Measuring Dietary Fibre

by Barry V. McCleary

Interest in dietary fibre is undergoing a dramatic revival, thanks in part to the introduction of new carbohydrates as dietary fibre components. Much emphasis is being placed on determining how much fibre is present in a food. Linking a particular amount of fibre to a specific health benefit is now an important area of research.

Total Dietary Fibre

The term "dietary fibre" first appeared in 1953 and referred to hemicelluloses, celluloses and lignin (1). In 1974, Trowell (2) recommended this term as a replacement for the no longer acceptable term "crude fibre." Burkitt (3) has likened the interest in dietary fibre to the growth of a river from its first trickle to a mighty torrent. He observes that dietary fibre "was first viewed as merely the less digestible constituent of food which exerts a laxative action by irritating the gut," thus acquiring the designation "roughage", a term which was later replaced by "crude fibre" and ultimately by "dietary fibre." Various definitions of dietary fibre have appeared over the years, partly due to the various concepts used in deriving the term (i.e. origin of material, resistance to digestion, fermentation in the colon etc.), and partly to the difficulties associated with its measurement and labelling (4). The principle components of dietary fibre, as traditionally understood, are non-starch polysaccharides, which in plant fibre are principally hemicelluloses and celluloses, and the noncarbohydrate phenolic components, cutin, suberin and waxes, with which they are associated in Nature. In 1976, the definition

of dietary fibre was modified to include gums and some pectic substances, based on the resistance to digestion of these components in the upper intestinal tract. For the purposes of labelling, Englyst and collaborators (5) proposed that dietary fibre be defined as "nonstarch polysaccharides (NSP) and lignin in the diet that are not digested by the endogenous secretions of the human digestive tract." Methods were concurrently developed to specifically measure NSP (6).

An alternative, and generally accepted method for the measurement of dietary fibre, is AOAC Method 985.29 (7), which has been modified to allow measurement of soluble and insoluble components (8), and to allow the use of alternative buffers (AOAC method 991.43). In principle, samples are treated with petroleum ether (if necessary) to remove fat, and then with enzymes to depolymerize starch and protein, which are subsequently removed in an ethanol precipitation step. This method is the culmination of the work of several research groups over many years. A major difference between this method and the NSP procedure is that the AOAC procedure includes resistant starch. Resistant starch should be included under the "umbrella" of dietary fibre, because it behaves in a manner similar to other dietary fibre components, in that it resists digestion in the small intestine and it is largely fermented in the colon, producing short-chain fatty acids.

The successful application of AOAC Method 985.29 to the measurement of dietary fibre requires the use of highly purified

enzymes of defined activity. Although this has always been implied, specific details were not available until recently. In a detailed study of the enzymes used in AOAC Method 985.29, the author (9) defined the requirement for activity of the three enzymes used (α-amylase,

amyloglucosidase and protease) and identified contaminating activities which can lead to significant underestimation. Specifically, cellulase contamination of amyloglucosidase preparations gives significant underestimation of β-glucan, and the concentration of α-amylase employed in the assay will alter the hydrolysis of "resistant starch." Methods which can be used to check and standardize the enzymes employed, and to check for the presence of contaminating activities were described.

The classical methods for the analysis of dietary fibre do not determine inulin, oligofructose and other resistant oligosaccharides (RO) accurately or reliably, due to the fact that these components are not precipitated in the ethanol treatment step in the AOAC procedures. Consequently, modifications to AOAC procedures have been developed for the measurement of oligosaccharides such as oligofructose,

polydextrose and galacto-oligosaccharides. These specific procedures may involve analysis of the whole sample or aqueous-ethanol extracts of the sample. In either case, enzymic hydrolysis is employed, and the purity, activity and specificity of these enzymes are essential for accurate

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and specific analyses. AOAC Method 985.29 is ideally suited to general applications in the measurement of dietary fibre. However, for specific dietary fibre components, new or modified methods have been, or need to be developed. Specific examples of particular analytical requirements for the measurement of mixed-linkage β-glucan, resistant starch, oligofructose and inulins, galacto-oligosaccharides and polydextrose will be discussed.

Specific Dietary Fibre Components:

1,3:1,4 β-D-Glucan (Mixed-linkage β-glucan).

Plant-derived dietary fibre is generally considered to include all plant components except protein, non-resistant starch, lipids and low molecular-weight materials not precipitated by 80% ethanol. The first of this mixture

of components to attract individual attention was mixed-linkage ß-glucan (ß-glucan) from cereal grain sources, particularly barley and oats. The interest in this polysaccharide was catalyzed by the numerous health claims concerning the use in human nutrition of oat fibre products rich in ß-glucan (10). A specific procedure for the measurement of this component was developed in 1985 (McCleary and Glennie-Holmes (11) (Figure 2), and then further simplified in 1991 (12). In principle, ß-glucan in the sample is hydrated by cooking a slurry of the flour, followed by depolymerization with a specific 1,3:1,4 ß-glucanase (lichenase) enzyme. The oligosaccharide reaction products are then hydrolyzed to glucose with a highly purified ß-glucosidase, and the glucose is measured enzymically with glucose oxidase/peroxidase reagent. Since the procedure is used to measure trace levels of ß-glucan in cereal and food products, which also contain high levels of other glucose containing oligosaccharides (malto-oligosaccharides and sucrose), as well as polysaccharides such as starch, the enzymes need to be very pure. The procedure developed and supplied by Megazyme was adopted in 1986, and is now the International standard method for the measurement of ß-glucan (AOAC 995.16; AACC Method 32-23; ICC Standard No. 166; EBC Methods 3.11.1, 4.16.1 and 8.11.1). The method has been adapted for the analysis of ß-glucan in cereal and food products and in liquid materials such as wort and beer.

Resistant Starch

Until recently, it was generally thought that starch was completely digested in the small intestine. However, it is now recognized that there is a portion (resistant starch; RS) which resists digestion, passes into the large intestine and is fermented there. Three types of resistant starch have been identified, (I) physically trapped starch, (II) resistant starch granules and (III)

retrograded starch. Physically trapped starch granules are locked within the food matrix, such that digestive enzymes are prevented or delayed from having access to them. Resistant starch granules, as present in raw potatoes and green bananas, resist attack by α-amylase, which is probably due to the crystalline nature of the starch. From an industrial and food technology perspective, the most interesting resistant starches are those which are produced through retrogradation of gelatinized starches. Retrogradation is generally attributed to the amylose portion of the starch, so starches high in amylose are particularly amenable to the production of resistant starch.

Since resistant starch is defined as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (13, 14), any analytical method for RS should take into account all the starch and α-dextrins covered in this physiological definition. Furthermore, the method should be validated using *in vivo* data from healthy human subjects. The various procedures for the measurement of resistant starch have been summarized and discussed by Champ *et al.* (15). The methods can be broadly grouped into two approaches. In one approach (16, 17), samples are analyzed for total starch and for soluble starch, and the latter is subtracted from the former to give resistant starch. The major inherent problem with this approach, analytically, is that the content of resistant starch is generally a small proportion of total starch. Thus, the resistant starch value is obtained by subtracting one large analytical value (for non-resistant starch) from another similar large value (for total starch), meaning that analytical precision would be greatly reduced. In the second approach (18, 19), the sample is treated with starch degrading enzymes to remove the non-resistant starch, which is washed away from the residue. The residue is then analyzed. By defini-

tion, this approach must be analytically more precise. The procedure should be used in conjunction with AOAC Method 985.29 to determine total dietary fibre in the sample.

In most TDF procedures, the hydrolytic conditions are quite severe, resulting in underestimation of resistant starch. If the final total dietary fibre value is to be the sum of TDF determined by AOAC Method 985.29, and resistant starch determined by a method such as that of Champ (15, 19), then the amount of resistant starch measured in AOAC Method 985.29, must be allowed for or removed (otherwise it will be counted twice). A possible way to handle this is to pre-treat samples with DMSO prior to TDF analysis by AOAC Method 985.29. The DMSO treatment should dissolve all of the starch, allowing complete hydrolysis by α-amylase and amyloglucosidase, and subsequent removal in the alcohol treatment step. Of course, such a treatment will alter the ratio of soluble to insoluble fibre as determined by AOAC Method 991.43. A DMSO treatment step is used in AOAC Method 996.11 for the measurement of total starch (20).

Oligofructan and Inulin

Fructans are widely distributed in the plant kingdom. They are defined as any compound in which one or more fructosyl-fructose linkages constitute a majority of the linkages. They are generally classified as inulin, levan or graminan types, based on linkage types. In Nature, inulin occurs as a series of oligosaccharides with degrees of polymerization from 2 to 60. It consists principally of linear chains of fructosyl units linked ß(2-1), ending with a glucosyl unit. Inulin and oligofructose resist hydrolysis by human digestive enzymes, but are fermented in the large intestine. They thus have the same physiological action as dietary fibre.

In recent years, interest in the measurement of fructans such as inulin and oligofructose has been

stimulated by applications made to regulatory authorities for acceptance of fructans as dietary fibre components for food labelling purposes. Since fructans are largely soluble in 80% ethanol, they are not significantly measured in currently used dietary fibre methods. Several methods have now been developed for the specific measurement of fructans, and these rely on the complete hydrolysis of the oligofructose and inulin to fructose and glucose, which is measured either instrumentally or chemically. In some procedures, the separate quantities of fructan, sucrose, glucose and starch are determined by hydrolysis with specific enzymes, followed by chromatographic procedures (AOAC Method 997.08)(21). In a procedure developed in the author's laboratory (22), fructan is measured chemically, after removal of sucrose and starch through enzymic hydrolysis, followed by borohydride reduction. Sucrose is hydrolyzed with a specific sucrase that, unlike invertase, has no action on fructo-oligosaccharides (even as small as kestose), and starch and maltosaccharides are hydrolyzed with a combination of ß-amylase, pullulanase and maltase. The released glucose and fructose is reduced to sugar alcohols with borohydride, and the inulin and oligofructose are then hydrolyzed to fructose and glucose with a mixture of *exo-* and *endo-*inulinase enzymes. The released sugars are measured with a reducing-sugar method. The specific advantages of this method are that highly purified enzymes are used and the assay can be performed with basic laboratory equipment. The method has been successfully evaluated in an interlaboratory study, and has been adopted first action by AOAC INTERNATIONAL (999.03).

In the AOAC dietary fibre method (985.29) some inulin is precipitated with 80% ethanol. Thus, if total fructan is determined separately and added to the TDF value obtained by the

AOAC procedure, there will be an overestimate of the total dietary fibre content of the sample. Consequently, Method 985.29 should be modified to include a fructanase treatment (to depolymerize the higher degree of polymerization fructan) before the ethanol precipitation step. The fructanase enzyme preparation must be devoid of enzymes active on other dietary fibre components, such as ß-glucan, arabinoxylan and pectin. Quemener and coworkers (23) recommended the use of heat-treated Fructozyme® (Novo Nordisk). While this treatment removes most of the pectinase activity, ß-glucanase is not inactivated and use of this enzyme preparation results in significant depolymerization and underestimation of ß-glucan in DF samples (22). Enzyme contaminants in Fructozyme® can be removed chromatographically, and such a preparation is available commercially (22).

Polydextrose

Polydextrose is prepared by vacuum thermal polymerization of glucose, using sorbitol as plasticizer and citric acid as catalyst (24). This random polymerization and branching yields various types of glucosidic bonds in the structure with α-1,6 bonds predominating. The compound includes covalent linkages to sorbitol and citric acid, and has an average degree of polymerization of 12. Polydextrose is an approved food additive in over 40 countries. It is not hydrolyzed by normal human digestive enzymes, but it is fermented in the colon like many other fibres

Polydextrose is not quantitatively measured with AOAC Method 985.29 because, as is the case with other resistant oligosaccharides (RO), it is not completely precipitated with 80% ethanol. Because of the complex chemical nature of the compound, measurement cannot be achieved through specific enzymic hydrolysis. Consequently, analytical procedures depend on aqueous extraction of the sample

being analyzed, followed by enzymic hydrolysis of other oligosaccharide and polysaccharide materials that are likely to interfere in subsequent chromatographic quantitation. Highly purified enzymes active on starch, cellulose, pectins and gums (e.g. galactomannans), have been employed (24).

Galacto-oligosaccharides

Galacto-oligosaccharides [α-D-Glc$p$(1-1)-ß-D-Gal$p$-containing oligosaccharides) have been shown to be beneficial to human health through promotion of the growth of bifidobacteria in the large intestine. The oligogalactosylated glucoses are prepared from lactose through the transgalactosylating activity of ß-galactosidase. Various parameters, such as the source of the enzyme, substrate concentration, pH and temperature influence the transgalactosylation and thus, the final yields of the different products. Typically, the reaction products are composed of about 60% transgalacto-oligosaccharides (mainly tri-, tetra- and pentasaccharides) and about 40% of a mixture of lactose, glucose and galactose.

Alternative galacto-oligosaccharides are the galactosyl-sucrose oligosaccharides of plant origin, namely raffinose, stachyose and verbascose. These oligosaccharides consist of sucrose, to which an extending chain of 1,6 α-linked galactosyl residues are attached to C-6 of the glucosyl residue. Raffinose is a major by-product of sugar crystallization from sugar beet extracts and it has been shown to promote the proliferation of bifidobacteria in the human intestinal microflora.

Acceptance of galacto-oligosaccharides as soluble dietary fibre components requires the development of analytical procedures that will specifically measure these components in food mixtures. The lactose-derived ß-galacto-oligosaccharides are effectively hydrolyzed with *Aspergillus niger* ß-galactosidase,

while the galactosyl-sucrose oligosaccharides are rapidly and quantitatively hydrolyzed by *A. niger* α-galactosidase. The released sugars can be measured, either chromatographically, or using various colourimetric procedures. In both cases, the specificity of the procedure depends on the purity of the enzyme used. A procedure for the measurement of transgalacto-oligosaccharides is the subject of an upcoming AOAC International, interlaboratory study.

In conclusion, success in the introduction of "new" dietary fibre components will be dependent on identifying a specific health benefit of the component, as well as providing an analytical procedure that will allow accurate measurement of this component in complex food mixtures. ♦

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