DETERMINING FIBER IN CEREALS

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Fiber was determined on cereal samples by three methods: crude fiber, acid-detergent fiber, and a new buffered acid-detergent fiber. Results by the new method are the highest, since less cellulose is lost from the sample. The method appears to remove most of the starch and protein from the samples.

Recent interest in the nutritional function (in the diets of humans) of the fibrous component of foods of plant origin has prompted a new look at methodology used to determine food fiber. Virtually all the fiber data in the literature were produced by the crude fiber method, which originated in the early nineteenth century (1) and has had only minor modifications since its development. Crude fiber is essentially the residue left after sequential hot digestion with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions. This determination underestimates, in varying degrees, the amount of material left undigested by humans (2). Therefore, it is generally agreed that the crude fiber

value cannot be considered an accurate estimate of dietary fiber, but is only a rough indicator.

Dietary fiber is variously defined in the literature as the remnants of vegetable cell walls which are not hydrolyzed by alimentary enzymes of man (3); as unavailable carbohydrate and lignin (4); and as plant material which is resistant to digestion by the secretions of the human gastrointestinal tract (5). From these definitions, dietary fiber can be considered a complex entity; and estimation by chemical methodology becomes limited to the relation between fibrous components isolated by chemical reagents and the indigestible residue.

Southgate (6) developed a fractionation procedure for isolating the main components of cereal fiber, which are cellulose, hemicellulose, and lignin. The method is time-consuming but does provide data on the composition of fiber. Van Soest (7) and Van Soest and Wine (8) studied the use of detergents in both acid and neutral solutions to determine fiber in feeds and forages for animal nutrition. According to Van Soest (7), fiber isolated by acid-detergent solutions contains cellulose and lignin; and fiber isolated by neutral-detergent solutions contains cellulose, hemicellulose, and lignin. However, filtering and removal of starch and protein are more difficult by the neutral-detergent fiber method, especially in samples of high starch content. The Van Soest (7) method for acid-detergent fiber and lignin has been accepted as an official method for feed by the AOAC (9). The acid-detergent fiber method is a rapid procedure and has been found to approximate values for cellulose and lignin as determined by the Southgate method (10). However, the acid-detergent fiber method uses 1.0*N* sulfuric acid in the detergent solution and also tends to give low estimates of the fiber of food samples. Thus, a method was sought with hydrolytic action milder than sulfuric acid, but more efficient than neutral solutions. A modified procedure was developed by using a hydrochloric acid-potassium chloride buffer solution as a solvent for the detergent. The buffer solution is a much less corrosive reagent than 1.0*N* sulfuric acid, and is within the pH range of the human stomach digestive medium. This paper reports the development of the modified acid-detergent fiber procedure and the results obtained on cereal samples analyzed by three methods.

MATERIALS AND METHODS

Samples of hard red winter wheat milling fractions, processed bran, rolled oats, xylan, and commercial cellulose were analyzed by the crude fiber method, the acid-detergent fiber method, and the buffered acid-detergent fiber procedure. Procedures for the crude fiber and acid-detergent fiber methods are found in the official methods of the AOAC (9).

Buffered Acid-Detergent Fiber Procedure

Reagents. Prepare a buffer solution of pH 1.5–2.0 by mixing 260 ml 0.2*M* HCl and 1000 ml 0.2*M* KCl. Dissolve 20 g hexadecyltrimethylammonium bromide in 1 liter of buffer solution.

Apparatus. Refluxing apparatus similar to that used for crude fiber determinations, Berzelius beakers, gooch crucibles with asbestos mat and vacuum filtering device, desiccator with desiccant equal to or better than 4–8 mesh Drierite.

Procedure. If sample contains more than 2% fat, extract with petroleum ether before determining fiber. Weigh 1-g sample, ground to pass 1-mm screen, into a Berzelius beaker; add 75 ml buffered acid-detergent solution; mix sample into suspension; and heat to boiling on a hot plate in 4–5 min. Transfer beaker to a well-controlled hot plate set to boil solution very gently. Cover beaker with a watch glass or condenser and gently reflux for 1 hr. Remove beaker from refluxing apparatus and vacuum-filter hot solution through tared gooch crucible with asbestos mat. Wash fiber with 50–60 ml hot water and finally with 25–30 ml acetone. After fiber has been sucked dry by vacuum, dry crucible and fiber in oven at 110°C overnight. Cool in desiccator and weigh. Calculate percentage of fiber to dry basis as follows:

$$\frac{W_2 - W_1}{S} \times 100 = \% \text{ fiber}$$

where W_2 = dried weight of crucible, asbestos mat + fiber,
W_1 = dried weight of crucible and asbestos mat, and
S = oven-dried sample weight.

TABLE I

Comparison of Fiber Determinations by Three Methods

Sample	Crude Fiber %	Acid-Detergent Fiber, %	Buffered Acid-Detergent Fiber, %
HRW			
Wheat	2.72	3.24	4.09 ± 0.18[a]
Bran	10.21	12.96	17.03 ± 0.36
Shorts	5.89	8.33	11.18 ± 0.39
Low-grade flour	0.42	0.46	0.60 ± 0.02
Patent flour	0.14	0.0	0.06 ± 0.05
Germ	2.63	2.92	4.26 ± 0.02
Processed bran	8.59	9.45	18.18 ± 0.29
Rolled oats			
1	1.82	1.76	4.02 ± 0.39
2	2.13	1.86	4.98 ± 0.23
3	1.63	2.17	5.96 ± 0.54
4	1.57	1.81	3.36 ± 0.13
5	1.80	1.86	3.38 ± 0.16
6	1.48	1.94	3.18 ± 0.27
Commercial cellulose	71.0	82.5	97.5 ± 1.70
Hemicellulose (xylan)	0.7	0.8	11.8 ± 0.9

[a]Standard deviation.

The protein contents of the samples and the fiber were determined by the Kjeldahl method (9). Protein was calculated by using nitrogen × 5.7 for wheat and its products, and nitrogen × 6.25 for the rolled oats samples.

RESULTS AND DISCUSSION

The results obtained by the three methods are compared in Table I. The acid-detergent fiber procedure, as developed by Van Soest (7), generally produced higher values than the crude fiber method, although the differences between the two methods were less on the rolled oats samples. Results by the buffered acid-detergent fiber procedure were much higher. Values are the average of three determinations with standard deviations shown. The standard deviations for the buffered acid-detergent method are within the range of those generally encountered in the crude fiber and acid-detergent fiber methods. A recent collaborative study of the acid-detergent method reported a mean duplicate error of 0.40 ± 0.31 (11). A survey of the AACC National Check Sample Service results for the years 1972 through 1974 shows standard deviations for crude fiber determination ranging from 0.28 to 0.68.

The buffered acid-detergent method does not remove as much of the cellulose from the sample as the other two methods do. This is shown by the commercial cellulose sample, where recovery was 71.0% in the crude fiber method, 82.5% in the acid-detergent fiber method, and 97.5% in the buffered acid-detergent procedure. Recovery of the hemicellulose, xylan, was 0.7, 0.8, and 11.8%, respectively. The loss of cellulose is believed to be caused by the sulfuric acid and alkali used in the crude fiber method and the 1.0*N* sulfuric acid used in the acid-detergent fiber method. It is generally known that hemicellulose is at least partly hydrolyzed by both acid and alkaline solutions, which explains the loss of xylan in all three procedures.

The hydrochloric acid-potassium chloride buffer apparently serves as well as sulfuric acid in hydrolyzing starch and assisting the detergent in protein hydrolysis and dissolution. A sample of wheat starch subjected to the procedure left no residue, and the small residue left from the flour samples indicates that the buffered detergent solution is capable of removing most of the starch from the samples.

When subjected to the new procedure, a sample of crude gluten containing 89.4% protein left a fiber residue of 0.5% which contained 0.6% protein. Over 99% of the gluten was dissolved. Table II shows protein residues in the buffered acid-detergent fiber compared to the protein content of the sample. Ninety-five per cent or more of the protein is removed from all the samples except four of the processed samples. The higher protein residue in the fiber of these samples may be explained by the effect of heat in the processing of the cereals. Van Soest (12) studied the effect of heating and drying on the yield of fiber and lignin in forages, and found that temperatures above 50° C significantly increased the yield of acid-detergent fiber. The effect is attributed to the nonenzymatic browning reaction. It is also generally known that toasting and drying of cereals cause polymerization of proteins and cross-linking which affect the availability of the proteins to digestion (13). It can also affect protein dissolution by acid-detergent. Therefore it is possible that part of the protein in processed cereals may be bound to the cellulose and lignin and become part of the fiber. Even in unprocessed

TABLE II
Protein Residue in Buffered Acid-Detergent Fiber Compared with Protein Content of Sample

Sample	Sample Protein %	Protein Residue in Buffered Acid-Detergent Fiber, %	Protein Removed from Sample, %
HRW			
Wheat	12.3	0.3	97.6
Bran	16.3	0.8	95.1
Shorts	18.0	0.7	96.1
Low-grade flour	12.4	0.2	98.4
Patent flour	10.5	<0.1	99.9
Germ	31.9	0.2	99.3
Processed bran	11.2	3.9	65.2
Rolled oats			
1	16.3	1.3	92.1
2	17.1	2.5	85.4
3	23.9	3.4	85.8
4	17.9	0.9	95.0
5	17.9	0.9	95.0
6	19.1	0.8	95.8
Wheat gluten	89.4	0.6	99.3

products, some natural structural binding may occur. In Table II, note the higher protein residue in the fiber from bran as compared to the fiber from wheat and germ. The question of whether these bound constituents should be included in dietary fiber will have to be resolved by human nutritional studies. Digestibility determinations have shown values considerably higher than crude fiber estimations of indigestible materials (2). While some of this difference may be attributed to the loss of cellulose and hemicellulose in the fiber determinations, some of it may also be caused by the unavailability of part of the protein, either through processing or natural structural binding.

Although some of the hemicellulose is still lost in the new procedure, this modified acid-detergent fiber method is an improvement over the use of strong sulfuric acid solutions. The buffered acid-detergent solution removes most of the starch and protein, while breaking down very little of the cellulose, and thereby produces more realistic values for cereal fiber. Recent human studies by Southgate (4,14) have shown some apparent digestibility of hemicellulose and, to a lesser extent, cellulose. As knowledge of the fate of hemicellulose and cellulose in the digestive tract increases, the relationship of fiber isolated by chemical methodology to dietary fiber will be more clearly understood.

Loss of lignin is probably minimal, since lignin withstands treatment with 72% sulfuric acid in the isolation procedures of Van Soest (7) and Southgate (6). Therefore, it should be little affected by the buffer solution.

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