents have less matrix to penetrate. Furthermore, fibrous residues are filtered on coarse porosity membranes suggesting that fine fiber particles may be washed out of the residue or plug the filter membrane. These factors explain why finer particle sized feed materials results in lower fiber values. However, a compromise is necessary between fine grinding to increase extraction efficiency and coarse grinding to prevent loss of fiber particles and plugging of the filtration vessel. The AOAC

Official Fiber Methods recommend grinding through a 1 mm screen using a cutting mill (e.g. Wiley). The centrifugal mills (e.g., Retsch ZM200 or Fritsch P-14) or cyclone mills (e.g., Foss Cyclotec or Udy) generate particle size distributions that are smaller than a cutting mill when similar size screens are used because centrifugal mills force the particles through the screen at an angle instead of allowing the particles to drop through the screen as in the cutting mill. Using the same size screen, cyclone and centrifugal mills will produce an average particle size that is one half that of cutting mills, resulting in slightly lower fiber values and greater filtering difficulties during detergent analyses. It is recommended to use a 2 mm screen with a cyclone or centrifugal mill. The objective of particle size reduction should be to obtain fiber analytical samples that average 1 mm particle size with minimal particle size distribution.

Standardizing Reagents. To provide consistent and accurate fiber values, the reagents and solutions need to be standardized. Commercially purchased fiber solutions should be periodically checked to see that they are within their specifications regarding normality or pH.

Measurement of CF depends upon the use of 0.255N sulfuric acid and 0.313N sodium hydroxide. These normalities are not to vary by $> \pm 0.005$N. Measurement of ADF depends on the use of 1N sulfuric acid which should not vary by $> \pm 0.004$N. The normalities are verified by titrating an aliquot of the solution against a standardized base or acid. If the solution is not within its specified range, then adjust the normality by adding water or concentrated acid or base; and recheck the normality by titration.

Care needs to be taken when preheating the CF acid and base solutions prior to adding them to the reflux vessel. Boiling of the solutions or prolonged simmering before addition will result in the loss of water and an increase in the concentration of the acid and base. A large quantity of solution should be preheated in a vessel equipped with a reflux condenser or heated using the heat exchanger described in AOAC Official Method 962.09C(f).

Neutral detergent (ND) solution should be standardized to a pH of 6.9 to 7.1 If pH differs by more than 0.2 from 7.0, check reagents to determine if the wrong chemicals were used and consider discarding the neutral detergent solution. If pH is between 6.8 and 7.2, an adjustment of pH must be performed by adding either HCl or NaOH to obtain a pH of 7.00.

The amylase solution used in the NDF analysis should be standardized so that the amount of enzyme solution added at boiling and during the first (and second) filtration step removes all traces of starch from the fritted disk of Gooch crucibles. See AOAC Official Method 2002.04C(e) for the standardization procedure. The alpha-amylase used needs to be heat-stable.

Sodium sulfite should be added to each test portion before refluxing in the NDF procedure. It is important for the removal of protein from NDF and is especially critical in the removal of nitrogenous contamination from cooked or heated feeds, animal byproduct feeds, and fecal or digesta materials.

Test Portion Sizes. The ratio of test portion to digestion solution can have a small, but significant, effect on fiber analyses. The standard ratio for fiber analysis is 1.0 g of test material per 100 mL of solution. (ANKOM detergent test portion size is 0.45 to 0.5 g.) The selection of

the test portion weight is a compromise among extraction efficiency, reagent cost, weighing errors, and sampling errors (e.g., selecting a higher ratio of coarse particles than fines). Larger test portions increase reagent costs when maintaining the same material:solution ratio. Smaller test amounts magnify any weighing errors. For example, if the residue weighs 0.01 g with a weighing error of 0.0002 g, the error is 2%; however, if the residue weighs only 0.002 g, the same error is 10%. The lower the fiber content, the greater test portion is needed to maintain confidence (or relative error) in the residue weight.

Varying RefluxTimes and Temperatures. Extraction of fiber is both time and temperature dependent. As the time and temperature increase, the amount of fibrous residue recovered decreases with the exception of NDF as the amount of residue plateaus when the non-fibrous components are solubilized. It is critical to each method that the time of refluxing from the onset of boiling be closely adhered to. Refluxing should be at a temperature that causes a rolling agitation of feed particles. Heating units for individual beakers or reflux columns should be calibrated to bring 100 mL of water at room temperature to a boil in 3 to 4 minutes. When refluxing in Berzelius beakers, the beakers need to be placed on the hot plates with a staggered time between each beaker placement to ensure that the reflux time is consistent for each beaker. This time is determined by the amount of time it takes to filter a test solution. Because the ANKOM system performs digestion under pressure, boiling does not occur so agitation must be accomplished mechanically by the instrument. The use of an anti-foaming agent, such as n-octanol, may be needed with the detergent solutions.

Incomplete Transfer of Residues to the Crucible. The greatest source of error is the loss or incomplete transfer of all fibrous residues from the Berzelius beaker to the crucible. Sometimes residues adhere to the sides or bottom of the beaker. These residues must be freed before they can be transferred. At other times, the last drop from the beaker is allowed to flow down the outside of the beaker when it is turned upright after pouring its contents into the crucible. The beaker should be kept inverted over the crucible and be rinsed with a fine stream of hot water to transfer all particles. If the beaker must be turned upright during transfer, it is critical to wipe the last drop from the lip of the beaker onto the lip of the crucible. Often this last drop contains significant fiber because particles have settled in the beaker during transfer. Transfer should be so complete that beakers do not need to be washed between uses. Beakers should be checked routinely for cleanliness to insure that previous transfers were complete.

Filtration Using Crucibles. Several factors are important in making filtration of fiber residues effective and efficient. Normally, minimum filtration vacuum should be used to prevent plugging the filter membrane with fiber residues and losing fine particles. The vacuum source should be constant and have reserve capacity. It is also important that the vacuum manifold and vacuum lines be constructed to minimize the trapping of foam that will greatly reduce the effective vacuum at the crucible.

AOAC Official Method 2002.04B(c) describes a manifold that minimizes vacuum leaks and foam in the system, yet is durable and economical to construct. The manifold is designed for Gooch crucibles, but can easily be modified for use with Buchner funnels or paper funnels. The basic design fits crucibles tightly and allows back flushing of problem crucibles by removing and reinserting them into the holder.

The choice of filtration vessel is a compromise between filtration ease and fiber recovery. Coarse membranes will allow some fine fiber particles to be lost, but fine membranes often plug, making filtration difficult. The retention size of some common filtration vessels indicates the potential variation that can occur:

Vessel or Membrane	Retention Size (micrometers)
Extra coarse fritted disk, Gooch crucibles	170-220
FiberTec P0 special crucible	160-250
FiberTec P1 special crucible	90-150
California Buchner funnel with 200 mesh screen	70-85
FiberTec P2 standard crucible*	40-90
Coarse fritted disk, Gooch crucible (50 ml)*	40-60
FiberTec P3 special crucible	14-40
Whatman 41/54/541 filter paper	20-25
Medium fritted disk, Gooch crucible	10-15
Whatman 40 filter paper	8
Fine fritted disk, Gooch crucible	4.0-5.5
Whatman GF/D glass microfibre filters	2.7
Very fine fritted disk, Gooch crucibles	2.0-2.5

*Recommended crucible for ADF and NDF analyses.

Check the filtration rate of crucibles by measuring the time it takes for 50 mL of water to pass through each crucible without vacuum. It should take approximately 180 seconds. If it takes less than 120 seconds, check the crucible to insure it is not cracked and leaking. If it takes longer than 240 seconds, clean the crucible with acid (see below) and measure again. If it still takes 240 seconds, clean with alkali (see below). If cleaned crucibles take longer than 240 seconds, discard them because they will cause filtration problems. The filtration rate should be checked on all new crucibles before use. The filtration rate of each crucible should be checked at least annually.

Filtration difficulties also can be caused by gradual plugging of the fritted disks of crucibles with fine particles or ash after repeated use. Crucibles can be easily cleaned by pulling hot water through the fritted disk in reverse of normal filtration flow. Crucibles also can be cleaned by ashing for 5 hours at 500-525°C, and then back flushing with hot water.

Occasionally, crucibles can be cleaned with 6N HCl and/or an alkaline cleaning solution containing 5 g of disodium EDTA, 50 g of trisodium phosphate, and 200 g of potassium

hydroxide per liter of water. The crucibles should be allowed to soak in either solution for 30 minutes and the alkaline solution should be used with heat at 70-80°C. The alkaline treatment can weaken the glass so use it only on crucibles that do not filter normally. See AOAC Official Method 2002.04C(h).

Filtration Using Filter Bags. Filter Bag Technology (ANKOM Technology, Macedon, NY) is designed to allow for filtration to take place continuously during solubilization in crude fiber and detergent solutions. The F57 filter bag is designed to retain fine particles milled according to Official Methods (1 mm screen with a cutting mill or 2 mm screen with a cyclone or centrifugal mill). The F58 filter bag is designed to retain finer particles produced by finer milling. The use of a blank bag during the analysis will alert the user to potential bias. F57 blank bags should produce blank bag correction values from 0.9940 to 0.9980 depending upon the method involved. Blank bag correction factors greater than 1.0000 indicate loss during the digestion process. If fiber loss is found or a smaller grind size is desired then the F58 bag should be used.

Washing Residues with Hot Water and Acetone. The most common error made by fiber analysts is incomplete washing of fiber residues to remove the fiber solutions and soluble feed components. All too often, residues are rinsed, rather than soaked, during the washing steps. Feed particles are filled with voids that can trap solutions and components. These voids cannot be washed free of contaminants by simply rinsing the outside of the particle. The laws of mass action must be used to equilibrate the liquids within the void with clean wash water on the outside of the particle. This is a time-dependent process. Thus, fibrous residues must be soaked in 30-40 mL of clean hot water (95-100°C) for at least 2 minutes (preferably 5 minutes) each time to remove the fiber solution and soluble compounds trapped in the voids of particles. The larger the volume of water and the longer the time of soaking, the more complete will be the extraction of soluble contaminants of fiber.

The same principles are true for acetone washes used to remove residual lipids (fats) from the fiber residue. Simply washing the outside of particles with acetone will not extract all the lipid. Both the time and amount of clean acetone are important. A minimum of 20 mL of acetone for 2 minutes (5 minutes preferred) is needed Do not add acetone before all rinse water has been removed. Although this will occasionally improve filtering, it does not remove detergent or detergent solubles from residues. Adding acetone before water washing is complete will give inflated fiber values.

It is especially important that all traces of acid be washed from ADF residues and filtration vessels. With crucibles it is desirable to rinse the underside of the crucible, and with filter paper it is wise to rinse the edges of the paper. If residual acid remains, it will migrate to the edges of particles and become concentrated during drying. The concentrated acid will char the fiber or filter paper during drying. Charring signifies oxidation and loss of organic matter resulting in low residue weights.

The removal of acid from the ANKOM Filter Bags is also important. The utilization of four hot, fresh water rinses should sufficiently remove the acid. However, the water of the fourth rinse

may be checked by the use of litmus or pH paper during the final minute of the rinse to ensure the acid's removal.

Drying and Weighing Fiber Residues. Filtration vessels with fiber residue should be placed in the oven all at one time at the end of the day. This prevents moisture from wet vessels placed in the oven from contaminating vessels that have been dried in the oven. Vessels should remain in the oven (100-105°C) until they achieve a constant dry weight. This normally takes 8 hours or overnight drying.

Residual acetone from the ADF and NDF filtration vessel should be removed as completely as possible either by vacuum or allowing to stand in an operating hood before placing the filtration vessels in an oven (to avoid an explosion).

Weighing technique is critical for obtaining dry weights of fiber residues. If too many filtration vessels are placed in the desiccator at one time, if the desiccator lid is held open during transfer from the oven or weighing, or if the desiccant is the wrong type or is not changed often, dry weights obtained using a desiccator are incorrect regardless of the oven temperature or drying time. If the hot weighing technique is being used, one needs to be consistent in the use of this technique.

With Filter Bag Technology, generally a large number of test portions are extracted at the same time. If the bags are placed in a desiccator after drying, each time the lid or door is opened to remove a bag, moist, ambient air is introduced. Because the desiccator is opened up to 24 times, the moisture can more readily affect the remaining bags. If a collapsible, ANKOM desiccant pouch is utilized, the air can be pushed out of the pouch each time a Filter Bag is removed. This will eliminate the introduction of large amounts of moist air that could affect the remaining Filter Bags and allow for a more accurate and precise result.

Calculation and Dry Matter Errors. Although it is rare, laboratories have been known to have errors in the equations used to calculate results. The most common source of discrepancies in fiber results among labs is due to differences in dry matter estimates and the variation associated with adjusting fiber values to a dry matter basis.

Determining Fiber in Difficult-to-Filter Materials

Any test solution that takes more than 10 minutes of filtration time under vacuum should be discarded because the results will be inaccurate. Instead, rerun the test material using one of the following modifications. Several modifications can be used on any or most materials that are difficult to filter:

1. Reduce the test weight amount. This will increase the errors associated with weighing but it often is the best approach to use with difficult materials.

2. Use filter aids.
   - Glass wool (about 0.25 g) or glass microfibre filter mats (Whatman GF/D, 4.25 cm) will keep gelatinous materials and ash or fine residues from plugging the fritted disk of the crucible.
   - Celite, Diatomaceous earth, acid washed, Celite 545 AW, or equivalent. Make sure that the Celite used is washed with acid and ashed at 525 ± 15ºC before use, otherwise there can be some weight loss obtained from the Celite.
3. Back-flush the crucible by removing, then reinserting it into the crucible holder to force air back through the fritted disk.

High Fat Materials. Pre-extract materials containing >5% fat with a suitable solvent such as acetone to remove some of the lipids before fiber analysis. If there is a presence of fat globules floating on the surface of the solutions or the wash water, repeat the analysis by first pre-extracting the fat.

High Starch Materials. Starch is a major cause of filtration problems during NDF analysis. A milky or opaque appearance of the neutral detergent solution indicates high starch. If filtration is difficult, add additional amylase solution to the crucible. Many times this will unplug the fritted disk and allow filtration. Shorten soaking times to the minimum to keep soaking solutions as hot as possible >85°C.

High Pectin, Mucilage, or Glycoprotein Materials. Pectic substances are suspected if fiber residue has a glossy, translucent sheen and filtration becomes more difficult with each water soak. Fiber residues from these materials must be kept hot to filter readily. Decrease soaking time to a minimum and keep rinse water at boiling temperature. Preheat the crucible by filling it with hot water before beginning to transfer the residue. Do not let residues settle in the beaker before transferring to the crucible; instead transfer as quickly as possible. Adding glass wool, glass filter mats, or Celite to the crucible helps to keep the gelatinous residue from plugging the filter. Adding acetone before the last water wash has been completely removed (less than 5 mL of water remaining in the crucible) can salvage some samples, but recognize that acetone will precipitate any residual detergent in the residue.

High Ash, Fecal, or Digesta Materials. Fecal materials can be especially difficult to filter. It appears that fine particles in these residues plug the pores of the filtering vessel and slows or prevents evacuation. Using microfibre filter mats or Celite is usually essential to the determination of NDF in these materials. Filtration also can be enhanced by allowing the residue to settle in the beaker for 1-2 minutes after it has been removed from the refluxing apparatus and carefully decanting the liquid from the beaker with minimal transfer of particles to the crucible. It helps to slowly transfer the liquid under vacuum in a way that does not cover the entire surface of the filter mat. If the crucible begins to plug during the washing step, carefully scrape the surface of the mat to provide a new surface for filtration. Patience and minimum vacuum during the transfer step are important in obtaining accurate results with these materials.

Factors Resulting in Variation in Total Dietary Fiber Analyses

The techniques used for the quantitative analysis of dietary fiber can be divided into two groups, enzymatic / gravimetric - and enzymatic / fractionation - methods. For routine analysis of dietary fiber the enzymatic/gravimetric approach is more convenient and less expensive. TDF, IDF and SDF are determined gravimetrically after treatment with different enzymes according to AOAC approved methods. The fractionation methods are mostly used for research purposes, where individual fiber monomers can be characterized. After enzymatic incubation the dietary fiber constituents are quantified by HPLC, GLC or colorimetric.

There are a number of traditional AOAC methods available for measuring insoluble dietary fiber (IDF) (AOAC 991.42 & 991.43) and soluble dietary fiber (SDF) (AOAC 991.43 & 993.19), but these methods cannot measure all non-digestible carbohydrates with a degree of polymerization (DP) < 10. A method (AOAC 2011.25) currently is available for the measurement of IDF, SDF, & TDF to include resistant starch and the water:alcohol-soluble non-digestible oligosaccharides and polysaccharides of DP >3. This method combines the key attributes of AOAC 985.29 and its extensions (AOAC 991.42 & 993.19), AOAC 991.43, AOAC 2001.03, and AOAC 2002.02. Duplicate substrates are incubated with pancreatic alpha-amylase and amyloglucosidase for 16 hours at 37°C while mixing to maintain continuous suspension. Non-resistant starch is solubilized and hydrolyzed to glucose and maltose. The reaction is terminated by pH adjustment and heating. Protein in the test material is digested with protease. For the measurement of IDF, the digestate is filtered and the IDF is determined gravimetrically after correction for protein or ash in the residue. For the measurement of water-soluble but water:alcohol-insoluble dietary fiber (SDFP), ethanol is added to the filtrate of the IDF. The precipitated SDFP is captured by filtration and determined gravimetrically after correction for protein or ash in the precipitate. Non-precipitable, water:alcohol-soluble dietary fiber (SDFS) in the filtrate is recovered by concentrating the filtrate, deionizing through ion exchange resins, concentrating, then quantifying by liquid chromatography or, alternatively, by concentrating the filtrate and simultaneously deionizing and quantifying by liquid chromatography. This method quantifies, for all practical purposes, all components of dietary fiber present in a substrate.

Many of the factors causing variation in dietary fiber are similar to those in the crude and detergent fibers. The following are some of the issues vital to the dietary fiber testing process.

High Fat Materials. Pre-extract materials with a suitable solvent to remove some of the lipids in products with a fat content > 10% before fiber analysis.

Difficult Filtering. Filtration times of more than one hour are not uncommon. Techniques to reduce long filtration times are reducing the test portion weight or scraping the top surface of the diatomaceous earth bed. Higher filtration time frames tend to produce inconsistent and artificially high fiber values.

Agitation During Digestion. Most water baths do not provide sufficient agitation during the enzymatic digestion. This reduces the solubilization of non-fibrous components which causes artificially high and inconsistent fiber values.

Waterbath Temperature Control. The dietary fiber procedures have very specific temperature requirements. Temperature is fairly easy to control. However, not all commonly used waterbaths are capable of maintaining the higher temperatures required by the procedures.

Single Test Portion Process (or splitting fiber residues). Dietary fiber procedures call for the duplication of each test material due to the fact that the fiber residue requires both an ash and protein correction. To increase throughput, it is not unusual for a lab to analyze only a single test portion and then split the fiber residue in half with one half for ash correction and the other half for protein correction. This splitting introduces error which leads to higher variability in results.

Technician Variability. Dietary fiber analysis is much more labor-intensive and has many more steps (> 40) in the testing process than do crude or detergent fiber. Each additional step provides opportunity for variation from technician to technician. A specially trained analyst with excellent technical skills is critical for accurate and precise results. Variation can occur in each step of this multi-step process. Seven to 15 labs participated in the interlaboratory evaluation of this method, and results were sufficiently robust for AOAC official method status to be conferred.

Quality Assurance/Quality Control

It is imperative that laboratories validate or verify any and all fiber method(s) in their own laboratory and in the hands of their own chemists before placing it in the production mode. Please refer to Section 5.4 of the *AAFCO 2014 Quality Assurance Quality Control Guidelines for Feed Laboratories* (available at http://www.aafco.org/Publications/QA-QC-Guidelines-for-Feed-Laboratories) for greater detail on selection and in-house verification and validation of methods. The verification or validation should be repeated periodically and especially when training new personnel or installing new equipment.

The inclusion of quality control checks is vital to monitor systematic and random error in fiber methods. Section 5.9 and Table 3 of the *AAFCO 2014 Quality Assurance Quality Control Guidelines for Feed Laboratories* (available at http://www.aafco.org/Publications/QA-QC-Guidelines-for-Feed-Laboratories) provides a listing of quality control checks frequently used in feed laboratories. For fiber methods, routine quality control checks should include a laboratory reagent blank, laboratory control sample(s) and incorporation of replicates. The laboratory should have procedures in place for evaluating quality control results and dealing with results that are unacceptable or non-conforming.

Sources of laboratory control materials that have consensus values are:

- Animal feed with values for CF, ADF and NDF – AAFCO Proficiency Testing Program Animal Feed Scheme http://www.aafco.org/Laboratory/Proficiency-Testing-Program

- Forage with values for ADF and NDF – NFTA (National Forage Testing Association) Check Sample http://foragetesting.org/index.php
- Pet food with values for CF -- AAFCO Proficiency Testing Program, Pet Food Scheme http://www.aafco.org/Laboratory/Proficiency-Testing-Program
- Soybean and distillers dried grains with values for CF – AOCS (American Oil Chemists Society) laboratory proficiency program https://www.aocs.org/attain-lab-services/laboratory-proficiency-program-(lpp)/laboratory-proficiency-program-series
- AACC (American Association of Cereal Chemists) Check Sample Program for dietary fiber http://www.aaccnet.org/resources/checksample/Pages/default.aspx

Other quality controls are maintaining a log of reagent preparations and amylase standardization. Check the normality and pH of each batch or lot of solutions and adjust as needed. Determine activity of amylase stock solutions every 6 months during storage and adjust amylase working solutions accordingly.

General References:

Mertens, D.R. 1992. *Critical conditions in determining detergent fibers*. Proc. NFTA Forage Analysis Workshop, Sept. 16-17, Denver, CO. pp. C1-C8.
http://www.foragetesting.org/lab_procedure/appendix/appendixF.htm

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