Dietary Fiber Analysis: Challenges of Automation

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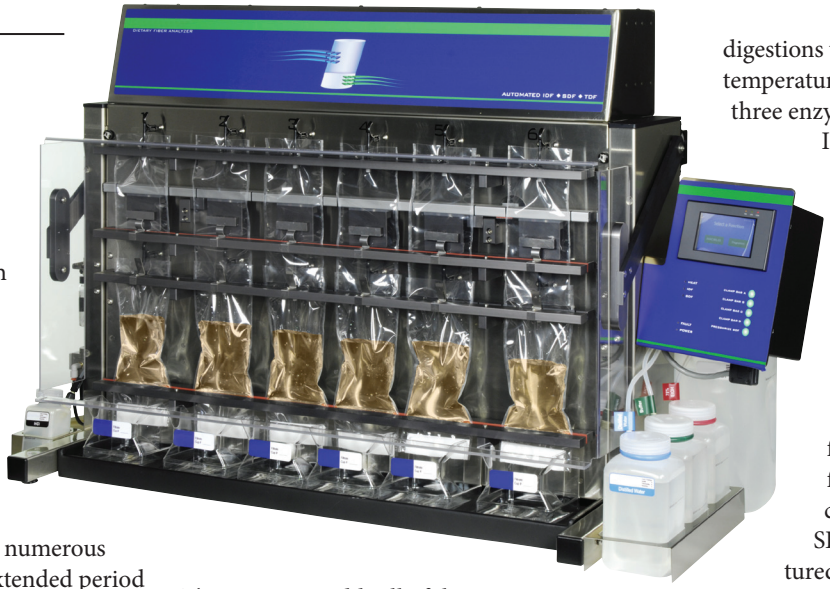

As more is understood about the human health benefits of dietary fiber (DF), interest in high-fiber foods continues to grow. This increased interest has created a greater demand for analysis of fiber in foods. Given that the current methods for determining DF include numerous manual steps that take an extended period of time to complete, there is significant interest in automating the process. Toward this end, an automated instrument has been developed that eliminates most of the labor-intensive aspects of the conventional method.

There are numerous official DF methods based on enzymatic gravimetric processes that use beakers, water baths, and vacuum-assisted filtration. AACC International Approved Method 32-07.01/ AOAC Method 991.43 (1) was chosen as the best candidate for automation because it is widely used and recognized as one of the most effective methods for determining DF. The fact that a portion of the fiber is precipitated during the analysis required a radically different design approach.

To automate this type of a method, alternatives to conventional glassware were necessary. Glass beakers were replaced with flexible vessels that have integrated polymer filters instead of beakers and fritted glass crucibles. These changes in the apparatus significantly improved the transfer and filtration of samples, enabling the process to be completely automated. The only significant technician action in the process is to weigh the test samples and filter vessels and insert them in the instrument. The computer adds all of the necessary chemicals at the proper times while continuously heating and mixing the samples. The digestion times and process temperatures are monitored and controlled by the computer. Incorporating a flexible filter vessel design allows the instrument to filter samples automatically, while virtually eliminating plugging. This new automated instrument produces the same digestion and filtration conditions as the reference method and produces equivalent DF values.

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Method Background

AACCI Approved Method 32-07.01 is designed to simulate the enzymatic digestion that occurs in the gastrointestinal tract of humans. Using this method, insoluble DF (IDF), soluble DF (SDF), and total DF (TDF) can be determined. This method was chosen over the earlier AACCI Approved Method 32-05.01/ AOAC Method 985.29 because it removes one pH adjustment and eliminates the coprecipitation problem experienced with the phosphate buffer. Although it is an improvement, AACCI Approved Method 32-07.01 still requires a series of enzymatic digestions with two changes in temperature and pH. Following three enzyme incubations, the IDF fraction is isolated by filtration through a vacuum-assisted filtering crucible with a diatomaceous earth filter mat. The filtrate is then mixed with heated ethanol to precipitate the SDF fraction. After waiting for flocculation to occur, the precipitated SDF is subsequently captured by a second filtration step. Both the IDF and SDF fractions caught in the filters are rinsed numerous times using heated water and/ or ethanol. Throughout the method eight different solutions are carefully measured and added to the sample. During the enzymatic digestion phase the samples are continuously agitated at temperatures of 95°C and then at 60°C.

This multistep method requires technicians to perform more than 35 manual steps that include numerous transfers and filtrations. Skilled technicians are required to ensure the process is done consistently and correctly. Plugged filters often waste time and energy. Space requirements for the conventional method are also large due to the amount of glassware and the multiple water baths that are required. Because the filtration rates of some samples are slow, the process tends to bottleneck, causing interruptions in consistent process timing. Some of the largest errors occur during the points in the filtration process where filters frequently plug and rinsing of the fiber is incomplete. It is not uncommon for samples to take considerable time to filter, causing technicians to

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leave the vacuum-assisted filtration and perform other work while waiting for the liquid to drain. Slow filtration is typically mitigated by scraping the surface of the diatomaceous earth to expose new filter area. This action may cause problems, however, if the scraping is too deep and the glass frit is exposed. Likewise, the diatomaceous earth mat may be disturbed if the liquid is transferred to the filter too quickly. Anytime the filtering surface is compromised there is a potential for low values. Automating this method eliminates numerous errors associated with technician variability, increasing the accuracy and precision of DF determinations.

The Challenge of Automation

DF analysis has been performed by analytical chemists for more than 30 years. During this time, instruments have been developed that offer some improvements over standard glassware systems. These instruments have focused on reproducing the conventional beaker and filtration process in a more compact and user-friendly system. However, they still require technicians to manually transfer liquids and use vacuum-assisted filtration. The benefits of such instruments to technicians is marginal. An automated instrument that performs the DF analysis method without significant technician involvement has not been successfully accomplished until now. In 2007 research and development began on an automated instrument that could perform DF analysis with strict adherence to all aspects of the reference method to guarantee equivalent results.

Computer-controlled instruments have shown great benefits over time for controlling processes and performing repetitious tasks, including accurate delivery of chemical solutions and monitoring temperature and time. However, steps in analytical methods that require the judgment of a technician are difficult to replicate using an instrument. Even simple observational tasks that are performed by technicians can be a challenge to automate. An example of this in the DF reference method is the transfer of fiber from the beaker to the filter and the subsequent filtration to separate the liquid from the solids.

The problems encountered when trying to automate the beaker and filtration process are not unique to fiber methods. Some of the most common analyses done in laboratories today involve extracting or digesting a sample in a beaker using heat and agitation. The resulting mixture of liquid and solids then needs to be separated through some type of filter. This quantitative transfer of a liquid/solid solution from a beaker to a filter, while drawing a vacuum, is relatively simple for a technician. Whether rinsing the wall of the beaker or adjusting the rate of vacuum, the observational skills of technicians are essential. Engineers have not found a reliable way to replace the visual input of technicians for this task. Have you ever seen a robot scrape the filter mat when it plugs? Although it is theoretically possible to reproduce these human faculties with machinery, it is impractical. Therefore, an entirely different approach to solving the beaker and filtration issue was needed.

Fig. 1. Dual-compartment insoluble dietary fiber filter bag has an upper chamber for enzyme digestion and a lower section for filtration.

Dual-Compartment Filter Chamber

Since the reference DF method is gravimetric, the transfer steps are critical. Any fiber that is left behind will result in erroneously low values. However, the method only allows two rinses with 10 mL of water each. Any additional water would require dilution at 4:1 with ethanol to maintain the proper ratio necessary for the precipitation phase. The limited amount of rinsing makes a mechanized transfer unreliable. As a result, a dual-compartment filtration vessel was developed that has a digestion chamber (formerly a beaker) integrated with a filter (Fig. 1). This vessel, called an IDF filter bag, contains an upper chamber constructed from a lightweight, flexible film that is connected to a lower filter section constructed from a polymer. The entire filter bag is used once during the analysis and then consumed in the required protein or ash corrections. To keep the liquid or sample in the upper chamber during the digestion phase, the instrument temporarily seals the bag by clamping it closed above the filter. After the digestion step is completed, the clamp is opened, and the liquid/solid mixture is released directly into the filter. Instead of using a vacuum, pressure is used to push the liquid through the filter. One benefit of this immediate transfer is that the liquid solution does not cool, which would allow dissolved components to precipitate. Likewise, the prescribed 70°C water rinses are performed immediately, reducing the risk of undesirable precipitation. The walls of the upper digestion chamber are washed uniformly with water rinses according to the reference method and then with ethanol rinses. The combination of water and ethanol rinses forces almost all of the fiber to wash down to the filter. By incorporating the digestion chamber and the filter, particles that fail to wash down are still accounted for because the entire bag is weighed. The invention of a consumable filter bag solved numerous difficulties associated with automating this method.

Although there are benefits to the dual-compartment filter bag, it also creates new challenges. Heating and mixing the solution in the filter bag requires a new approach. The reference method does not

provide detailed requirements on agitation. It only specifies that "continuous agitation" be used. Although shaker water baths are typical, the rate and type of agitation can vary between laboratories. Because the rates at which components dissolve are directly related to agitation, standardization would be beneficial. The design that evolved uses heated paddles to mix and heat the samples. By constructing the upper chamber of the filter bag from a thin walled, flexible polymer, it is possible to conduct heat from the paddle into the solution. Sensors are embedded in the paddles to provide temperature feedback and maintain consistent temperatures of up to 95°C. Accomplishing the proper conditions for this analysis without penetrating the bag was important to reduce the complexity of the design. With no mixing bars inserted in the digestion chamber, there is no risk of fiber entrapment or obstruction of rinsing.

SDF Filter Bag

The same efficiencies accomplished using a filter bag during the digestion phase of the method were achieved by constructing a filter bag for the soluble fraction precipitation phase. The SDF filter bag is constructed in a fashion similar to the IDF bag, but its upper chamber is larger, and the filter section is constructed from a different polymer. The opening of the SDF bag is large enough to allow the bottom of the IDF filter to fit inside. This ensures the filtrate is quantitatively transferred into the upper chamber of the SDF filter bag without the need for assistance from a technician (Fig. 2). Heated 95% ethanol is pre-inserted into the upper chamber of the SDF bag so it will immediately mix with the filtrate. The temperatures required for each phase of the analysis are easily maintained because each sequential step is automatically completed without delay. As a result, this process may be more reliable than the manual method because of the inevitable interruptions experienced by most technicians. After the precipitation phase is completed, a lower clamp separating the upper chamber from the filter is released, and the liquid is allowed to pass into the filter and drain. Once again pressure is used to force the liquid through the filter. The filtrate is captured in individual containers should further analysis be desired. After a series of rinses, the SDF filter bag is removed, dried, and weighed. Protein corrections are performed by either cutting the filter open and pouring out the residue with the diatomaceous earth or by consuming the entire filter section. To determine the ash correction, the entire bag is consumed.

Fig. 2. Transfer of digested sample filtrate into the soluble dietary fiber filter bag for the precipitation phase.

Filter Design

Filtration equivalence with the reference method, relative to particle retention, was considered one of the most critical aspects of the filter design. The development of a filter with a superior flow rate was central to the design and function of the entire instrument. It was imperative to improve the filtration process to eliminate the clogging problems associated with the small surface area of conventional filtration crucibles. The new filter needed to be flexible and able to be attached to the polymer film of the upper chamber. The filter also needed to be lightweight and chemically inert and contain negligible amounts of nitrogen and ash.

The reference method calls for the use of 60 mL coarse fritted glass crucibles (or equivalent) precoated with diatomaceous earth to reduce porosity. The successful design of new filters required a consistent, fine porosity that achieved the same size particle retention. Studies were conducted to establish the size of the particles retained in the reference method's filter, and the results were used to design the new filters. Due to the different characteristics of the liquids being filtered, two different polymer matrices were chosen for the IDF and SDF filters. The filter matrix used in the IDF bag requires a less hydrophobic polymer because of the aqueous solution that passes through the filter. Filter clogging is caused by limitations in the fill capacity of the filter—the finer the filtering pores the lower the flow rate and the easier it is to plug. By designing a more three-dimensional filter, surface area is increased, and liquid flow rates are enhanced. Some polymers are more hydrophobic and would not produce the desired flow rates for the aqueous solution. The final design resulted in a larger filter surface area and demonstrated performance that was superior to the crucibles used in the reference method. Both filter matrices were designed with a consistent pore size, high flow-through rate, and acceptable fill capacities. Consistently retaining all fine particles and greatly reducing the time needed for the liquid to pass through makes manual intervention unnecessary. Even a slow filtering sample,

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Table I. Results of Youden's ruggedness study of dietary fiber analysis using AACC International Approved Method 32-07.01[a]

Variable	Level 1 (Low)	Level 2 (High)	Cereal			Oat Bran			Carrot		
			Low	High	Difference	Low	High	Difference	Low	High	Difference
Insoluble dietary fiber (IDF)											
Sample size	0.44–0.46 g	0.54–0.56 g	36.8	36.9	0.05	8.4	8.3	–0.10	19.5	19.4	–0.14
Amylase temp.	85°C	97°C	36.9	36.8	–0.13	8.6	8.1	–0.52	20.1	18.8	–1.28*
AMG pH	4.2–4.4	4.8–5.0	37.0	36.7	–0.33	8.5	8.2	–0.36	19.5	19.5	0.01
IDF water rinses	2	3	36.9	36.8	–0.16	8.3	8.3	–0.03	19.6	19.3	–0.25
EtOH 78% rinses	2	4	36.8	36.9	0.05	8.2	8.4	0.18	19.5	19.4	–0.09
EtOH/water ratio	3.5:1	4.5:1	36.8	36.8	0.01	8.2	8.4	0.19	19.4	19.5	0.14
Flocculation time	30 min	60 min	36.9	36.8	–0.03	8.4	8.3	–0.13	19.4	19.5	0.11
Soluble dietary fiber (SDF)											
Sample size	0.44–0.46 g	0.54–0.56 g	3.6	3.5	–0.10	8.9	9.2	0.33	4.7	4.8	0.11
Amylase temp.	85°C	97°C	3.5	3.7	0.20	9.0	9.1	0.03	4.1	5.4	1.31*
AMG pH	4.2–4.4	4.8–5.0	3.4	3.8	0.38	8.8	9.4	0.59	4.7	4.8	0.08
SDF water rinses	2	3	3.5	3.6	0.06	9.2	9.0	–0.20	4.8	4.7	–0.11
EtOH 78% rinses	2	4	3.8	3.4	–0.36	9.2	8.9	–0.28	4.7	4.8	0.05
EtOH/water ratio	3.5:1	4.5:1	3.5	3.7	0.19	9.0	9.1	0.11	4.7	4.7	0.00
Flocculation time	30 min	60 min	3.6	3.5	–0.05	9.1	9.0	–0.08	4.9	4.6	–0.27

[a] Values followed by an * are significantly different at $P < 0.01$.

such as oat bran, generally filters in less than 5 min.

Ruggedness

Throughout the design of the automated instrument, it was imperative that all variables in the reference method be clearly understood. Therefore, in addition to studying agitation and filtration, variables

Table II. Comparison of results for oat bran analysis using the AACC International dietary fiber (DF) reference standard (method 32-07.01) and the ANKOM automated analyzer

	Insoluble DF	Soluble DF	Total DF
Reference standard			
Average	8.7	7.7	16.7
Standard deviation	1.01	1.00	1.13
Automated analyzer			
Average	7.8	8.3	16.8
Standard deviation	0.44	0.41	0.1

such as digestion temperature, pH, ethanol concentration, sample size, and rinse methods, as well as others, were thoroughly investigated. In addition to studying the general ruggedness of these variables, a statistical procedure, Youden's ruggedness test, was used. This statistical test allows seven variables at two levels to be evaluated using eight studies. The objective is to use two levels that have an acceptable range for the conditions specified by the method. Some of the results of the ruggedness studies are presented in Table I. The results indicate that the method was generally rugged within the two levels evaluated. The only variable that showed a significant effect was the amylase digestion temperature when it was lowered to 85°C (the reference method requires 95–100°C). Both the IDF and SDF results for carrots were significantly different ($P < 0.01$). The IDF results were higher, and the SDF results were lower. This was probably due to soluble fiber not being dissolved completely during the digestion phase and being captured in the IDF fraction. This demonstrates that even a drop in temperature of 10 degrees Celsius can produce erroneous results.

Comparative Results

Table II presents data comparing results using the automated instrument with reported results using the AACCI reference standard (Approved Method 32-07.01) for analysis of oat bran. Results produced by the instrument were consistent with the average reported results from multiple laboratories using the reference standard. When directly measuring TDF, the instrument results agreed with the reference standard. The IDF results produced by the instrument were generally lower, while the SDF values were higher. As noted earlier, this may be due to more soluble fiber being dissolved and then recovered in the SDF fraction.

Numerous other sample types have been evaluated and also show results consistent with the reference method.

Conclusions

AACCI Approved Method 32-07.01/AOAC Method 991.43 was successfully automated from enzymatic incubations and IDF filtrations through SDF precipitation and subsequent SDF filtration. The successful design was accomplished by solving a variety of problems associated with the multiple manual steps included in the reference method. The key to solving these problems and enabling automation was the invention of the flexible, convertible dual-chambered vessel in the shape of a bag. Composed of an upper reaction section and a lower filter section that are temporarily sealed to separate them, the filter bag enables automatic transfer. By having the reaction and filtration occur in one vessel, gravimetric fiber determinations are made possible. The automated instrument adheres to the specifications of the reference method, producing accurate and precise DF analysis results. It should be noted that the design of the system for DF lends itself to automation of other analytical methods. Presently, the current design is also being applied to determining DF using AACCI Approved Method 32-45.01 protocols.

Reference

1. AACC International. *Approved Methods of Analysis*, 11th ed. Method 32-07.01. AACC International, St. Paul, MN, 2012.

Andrew R. Komarek is the owner and president of ANKOM Technology Corp., a manufacturer of analytical instruments that serves, among others, the food, feed, and biofuel industries. Since founding the company in 1986, his primary focus has been on automating labor-intensive analytical methods. Personally leading the research and development of new products over the last 25 years, Andrew has been awarded numerous U.S. and international design and technique patents. Since developing filter bag technology in 1993, ANKOM has sold proprietary products such as the A2000 fiber analyzer, the XT15 fat extractor, and the RF gas production system in more than 95 countries. Andrew holds a B.S. degree in mechanical engineering from the University of Buffalo. Andrew is an AACC International member and can be reached at akomarek@ankom.com.