

## Two Issues in Dietary Fiber Measurement

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As a member of the AACC Board of Directors, I was asked to read and comment on the "The Definition of Dietary Fiber" report prepared on behalf of the AACC Dietary Fiber Definition Committee. While I completely agree with the definition, there are two topics covered in the report that I would like to specifically address: the importance of enzyme purity and activity and the measurement of resistant starch.

### Enzyme Activity and Purity

Of these topics, the easiest to deal with is the importance of enzyme purity and activity. As a scientist actively involved in polysaccharide research over the past 25 years, I have come to appreciate the importance of enzyme purity and specificity in polysaccharide modification and measurement (7). These factors translate directly to dietary fiber (DF) methodology, because the major components of DF are carbohydrate polymers and oligomers. The committee report published in the March issue of CEREAL FOODS WORLD refers only to the methodology for measuring enzyme purity and activity (8) that led up to AOAC method 985.29 (2). In this work, enzyme purity was gauged by the lack of hydrolysis (i.e., complete recovery) of a particular DF component (e.g.,  $\beta$ -glucan, larch galactan, or citrus pectin). Enzyme activity was measured by the ability to completely hydrolyze representative starch and protein (namely wheat starch and casein). These requirements and restrictions on enzyme purity and activity were adequate at the time the method was initially developed and served as a useful working guide. However, it was recognized that there was a need for more stringent quality definitions and assay procedures for enzymes used in DF measurements.

For example, accurate measurement of  $\beta$ -glucan (as a component of the dietary fiber complex) was impeded for many years by the presence of a cellulase contaminant in the amyloglucosidase used in total dietary fiber (TDF) assays. Although this contamination resulted in a 10–20% underestimation of  $\beta$ -glucan, its significance was not recognized by the recovery experiments that have been traditionally used to check enzyme purity. Possibly, analysts simply assumed that this was a limitation of the method. In fact, when amyloglucosidase preparations essentially devoid of cellulase are used, the recovery of  $\beta$ -glucan is quantitative, demonstrating the robust nature of the TDF assay procedure. Simple viscometric and dyed substrate-based procedures have been developed for assaying cellulase ( $\beta$ -glucanase) (6), and their use helps ensure that cellulase contamination of amyloglucosidase is not a problem. The potential for introducing contaminating enzyme activities will become more pronounced as the TDF procedure is adapted to accommodate the measurement of "new" DF components. For example, inulin (fructan) measurement requires the use of a specific procedure (AOAC methods 997.08 and 999.03 [2] and AACC Ap-

proved Method 32.32 [1]) because it is only partially precipitated by alcohol in the official TDF procedures. This, in turn, requires that all fructan in the alcohol insoluble residue in the TDF assay is removed. Quemener and coworkers (9) recommended the use of a heat-treated, commercially available, industrial enzyme preparation with inulinase activities at the amyloglucosidase treatment step of the TDF procedure. This preparation removes the fructan, but it also contains a very active cellulase that is not inactivated by the heat treatment, which results in hydrolysis and subsequent under estimation (by as much as 50%) of the  $\beta$ -glucan present in the sample. McCleary (6) discussed other examples of problems caused by variations in enzyme activity, specificity, and purity. Undoubtedly, other problems will arise in the future. For this reason, there is a need for accurate and reliable procedures to assay enzyme purity and activity, particularly when analyzing for a diverse mixture of substances that are classified as dietary fiber.

### Resistant Starch

The committee report includes RS as a DF. RS is a natural component of plant materials. It can be the product of "purposeful synthesis" by selecting maize varieties that produce high-amylose starches or by cooking processes aimed at inducing retrogradation and crystallization. As a natural component, RS should not be grouped with such chemically produced materials as methyl cellulose, hydroxy propyl methyl cellulose, or starch ethers and esters. Given the important role that RS plays and will continue to play in our diet as a DF, I think it should be listed as a separate major grouping, rather than being grouped under the heading "Analogous Carbohydrates."

In the past, the measurement of RS has caused considerable problems and debate. Should, for instance (as proposed in the committee report), RS be simply considered that proportion of starch that is measured in the official AOAC/AACC DF methodology? Alternatively, should it be treated more seriously, and should an attempt be made to measure the amount of starch that is physiologically significant as DF (i.e., the amount that is "resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine")? If the latter is the objective (as I think it should be), then RS should be measured either by in vivo methodology, using ileostomy subjects, or by the intubation procedure. Alternatively, one could use in vitro procedures that simulate, as closely as possible, in vivo tests and that yield results in line with the currently available in vivo data across a range of samples. Such in vitro tests have been developed by Berry (3), Englyst and coworkers, Champ and coworkers, and Goni and coworkers (Champ and coworkers [4]).

If RS is measured by a procedure such as that of Berry (3) or Champ (4), how will the values obtained be incorporated into TDF values? How will the residual starch measured in the AOAC/AACC TDF procedure be accounted for or removed? In terms of removal, there are two options: pretreatment of the sample with dimethyl sulfoxide (DMSO) or KOH (followed by neutralization) before  $\alpha$ -amylase treatment, to ensure complete hydrolysis and removal of starch. DMSO pretreatment may be preferred, because

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it has limited effects on the other polysaccharides present (other than increased solubilization). KOH treatment alters pectins and breaks diferulate linkages in certain polysaccharides, such as arabinoxylans, leading to altered recovery of these components. The choice of method is an interesting challenge.

An alternative approach, and one under intensive investigation in our laboratory, is to make minor alterations to the official TDF procedure so it will more accurately measure and reflect the real RS content of a particular sample. At the time the seeds of the current official method were being sown, the aim of the methodology was to completely remove protein and starch. We now know that protein removal is not complete, resulting in the need to measure the protein content of the assay residue. It is now also well known that treatment with  $\alpha$ -amylase at 95–100°C does not remove all of the starch, particularly for the newly designed high-amylose starches. Furthermore, with resistant starches, the measured level of DF (AOAC/AACC methodology) is significantly affected by the level of  $\alpha$ -amylase used (6). This result alone indicates that it is possible to fine-tune the official method so the determined levels of DF for resistant starches more accurately reflects data obtained from in vivo studies. We have proposed that the use of lower incubation temperatures for  $\alpha$ -amylase, more in the range of the gelatinization temperature of most "normal" starches, could yield DF values for RS more in line with in vivo data. To this end, a range of RS samples was analyzed by AOAC method 985.29 (AACC Approved Method 32.05 [1]) with  $\alpha$ -amylase incubations at 95–100°C (standard), 80°C, and 70°C. The results obtained have been compared with values obtained using an in vitro assay employing elements of the methods of Champ and coworkers (4) and Goni and coworkers (5) (using pancreatic  $\alpha$ -amylase and amyloglucosidase at pH 6, 37°C, 16 hr of incubation with shaking) (McCleary and Monaghan, unpublished). From the data shown in Table I, it is evident that there is good agreement between DF values obtained at 70°C and those obtained with the pancreatic  $\alpha$ -amylase in vitro assay. Moreover, the results are in good agreement with in vivo data.

#### Improving Accuracy of Measurements

In conclusion, I believe that it is essential that we actively pursue analytical methodology that will accurately measure the component of starch that is resistant to digestion and absorption in the ileum. There may be a need for two formats. One of these would be for natural (fruits and vegetables) and cereal products, both analyzed in the form in which they are to be consumed.

**Table I. Total Dietary Fiber (TDF) and Resistant Starch (RS) Values Determined for a Range of Starch Samples\***

Sample details	TDF (%)			RS (% g/g sample) (Method E)
	95–100°C	80°C	70°C	
High-amylose starch				
Sample A	25.7	35.3	49.5	52.0
Sample B	23.0	35.5	55.0	51.7
Retrograded high-amylose starch				
Sample A	51.4	61.7	58.8	45.7
Sample B	32.3	37.2	39.2	42.0
Sample C	34.3	37.1	40.0	40.9
Potato amylose	29.6	33.9	36.1	44.2
Regular maize starch	0.0	0.4	0.5	0.5

\* TDF values were determined using AACC Approved Method 32.05 (AOAC method 985.29) (1,2) with the modification that  $\alpha$ -amylase incubations were performed at 95–100°C (standard method), 80°C, and 70°C. RS was determined using a modification of the Berry, Champ, and Goni procedures. Samples were incubated in a shaking water bath for 16 hr with optimized levels of pancreatic  $\alpha$ -amylase and amyloglucosidase at pH 6.0 (McCleary and Monaghan, unpublished). High-amylose starch samples A and B are commercially available materials, as are retrograded high-amylose starch samples A, B, and C.

A second format for products that will be processed before consumption may also be required. The important issue presently is that we recognize the problems associated with the accurate measurement of resistant starch and that research efforts in this area continue.

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