

Appendix C

Development and Evolution of Methods Used to Extract and Measure Dietary Fiber

Two general types of methods have been developed for isolating and analyzing dietary fiber: enzymatic-gravimetric and enzymatic-chemical. The food components isolated vary depending on the method used. Both the enzymatic-gravimetric and enzymatic-chemical methods have undergone a number of modifications and improvements, most occurring over the last 20 years. The enzymatic-gravimetric approach attempts to reflect the material that enters the large intestine by removing starch, protein, and fat and obtaining a residue that is then dried and weighed. A correction is made for any remaining protein and ash, and the result is expressed as a proportion of the starting material. The enzymatic-chemical approach chemically characterizes the carbohydrate content of fiber after the removal of available carbohydrate (monosaccharides, disaccharides, and starch) and fat. A number of different procedures have been developed to enable carbohydrates to be measured as their constituent monosaccharides or as groups of monosaccharide types. The current available methods and their various formats and major modifications are outlined in Tables 2 and 4.

ENZYMATIC-GRAVIMETRIC METHODS

The gravimetric approach began with the measurement of crude fiber, developed at the Weende Research Station in Germany in the latter half of the nineteenth century (Henneberg and Stohmann, 1860). The method comprised of treatment of plant material with acid and alkali, resulting in a residue. The method became well established during the early part of the twentieth century,

and a modification of the original method was later adopted by the Association of Official Analytical Chemists International (AOAC) as a method for measuring fiber in animal feeds (AOAC, 1995). The crude fiber method was used for the determination of the fiber content for the U.S. Food Composition tables in the 1970s (Watt, 1976). It continues to be used in some regions of the world as well as the animal feed industry. However, its usefulness is severely limited by the loss of all soluble polysaccharides, some insoluble polysaccharides, and some lignin, and the inclusion of some nitrogenous material in the remaining residue.

In the 1960s, Van Soest and colleagues introduced the use of detergents to solubilize protein. The Acid Detergent Fiber (ADF) method, which was adopted for animal feeds, utilizes strong acid to hydrolyze all polysaccharides except cellulose and lignin, which are therefore the only components in ADF (Van Soest, 1963). Other cell wall polysaccharides are not included in this method, limiting its usefulness for human nutrition in the same way as crude fiber.

Recognizing the need to describe and include other cell wall constituents, Van Soest and Wine (1967) developed the Neutral Detergent Fiber (NDF) method, which measures all insoluble cell wall material. This proved to be a better predictor of the nutritional value of dietary fiber in animal feeds than crude fiber. In the 1970s, the use of the NDF method spread to human nutrition, but its utility remained limited because it did not include soluble fiber components nor did it remove all starch.

With growing interest in dietary fiber in human nutrition in the 1970s and the development of a physiological role for this dietary component, there was a need for an analytical method that measured insoluble cell wall and soluble fiber components. German researchers introduced the use of enzymes during the nineteenth century to remove available carbohydrate, and this approach was used by Williams and Olmsted (1935) in the United States in an effort to measure the indigestible material in a more physiological way in their human studies. Building on this work, a number of investigators, such as Asp and Johansson (1981), Furda (1977), Hellendoorn and colleagues (1975), and Schweizer and Würsch (1979), developed analytical approaches that reflected the "nondigested" fraction of the diet, including soluble material as well as insoluble components. Prosky and coworkers (1985) published a method that was based on the work of these various investigators, and it was subsequently adopted by AOAC as AOAC method 985.29. The method provides a measure of total dietary fiber by enzymatic removal of available starch and solubilization and extraction of a portion of the protein; the remaining residue is dried, weighed, and corrected for crude protein and ash contents. An initial step is added to remove fat if it is present at concentrations of 10 percent or more. The method is relatively rapid and easy to perform and has been automated to enable a large number of samples to be assessed. It has been adopted as an official method for dietary fiber analysis by many countries.

The method was extended to the determination of soluble and insoluble dietary fiber as the need to measure these components was recognized (Prosky et al., 1988, 1992, 1994). Other related methods were subsequently validated by AOAC collaborative studies and approved as official by AOAC. Lee and co-workers (1992) substituted MES-TRIS buffer in place of the original phosphate buffer, and in doing so, generated the new AOAC method 991.43. Li and Cardozo (1994) introduced a simpler method (AOAC method 993.21) for foods that contain little or no starch, such as fruits and some vegetables. Mongeau and Brassard (1993) took a somewhat different approach, using a modified NDF method to measure insoluble fiber and a new approach to analyze for the soluble fiber fraction (AOAC method 992.16).

As shown in Table 4, there are two ways of deriving soluble fiber: (1) by direct analysis (Mongeau and Brassard, 1993), and (2) by subtraction of insoluble fiber from total fiber (Englyst and Hudson, 1987; Quigley and Englyst, 1992).

ENZYMATIC-CHEMICAL METHODS

It was recognized early on that significant proportions of the carbohydrate, which are resistant to human digestive enzymes, are soluble in nature and lost when fiber is recovered by filtration. McCance and Lawrence (1929) developed a method for "unavailable carbohydrates" which involved reflux with strong acid, followed by colorimetric determination of reducing sugars and pentoses.

During the 1950s, Southgate continued to develop this chemical approach to fiber measurement, extending McCance and Lawrence's work by introducing a series of extraction steps followed by hydrolysis of polysaccharides and subsequent colorimetric analysis of component monosaccharides. He published his procedure for unavailable carbohydrates in 1969 (Southgate, 1969). Southgate recognized that a crucial step was the complete removal of starch since incomplete removal would result in overestimation of glucose-based dietary fiber. The Southgate method was modified for human nutrition during the 1970s and became incorporated in the United Kingdom nutrient tables in the Fourth Edition of McCance and Widdowson's *The Composition of Foods* (Paul and Southgate, 1978).

Although the method provided considerable information on monosaccharide groups (hexoses, pentoses, and uronic acids), Southgate recognized that a colorimetric assay did not distinguish individual monosaccharides, and recommended that gas chromatography (GC) or high-performance liquid chromatography (HPLC) be employed (Southgate, 1981). In addition, there remained difficulties with the removal of starch, which gave inflated values for many individual food items with high starch content such as starchy vegetables, legumes, and grains. Englyst and coworkers (1982) published a procedure extending Southgate's work for the measurement of nonstarch polysaccharides using GC. The method in-

volved more complete removal of available starch and allowed for determination of the different monosaccharides present as constituents of dietary fiber in food products. It also allowed for separation of cellulose from noncellulosic polysaccharides, and soluble from insoluble polysaccharides. Hence, the method provided considerable detail on the polysaccharide components of human foods.

Several modifications have been made to the 1982 Englyst method. One of these was the removal of resistant starch, which was identified in the early 1980s (Englyst and Cummings, 1984). Resistant starch consists of (1) starch that is not physically accessible to digestive enzymatic hydrolysis; (2) retrograded starch that has been rendered resistant to hydrolysis by processing or by cooking and cooling; and (3) uncooked starch in granules that is not accessible to enzymatic hydrolysis unless it is gelatinized by heating (Englyst et al., 1992a). Englyst and Cummings (1984) removed resistant starch from the nonstarch polysaccharide component in a method using dimethyl sulfoxide. Since resistant starch is created by cooking and processing, the method ensured that foods could be assessed using nonstarch polysaccharide values of ingredients by the use of recipes, and that each food product did not have to be individually measured to obtain an accurate value.

In response to criticism that the method was too time consuming, Englyst and Hudson (1987) developed an alternative colorimetric method for the measurement of the component monosaccharides. Englyst made the procedure faster in another modification, with a more rapid procedure for the removal of starch (Englyst et al., 1992b). HPLC methods were developed for the measurement of uronic acids (Englyst et al., 1994; Quigley and Englyst, 1994).

Prior to Englyst's work, Theander and Åman (1979) developed a chemical method that used GC to measure soluble and insoluble fiber components. This method was later modified to improve starch hydrolysis (Theander and Westerlund, 1986) and to measure the fiber-derived monosaccharides by HPLC (Shinnick et al., 1988). The procedure does not remove resistant starch and measures lignin separately as Klason lignin, material insoluble in 72 percent sulfuric acid (Goering and Van Soest, 1970). Originally, the method did not rely on ethanol precipitation of solubilized fiber components, but rather recovered them from the soluble fraction by dialysis with a molecular weight cutoff of 12,000 to 14,000 daltons. Subsequently, the procedure was simplified and made more rapid by precipitating solubilized fiber components with 80 percent ethanol (Theander et al., 1994).

COMPONENTS INCLUDED IN EACH METHOD OF ANALYSIS

A list of potential components of fiber included or not included in each analysis is provided in Table 2.

Nonstarch Polysaccharides

All the current methods include all nonstarch polysaccharides that precipitate in 78 to 80 percent ethanol. Polysaccharides that do not precipitate in ethanol are not included in any of the existing methods. Polysaccharides that are excluded by ethanol precipitation include inulin, other fructans, modified cellulose, and some arabinogalactans.

Lignin

All methods except those of Englyst (Englyst and Cummings, 1984; Englyst and Hudson, 1987; Quigley and Englyst, 1992) for nonstarch polysaccharides include lignin. In the enzymatic-gravimetric methods, this is included as part of the residue after filtration. In the enzymatic-chemical methods of Theander and coworkers (1994) and Southgate (1969), it is analyzed as a separate component, using the Klason lignin method (Goering and Van Soest, 1970). This method measures native lignin, but can also include tannins, cutins, and Maillard reaction products (Theander et al., 1995).

Resistant Starch

Resistant starch is not included in the Englyst methods for nonstarch polysaccharides (Englyst and Cummings, 1984; Englyst and Hudson, 1987; Quigley and Englyst, 1992), since it is removed using dimethyl sulfoxide. In all the other methods, a proportion of resistant starch is included in the analysis for dietary fiber, largely as retrograded amylose. However, this proportion of resistant starch is not constant for different foods made from the same ingredients, as retrograded amylose is created by cooking and cooling food and through food processing. Since resistant starch has many physiological properties similar to those of dietary fiber, there is a need for a uniform method for its analysis.

There are currently a number of methods available for measurement of resistant starch, although none have been submitted for evaluation by the approved methods process of the AOAC. Several of these were developed during the European Resistant Starch (EURESTA) program which involved 40 research groups in 11 countries, all involved in resistant starch research from 1990 to 1995 (Asp et al., 1996). Björck and colleagues (1986) reported the starch remaining in the dietary fiber residue from the Asp enzymatic-gravimetric procedure, using potassium hydroxide (Asp et al., 1983). Englyst and colleagues (1992a) calculated resistant starch as the difference between available starch and total starch. Muir and O'Dea (1992) developed a method based on more physiological influences, as did Åkerberg and colleagues (1998).

TABLE 4 Methods of Fiber Analysis

Reference (Method)	Procedure Type	Measures
Asp et al., 1983	Enzymatic-gravimetric	Soluble dietary fiber Insoluble dietary fiber Total dietary fiber
Craig et al., 2000 (AOAC 2000.11)	Enzymatic-ion exchange chromatographic	Polydextrose
Englyst and Cummings, 1984	Enzymatic-gas chroma- tographic	Total nonstarch polysaccha- rides Individual constituent sugars
Englyst and Hudson, 1987	Enzymatic-colorimetric	Soluble nonstarch polysac- charides, by difference Insoluble nonstarch polysac- charides Total nonstarch polysaccha- rides
Gordon and Ohkuma, in press (AOAC 2001.03)	Enzymatic-gravimetric liquid chromatographic	Total dietary fiber including low molecular weight re- sistant maltodextrins
Hoebregs, 1997 (AOAC 997.08)	Enzymatic-ion exchange chromatographic	Fructans
Lee et al., 1992 (AOAC 991.43)	Enzymatic-gravimetric using MES-TRIS buffer	Soluble dietary fiber Insoluble dietary fiber Total dietary fiber
Li and Cardozo, 1994 (AOAC 993.21)	Enzymatic-gravimetric (For foods and food products with $\leq 2\%$ starch)	Total dietary fiber
McCleary et al., 2000 (AOAC 999.03)	Enzymatic-spectrophoto- metric	Fructans
Mongeau and Brassard, 1993 (AOAC 992.16)	Enzymatic-gravimetric	Soluble dietary fiber Insoluble dietary fiber Total dietary fiber
Prosky et al., 1985	Enzymatic-gravimetric	Total dietary fiber
Prosky et al., 1992 (AOAC 991.42)	Enzymatic-gravimetric	Insoluble dietary fiber

Total Dietary Fiber Determination	Concerns
Calculated as the weight of fiber residue less the weight of protein and ash or calculated as the sum of soluble and insoluble fiber	
Not applicable	
Calculated as the sum of the monosaccharides	Does not estimate lignin
Sum of hexoses, pentoses, and uronic acids	Does not estimate lignin
Calculated as the sum of insoluble fiber, high molecular weight and low molecular weight soluble fibers	
Not applicable	
Measured by independent analysis or calculated as the sum of soluble fiber and insoluble fiber	
Calculated as the weight of fiber residue less the weight of protein and ash	
Not applicable	
Calculated as the sum of the soluble and insoluble fiber	
Calculated as the weight of fiber residue less the weight of protein and ash	
Not applicable	

continued

TABLE 4 Continued

Reference (Method)	Procedure Type	Measures
Prosky et al., 1994 (AOAC 993.19)	Enzymatic-gravimetric	Soluble dietary fiber
Quigley and Englyst, 1992	Enzymatic-high performance liquid chromatographic	Soluble nonstarch polysaccharides, by difference Insoluble nonstarch polysaccharides Total nonstarch polysaccharides Individual constituent sugars
Schweizer and Würsch, 1979	Enzymatic-gravimetric	Soluble dietary fiber Insoluble dietary fiber Total dietary fiber
Southgate, 1969	Enzymatic-colorimetric	Soluble dietary fiber Insoluble dietary fiber Total dietary fiber
Theander and Åman, 1979	Enzymatic-gas chromatographic	Insoluble neutral polysaccharides Soluble neutral polysaccharides Insoluble uronic acids Soluble uronic acids Klason lignin Total dietary fiber
Theander and Westerglund, 1986	Enzymatic-gas chromatographic	Insoluble neutral polysaccharides Soluble neutral polysaccharides Insoluble uronic acids Soluble uronic acids Klason lignin Total dietary fiber
Uppsala Method of Theander et al., 1995 (AOAC 994.13)	Enzymatic-gas chromatographic	Neutral polysaccharides Uronic acids Klason lignin Total dietary fiber

Total Dietary Fiber Determination	Concerns
Not applicable	
Calculated as the sum of the monosaccharides	Does not estimate lignin
Calculated as the sum of soluble and insoluble fiber	
Sum of hexoses, pentoses, uronic acids, and lignin	Incomplete removal of starch; not specific with respect to individual sugars (Theander and Westerlund, 1986)
Calculated as the sum of neutral polysaccharides, uronic acids, and Klason lignin	
Calculated as the sum of neutral polysaccharides, uronic acids, and Klason lignin	
Calculated as the sum of neutral polysaccharide residues, uronic acid residues, and Klason lignin	

Champ (1992) has published a method based on extensive use of amylase, resulting in a direct method for resistant starch, rather than by difference of total starch and residual starch. More recently, McCleary (2001b) modified and simplified the method (AOAC method 996.11) of Gofii and colleagues (1996), which closely reflects conditions in the small intestine. Comparisons between methods tend to produce similar results, although not for all types of foods (Champ et al., 2001; McCleary, 2001a). Digestion and absorption in the human gastrointestinal tract is a dynamic process in which many enzymes, including proteases and amylases, work in concert to disrupt the three dimensional and then the intermolecular relationships in foods. Macromolecules are hydrolyzed as they become exposed, and hydrolytic products are rapidly absorbed. The successful analytical method for resistant starch will be one that mimics this process in the human gastrointestinal tract, so that the analytically determined value reflects starch not assimilated in the human.

Oligosaccharides

Compounds of chain length less than 10 monosaccharide units are generally soluble in 78 to 80 percent ethanol, do not precipitate, and are not included in any of the analytical methods for dietary fiber. However, many of these behave physiologically in a similar way to polysaccharides, and hence there has been a need to analyze for them. Quigley and Englyst (1992) published an HPLC method for oligosaccharides from a number of sources, and methods have also been or are currently being developed for specific oligosaccharides, such as fructooligosaccharides (Hoebregs, 1997; McCleary and Blakeney, 1999; McCleary et al., 2000), and galactooligosaccharides (de Slegte, in press). The method for galactooligosaccharides has received AOAC approval (AOAC method 2001.02).

Fructans, Inulin, and Oligofructose

Inulin, a polymer of fructose found in a small number of vegetables, fruit, and grains, is soluble in 78 to 80 percent ethanol and is therefore not detected by any of the currently AOAC approved dietary fiber methods. The hydrolysis product of inulin is oligofructose, also called fructooligosaccharide, and is similarly soluble in ethanol and not included as fiber by any method for fiber analysis. Because of widespread interest in these compounds, a number of methods have been developed for their measurement in foods (Hoebregs, 1997; McCleary and Blakeney, 1999; McCleary et al., 2000). These entail hydrolysis to fructose and glucose, which are then measured by a variety of methods.

Polydextrose

Polydextrose is a synthesized polysaccharide created by thermal polymerization of glucose. It is not precipitated with 78 to 80 percent ethanol and is therefore not included in the analysis of dietary fiber by any of the existing methods. There are specific methods for the analysis of polydextrose, which have been developed and improved since the development of the polysaccharide. Of these, the most comprehensive is that using HPLC, which has recently gained AOAC approval (AOAC method 2000.11) (Craig et al., 2000).

Modified Cellulose

There are a number of modified cellulose compounds, such as methyl cellulose, carboxymethyl cellulose, and derivatives of these, which are soluble and do not precipitate in 78 to 80 percent ethanol. Several of these compounds are used as laxative agents and in a variety of food products such as salad dressings, icings and toppings, desserts, and baked goods (Sandford and Baird, 1983). They have many of the same physiological properties as dietary fiber.

Resistant Maltodextrins

Resistant maltodextrins are produced through various acid/pressure processes, are not susceptible to enzymatic hydrolysis, and have similar properties to fiber, but they do not precipitate in 78 to 80 percent ethanol and are therefore not included in any current analytical method for dietary fiber. Currently, there is an enzymatic-gravimetric-liquid chromatographic method for the analysis of resistant maltodextrins, and the method of Gordon and Ohkuma (in press) has gained the approval of the AOAC for its analysis (AOAC method 2001.03).

Chitin and Chitosan

Chitin and chitosan are polysaccharide-containing materials with chemical structures similar to cellulose and are derived mainly from the outer shell of crab and other sea creatures. Because of the high polysaccharide content, these materials may have physiological properties similar to dietary fiber. Some of these compounds are insoluble in 78 to 80 percent ethanol and therefore analyze as dietary fiber by all current dietary fiber methods. However, the proportion of the total that can be analyzed as dietary fiber depends on the manner and extent of processing of the source material prior to analysis.

Chondroitin

Chondroitin and chondroitin sulfate are polysaccharide-containing compounds found in connective tissues of animals, particularly blood vessels, bone, and cartilage. Some of these compounds precipitate in 78 to 80 percent ethanol and therefore analyze as dietary fiber by current methods.

Noncarbohydrate Components

Because of the nonspecific nature of the gravimetric methods, these methods include components that are not carbohydrate. Hence, the methods of Lee (Lee et al., 1992), Mongeau and Brassard (1993), Prosky (Prosky et al., 1985, 1988, 1992, 1994), and earlier versions of any of these include lignin, cutin, tannins, and Maillard reaction products as well as other less well-characterized compounds. The contribution of these components in most unrefined foods is very small, but processing can increase their presence through complexing in various ways. Maillard reaction products, for example, are generated through heating, and therefore application of heat through processing or cooking will increase their contribution to dietary fiber content. However, in most instances the increase in dietary fiber content caused by heat-generated Maillard reaction products is insignificant. The enzymatic-chemical methods of Englyst (Englyst and Cummings, 1984; Englyst and Hudson, 1987; Quigley and Englyst, 1992), Southgate (1969), and Theander (Theander and Åman, 1979; Theander and Westerlund, 1986; Theander et al., 1995) do not include these noncarbohydrate components in the analysis of polysaccharides because these procedures analyze carbohydrate directly. However, Klason lignin included in the dietary fiber values obtained by the Theander methods (Theander and Åman, 1979; Theander and Westerlund, 1986; Theander et al., 1995) include some tannins and Maillard reaction products.

SUMMARY

In some countries, isolated/purified fibers are specified as dietary fiber if they analyze as dietary fiber by the accepted fiber methods. Examples of substances that have been extracted from plant materials include cellulose, hemicellulose, gums, and pectins. These have traditionally been considered dietary fiber and are captured in the existing dietary fiber methods. Other unabsorbable carbohydrates have been chemically synthesized or made resistant through physical or chemical modifications and include resistant starch, resistant maltodextrin, polydextrose, and hydroxymethylcellulose. Some of these synthesized compounds may also appear naturally in foods, such as resistant starch. Other unavailable carbohydrates that may have physiological effects, such as inulin,

are not measured by most accepted dietary fiber methods and have not been included as dietary fiber. The technology is available to synthesize an infinite number of these food components. Unavailable carbohydrates with fiber-like properties may be manufactured de novo, modified, or isolated from existing fiber sources for incorporation into foods or supplements. The challenge of defining dietary fiber is that potential sources that may either meet chemical definitions or physiological endpoint requirements are expanding at a fast rate and challenge the existing methods to determine fiber.

