Review

Evolution of a Definition for Dietary Fiber and Methodology to Service this Definition

Barry V. McCleary* and Jodi Cox

Megazyme, Bray Business Park, Southern Cross Road, Bray, County Wicklow, Ireland.

The definition of dietary fiber has been evolving over the past 70 years. The changing definitions reflect our better understanding of the types and physiological functions of dietary fiber. Initial definitions focused on “the remnants of the plant cell walls which are not hydrolyzed by the digestive enzymes of man” and appropriate analytical methods were developed and implemented. More recently, the role of resistant starch and non-digestible oligosaccharides (NDO) as dietary fiber components has been recognized. Incorporation of these has required the development of a host of other methodologies to measure specific dietary fiber components such as fructo-oligosaccharides, galacto-oligosaccharides, resistant maltodextrins, resistant starch and others. Having these specific methods is useful for product manufacturers, but not necessarily for regulators because some of the specific component may also be partially measured by the “gold standard” fiber method, the Prosky method (AOAC Method 985.29). It is thus not possible to simply sum the various specific components with the value obtained with AOAC Method 985.29, as this will lead to “double counting” and thus overestimation of fiber content. To resolve this problem, and allow measurement of all dietary fiber components, an integrated method for measurement of total dietary fiber (AOAC Method 2009.01/AACCI method 32-45.01) was developed and adopted. Evaluation of this method over the past 8 years identified some aspects of the method that could be improved. Modifications have been made and incorporated into a Rapid Integrated Total Dietary Fiber (RINTDF) method, and this method has been subjected to interlaboratory evaluation under the auspices of ICC International and AACC International.

Recognizing the importance of dietary fiber

The term “dietary fiber” (DF) was introduced by Hipsley[1] in 1953 to cover the non-digestible constituents of plants that make up the plant cell wall, including cellulose, hemicellulose and lignin. This definition was broadened by Trowell *et al*. in 1972[2] and refined in 1976[3] to become primarily a physiological definition, based on edibility and resistance to digestion in the human small intestine; the definition included indigestible polysaccharides such as gums, modified celluloses, mucilages and pectin.

Several research groups worldwide worked to develop a method to service this definition, and this work was crystallised into the so-called “Prosky method” (AOAC Method 985.29)[4] (Figure 1). In this method, starch and protein are enzymatically removed by hydrolysis to fragments, followed by alcohol precipitation of the higher molecular weight soluble dietary fiber (HMWSDF) fragments [more recently termed “soluble dietary fiber that precipitates in the presence of 78% aqueous ethanol (SDFP)], and this is recovered along with insoluble dietary fiber (IDF) by filtration. Replicate residues are washed, dried and weighed and either analysed for ash or protein. Ash and protein are subtracted from residue weights to give “total dietary fiber” (TDF), comprising SDFP and IDF. In this method some but not all, of the resistant starch (RS) fraction is measured. Subsequently, AOAC Official Method 985.29 (“Total Dietary Fiber in Foods; Enzymatic-Gravimetric Method”) was extended to allow measurement of total, soluble and insoluble dietary fibre in foods (AOAC Official Method 991.43)[5]. Various other modifications of these methods were developed, notably AOAC Method 2001.03

* Corresponding author
Megazyme, Bray Business Park, Southern Cross Road, Bray, County Wicklow, Ireland.
E-mail　barry@megazyme.com

(the Matsutani method)[6], which also allows measurement of non-digestible oligosaccharides (NDO) [also referred to as "soluble dietary fiber that remains soluble in the presence of 78% aqueous ethanol" (SDFS)][7]. These methods are all enzymatic/gravimetric methods and the official status of each is summarised in Table 1 and acronyms are listed in Table 2.

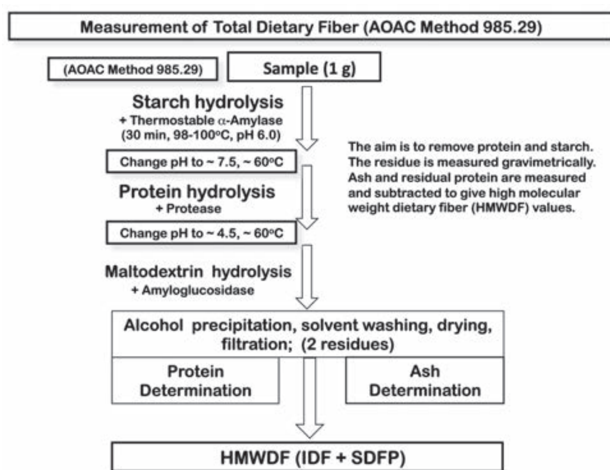

Figure 1. A schematic representation of the Prosky method (AOAC Method 985.29) for total dietary fiber determination.

Concurrently, in the UK, Englyst and colleagues[8,9], extended the work of Southgate[10], and developed methods for measurement of non-starch polysaccharides (NSP). In these procedures starch is specifically removed from the sample by complete dissolution in dimethyl sulphoxide (DMSO) followed by enzymatic hydrolysis of the starch to mainly glucose, ensuring subsequent removal by washing with aqueous ethanol. The NSP is recovered, acid hydrolysed and analysed by gas-liquid chromatography, high performance liquid chromatography or colourimetrically. These authors clearly did not consider RS to be important as a dietary fiber component, eventhough it passes through the small intestine and is fermented in the colon.

In Japan, Innami[11] and colleagues, noting that there was considerable confusion about fiber-related terms, organised an academic conference in 1980 to discuss the various definitions of dietary fiber. At that meeting, Kiriyama[12] proposed the use of the term "luminacoids". By definition, the term "luminacoids" means "dietary components which are not digested and/or absorbed in the human small intestine and which exert physiological effects that are useful in maintaining good health via the gastrointestinal tract". Included in these components

Table 1. Codex Alimentarius, AOAC International, AACC International methods for the analysis of dietary fiber.

AOAC Method	AACC Method	Codex Type Method	What is measured
985.29	32-05.01	I	HMWDF (IDF + SDFP)
991.43	32-20.01	I	IDF in foods
993.19	-	I	SDFP in foods
991.43	32-07.01	I	IDF and SDFP separately
994.13	32-25.01	I	HMWDF; provides sugar composition and Klason lignin
2001.03	32-41.01	I	HMWDF and SDFS in foods devoid of resistant starch
993.21	-	I	Total HMWDF in samples with > 10 % fibre and < 2 % starch
2009.01	32-45.01	I	HMWDF and SDFS in all foods
2011.25	32-50.01		IDF, SDFP and SDFS in all foods
995.16	32-23.01	II	(1-3)(1-4)-β-Glucan in cereals, feeds and foods
997.08	32-31.01	II	Fructans and FOS
999.03	32-32.01	III	Fructans and FOS (underestimates highly depolymerised FOS
2000.11	32-28.01	II	Polydextrose
2001.02	32-33.01	II	Trans galacto-oligosaccharides
2002.02	32-40.01	II	Resistant starch (RS_2 and RS_3)

Table 2. Definition of dietary fiber terminology.

TDF	Total dietary fiber = HMWDF (IDF + SDFP) + SDFS
IDF	Insoluble dietary fiber
HMWDF	Higher molecular weight dietary fiber = IDF + SDFP.
SDFP	Dietary fiber soluble in water but insoluble in 78 % aqueous ethanol = High molecular weight soluble dietary fiber (HMWSDF).
SDFS	Dietary fiber soluble in water and also soluble in 78 % aqueous ethanol = non-digestible oligosaccharides (NDO).

are dietary polysaccharides including resistant starch, resistant maltodextrins, oligosaccharides, sugar alcohols, resistant proteins and other components. Importantly, digestion-resistant oligosaccharides down to and including disaccharides are included[12].

Over the past 15 years, recognition that RS and NDO also act as dietary fiber, has led to more inclusive dietary fiber definitions:
The American Association of Cereal Chemists International (AACCI) in 1998 began a critical review of the state of dietary fiber science including its definition. In 2001[13] the following definition was published:
"Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation."

The Food Nutrition Board (FNB) of the Institute of Medicine of the National Academies (USA) proposed (2001)[14] a definition that they considered would encompass current and future nondigestible carbohydrates in the food supply that could be defined as fiber, namely:

1. *Dietary Fiber* consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants.
2. *Added Fiber* consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in humans.

   Total Fiber is the sum of *Dietary Fiber* and *Added Fiber.*

These types of fiber were distinguished on the basis that *dietary fiber* consists of nondigestible food plant carbohydrates and lignin in which the plant matrix is largely intact. The definition recognized that the three-dimensional plant matrix is responsible for some of the physicochemical properties attributed to dietary fiber. Thus, mechanical treatment would still result in intact fiber and resistant starch that is naturally occurring and inherent in a food or created during normal processing of a food, would be categorized as *Dietary Fiber*. Oligosaccharides that fall under the category of *Dietary Fiber* are those that are normally constituents of a dietary fiber source, such as raffinose, stachyose, and verbascose in legumes, and the low molecular weight fructans in foods, such as Jerusalem artichoke and onions.
Added Fiber consists of isolated or extracted nondigestible carbohydrates that have beneficial physiological effects in humans. These include fibers that have been isolated or extracted using chemical, enzymatic or aqueous steps or chemically modified. Manufactured resistant starch and animal-derived, non-digestible carbohydrates are included. Isolated, manufactured, or synthetic oligosaccharides of three or more degrees of polymerization are included, but nondigestible monosaccharides, disaccharides, and sugar alcohols are not included because they fall under “carbohydrates” on the food label. In 2002, the term “Added fiber” was modified to “Functional Fiber”

From the 27th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSD; ALINORM 06/29/26), Bonn Germany, 21-25th November, 2005[15], a DF definition was proposed that is similar in many respects to that proposed by AACC International, namely:
“Dietary fibre means carbohydrate polymers with a degree of polymerization (DP) not lower than 3 which are neither digested nor absorbed in the small intestine. A degree of polymerization not lower than 3 is intended to exclude mono- and disaccharides. It is not intended to reflect the average DP of the mixture. Dietary Fibre consists of one or more of; edible carbohydrate polymers naturally occurring in the food as consumed; carbohydrate polymers which have been obtained from raw materials by physical, enzymatic or chemical means; synthetic carbohydrate polymers.”

At an FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition held in Geneva on 17-18th July, 2006[16] (as discussed at the 28th session of Codex Committee on Nutrition and Food for Special Dietary Uses)[17], the participants concluded that the definition of dietary fiber should be more closely linked to fruits, vegetables and wholegrain cereals. To achieve this aim, they stated that the definition should include 1) a source element identifying that the dietary fiber is an intrinsic component of these food groups, and 2) a chemical element identifying the component to be measured. The following definition was proposed; ***“Dietary fibre consists of intrinsic cell wall polysaccharides”***. In rationalizing this decision and definition, many points were discussed, including their statement that NSP can be measured specifically and that NSP relates directly to the content of plant cell walls, and the other beneficial substances they contain such as micronutrients and phytochemicals. Since the focus of this FAO/WHO proposed definition[17] is based on the measurement of plant cell walls, rather than on carbohydrates that are not digested or absorbed in the

human small intestine, it does not address the sole reason for which there is an interest in dietary fiber, i.e. 'the belief that dietary fiber contributes positively to the health/quality of life of the consumer'.

Based on the recommendation for endorsement of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) in November 2008, a definition for dietary fiber was adopted in June 2009 by the Codex Alimentarius Commission (CAC)[18]. The definition lists three categories of carbohydrates that are not hydrolyzed by the endogenous enzymes in the small intestine of humans. However, the definition left the decision concerning the inclusion, or otherwise, of oligosaccharides with degrees of polymerization (DP) in the range of 3 and 9 to the discretion of national authorities and left the 'physiological effect(s) of benefit to health' as undefined[19]:

"Dietary fiber consists of carbohydrate polymers[a] with ten or more monomeric units[b], which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories: edible carbohydrate polymers naturally occurring in the food as consumed; carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities, and; synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

a When derived from a plant origin, dietary fiber may include fractions of lignin and/or other compounds when associated with polysaccharides in the plant cell walls and if these compounds are quantified by the AOAC gravimetric analytical method for dietary fiber analysis: fractions of lignin and the other compounds (proteic fractions, phenolic compounds, waxes, saponins, phytates, cutin, phytosterols, etc.) intimately "associated" with plant polysaccharides in the AOAC 991.43 method.

b Decision on whether to include carbohydrates of 3 to 9 monomeric units should be left up to national authorities."

Since the Codex definition of dietary fiber will be the reference definition in most countries, an urgent need existed for methodology that could service this definition i.e. a method that could measure dietary fiber as defined by AOAC Method 985.29, but with an accurate measure of resistant starch and accurate and reliable measurement of NDO.

Development of an integrated total dietary fiber method to support the Codex Alimentarius definition of dietary fiber

a. Background research

By 2005, it was well known that AOAC Methods 985.29 and 991.43 did not quantitatively measure resistant starch and measured little or none of the NDO. Methods were thus developed for measurement of specific NDO such as fructo-oligosaccharides (FOS), AOAC Methods 997.08[20] and 999.03[21]; Polydextrose®, AOAC Method 2000.11[22]; Fibersol 2®, AOAC Method 2001.03[23]; galacto-oligosaccharides, AOAC Method 2001.02[24], and for the accurate measurement of resistant starch, AOAC Method 2002.02[25]. At that time it had been considered that the best way to measure total dietary fiber was to measure dietary fiber by the Prosky method (AOAC Method 985.29) and the individual components such as FOS and resistant starch by the specific methods, and then simply add these together. However, in doing this, another problem was introduced, that of double counting. This is shown schematically in Figure 2. For example, AOAC Method 985.29 measures a proportion of resistant starch so if resistant starch is measured separately using AOAC Method 2002.02 and added to the DF value obtained using AOAC method 985.29, there will be overestimation of total dietary fiber. The need for a single, integrated procedure for the measurement of all fiber components as defined by Codex Alimentarius, was apparent.

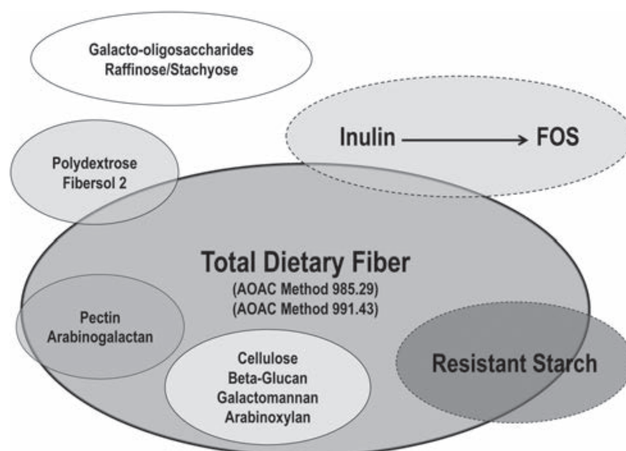

Figure 2. Components measured and not measured with AOAC Method 985.29.

Of all dietary fiber components, the most difficult to measure is resistant starch, and this is because the value

obtained is a consequence of the incubation conditions used. It is essential that the conditions of hydrolysis in the reaction tube or bottle simulate, as much as possible, hydrolysis conditions in the human small intestine. In developing an appropriate method, conditions such as incubation temperature, pH and time and enzymes employed must be considered. A method for measurement of RS developed by McCleary *et al.*[25] employs pancreatic α-amylase (PAA) and amyloglucosidase (AMG), and was optimized using a set of food and starch samples with defined resistant starch values (based on ileostomy studies). This method was successfully evaluated in an AOAC International (Method 2002.02)/AACC International (Method 32-40.01) interlaboratory study involving 39 laboratories[25].

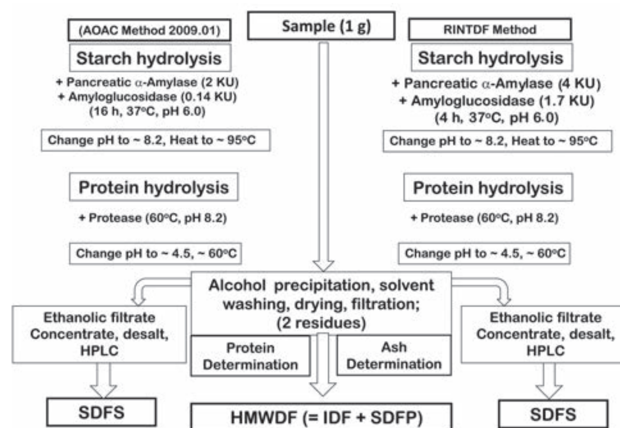

Figure 3. A schematic representation of the integrated TDF procedure (AOAC Method 2009.01) (LHS) and the RINTDF total dietary fiber procedure (RHS).

b. An integrated method for the measurement of total dietary fiber

In developing an integrated procedure for the measurement of total dietary fiber (INTDF)[26], the aim again, was to simulate *in vivo* conditions in the human small intestine. Thus, incubation conditions were the same as those employed in the RS method. The amounts of all components (including sample and enzymes employed) were increased ten-fold so that the sample size (1 g) employed was sufficient for gravimetric analysis methodology. An outline of this method is given in Figure 3 (left hand side). Samples are incubated with 2 Kilo-units (KU) of PAA and 0.14 KU of AMG with shaking or stirring of the incubation mixture at pH 6.0 and 37℃for 16 h. The pH of the incubation solution is increased to pH 8.2 (a pH at which AMG is not active) and then PAA and AMG are inactivated, and protein in the sample denatured, by heating the samples to ~ 95℃. On cooling to 60℃, denatured protein is hydrolyzed to peptides with protease and after pH adjustment to ~ 4.5, ethanol is added to a concentration of 78% to precipitate SDFP. Insoluble fractions are recovered by filtration and washing, dried and weighed. These are analyzed for protein and ash and higher molecular weight dietary fiber (HMWDF) determined by subtracting protein and ash weights from residue weights. An aliquot of the alcoholic filtrate is concentrated to dryness, re-dissolved in water, desalted and analyzed for SDFS by chromatography on a Waters Sugar-Pak® HPLC column. This method was evaluated under the auspices of AOAC International and was accepted as AOAC Method 2009.01 (HMWDF plus SDFS)[27] and AOAC Method 2011.25 [IDF, SDFP and SDFS][28].

Determination of non-digestible oligosaccharides using pancreatic α-amylase and intestinal disaccharidase

In 2014, Tanabe *et al.*[29] stated that in the measurement of non-digestible oligosaccharides, a mixture of PAA and purified porcine intestinal disaccharidase gave more effective hydrolysis of digestible oligosaccharides than the PAA plus AMG employing in AOAC Method 2009.01, and thus gave more accurate measurement of non-digestible oligosaccharides. However, this difference in determined values can be attributed mainly to the levels of remaining disaccharides (which are included in the luminacoid method). The enzyme mixture used by Tanabe *et al.* [29] completely hydrolyses both sucrose and palatinose (isomaltulose; 6-O-α-D-glucopyranosyl-D-glucose)] to monosaccharides, whereas these are not hydrolyzed in AOAC Method 2009.01. Under the Codex definition of dietary fiber, disaccharides are not included, so hydrolysis of these is of no consequence as they are separated by HPLC and not included in the calculation of DF. With this considered, the match between the results of Tanabe *et al.* [29] and those with AOAC Method 2009.01 for NDO, is very good. The one significant difference is isomalto-oligosaccharides (IMO). The enzymes employed by Tanabe *et al.* [29] give complete hydrolysis of the IMO to monosaccharides. In contrast, with AOAC Method 2009.01, ~ 30% of IMO is not hydrolyzed. More extensive hydrolysis (~ 90%) of IMO is achieved under the conditions of incubation employed in the RINTDF method (below). While the results with the porcine small intestine enzymes on non-digestible and digestible oligosaccharides are academically interesting, it should be noted that the production of these enzymes for routine use in dietary fiber analysis is impossible.

Development of the Rapid Integrated Total Dietary Fiber (RINTDF) Method

Since its publication in 2007[26], the INTDF method has been applied to a wide range of samples and ingredients and several challenges/concerns have been identified. Firstly, the incubation time with PAA plus AMG employed was 16 h (in line with AOAC Method 2002.02 for RS). This was considered not to simulate physiological conditions. Based on numerous published studies, a more likely residence time for food in the small intestine is 4 +/- 1 h[30-35]. Secondly, most commercially available FOS contain the trisaccharide, fructosyl-β-(2-1)-fructosyl-β-(2-1)-fructose (F3). This oligosaccharide elutes from the Waters Sugar-Pak® column at the same point as disaccharides such as lactose, maltose and sucrose, so it is not included in the analytical value for SDFS (DP ≥ 3). Thirdly, on hydrolysis of products containing high starch levels, including certain breads and rice products, various maltodextrins are produced as by-products of the incubation, which are very resistant to further hydrolysis by AMG or PAA[36]. However these oligosaccharides are readily hydrolysed[37] by a mucosal α-glucosidase preparation from the small intestine of pig, indicating that they should not be included in the SDFS fraction of dietary fibre (DF). A fourth challenge experienced in the use of AOAC Method 2009.01 is the considered underestimation of phosphate cross-linked starch (Resistant Starch 4, RS_4, e.g. FiberRite®, Fibersym®). Much higher DF values for FiberRite® and Fibersym® are obtained[38] using the Prosky TDF method (AOAC Method 985.29)[9]. A fifth concern in the use of AOAC Method 2009.01, relates to the use of sodium azide as a preservative in the buffer. While the concentration employed is low (0.02 % w/v), it is still of concern to analysts. With the extended incubation conditions (16 h) employed in AOAC Method 2009.01, inclusion of an antimicrobial agent was considered to be essential.

a. Optimization of levels of PAA plus AMG used in the incubations.

Numerous studies in literature indicate that the time of residence of food in the small intestine is 4 ± 1 h[30-35].

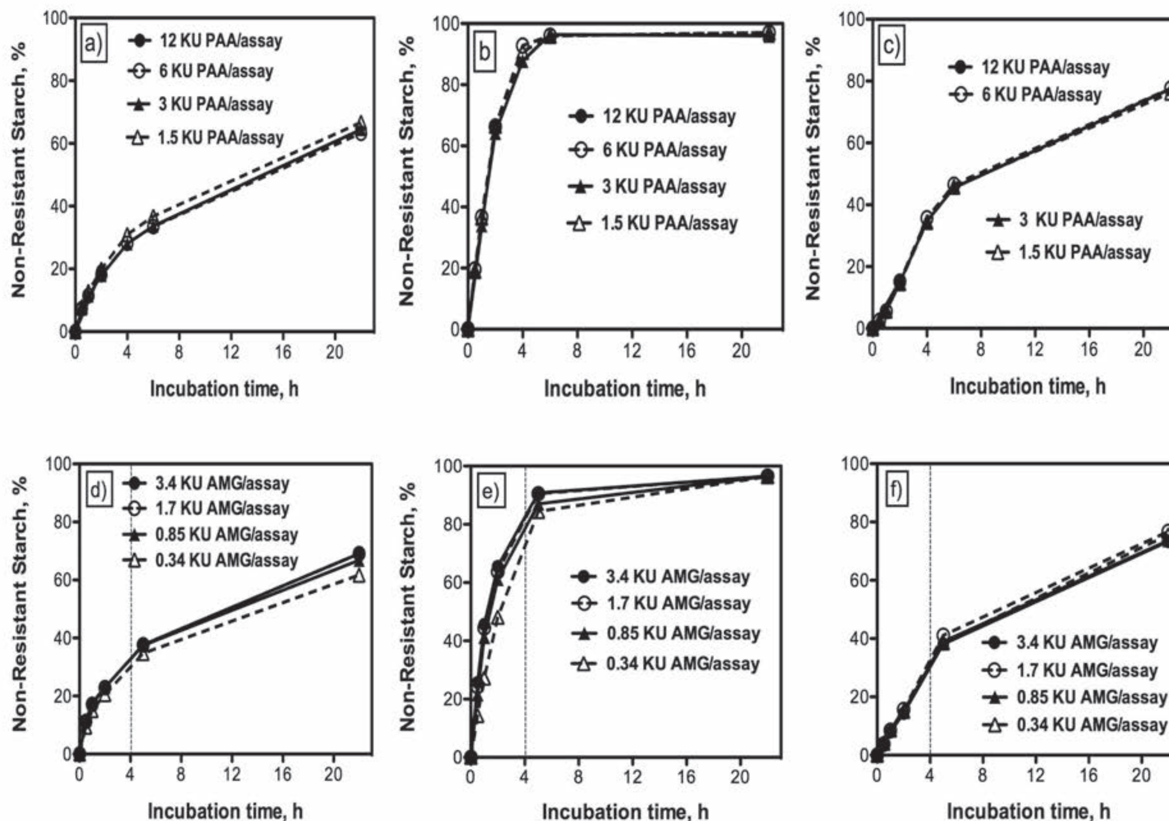

Figure 4. The effect of the concentration of PAA and of AMG on the extent of hydrolysis of Hylon VII® (a & d), RMS (b & e) and Fibersym® (c & f) in Fisherbrand® bottles with magnetic stirring at 170 rpm and at 37℃ and pH 6.0. Samples a - c were incubated with 1.7 KU AMG and varying levels of PAA. Samples d - f were incubated with 6 KU of PAA and varying levels of AMG. Samples were analysed for free D-glucose.

With this in mind, a range of experiments with varying conditions of incubation, reaction pH and enzyme concentrations were performed.

Initial hydrolysis conditions simulated those employed by Englyst *et al.*[39] in their research on hydrolysis of starches and development of the NSP analysis procedure. Incubations were performed in tubes with agitation by lateral shaking at 150 rpm in the presence or absence of marbles and guar gum (as employed by Englyst *et al.*[39]), or in Fisherbrand 250 mL, wide-mouthed bottles with either stirring or agitation by swirling the bottles during the incubation. The rates of hydrolysis of various starch samples, including high amylose maize starch (Hylon VII®); wheat starch (WS); native potato starch (NPS) and phosphate cross-linked starch (Fibersym®; RS_4), were essentially the same, independent of the agitation method employed or the pH (5.2 or 6.0). The effect of the concentration of PAA and AMG on the rates of hydrolysis of Hylon VII® (a and d), RMS (b and e) and Fibersym® (c and f) in stirred containers is shown in Figure 4. Concentrations of AMG of 1.7 KU per assay and of PAA of 4 KU per assay are saturating and were adopted. The determined levels of dietary fibre for a range of samples containing resistant starch using these incubation conditions compared to those employed in the INTDF method are shown in Table 3. For all samples analysed, except Hylon VII® and Fibersym®, the HMWDF values were very similar. A much higher value was obtained for Fibersym® (~ 60% w/w) and a significantly higher value for Hylon VII®. Values obtained for Fibersym® with the RINTDF method are still much lower than values obtained with AOAC Method 985.29 (~ 86% w/w). However, since these high values can only be obtained with methods that employ incubation with thermostable α-amylase at 97-98℃, it is clear that these are method dependent and have little relevance to actual values experienced under physiological conditions. Since the RINTDF method simulates physiological conditions in the human small intestine, we consider the values obtained with this method to be more accurate. Furthermore, since RS_4 is a manufactured product, there is likely to be batch-to-batch variation in DF levels, so each lot of product should be analysed with the RINTDF method.

b. Measurement of fructo-oligosaccharides (FOS) and other oligosaccharides

In the development of the INTDF procedure[26], we chose to use a Waters Sugar-Pak® HPLC column with D-sorbitol as the internal standard. Glycerol was not used because the enzymes employed in the incubations contained glycerol as a stabiliser. It was subsequently found that certain commercially available FOS contain high levels of the trisaccharide, fructosyl-β-(2-1)-fructosyl-β-(2-1)-fructose (inulinotriose), which chromatographs on the Waters Sugar-Pak® HPLC column at the same point as the disaccharides, maltose, sucrose and lactose, which makes accurate measurement of FOS difficult. Complete separation of F3 from sucrose and lactose is readily achieved on TSKgel® G2500PW_{XL} columns, however, sorbitol cannot then be used as the internal standard because it elutes at the same point as glucose. Since glycerol was the internal standard of choice, a decision was made to delete this stabiliser from enzyme preparations. Consequently, a PAA/AMG powder mixture is used with dissolution just before use. The solution can be stabilised as

Table 3. HMWDF, SDFS and TDF values obtained for a range of samples using the INTDF and RINTDF methods.

	AOAC Method 2009.01 (INTDF Method)			RINTDF Method		
Sample	HMWDF % w/w	SDFS % w/w	TDF (HMWDF + SDFS) % w/w	HMWDF % w/w	SDFS % w/w	TDF (HMWDF + SDFS) % w/w
Wholemeal bread	12.4	1.8	14.2	12.0	1.5	13.5
Oat bran	18.9	0.6	19.5	19.9	1.4	21.3
Weetabix	9.8	2.8	12.6	10.1	1.4	11.6
Kellogg All Bran	26.6	3.9	30.5	28.1	3.6	31.7
Whole wheat pasta	9.9	2.8	12.7	10.1	2.2	12.3
Semi-ripe banana	30.2	0.9	31.1	30.2	1.4	31.6
Sweet corn (tinned)	12.7	0.4	13.1	12.4	0.5	12.9
Garden peas (tinned)	29.1	1.4	30.5	29.1	2.2	31.3
Broccoli	28.1	0.4	28.5	29.7	0.6	30.3
Carrots	21.8	0.6	22.4	22.2	1.2	23.4
Fibersym®	**28.6**	1.1	29.7	**59.2**	1.0	60.2
Hylon VII®	**48.6**	0.5	49.3	**58.8**	0.0	58.8
Polydextrose®	1.5	83.3	84.8	1.1	83.3	84.4

Table 4. Recovery of oligosaccharides of DP ≥ 3 in original samples and on incubation of the samples according to AOAC 2009.01 and the RINTDF method*.

	Recovery of Oligosaccharides of DP > 3 as a percentage of total carbohydrate in the sample (% w/w)		
Sample	Original Oligosaccharides	AOAC Method 2009.01** (INTDF)	RINTDF Method**
Neosugars®	93.0	92.9	92.8
Raftilose P95® ***	89.0	76.2	88.2
Polydextrose®	84.3	85.1	82.5
Fibersol 2®	88.5	83.4	82.4
Galacto-oligosaccharides	76.0	70.6	72.0
Xylo-oligosaccharides	78.0	78.6	76.2
Raffinose	99.0	99.0	98.0
Isomalto-oligosaccharides AdvantaFiber®	65.4	29.0	10.8

* Calculated from HPLC patterns as areas under the peaks for oligosaccharides of DP ≥ 3 as a percentage of combined area for all peaks from the sample.
** from McCleary *et al.* [41].
*** Raftilose P-95 (Lot PEOHS7DHS7) which has a high content of inulinotriose.

an ammonium sulphate suspension for up to three months, allowing bulk preparation and suitable storage. Protease is used as a stabilised ammonium sulphate suspension. The recovery of FOS and other oligosaccharides run through the RINTDF procedure and the INTDF procedure (AOAC Method 2009.01) are compared to the contents in the original preparations in Table 4. With the RINTDF procedure, the values obtained for all oligosaccharides (except IMO) are very similar to those obtained for the original preparations, showing that the enzymes employed are suitable for use. The level of measured dietary fiber in Raftilose P-95 is higher with the RINTDF procedure than with the INTDF method because in the former method, TSKgel® G2500PW$_{XL}$ columns are employed, which give complete separation and allow accurate measurement of all FOS. With the RINTDF method, the DF value for IMO is 10.8 % w/w. This is similar to the values obtained with both AOAC Method 985.29 and 991.43 (~ 8% w/w). The pancreatic α-glucosidase/PAA enzyme mixture employed by Tanabe *et al.*[29] gave complete hydrolysis of IMO to monosaccharides (i.e. a DF value of zero).

c. Low molecular weight branched maltodextrins produced on hydrolysis of non-resistant starch

On incubation of samples containing high levels of starch (e.g. rice and white bread) under the conditions described in AOAC Method 2009.01, small amounts of branched maltodextrins namely, a heptasaccharide, 6^3,6^5-di-α-D-glucosyl maltopentaose, with lesser quantities of the tetrasaccharide, 6^3-α-D-glucosyl maltotriose[37] are produced. These compounds, particularly the heptasaccharide, are highly resistant to hydrolysis by PAA and AMG, but are readily hydrolysed by a mucosal α-glucosidase preparation from the small intestine of pig[37], and thus cannot be considered to be dietary fiber. In the RINTDF procedure, these oligosaccharides are not produced due to the higher levels of PAA and AMG employed.

d. Deletion of sodium azide from the incubation buffer

In the extended incubation period in the INTDF procedure (16 h), it is important to include sodium azide as an antimicrobial agent. However, with an incubation time of just 4 h with the RINTDF method, sodium azide can be deleted from the buffer.

e. Preparation of the SDFS fraction for HPLC

Oligosaccharides in the SDFS fraction are separated on TSKgel® G2500PW$_{XL}$ columns and quantitated by refractive index (RI) detection. Salt in the sample must be removed. Traditionally, this has been performed by percolation of the sample through a column of a mixture of cation and anion resins; a tedious and time consuming operation. This can be greatly simplified by first removing ~ 95% of salt by mixing an aliquot of the sample with a mixture of cation and anion exchange resins in a tube[40]. Last traces of salt are then removed using Bio-Rad® de-ashing, HPLC pre-columns. Pre-removal of the bulk of the salt with resins before HPLC extends the useful life of the expensive Bio-Rad de-ashing columns by 10-15 fold. The effect of this process of sample desalting is clearly shown in Figure 5 where a mixture of glycerol and D-glucose was

mixed with the buffers used in the RINTDF procedure and then chromatographed through the TSKgel® G2500PW$_{XL}$ columns; a) before desalting; b) after desalting with resins and finally; c) with the desalted sample applied with Bio-Rad de-ashing pre-columns in place. Clearly, this process removes all salt, allowing accurate determination of applied samples.

All of these improvements in the INTDF method have been incorporated into the new Rapid Integrated Total Dietary Fiber (RINTDF) method[41]. This procedure is shown schematically in Figure 3 (right hand side) and directly compared to the INTDF method. From an operational point of view, this method is very similar to the INTDF method, except that higher concentrations of PAA and AMG are employed and the incubation time with PAA/AMG is reduced to 4 h, as opposed to 16 h in the INTDF method. Several other improvements as described above are incorporated which positively impact on the accuracy, efficiency and safety of the method.

Interlaboratory evaluation of the RINTDF method

RINTDF method was subjected to interlaboratory evaluation under the auspices of ICC and AACC International according to the protocols of AOAC International. Thirteen collaborating laboratories were involved and they analyzed sixteen samples that were provided as eight blind duplicates. The results of the study are shown in Table 5. In total, only 4 sets of data from the 104 sets submitted were statistically excluded as outliers. The dietary fiber content of the 8 test pairs ranged from 6.79 to 60.6%. TDF was calculated as the sum of HMWDF (IDF + SDFP) and SDFS. For TDF, the within laboratory variability (s_r) ranged from 0.29 to 0.74 and the between laboratory variability (s_R) ranged from 0.57 to 4.67. The within laboratory relative variability (RSD_r) ranged from 1.22 to 6.34% and the between laboratory relative variability (RSD_R) ranged from 2.64 to 13.38%. In previously adopted methods, the between laboratory

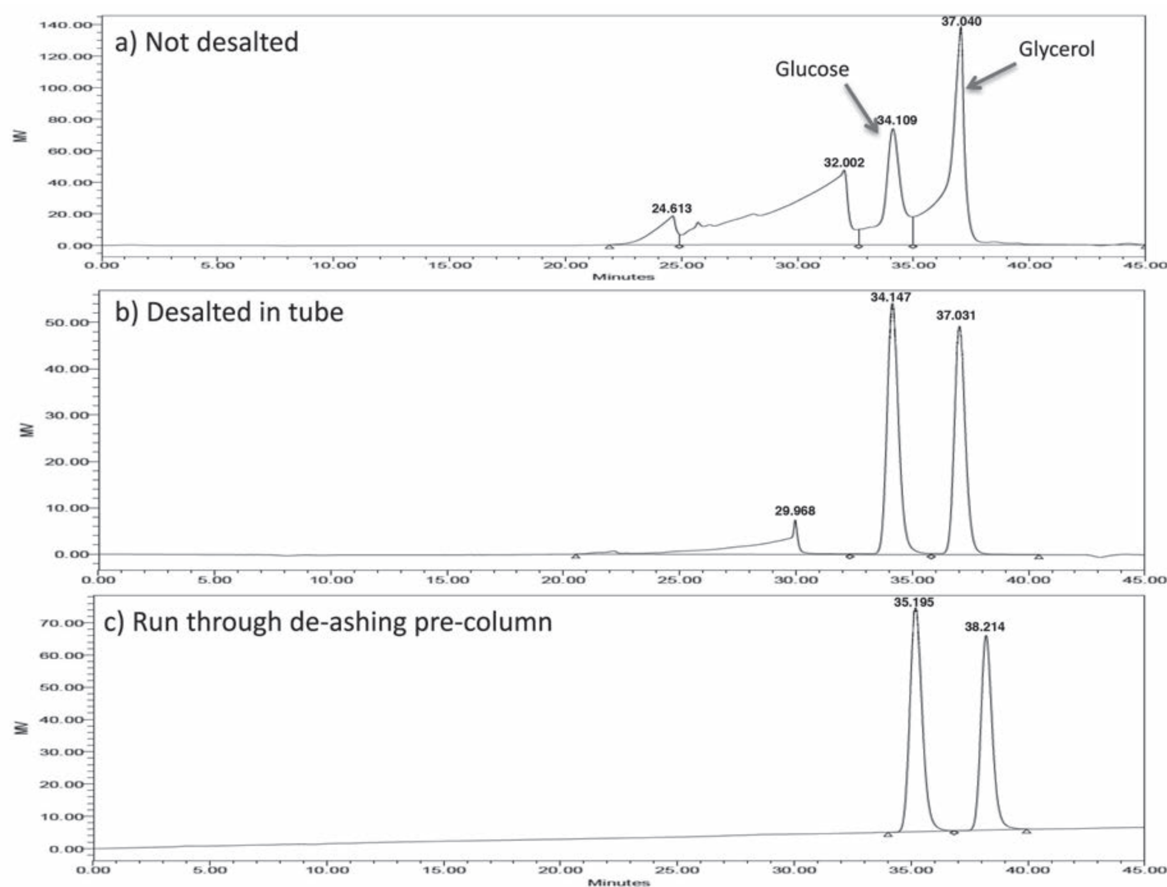

Figure 5. Chromatography on TSKgel® G2500PW$_{XL}$ columns of a) a non-desalted mixture of glucose and glycerol in RINTDF incubation buffer mixture; b) the same sample after desalting 5 mL with 1.5 g of Amberlite® FPA53 (OH^-) and 1.5 g of Ambersep® 200 (H^+) resins; and c) sample "b" chromatographed on the same TSKgel® G2500PW$_{XL}$ columns, but also with desalting pre-columns in place.

Table 5. Interlaboratory study results for total dietary fiber in foods (RINTDF method). Statistical evaluation according to AOAC International statistics format[a].

Sample/parameter	A & D	B & F	C & J	E & H	G & N	I & M	K & O	L & P
No. labs/analysts	12	13	12	12	13	13	12	13
Mean, %	60.62	23.70	29.37	6.79	16.15	19.28	21.09	10.76
S_r	0.74	0.67	0.36	0.29	0.39	0.29	0.43	0.68
S_R	4.67	0.99	0.78	0.91	0.85	1.74	0.57	0.86
$\%RSD_r$	1.22	2.81	1.22	4.32	2.41	1.51	2.05	6.34
$\%RSD_R$	7.70	4.17	2.64	13.38	5.29	9.01	2.72	8.02

Samples: A&D = Fibersym®; B&F = kidney beans (canned, washed and lyophilized); C&J = bran cereal; E&H = Defatted cookies containing FOS; G&N = oat bran; I&M = defatted cookies containing polydextrose and RS_2; K&O = dark rye crispbread;
L&P = whole meal bread.

[a] s_r; Within laboratory variability; RSD_r: within laboratory relative variability; s_R: between laboratory variability; and RSD_R: between laboratory relative variability.

variability (s_R) ranged from 0.04 to 9.49 and the between laboratory relative variability (RSD_R) from 1.58 to 66.25.

Collaborating laboratories:

[1] Megazyme, Bray, County Wicklow, Ireland.
[2] Medallion Laboratories/General Mills, Golden Valley, MN, USA.
[3] Agriculture and Agri-Food Canada/Agriculture et Agroalimentaires Canada, University of Manitoba –Winnipeg, Manitoba, Canada.
[4] Grain Growers Limited, PO Box 7, North Ryde, NSW, Australia.
[5] Sanitarium Development and Innovation Analytical Department, Cooranbong, NSW, Australia.
[6] CRDS Tienen, Central Department Research, Development and Services, Tienen, Belgium.
[7] Finnish Food Safety Authority Evira, Chemistry and Toxicology Research Unit, Helsinki, Finland.
[8] Matsutani Chemical Company, Itami City, Hyogo, Japan.
[9] NEOTRON SPA, Stradello Aggazzotti, Modena, Italy.
[10] Eurofins Food Testing Netherlands BV, Heerenveen, Netherlands.
[11] Kellogg Company, Battle Creek, MI, U.S.A.
[12] Japanese Food Research Laboratories, Japan
[13] Nestle, Food Science and Technology Carbohydrates, Nestlé Research Centre Lausanne, Switzerland.

The results of this study have been submitted to the technical committees of ICC, AACC International and AOAC International and are currently under review.

Conclusion

In conclusion, the RINTDF method is an improvement on AOAC Method 2009.01 and is suitable for measurement of total dietary fiber as defined by Codex Alimentarius. The method simulates physiological conditions in the human small intestine and all of the identified problems with AOAC Method 2009.01 (the INTDF method) have been resolved. The method is robust, reliable and reproducible as demonstrated by the results of the interlaboratory study.

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総　説

食物繊維定義の進展とこの定義を全うする測定法

Barry V. McCleary* and Jodi Cox

Megazyme, Bray Business Park, Southern Cross Road, Bray, County Wicklow, Ireland.

和文要旨

食物繊維の定義は過去70年に亘り進展してきた。変遷する定義は食物繊維のタイプや生理学的機能についての私たちの理解を反映している。最初の定義では“ヒトの消化酵素によって消化を受けない植物細胞壁の残渣”に焦点を当て，適切な分析方法が発展し，用いられた。つい最近になって，食物繊維構成要素としてレジスタントスターチや非消化性オリゴ糖類（NDO）が認識されるようになってきた。これらを組み込むにはフラクトオリゴ糖，ガラクトオリゴ糖，レジスタントマルトデキストリン，レジスタントスターチなどといった特定の食物繊維構成要素を測定する多くの他の方法の発展を必要としてきた。特定の成分のいくらかはまた，“ゴールドスタンダード”な繊維測定法であるプロスキー法（AOAC法985.29）によって部分的に測定されうる。従って，こうした特別な方法を得ることは，製造メーカーにとって有用であるが，規制側にとってはそうとは限らない。このような訳でAOAC法985.29で得られた値で種々の特定成分を単に合計することはできない。というのもこれでは繊維含量を“ダブルカウント”し，過大評価につながるからである。この問題を解決し，全ての食物繊維構成要素を測定するために，総食物繊維測定のために統合された方法（AOAC法2009.01/AACCI法32-45.01）が発展し採用された。過去8年に及ぶこの方法の評価は，その方法が改良されうるいくつかの側面を割り出した。改良がなされ，Rapid Integrated Total Dietary Fiber（RINTDF）法に取込まれ，ICC InternationalとAACC Internationalの下で研究室間評価を受けている状況にある。