To the Editor,

I read with interest the editorial message from Barry McCleary, AACC International president, concerning the definition and assay of dietary fiber in foods (Cereal Foods World 56(5):103, 2011). The editorial reads, in part,

In line with the recently adopted (2008) definition of total dietary fiber by Codex Alimentarius, a new integrated total dietary fiber method was developed by AACC Intl. members and validated through both AACC Intl. (Method 32-45.01) and AOAC International (Method 2009.01). This method has just received Type I classification by the Codex Committee on Methods of Analysis and Sampling (CCMAS) in Budapest (March 2011). This is the only dietary fiber method that allows accurate measurement of all dietary fiber components, including resistant starch and nondigestible oligosaccharides.

McCleary in Analytical and Bioanalytical Chemistry (389:291-308, 2007) assayed the levels of resistant starch (RS) in various samples of starches, resistant starch ingredients, legumes (raw), and cooked foods using the integrated total dietary fiber method and the resistant starch methods of AACC Intl. (Method 32-40.01) and AOAC Intl. (Method 2002.02). He showed that the integrated procedure gave low recovery of two granular (Type 2 RS) resistant starches, potato and green banana; both of which have been found to be 90–100% resistant to digestion in ileostomy patients (Englyst et al., British Journal of Nutrition 75:749-755, 1996). On the other hand, the integrated method gave high recovery of resistant starch from high-amylose maize (Hylon VII) (RS2).

The origin of digestion resistance in granular starches, such as those from potato and green banana, is not known. It may be that a protective “shell” (lack of surface pores) near the periphery of the granule serves as an impenetrable barrier to amylase. Thus, minor damage near the granule surface would permit enzymes to enter the granule, where digestion then occurs. Evidence of a protective “shell” in potato starch was the almost total destruction of its resistant starch content when its digest was stirred (4% resistant starch) at 38°C for 16 hr, as opposed to when its digest was shaken (77% resistant starch) (McCleary and Monaghan, Journal of AOAC International 85:665-675, 2002). Cross-linked resistant starches (Type 4 RS), produced commercially from potato or wheat starches, also are granular resistant starches, and they have been shown to possess a number of physiologic benefits (Thompson et al., Cereal Chemistry 88:72-79, 2011, and references therein). It appears likely that cross-linked zones in granular resistant starches prevent access of amylases to a disproportionately high level of unprotected starch molecules.

The gelatinization properties of cross-linked RS4 made from wheat starch indicates these granules are more friable than normal. Physical damage to granular resistant starch could occur in several steps of the integrated method, including freeze-drying, grinding, and agitation during digestion. In addition, the level of amylase activity (based on total digest volume) in the integrated total dietary fiber method has been increased (α-amylase + 65%, amyloglucosidase + 10%) compared with the level used in AACC Intl. Method 32-40.01 or AOAC Intl. Method 2002.02 for resistant starch. With some classes of resistant starch, digestion conditions may cause loss of resistant starches, as well as “slowly digestible” starches, that may survive the digestion conditions in other dietary fiber methods (e.g., AACC Intl. Method 32-07.01 or AOAC Intl. Method 991.43). In contrast to samples containing physically sensitive granular resistant starch, samples containing highly associated or retrograded amylase, such as RS3 type or high-amylose granular starches, when assayed for total dietary fiber by the integrated method, gave dietary fiber levels that matched the levels of resistant starch determined by AACC Intl. Method 32-40.01 or AOAC Intl. Method 2002.02. Apparently, the structural features that generate resistant starch in high-amylose starches and RS3 types, operate on a nano- rather than microscale level, so that physical damage to the “source” of resistance is much less likely than for granular resistant starch.

The compromises entailed in using a single integrated procedure for dietary fiber likely will be discussed at the workshop on fiber scheduled for October 16 at the 2011 AACC International Annual Meeting in Palm Springs, CA, which is mentioned in AACC Intl. President McCleary’s editorial. It appears that foods containing resistant starch in the form of fragile granules, or perhaps those containing amylase-lipid complexes (RS5 type), should be assayed for total dietary fiber by methods with mild grinding and mild digestion conditions, consistent with removal of rapidly digestible starch. Less harsh conditions would result in increased recovery of resistant starch and some “slowly digestible” starch. Both of these fractions are thought to be desirable in foods.

P. A. Seib
Professor Emeritus
Department of Grain Science and Industry
Kansas State University, Manhattan, KS, U.S.A.

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1 Consultant to MGP Ingredients Inc.
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Response to Sieb’s Letter

Advances in our understanding of the complexity of dietary fiber and that food components such as resistant starch, fructans, polydextrose, and resistant maltodextrins are part of dietary fiber in the diet has led to an updated definition being adopted by the Codex Alimentarius Commission along with an updated total dietary fiber method (McCleary, Analytical and Bioanalytical Chemistry 389:291-308, 2007; AOAC Intl. Method 2009.01 and AACC Intl. Method 32-45.01).

This reclassification of dietary fiber poses significant analytical challenges. Low molecular weight soluble dietary fiber (SDFS—the resistant oligosaccharides that do not precipitate in the presence of 78% aqueous ethanol) is recovered and analyzed by HPLC. Resistant starch is measured as part of insoluble dietary fiber (IDF). Measurement of resistant starch is complicated by the fact that additional types of resistant starch are being produced and catalogued under the general heading of “resistant starch.” The term “resistant starch” was first introduced by Englyst et al. (European Journal of Clinical Nutrition 46:S33-S50, 1992) and was subdivided into RS1 (physically trapped starch as found in coarsely ground or chewed cereals, legumes, and grains), RS2 (resistant starch granules or nongelatinized starch granules that are highly resistant to digestion by α-amylase until
gelatinized [e.g., uncooked potato, green banana, and high-amylose starch], and RS₃ (retrograded starch polymers, mainly amylose, that are produced when starch is cooled after gelatinization). More recently, Brown et al. (*Food Australia* 50:603-622, 1998) introduced the classification RS₄ to cover chemically modified and cross-linked starches, and the classification RS₅ has been introduced for amylose-lipid complexes.

RS₁, RS₂, RS₃, and RS₄ are all native starches and, thus, under the appropriate conditions can be enzymically hydrolyzed to dextrins and ultimately to glucose, allowing quantitation by this methodology. RS₄, in contrast, is very resistant to enzymic hydrolysis, even if it is initially dissolved or dispersed in 2M potassium hydroxide. The question that should be asked is if RS₄ should actually be classified as starch, let alone as resistant starch. At what point is a polymer referred to as a “chemically modified polysaccharide” rather than as the native polysaccharide—especially if it has few properties in common with the native polysaccharide?

In developing the resistant starch method (McCleary and Monaghan, *Journal of AOAC International* 85:665-675, 2002; AACC Intl. Method 32-40.01 and AOAC Intl. Method 2002.02), the aim was to have a robust in vitro procedure that gave results for resistant starch that are in line with results obtained by in vivo methods. For a dietary fiber method to accurately measure resistant starch, it was concluded that the method should be based on the steps and procedures in the resistant starch method. However, some changes had to be introduced. First, sample size had to be increased from 0.1 to 1.0 g so there would be enough residue weight to measure gravimetrically. Second, a purer pancreatic α-amylase was required to minimize its contribution to residue weight and to remove the lactose carrier found in many preparations. Amyloglucosidase had to be essentially devoid of cellulase and xylanase to prevent any depolymerization and loss of β-glucan and/or arabinoxylan in cereal samples during the extended incubation. Third, a heating step had to be introduced into the method to denature protein in the sample, allowing its depolymerization by protease and subsequent removal from the insoluble fraction at the alcohol precipitation step.

Of course, incubation of an aqueous suspension of the sample at elevated temperature will result in solubilization of some resistant starches, particularly in raw potato and green banana. In AOAC Intl. Method 985.29 (Prosky method) and other methods employing thermostable α-amylase, this solubilized resistant starch is hydrolyzed and completely removed. In the integrated dietary fiber method (AOAC Intl. Method 2009.01 and AACC Intl. 32-45.01), in which pancreatic α-amylase is used, this enzyme is inactivated at a temperature lower than that required to gelatinize the starch. Also, the incubation is performed at a pH where amyloglucosidase has essentially no activity. Consequently, the solubilized resistant starch remains intact, and most (but not all) is precipitated upon addition of ethanol to 78% (vol/vol) and is recovered in the IDF fraction. For example, potato starch, which analyzes as 64.9% resistant starch when using the AACC resistant starch method (Method 32-40.01), gives a value of 56.8% when using the integrated procedure and a value of just 0.9% when using the traditional dietary fiber procedure (AOAC Intl. Methods 991.43 and 985.29).

One thing is very clear—when analyzing the dietary fiber content of resistant starch-containing samples, the samples must be analyzed, as much as possible, “as eaten.” If foods containing native potato or banana starches are cooked or exposed to physical shear, most of the resistant starch is rendered nonresistant.

As stated by Paul Seib, the amount of pancreatic α-amylase used per weight of sample in the integrated dietary fiber method (AACC Intl. Method 32-40.01) is higher that used in the resistant starch method (AACC Intl. Method 32-45.01); however, the increase is 33%, not 65% as stated in his letter. The level of amyloglucosidase did not change, contrary to the comment in his letter. This increase in α-amylase concentration has no effect on the measured value of resistant starch, as shown in Figure 2 in McCleary and Monaghan (*Journal of AOAC International* 85:665-675, 2002). Pancreatic α-amylase levels of 5–20 mg/mL (15–60 Ceralpha units/mL) showed essentially the same hydrolytic patterns for resistant and nonresistant starches. The authors simply considered it more prudent to incorporate the higher levels to guard against possible loss of enzyme activity with prolonged storage. In our laboratory, we have not analyzed RS₅ (amylose-lipid complexes), so we cannot comment on the effects of sample handling, e.g. milling and freeze-drying, for such materials. However, neither freeze-drying nor milling through a 0.5- or 1.0-mm screen has had any effect on the resistant starch or starch damage values for any food or starch samples we have analyzed. Clearly, it would make a difference for values for coarsely milled grain samples.

In the measurement of a mixture as complex as dietary fiber, especially now with the inclusion of resistant starch and nondigestible oligosaccharides, compromises have to be made in analytical methodology. The strengths and limitations of the integrated dietary fiber method will form the basis of a lively discussion at the fiber workshop on October 16 at the 2011 AACC International Annual Meeting in Palm Springs, CA. I hope to see you there.

Barry McCleary
AACC International President
CEO, Megazyme International Ireland
Bray, Co. Wicklow, Ireland