Collaborative Study Report: Determination of Insoluble, Soluble, and Total Dietary Fiber (Codex Definition) by an Enzymatic-Gravimetric Method and Liquid Chromatography

Barry V. McCleary,[1] Jonathan W. DeVries,[2] Jeanne I. Rader,[3] Gerald Cohen,[4] Leon Prosky,[5] David C. Mugford,[6] Martine Champ,[7] and Kazuhiro Okuma[8]

ABSTRACT

A method for the determination of insoluble, soluble, and total dietary fiber (IDF, SDF, and TDF, respectively), as defined by the Codex Alimentarius Commission, was validated for foods. Based on the principles of AACC Intl. Approved Methods 32-05.01, 32-07.01, 32-41.01, and 32-40.01 (1), the method quantitates water-insoluble and water-soluble dietary fiber. This method extends the capabilities of the previously adopted AACC Intl. Approved Method 32-45.01 (2,9) (Total Dietary Fiber in Foods, Enzymatic–Gravimetric–Liquid Chromatographic Method). (This method is applicable to plant materials, foods, and food ingredients consistent with the 2009 Codex definition [ALINORM 09/32/REP] [3], including naturally occurring, isolated, modified, and synthetic polymers meeting this definition.) In 2007, McCleary (8) described a method of extended enzymatic digestion at 37°C designed to simulate human intestinal digestion followed by gravimetric isolation and quantitation of HMWDF (high molecular weight dietary fiber) and the use of liquid chromatography (LC) to quantitate LMWSDF (low molecular weight soluble dietary fiber). The use of the terms HMWDF and LMWSDF in this context is a bit misleading, because the method does not quantitate dietary fiber on the basis of molecular weight, but rather on the basis of solubility in a solution of one part water and four parts alcohol. Thus, HMWDF actually consists of dietary fiber that is insoluble or precipitates in the water-alcohol mixture, and LMWSDF is dietary fiber that remains soluble in the same mixture. The method reported here quantitates water-insoluble dietary fiber (IDF) separately from water-soluble dietary fiber (SDF). SDF includes the dietary fiber that precipitates (SDFP) in the presence of 78% aqueous ethanol or IMS (industrial methylated spirits) and dietary fiber that remains soluble (SDFS) in 78% aqueous ethanol (formerly termed LMWSDF). The method, thus, quantitates the complete range of water-insoluble and water-soluble fractions (by inclusion of the fractionation steps of AACC Intl. Approved Methods 32-20.01 and/or 32-07.01) of dietary fiber components from resistant starch (by utilizing the digestion conditions of AACC Intl. Approved Method 32-40.01) to digestion-resistant oligosaccharides (by incorporating deionization and LC procedures similar to those of AACC Intl. Approved Method 32-41.01). A further option for increased laboratory productivity using on-line, simultaneous deionization and LC quantitation has recently been published (10) and has been incorporated as an option in the new method. The method was evaluated through an AACC International/AOAC International collaborative study. A total of 22 laboratories participated, with 19 laboratories returning valid assay data for 16 test portions (8 blind duplicates) consisting of samples with a range of traditional dietary fiber, resistant starch, and nondigestible oligosaccharides. The dietary fiber content of the eight test pairs ranged from 10.45 to 29.90%. Digestion of samples under the conditions of AACC Intl. Approved Method 32-40.01 followed by the isolation, fractionation, and gravimetric procedures of AACC Intl. Approved Methods 32-05.01and 32-07.01 resulted in quantitation of IDF and water-soluble but water-alcohol–insoluble dietary fiber (SDFP). The filtrate from the quantitation of SDFP was concentrated, deionized, concentrated again, and analyzed by LC to determine water-alcohol–soluble dietary fiber (SDFS), i.e., all dietary fiber polymers with DP ≥ 3, consisting primarily, but not exclusively, of oligosaccharides. SDF was calculated as the sum of SDFP and SDFS. TDF was calculated as the sum of IDF and SDF. For IDF the within laboratory variability (s_r) ranged from 0.18 to 0.71, and the between laboratory variability (s_R) ranged from 0.42 to 2.24. For SDF, s_r ranged from 0.28 to 1.03, and s_R ranged from 0.85 to 1.66. For TDF, s_r ranged from 0.47 to 1.41, and s_R ranged from 0.95 to 3.14. This is comparable to other official and approved dietary fiber methods. The study directors recommended this method be granted First Approval status by AACC International. Method 32.50.01 was approved in August 2011.

Dramatic increases in the utilization of fiber analyses in fiber research and the marketing, research, and development of food products have accompanied increases in public awareness of the health benefits of high-fiber foods over the past several decades. The relationships of particular health benefits to the insoluble and soluble fractions of dietary fiber have been established (16). As researchers have discovered and elucidated additional dietary fiber sources, not only has there been a need to update the definition of dietary fiber, but also to update the methodologies that support this definition. AACC International has been a leader in providing approved methods of analysis consistent with the state of dietary fiber science (7). In the 1970s, Trowell and fellow dietary fiber researchers (12–15) published a definition that was later adopted through consensus by AOAC Intl. and AACC Intl. following an international survey by Prosky et al. (11) in the late 1970s:

> Dietary fiber consists of the plant polysaccharides and lignin which are resistant to hydrolysis by digestive enzymes of man. This definition defines a macro constituent of foods which includes cellulose, hemicellulose, lignin, gums, modified celluloses, mucilages, oligosaccharides, and pectins and associated minor substances such as waxes, cutin, and suberin.

[1] Megazyme International, Bray, Co. Wicklow, Ireland.
[2] Medallion Laboratories/General Mills, Golden Valley, MN, U.S.A.
[3] U.S. Food and Drug Administration, College Park, MD, U.S.A.
[4] Kraft Foods (retired), New City, NY, U.S.A.
[5] U.S. Food and Drug Administration (retired), Rockville, MD, U.S.A.
[6] BRI Australia Pty. Ltd., North Ryde, NSW, Australia.
[7] UMR PhAN, INRA, CRNH, Nantes, Cedex 1, France.
[8] Matsutani Chemical, Research Laboratory, Itami City, Japan.

doi:10.1094/CFW-56-6-0238

The methodology approved by AACC International (i.e., AACC Intl. Approved Method 32-05.01, its extension AACC Intl. Approved Method 32-20.01, and methods that produce equivalent results [AACC Intl. Approved Methods 32-07.01, 32-06.01, and 32-25.01]) adequately quantitates the dietary fiber food fractions that were known at the time the Trowell et al. (12–15) definition was adopted. (Note, AACC Intl. Approved Methods 32-05.01 and 32-07.01 quantitate total and insoluble dietary fiber, respectively. Soluble dietary fiber can be calculated using the chemistry principles of AACC Intl. Approved Method 32-05.01. AACC Intl. Approved Method 32-07.01 incorporates the quantitation of insoluble and soluble dietary fiber along with total dietary fiber.) As the science of dietary fiber has advanced, however, this approved methodology has proven insufficient to quantitate all of the newly identified dietary fiber components. Advances in understanding the complexity of dietary fiber, including the fact 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 that has been adopted by the Codex Alimentarius Commission and an updated AACC Intl. Approved Method for Total Dietary Fiber (Method 32-45.01). Recently, international authorities on the definition of dietary fiber, working through the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCFNSDU), updated the terminology of the dietary fiber definition (3) and recommended its adoption by the Codex Alimentarius Commission (CAC). The CAC adopted this definition during its 2009 session (4). The wording underwent minor editing of the first footnote during the 2009 session of CCNFSDU (5) and the 2010 session of CAC (6). Codex defines dietary fiber as

> 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,
> - 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 fibre may include fractions of lignin and/or other compounds associated with polysaccharides in the plant cell walls. These compounds also may be measured by certain analytical method(s) for dietary fibre. However, such compounds are not included in the definition of dietary fibre if extracted and re-introduced into a food.
>
> [b] Decision on whether to include carbohydrates of 3 to 9 monomeric units should be left up to national authorities.

With the possible exception of the synthetic carbohydrate polymers, the food components included in the Codex definition match those of the more broadly stated Trowell et al. (12–15) definition. Therefore, the currently adopted approved methods can be readily applied, although the same issues of the extra expense of carrying out multiple assays, the need for mathematical summations, and the carrying out of special procedures to avoid double counting apply. The single approved method that quantitates the entirety of components included in the Trowell and Codex definitions was validated and adopted as AACC Intl. Approved Method 32-45.01. Extending this method to quantitate the insoluble and soluble dietary fiber fractions is the important next step to provide a continuum of methodology to meet the needs of the dietary fiber research, regulation, and labeling communities. The principles for delineating the water-insoluble and water-soluble fractions of dietary fiber were first validated for AACC Intl. Approved Methods 32-05.01 and 32-20.01 and later incorporated as part of the validation for AACC Intl. Approved Method 32-07.01. Applying these principles to AACC Intl. Approved Method 32-45.01 provides a robust, time-proven method suitable for the purpose. AACC Intl. Approved Method 32-50.01 has been published in the AACC International *Approved Methods of Analysis* revised 11th edition (2).

Precollaborative Ruggedness Testing

Based on the successful study that resulted in the adoption of AACC Intl. Approved Method 32-45.01, it was determined that additional precollaborative ruggedness testing of this method was not needed.

Collaborative Study Protocol

Eight food samples were selected for the collaborative study. Because the method under consideration incorporates resistant starch and nondigestible oligosaccharides into a more traditional dietary fiber methodology, the samples for this collaborative study were chosen to be challenging, i.e., with an emphasis on quantitating products high in resistant starch (legumes, a resistant starch ingredient, and whole-grain products) and products with typical levels of nondigestible oligosaccharides. Methods designed to quantitate dietary fiber have been thoroughly studied and validated since 1980 (AACC Intl. Approved Methods 32-05.01, 32-20.01, 32-07.01, etc.), matching the Trowell et al. (12–15) dietary fiber definition of the time. Inclusion of components such as resistant starch and nondigestible oligosaccharides in the Codex Alimentarius definition indicates that updated testing procedures must include the capability to accurately quantitate these components.

Moist samples were freeze-dried before grinding. All samples were ground to the method-specific size and homogenized by thorough mixing before being subdivided into polyethylene bottles and sealed. Samples, copies of the method, electronic report sheets, Excel-based calculators, and sample-storage instructions, along with an adequate supply of enzymes and deionizing resins, were shipped to collaborating laboratories by express overnight shipment.

A total of 22 laboratories reported data for the collaborative study samples. One laboratory utilized high-pressure anion-exchange chromatography with electrochemical detection instead of the prescribed LC method and, therefore, could not obtain accurate data for dietary fibers that are soluble in the water-alcohol solution. Another laboratory that had not previously performed dietary fiber analyses and that reported difficulties measuring the water-alcohol–soluble dietary fibers reported results that were significantly lower than those obtained by the other laboratories in all three categories, i.e., for IDF, SDF, and TDF. The source of the discrepancy could not be determined. A third laboratory was able to complete only 10 of the 16 samples. Data from these three laboratories are not included in the data tables or statistical analyses. In addition, four laboratories did not quantitate the IDF and SDF fractions of the TDF. The data from these four laboratories are included with the data from the five laboratories that both quantitated IDF and SDF and measured TDF directly.

Table I. Collaborative study data for insoluble dietary fiber (% IDF) reported by laboratories[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1	25.90	25.10	5.30	5.00	10.10	10.80	1.20	1.20	8.50	8.20	13.90	14.20	11.80	11.70	10.30	10.30
2	24.59	26.00	4.91	4.33	7.64	7.00	1.26	1.33	7.81	7.88	12.06	13.32	9.48	10.25	9.40	9.34
3	26.60	25.01	4.72	4.51	7.30	9.03	1.21	1.11	7.79	7.65	11.31	10.45	10.90	10.76	8.86	8.57
4	25.39	25.18	5.09	4.91	12.40	13.15	1.16	1.19	8.31	8.32	10.59	10.81	13.33	13.46	10.24	10.27
5	27.28	27.28	5.43	5.76	10.38	11.16	1.68	1.89	9.20	9.33	12.60	13.02	13.25	13.98	11.64	11.10
6	26.91c	30.52c	4.13	4.29	10.40	8.73	0.54	0.52	7.68c	11.08c	37.94sg	38.32sg	16.10dg	16.93dg	15.77sg	15.04sg
7	24.70	25.51	5.35	5.16	8.78	8.71	1.23	1.51	8.44	8.65	11.55	11.46	11.92	11.99	10.31	10.82
8	26.15	25.81	5.02	5.42	9.30	11.35	0.91	0.94	12.38c	10.34c	11.69	13.16	25.81c	12.24c	13.65c	10.14c
9	23.95	24.44	4.43	4.25	7.27	7.50	0.71	0.28	8.18	7.66	10.29	10.38	9.95	10.10	8.90	8.77
10	27.77	27.58	4.06	3.89	10.39	10.21	0.07	0.20	8.08	7.85	11.40	10.89	16.25dg	16.64dg	10.09	10.10
11	27.32	27.09	5.16	5.15	12.41	11.61	1.08	1.18	8.74	8.73	11.03	11.50	11.79	11.53	10.60	10.27
12	24.41	24.54	4.48	4.40	3.42	4.87	1.00	0.96	7.56	8.02	10.76	10.43	11.65	10.62	10.10	9.12
13	23.63	24.00	4.81	4.74	8.18	8.42	1.01	1.06	7.99	8.18	11.49	11.82	12.23	11.92	9.36	9.77
14	26.24	26.97	5.08	4.91	10.69	10.78	1.18	1.54	8.58	8.97	11.52	10.87	11.40	11.85	10.06	10.40
15	25.7	26.5	4.6	4.4	7.6	7.3	1.2	1.1	8.1	8.0	–	–	10.3	10.3	9.6	9.1

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. –: laboratory reported no result for the sample; c: Cochran test outlier on IDF; sg: single Grubbs test outlier on IDF; and dg: double Grubbs test outlier on IDF.

Table I-A. Statistical data for insoluble dietary fiber[a]

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
No. of labs	14	15	15	15	13	13	12	13
Mean (%)	25.74	4.79	9.23	1.05	8.26	11.63	11.52	9.90
s_r	0.52	0.18	0.71	0.13	0.19	0.48	0.33	0.29
s_R	1.23	0.47	2.24	0.42	0.48	1.12	1.21	0.77
RSD_r	2.03	3.76	7.67	12.25	2.26	4.11	2.87	2.98
RSD_R	4.76	9.82	24.35	39.64	5.81	9.65	10.53	7.80
HORRAT	1.94	3.11	8.51	9.98	2.00	3.49	3.80	2.75

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. s_r: within laboratory variability; s_R: between laboratory variability; RSD_r: within laboratory relative variability; and RSD_R: between laboratory relative variability.

Table II. Collaborative study data for soluble dietary fiber (% SDF) measured using manual deionization reported by laboratories[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1	3.00	2.72	4.57	4.80	16.95	17.52	10.58	10.82	12.53	12.02	3.22	3.15	11.10	11.34	6.95	7.51
2	3.34	3.32	5.70	5.71	16.37	16.56	11.00	10.88	11.81	11.65	3.70	3.53	10.69	10.49	8.24	8.63
3	4.51c	8.22c	7.04	6.72	18.01	20.90	11.10	12.18	15.51	16.98	6.58	5.15	12.44	14.83	11.09	11.36
4	4.98	5.86	6.75	7.27	17.84	18.30	11.17	11.01	13.61	14.25	4.01	6.88	12.14	12.76	10.24	9.24
5	3.05	4.31	5.76	5.80	17.17	17.08	10.12	11.66	12.66	12.67	10.26c	4.58c	11.91	11.57	8.13	7.82
6	5.76	5.14	5.78c	7.41c	18.79	18.77	12.84	12.97	13.55	14.04	5.10	4.74	13.32	12.70	11.31c	14.74c
7	4.26	3.39	5.56	5.52	15.42	15.81	9.11	9.04	10.63	10.54	2.87	3.34	11.14	11.74	7.92	7.58
8	2.72	2.94	4.37	4.53	16.67	18.16	9.15	9.29	11.77c	14.60c	7.94	8.13	8.67	9.25	9.14	10.33
9	4.33	4.31	5.22	4.94	16.87	17.21	10.17	9.92	13.12	13.07	3.77	3.91	11.14	11.04	8.51	7.87
10	3.87	4.57	5.82	6.88	18.60	14.8	11.42	11.25	14.41	13.85	5.16	7.62	12.89	11.79	7.19	7.02
11	3.98	4.33	6.16	6.58	17.89	17.95	10.80	11.52	13.53	13.61	4.24	4.43	11.63	12.69	8.68	8.17
12	2.66	1.91	5.19	5.70	18.55	16.12	11.02	12.35	11.68	10.29	3.20	2.99	8.87	6.76	6.12	7.31
13	4.56	4.04	5.26	5.25	18.42	18.64	10.99	11.35	13.22	14.49	4.56	4.93	12.17	12.23	8.01	8.66
14	3.71	3.69	5.11	4.69	16.19	16.12	10.51	9.99	11.67	11.69	2.31	3.17	10.16	11.14	7.59	6.52
15	2.44	3.12	4.63	4.64	15.75	16.24	9.08	8.91	12.56	12.67	–	–	10.07	10.03	2.59sg	2.48sg

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. –: laboratory reported no result for the sample; c: Cochran test outlier on SDF; and sg: single Grubbs test outlier on SDF.

Table II-A. Statistical data for soluble dietary fiber measured using manual deionization[a]

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
No. of labs	14	14	15	15	14	13	15	13
Mean (%)	3.80	5.58	17.32	10.74	12.94	4.56	11.29	8.30
s_r	0.44	0.28	1.03	0.46	0.50	0.83	0.71	0.51
s_R	0.99	0.85	1.30	1.12	1.52	1.66	1.61	1.33
RSD_r	11.71	5.10	5.97	4.33	3.86	18.10	6.29	6.18
RSD_R	26.20	15.20	7.53	10.46	11.73	36.40	14.25	16.07
HORRAT	8.01	4.92	2.89	3.74	4.31	11.44	5.13	5.53

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. s_r: within laboratory variability; s_R: between laboratory variability; RSD_r: within laboratory relative variability; and RSD_R: between laboratory relative variability.

Statistical Treatment

Collaborating laboratory data were evaluated statistically according to AACC International protocols using software supplied by AOAC International. Of the 118 valid pairs (236 data points) of assay results reported for IDF, laboratories 1, 2, 3, 4, 5, 7, 9, 11, 12, 13, 14, and 15 had no statistical outliers, laboratory 10 had one statistical outlier, laboratory 8 had three statistical outliers, and laboratory 6 had five statistical outliers, for a total of nine statistical outlier pairs overall. The raw data and statistically paired data from the blind duplicate results for IDF reported by the collaborating laboratories are shown in Tables I and I-A, respectively. Outliers, and the reason for outlier removal, are indicated and footnoted in Table I. Of the 118 valid pairs of assay results reported for SDF, laboratories 1, 2, 4, 7, 9, 10, 11, 12, 13, and 14 had no statistical outliers, laboratories 3, 5, 8, and 15 had one statistical outlier, and laboratory 6 had two statistical outliers, for a total of six statistical outlier pairs overall. The raw data and statistically paired data from the blind duplicate results for SDF reported by the collaborating laboratories are shown in Tables II and II-A, respectively. Outliers, and the reason for outlier removal, are indicated and footnoted in Table II. Of the 118 valid pairs of assay results reported for TDF, laboratories 1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13, and 14 had no statistical outliers, laboratories 8 and 15 had one statistical outlier, and laboratory 6 had three statistical outliers, for a total of five statistical outlier pairs overall. The raw data and statistically paired data from the blind duplicate results for TDF reported by the collaborating laboratories are shown in Tables III and III-A, respectively. Outliers, and the reason for outlier removal, are indicated and footnoted in Table III.

Results and Discussion

To simulate food digestion in the small intestine, a combination of gentle shaking combined with enzymatic digestion at 37°C is used. Adjusting the pH of the digestion solution followed by temporary heating to 100°C destroys the amylase and amyloglucosidase activity and promotes partial denaturation of protein, providing for efficient protein digestion after cooling to 60°C. Incorporating the water-insoluble and the water-soluble, water-alcohol–insoluble dietary fiber segregation steps and the liquid chromatographic quantitation of the water-alcohol–soluble dietary fiber, as validated in previously adopted approved methods, completes the assay.

The raw data results for the dietary fiber collaborative study are shown in Tables I, II, and III for IDF, SDF, and TDF, respectively. Cochran and Grubbs outliers are noted in the tables. Tables I-A, II-A, and III-A show the statistical results obtained after removal of outliers for IDF, SDF, and TDF, respectively. As stated earlier, samples for this collaborative study were chosen to be challenging, i.e., with an emphasis on quantitating products with resistant starch and products with nondigestible oligosaccharides. As can be seen, the within laboratory variability (s_r) for IDF ranged from 0.18 to 0.71, and the between laboratory variability (s_R) ranged from 0.42 to 2.24. For SDF, s_r ranged from 0.28 to 1.03, and s_R ranged from 0.85 to 1.66. For TDF, s_r ranged from 0.47 to 1.41, and s_R ranged from 0.95 to 3.14. When compared with the statis-

Table III. Collaborative study data for total dietary fiber (% TDF) calculated as the sum of insoluble and soluble dietary fiber and measured using manual deionization reported by laboratories[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1	28.90	27.82	9.87	9.80	27.05	28.32	11.78	12.02	21.03	20.22	17.12	17.35	22.90	23.04	17.25	17.81
2	27.93	29.32	10.61	10.04	24.01	23.56	12.26	12.21	19.62	19.53	15.76	16.85	20.17	20.74	17.64	17.97
3	31.11	33.23	11.75	11.23	25.31	29.92	12.32	13.29	23.31	24.63	17.89	15.60	23.34	25.59	19.95	19.93
4	30.37	31.04	11.85	12.61	30.24	31.45	12.33	12.20	21.93	22.57	14.60	17.69	25.46	26.81	20.48	19.50
5	30.33	31.59	11.19	11.56	27.54	28.24	11.80	13.56	21.86	22.00	22.86	17.61	25.16	25.56	19.78	18.91
6	32.66	35.66	9.91	11.70	29.18	27.51	13.38	13.50	21.24c	25.12c	43.04sg	43.07sg	29.42	29.63	27.07c	29.79c
7	28.96	28.90	10.91	10.68	24.20	24.52	10.35	10.55	19.07	19.19	14.41	14.80	23.07	23.73	18.23	18.41
8	28.87	28.75	9.39	9.95	25.97	29.51	10.06	10.23	24.15	24.94	19.63	21.29	34.48c	21.49c	22.79	20.47
9	28.28	28.75	9.65	9.18	24.14	24.71	10.88	10.20	21.29	20.72	14.06	14.29	21.10	21.14	17.41	16.64
10	31.64	32.15	9.88	10.77	28.98	25.01	11.50	11.45	22.49	21.70	16.56	18.52	29.14	28.43	17.28	17.12
11	31.30	31.42	11.32	11.73	30.30	29.56	11.88	12.70	22.26	22.34	15.26	15.93	23.42	24.22	19.29	18.45
12	27.07	26.45	9.67	10.10	21.97	21.00	12.02	13.31	19.24	18.31	13.95	13.41	20.52	17.38	16.22	16.43
13	28.20	28.04	10.07	10.00	26.60	27.06	11.99	12.41	21.21	22.66	16.04	16.75	24.41	24.16	17.36	18.43
14	29.95	30.66	10.19	9.60	26.88	26.91	11.69	11.53	20.25	20.65	13.84	14.04	21.56	22.99	17.66	16.92
15	28.12	29.59	9.24	9.02	23.34	23.54	10.25	10.01	20.63	20.67	–	–	20.40	20.30	12.15sg	11.53sg

[a]Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. –: laboratory reported no result for the sample; c: Cochran test outlier on TDF; and sg: single Grubbs test outlier on TDF.

Table III-A. Statistical data for total dietary fiber calculated as the sum of insoluble and soluble dietary fiber and measured using manual deionization[a]

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
No. of labs	15	15	15	15	14	13	14	13
Mean (%)	29.90	10.45	26.55	11.79	21.37	16.39	23.71	18.40
s_r	0.86	0.47	1.39	0.49	0.52	1.41	0.87	0.64
s_R	2.05	0.95	2.74	1.10	1.72	2.37	3.14	1.56
RSD_r	2.88	4.51	5.25	4.17	2.43	8.60	3.65	3.47
RSD_R	6.85	9.11	10.31	9.30	8.04	14.48	13.23	8.47
HORRAT	2.85	3.24	4.22	3.37	3.19	5.51	5.33	3.28

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. s_r: within laboratory variability; s_R: between laboratory variability; RSD_r: within laboratory relative variability; and RSD_R: between laboratory relative variability.

tical results for dietary fiber for previously adopted approved dietary fiber methods (Table IV), the level and range of variability were similar to those for other dietary fiber methods and were influenced by the significant number of technique-dependent manual operations, each of which contributed to the overall variability of the final results. Repeatability, reproducibility, and HORRAT were within the range of performance characteristics typically found for dietary fiber methods in which a significant number of manual steps are necessary to perform the assay. In previously adopted methods, the between laboratory variability (s_R) ranged from 0.04 to 9.49, and the between laboratory relative variability (RSD_R) ranged from 1.58 to 66.25%. This is due to the fact that all previous dietary fiber methods are composed of a significant number of technique-dependent manual operations, each of which contributes to the overall variability of the final results. For comparison, the statistical characteristics of various AACC Intl. and AOAC Intl. approved methods are compiled in Table IV. The statistical characteristics of the new method, which combines steps from AACC Intl. Approved Methods 32-05.01 (and its extension 32-06.01), 32-07.01, 32-41.01 (1), and 32-40.01 (1), lie within the ranges of the statistical characteristics of the current AACC Intl. approved methods for dietary fiber.

A number of laboratories participating in the study measured TDF directly without quantitating the IDF and SDF fractions. The data provided by these laboratories are shown in Table V. The statistical results for measuring TDF directly are shown in Table V-A. Within laboratory variability (s_r) (0.41–1.26) and between lab variability (s_R) (1.24–5.27) were similar to the data obtained by summing IDF and SDF and within the ranges for the dietary fiber methods shown in Table IV. Five laboratories performed the analysis using both approaches, i.e., TDF was calculated by summing IDF and SDF, as well as measured directly. A comparison of the results is shown in Table VI. ANOVA (type II, sum of squares) was performed on the comparative data (Table VI-A). ANOVA showed no statistical difference between the two approaches. As can be seen in Figure 1, the results were in very good agreement. The statistical data showed very good agreement with the data from the collaborative study for AACC Intl. Approved Method 32-45.01 (the method is similar in all respects

Table IV. Comparable AACC International and AOAC International method data[a]

Method	Title	s_r	RSD_r	s_R	RSD_R	HORRAT
AACC Intl. 32-05.01	Total Dietary Fiber in Foods	0.15–0.99	0.56–66.25	0.27–1.36	1.58–66.25	0.76–17.46
AACC Intl. 32-20.01	Insoluble Dietary Fiber in Food and Food Products	0.41–2.82	0.86–10.38	0.62–9.49	3.68–19.44	1.73–8.68
AACC Intl. 32-07.01[b]	Insoluble Dietary Fiber in Food and Food Products	0.36–1.06	1.50–6.62	0.85–2.06	1.58–12.17	0.74–4.66
AACC Intl. 32-06.01	Total Dietary Fiber	0.18–1.01	1.48–14.73	0.22–2.06	4.13–17.94	1.84–4.62
AOAC Intl. 993.19	Soluble Dietary Fiber in Food and Food Products	0.49–1.15	1.74–5.93	0.79–2.05	2.41–7.01	1.13–2.83
AACC Intl. 32-25.01	Total Dietary Fiber (Determined as Neutral Sugar Residues, Uronic Acid Residues, and Klason Lignin)	0.32–2.88	1.80–6.96	0.52–4.90	4.80–11.30	2.32–4.20
AACC Intl. 32-41.01	Dietary Fiber Containing Supplemented Resistant Maltodextrin (RMD)	0.02–1.63	1.33–6.10	0.04–2.37	1.79–9.39	0.77–3.32
AACC Intl. 32-40.01	Resistant Starch in Starch and Plant Materials	0.08–2.66	1.97–4.12	0.21–3.87	4.58–10.9	1.44–3.74
AACC Intl. 32-45.01	Total Dietary Fiber in Foods	0.41–1.43	1.65–12.34	1.18–5.44	4.70–17.97	1.91–6.49

[a] s_r: within laboratory variability; RSD_r: within laboratory relative variability; s_R: between laboratory variability; and RSD_R: between laboratory relative variability.
[b] Samples were not dried and/or were desugared only.

Table V. Collaborative study data reported by laboratories for total dietary fiber (% TDF) measured directly[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1	28.51	29.58	9.78	9.51	25.80	25.30	11.51	11.52	20.50	19.85	14.81	14.11	20.90	23.16	17.69	17.70
2	27.42	27.86	10.09	9.53	23.49	23.06	11.75	11.71	19.10	19.02	15.25	15.71	19.65	20.23	17.13	17.46
3	27.56	27.32	9.74	10.10	23.69	24.84	8.49	8.89	20.98	22.15	14.62	15.69	22.48	22.68	17.50	17.50
4	28.07	27.76	11.89	12.88	27.88	29.34	13.13	14.01	23.10	21.77	29.09	27.29	23.25	20.89	17.10	13.19
5	27.33	27.81	9.93	9.97	23.54	24.52	10.04	10.01	20.90	23.50	14.27	15.64	20.25	19.17	15.56	17.14
6	27.79	29.04	9.02	8.55	23.24	23.30	10.12	10.40	19.75	19.97	14.68	14.17	20.74	19.90	11.86	11.73
7	26.67	27.42	8.56	8.45	21.84	22.13	6.20	7.03	18.81	18.51	12.77	12.53	19.23	19.33	15.06	15.12
8	25.70	29.35	10.24	11.25	25.64	24.32	11.68	11.19	22.03	20.32	23.78c	14.63c	20.19	20.19	16.80	16.29
9	28.40	31.80	11.42c	14.71c	26.15	24.72	14.72	15.63	27.26	24.30	35.69c	30.85c	23.56	27.12	19.61	22.06

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. c: Cochran test outlier on TDF.

Table V-A. Statistical data for total dietary fiber measured directly[a]

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
No. of labs	9	8	9	9	9	7	9	9
Mean (%)	28.08	9.97	24.60	11.00	21.21	16.47	21.27	16.47
s_r	1.26	0.41	0.70	0.39	1.11	0.72	1.19	1.16
s_R	1.31	1.24	1.95	2.54	2.30	5.27	2.10	2.58
RSD_r	4.50	4.16	2.83	3.57	5.23	4.38	5.61	7.04
RSD_R	4.67	12.41	7.95	23.11	10.86	32.00	9.89	15.66
HORRAT	1.93	4.39	3.22	8.29	4.30	12.20	3.92	5.97

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. s_r: within laboratory variability; s_R: between laboratory variability; RSD_r: within laboratory relative variability; and RSD_R: between laboratory relative variability.

except that the current method utilizes updated equipment for higher productivity), indicating the method described here provides results equivalent to the same level of confidence as those for AACC Intl. Approved Method 32-45.01. The analyst, thus, has a choice between this method and AACC Intl. Approved Method 32-45.01 (1) for measuring TDF directly.

Six of the laboratories involved in the study were able to equip themselves with the on-line deionization option to remove salts from the sample digests and filtrates as part of the HPLC assay of water-alcohol–soluble dietary fiber versus manual deionization prior to HPLC assay. The results for SDF measured using direct on-line deionization are shown in Table VII; the results for TDF are shown in Table VIII. Their respective statistical results are shown in Tables VII-A and VIII-A. For SDF, when using on-line deionization s_r ranged from 0.16 to 0.58, and s_R ranged from 0.40 to 1.99. For TDF, when using on-line deionization s_r ranged from 0.24 to 0.73, and s_R ranged from 0.37 to 2.41. Thus, on-line deionization showed slightly better precision than manual deionization. Tables IX and X show a comparison of SDF and TDF values, respectively, obtained using manual versus on-line deionization. These results are plotted in Figures 2 and 3 for SDF and TDF, respectively. As can be seen in Figures 2 and 3, on-line deionization showed a small (≈5% relative) bias to a lower value for some of the samples for both SDF and TDF. Further research is underway to discover the reason for this difference.

Twelve of the laboratories provided data for calculating SDF and TDF using the external standard method. Results for SDF and TDF using the external standard method of calculation are shown in Tables XI and XII, respectively. Tables XI-A and XII-A provide the statistical summaries of these results. As can be seen, the results of the external and internal standard methods agreed well, with the external standard method having less variability in some cases and the internal standard method having less variability in others.

Table VI. Collaborative study data for total dietary fiber (% TDF) measured directly versus TDF calculated as the sum of insoluble and soluble dietary fiber reported by laboratories that ran both methods[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1 (direct)	28.51	29.58	9.78	9.51	25.80	25.30	11.51	11.52	20.50	19.85	14.81	14.11	20.90	23.16	17.69	17.70
1 (sum)	28.90	27.82	9.87	9.80	27.05	28.32	11.78	12.02	21.03	20.22	17.12	17.35	22.90	23.04	17.25	17.81
2 (direct)	27.42	27.86	10.09	9.53	23.49	23.06	11.75	11.71	19.10	19.02	15.25	15.71	19.65	20.23	17.13	17.46
2 (sum)	27.93	29.32	10.61	10.04	24.01	23.56	12.26	12.21	19.62	19.53	15.76	16.85	20.17	20.74	17.64	17.97
3 (direct)	27.56	27.32	9.74	10.10	23.69	24.84	8.49	8.89	20.98	22.15	14.62	15.69	22.48	22.68	17.50	17.50
3 (sum)	28.87	28.75	9.39	9.95	25.97	29.51	10.06	10.23	24.15	24.94	19.63	21.29	34.48	21.49	22.79	20.47
4 (direct)	28.07	27.76	11.89	12.88	27.88	29.34	13.13	14.01	23.10	21.77	29.09	27.29	23.25	20.89	17.10	13.19
4 (sum)	27.07	26.45	9.67	10.10	21.97	21.00	12.02	13.31	19.24	18.31	13.95	13.41	20.52	17.38	16.22	16.43
5 (direct)	27.79	29.04	9.02	8.55	23.24	23.30	10.12	10.40	19.75	19.97	14.68	14.17	20.74	19.90	11.86	11.73
5 (sum)	28.12	29.59	9.24	9.02	23.34	23.54	10.25	10.01	20.63	20.67	–	–	20.40	20.30	12.15	11.53

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. –: laboratory reported no result for the sample.

Table VI-A. ANOVA of results for total dietary fiber (TDF) measured directly versus TDF calculated as the sum of insoluble and soluble dietary fiber

Main Effect	Sum of Squares	DF	Mean Square	*F* Ratio[a]	*P* Value
A: lab	129.604	4	32.401	10.77	0.0000
B: method	0.247724	1	0.247724	0.08	0.7747
C: sample	5,629.01	7	804.144	267.26	0.0000

[a]All *F* ratios are based on residual mean square error.

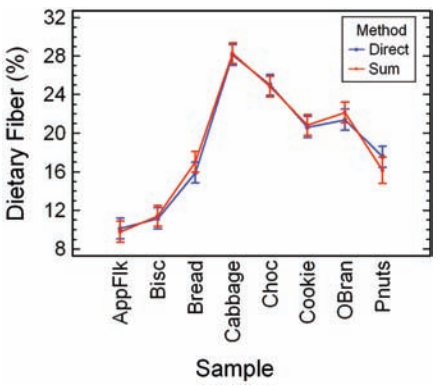

Fig. 1. Sample–method interaction for dietary fiber. Samples (left to right): mixed grains with apple flakes; biscuits containing fructooligosaccharides; whole-wheat bread with 2% α-cyclodextrin; cabbage; chocolate with fructooligosaccharides; defatted cookies with oat graham, polydextrose, and RS2 starch; oat bran; and peanuts.

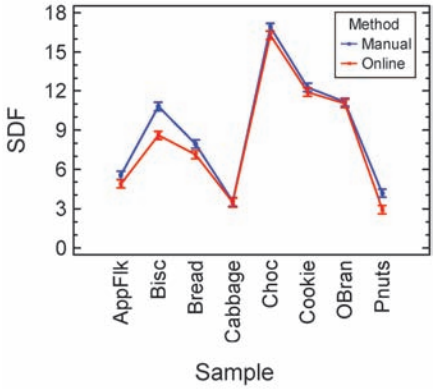

Fig. 2. Sample–method interaction for soluble dietary fiber (SDF). Samples (left to right): mixed grains with apple flakes; biscuits containing fructooligosaccharides; whole-wheat bread with 2% α-cyclodextrin; cabbage; chocolate with fructooligosaccharides; defatted cookies with oat graham, polydextrose, and RS2 starch; oat bran; and peanuts.

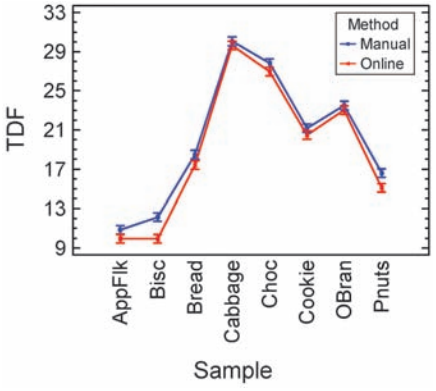

Fig. 3. Sample–method interaction for total dietary fiber (TDF). Samples (left to right): mixed grains with apple flakes; biscuits containing fructooligosaccharides; whole-wheat bread with 2% α-cyclodextrin; cabbage; chocolate with fructooligosaccharides; defatted cookies with oat graham, polydextrose, and RS2 starch; oat bran; and peanuts.

Table VII. Collaborative study data for soluble dietary fiber (% SDF) measured using on-line deionization[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1	2.68	2.43	4.32	4.78	17.30	18.10	10.49	10.43	12.50	12.40	2.34	2.53	11.19	11.54	8.32	8.73
2	4.43	4.18	4.86	4.85	16.48	15.69	5.95	6.11	12.62	12.55	3.12	3.23	10.96	11.03	6.76	6.53
3	2.88	3.79	5.33	4.93	15.94	14.86	9.61	9.25	11.61	11.24	3.25	3.33	11.22	10.56	5.39	6.07
4	3.60	3.88	4.57	3.86	14.54	15.15	9.72	9.49	10.63	10.99	2.36	2.82	10.41c	12.22c	7.26	7.55
5	2.94	3.32	5.04	4.88	16.59	15.49	6.95	5.44	11.17	11.69	2.63	2.51	10.47	10.39	7.93	8.05
6	3.40	3.90	5.34	5.66	17.61	17.69	9.54	10.19	12.67	13.03	3.57	3.67	11.22	11.26	6.27	6.41

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. c: Cochran test outlier on SDF.

Table VII-A. Statistical data for soluble dietary fiber measured using on-line deionization[a]

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
No. of labs	6	6	6	6	6	6	5	6
Mean (%)	3.45	4.87	16.29	8.60	11.93	2.95	10.98	7.11
s_r	0.34	0.29	0.58	0.49	0.24	0.16	0.24	0.26
s_R	0.64	0.49	1.24	1.99	0.83	0.49	0.40	1.07
RSD_r	9.98	5.94	3.55	5.73	2.00	5.28	2.18	3.64
RSD_R	18.63	10.12	7.60	23.12	6.96	16.73	3.64	15.08
HORRAT	5.61	3.21	2.89	7.99	2.53	4.92	1.31	5.07

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. s_r: within laboratory variability; s_R: between laboratory variability; RSD_r: within laboratory relative variability; and RSD_R: between laboratory relative variability.

Table VIII. Collaborative study data for total dietary fiber (% TDF) calculated as the sum of insoluble and soluble dietary fiber and measured using on-line deionization[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1	28.58	27.53	9.62	9.78	27.40	28.90	11.69	11.63	21.00	20.60	16.24	16.73	22.99	23.24	18.62	19.03
2	29.82	29.36	9.95	9.76	28.88	28.84	7.11dg	7.30dg	20.93	20.86	13.71	14.04	24.28	24.49	17.00	16.79
3	30.16	31.08	10.76	10.69	26.32	26.02	11.30	11.15	20.81	20.57	15.85	16.34	24.47	24.53	17.03	17.16
4	29.84	30.84	9.66	8.76	25.23	25.93	10.90	11.03	19.21	19.96	13.88	13.69	21.81c	24.07c	17.32	17.95
5	27.53	29.32	9.95	9.21	24.23	22.49	8.21dg	6.77dg	18.98	19.57	14.69	15.83	19.95	20.64	17.33	17.39
6	30.72	30.99	10.50	10.81	30.02	29.30	10.63	11.37	21.41	21.76	14.60	15.17	23.01	22.79	16.88	16.68

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. c: Cochran test outlier on TDF and dg: double Grubbs test outlier on TDF.

Table VIII-A. Statistical data for total dietary fiber calculated as the sum of insoluble and soluble dietary fiber and measured using on-line deionization[a]

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
No. of labs	6	6	6	4	6	6	5	6
Mean (%)	29.65	9.95	26.96	11.21	20.47	15.06	23.04	17.43
s_r	0.73	0.36	0.73	0.27	0.32	0.43	0.25	0.24
s_R	1.29	0.66	2.41	0.37	0.90	1.16	1.71	0.77
RSD_r	2.47	3.58	2.70	2.42	1.58	2.87	1.09	1.36
RSD_R	4.33	6.62	8.95	3.33	4.40	7.67	7.40	4.41
HORRAT	1.81	2.34	3.67	1.20	1.73	2.88	2.97	1.69

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. s_r: within laboratory variability; s_R: between laboratory variability; RSD_r: within laboratory relative variability; and RSD_R: between laboratory relative variability.

Table IX. Comparison of soluble dietary fiber measured using on-line deionization versus manual deionization[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1 (on-line)	2.68	2.43	4.32	4.78	17.30	18.10	10.49	10.43	12.50	12.40	2.34	2.53	11.19	11.54	8.32	8.73
1 (manual)	3.00	2.72	4.57	4.80	16.95	17.52	10.58	10.82	12.53	12.02	3.22	3.15	11.10	11.34	6.95	7.51
2 (on-line)	4.43	4.18	4.86	4.85	16.48	15.69	5.95	6.11	12.62	12.55	3.12	3.23	10.96	11.03	6.76	6.53
2 (manual)	4.98	5.86	6.75	7.27	17.84	18.30	11.17	11.01	13.61	14.25	4.02	6.88	12.14	12.76	10.24	9.24
3 (on-line)	2.88	3.79	5.33	4.93	15.94	14.86	9.61	9.25	11.61	11.24	3.25	3.33	11.22	10.56	5.39	6.07
3 (manual)	3.05	4.31	5.76	5.80	17.17	17.08	10.12	11.66	12.66	12.67	10.26	4.58	11.91	11.57	8.13	7.82
4 (on-line)	3.60	3.88	4.57	3.86	14.54	15.15	9.72	9.49	10.63	10.99	2.36	2.82	10.41	12.22	7.26	7.55
4 (manual)	3.71	3.69	5.11	4.69	16.19	16.12	10.51	9.99	11.67	11.69	2.31	3.17	10.16	11.14	7.59	6.52
5 (on-line)	2.94	3.32	5.04	4.88	16.59	15.49	6.95	5.44	11.17	11.69	2.63	2.51	10.47	10.39	7.93	8.05
5 (manual)	3.34	3.32	5.70	5.71	16.37	16.56	11.00	10.88	11.81	11.65	3.70	3.53	10.69	10.49	8.24	8.63
6 (on-line)	3.40	3.90	5.34	5.66	17.61	17.69	9.54	10.19	12.67	13.03	3.57	3.67	11.22	11.26	6.27	6.41
6 (manual)	3.98	4.33	6.16	6.58	17.89	17.95	10.80	11.52	13.53	13.61	4.24	4.43	11.63	12.69	8.68	8.17

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin.

Collaborator Comments

One collaborating laboratory expressed the opinion that new deionization columns should be used each time HPLC is performed, because they observed noisier HPLC performance when the deionization columns were regenerated and reused. Although the regeneration of the deionizing columns described in the method appeared to be effective in most laboratories, further testing led to the conclusion that reuse of resins is not advisable on an ongoing basis. With the new recommended column to contain the resins and the reduced quantity of resin used per sample, column repacking is simple, and the cost of resin is low.

One collaborator reported difficulty obtaining an HPLC pump capable of precise flow (pumps exhibited noisy baselines) at an EDTA solution flow rate of 0.05 mL/min; therefore, the work was done with a flow rate of 0.1 mL/min. The results were in line with those of the other laboratories in the study. Because the analytical column should be calcium saturated, the excess calcium salt flow should not be an issue.

Another collaborator comment concerned the use of Duran bottles rather than beakers for the enzymatic digestion steps in the method. The cap on a Duran bottle retains liquid that must be carefully recovered during filtering. It is also more difficult to transfer traces of dietary fiber from the neck of the bottle to the filter than it is with a beaker, as is used in AACC Intl. Approved Methods 32-05.01 and 32-07.01. Duran bottles were chosen, in conjunction with the shaker, to assure that the entire sample tested properly contacts the enzymes during digestion. Use of the bottle and shaker does not allow rings of sample to form above the solution and keeps the mixture homogeneous during the entire 16 hr of digestion. An ideal solution would be a vessel that is as easy to use as the beakers used in AACC Intl. Approved Method 32-05.01 for manual operations while assuring complete enzyme interaction with the sample during digestion. The study directors agreed there is a limitation in using Duran bottles, so a switch was made to Fisherbrand bottles, which are now part of the recommended procedure.

Table X. Comparison of total dietary fiber measured using on-line deionization versus manual deionization[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1 (on-line)	28.58	27.53	9.62	9.78	27.40	28.90	11.69	11.63	21.00	20.60	16.24	16.73	22.99	23.24	18.62	19.03
1 (manual)	28.90	27.82	9.87	9.80	27.05	28.32	11.78	12.02	21.03	20.22	17.12	17.35	22.90	23.04	17.25	17.81
2 (on-line)	29.82	29.36	9.95	9.76	28.88	28.84	7.11	7.30	20.93	20.86	13.71	14.04	24.28	24.49	17.00	16.79
2 (manual)	30.37	31.04	11.85	12.61	30.24	31.45	12.33	12.20	21.93	22.57	14.60	17.69	25.46	26.81	20.48	19.50
3 (on-line)	30.16	31.08	10.76	10.69	26.32	26.02	11.30	11.15	20.81	20.57	15.85	16.34	24.47	24.53	17.03	17.16
3 (manual)	30.33	31.59	11.19	11.56	27.54	28.24	11.80	13.56	21.86	22.00	22.86	17.61	25.16	25.56	19.78	18.91
4 (on-line)	29.84	30.84	9.66	8.76	25.23	25.93	10.90	11.03	19.21	19.96	13.88	13.69	21.81	24.07	17.32	17.95
4 (manual)	29.95	30.66	10.19	9.60	26.88	26.91	11.69	11.53	20.25	20.65	13.84	14.04	21.56	22.99	17.66	16.92
5 (on-line)	27.53	29.32	9.95	9.21	24.23	22.49	8.21	6.77	18.98	19.57	14.69	15.83	19.95	20.64	17.33	17.39
5 (manual)	27.93	29.32	10.61	10.04	24.01	23.56	12.26	12.21	19.62	19.53	15.76	16.85	20.17	20.74	17.64	17.97
6 (on-line)	30.72	30.99	10.50	10.81	30.02	29.30	10.63	11.37	21.41	21.76	14.60	15.17	23.01	22.79	16.88	16.68
6 (manual)	31.30	31.42	11.32	11.73	30.30	29.56	11.88	12.70	22.26	22.34	15.26	15.93	23.42	24.22	19.29	18.45

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin.

Table XI. Collaborative study data for soluble dietary fiber (SDF) calculated using the external standard approach[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1	2.92	2.62	4.28	4.49	14.80	16.20	9.79	10.40	11.70	11.60	3.20	2.98	10.95	11.13	6.31	7.03
2	3.35	3.31	5.60	5.68	16.47	16.49	9.04	8.78	10.65	10.18	3.03	2.73	10.25	10.06	8.02	8.45
3	4.54c	8.16c	8.31	7.37	19.61	18.41	13.98	12.88	16.31	16.73	6.57	5.80	12.39c	15.34c	11.78	13.56
4	5.04	6.41	6.28	6.72	15.33	17.57	11.58	11.35	13.29	14.19	4.04	6.46	13.61	12.12	10.19	9.22
5	2.77	3.65	4.98	4.41	11.19	13.23	5.56	7.53	9.13	7.23	2.39	3.69	11.29	11.00	5.65	5.87
6	5.19	5.32	5.36	7.34	15.15	17.55	11.16	12.21	13.38	13.57	4.97	3.92	12.45	12.50	9.75c	13.42c
7	4.48	3.59	5.56	5.99	16.74	18.09	10.39	10.32	12.21	11.85	3.18	3.65	11.16	11.91	8.11	7.89
8	4.31	4.32	4.04	4.27	16.03	16.63	9.82	8.74	13.45	13.82	3.40	3.81	11.00	10.94	8.91	7.73
9	3.73	4.06	5.15	5.83	13.99	14.27	8.55	9.99	11.60	12.65	3.91	4.18	11.46	11.96	7.40	7.01
10	4.58	4.11	5.74	4.97	19.82	15.28	11.39	9.37	14.84	12.69	4.45	4.54	11.98	12.13	7.46	7.48
11	3.74	3.65	5.47	4.81	13.42	16.74	10.99	9.70	12.04	12.06	2.27	3.07	10.18	11.25	7.47	6.24
12	2.37	3.12	4.61	4.64	15.80	16.24	9.09	8.91	12.59	12.68	–	–	10.04	10.03	2.55dg	2.48dg

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. –: laboratory reported no result for this sample; c: Cochran test outlier on SDF; and dg: double Grubbs test outlier on SDF.

Table XI-A. Statistical data for soluble dietary fiber calculated using the external standard approach[a]

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
No. of labs	11	12	12	12	12	11	11	10
Mean (%)	3.94	5.49	16.04	10.06	12.52	3.92	11.34	8.09
s_r	0.45	0.54	1.48	0.81	0.67	0.69	0.44	0.63
s_R	1.00	1.11	2.02	1.81	2.10	1.20	0.95	1.99
RSD_r	11.42	9.92	9.21	8.06	5.37	17.58	3.93	7.81
RSD_R	25.40	20.20	12.62	17.95	16.74	30.58	8.39	24.58
HORRAT	7.80	6.53	4.79	6.35	6.12	9.39	3.02	8.42

[a] Samples: A: cabbage; B: mixed grains with apple flakes; C: chocolate with fructooligosaccharides; D: biscuits containing fructooligosaccharides; E: defatted cookies with oat graham, polydextrose, and RS2 starch; F: peanuts; G: oat bran; and H: whole-wheat bread with 2% α-cyclodextrin. s_r: within laboratory variability; s_R: between laboratory variability; RSD_r: within laboratory relative variability; and RSD_R: between laboratory relative variability.

One collaborating laboratory commented that SDFS (dietary fiber that remains soluble in 78% aqueous ethanol) results might be biased due to the use of sorbitol in the internal standard method. The laboratory stated that sorbitol is naturally present in some foods and food products, primarily fruits, and in addition, the laboratory encountered difficulty in properly integrating the peak area of the sorbitol because it is not always baseline resolved from neighboring peaks. The study directors are aware of the presence of sorbitol in a number of fruits such as dried plums (prunes), apples (fresh and dried), and pears (fresh and dried). Sorbitol is also present at varying levels in polydextrose, a synthetic carbohydrate polymer that resists digestion and is used as a food bulking agent that provides reduced calories. The study directors agreed that for these foods and for foods with sorbitol added, low analytical results for SDF would be obtained. Therefore, a note has been added to the method to this effect. The study directors did not encounter problems in integrating the peak area of sorbitol. If a more appropriate internal standard can be identified, it will be evaluated.

One collaborator commented that they preferred to use the glass column-based deionization procedure used in AACC Intl. Approved Method 32-45.01 rather than the disposable plastic column-based deionization procedure provided with the new method. The laboratory is comfortable with the former procedure and also noted that occasionally the plastic columns clog. The study directors did not see an issue with using glass versus plastic for this procedure. The study directors also concluded that a single column with mixed-bed resin was preferable.

Another collaborator commented that the fructosyl trisaccharide (F3) derived from acid or enzymic hydrolysis of inulin elutes from the Sugar-Pak column slightly later than maltose and sucrose and at the same spot as lactose. At the time of the study, this trisaccharide was not available, and the amount present in the Raftilose P-95 product available to the study directors contained a relatively low amount of this trisaccharide. Consequently, the problem with this trisaccharide was not noticed. Further studies by the study directors have resolved this problem as part of sample preparation. Basically, an aliquot of the sample containing F3 is incubated with a thermostable α-glucosidase, which hydrolyzes both sucrose and maltose, allowing the amount of F3 to be determined accurately. For samples containing lactose, incubation with β-galactosidase removes this disaccharide. The determined area of F3 is then added to the area of oligosaccharides eluting earlier than maltotriose. A mixture of α-glucosidase and β-galactosidase will be made available. An alternative solution is chromatography of the oligosaccharide mixture on two size-exclusion LC columns (TSK-Gel, 30 cm × 7.8 mm) connected in a series (Sigma-Aldrich, part no. 808020), as described in AACC Intl. Approved Method 32-45.01.

Recommendation

The study directors recommended this method be granted First Approval status by AACC International. The method was approved in August 2011.

Acknowledgments

The study directors wish to thank the collaborators for all their efforts in completing this study: Tony Bajoras, Sneh Bandari, Manuel Barata, Kommer Brunt, Mary Camire, Steve Cui, Michael P. Dougherty, Anna Draga, Veronica Ernste-Nota, Annette Evans, Tiffany Gallegos, Gian Carlo Gatti, Juergen Hollmans, Yulai Jin, Kenichiro Kanaya, Martijn Leijdekkers, Kai Liu, Mike Marshak, Ursula Neese, Uwe Nienaber, Toyohide Nishibata, Stephan Pasaribu, Hayfa Salman, Peter Sanders, Alessandro Santi, Naomi Sloane, Monique Steegmans, Ruth Sublett, Asta Tervilä-Heikkinen, Heinz Themeier, José van de Laar, Janine van de Ven-Borgmans, Susan I. Whitaker, and Garrett Zielinski.

The study directors would also like to thank all of the organizations that supported this effort: Agriculture and Agri-Food Canada, Beneo-O'Rafti, Co-Sun Laboratories, Covance Laboratories, Danisco, DTS Food Laboratories, Eurofins Heerenveen Laboratories, Eurofins US Laboratories, Japanese Food

Table XII. Collaborative study data for total dietary fiber (TDF) calculated using the external standard approach[a]

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G		Sample H	
1	28.82	27.72	9.58	9.49	24.90	27.00	10.99	11.60	20.20	19.80	17.10	17.18	22.75	22.83	16.61	17.33
2	27.94	29.31	10.51	10.01	24.11	23.49	10.30	10.11	18.46	18.06	15.09	16.05	19.73	20.31	17.42	17.79
3	31.14	33.17	13.03	11.88	26.90	27.44	15.19	13.99	24.11	24.38	17.88	16.25	23.29	26.10	20.64	22.13
4	30.43	31.59	11.37	11.63	27.72	30.73	12.75	12.53	21.60	22.50	14.63	17.26	26.94	25.59	20.43	19.48
5	30.05	30.93	10.40	10.17	21.57	24.38	7.24	9.42	18.33	16.56	14.99	16.71	24.54	24.98	17.30	16.97
6	32.10	35.84	9.48	11.63	25.54	26.29	11.70	12.74	21.07	24.65	42.91sg	42.24sg	28.55	29.43	25.51dg	28.47dg
7	29.18	29.09	10.91	11.15	25.52	26.79	11.62	11.83	20.65	20.50	14.72	15.11	23.09	23.90	18.42	18.71
8	28.26	28.76	8.47	8.52	23.30	24.13	10.53	9.02	21.63	21.48	13.70	14.19	20.95	21.04	17.81	16.50
9	31.04	31.15	10.31	10.98	26.40	25.88	9.63	11.17	20.34	21.38	14.94	15.67	23.24	23.49	18.00	17.28
10	28.21	28.11	10.55	9.71	27.99	23.70	12.40	10.43	22.83	20.87	15.94	16.37	24.21	24.05	16.81	17.25
11	29.98	30.62	10.55	9.71	24.11	27.53	12.17	11.24	20.62	21.03	13.80	13.93	21.58	23.10	17.54	16.64
12	28.05	29.59	9.22	9.02	23.39	23.54	10.26	10.01	20.66	20.68	–	–	20.37	20.30	12.11dg	11.53dg

[a] Samples: A = cabbage; B = mixed grains with apple flakes; C = chocolate with fructooligosaccharides; D = biscuits containing fructooligosaccharides; E = defatted cookies with oat graham, polydextrose, and RS2 starch; F = peanuts; G = oat bran; and H = whole-wheat bread with 2% α-cyclodextrin. –: laboratory reported no result for this sample; sg: single Grubbs test outlier on TDF; and dg: double Grubbs test outlier on TDF.

Table XII-A. Statistical data for soluble dietary fiber calculated using the external standard approach[a]

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
No. of labs	12	12	12	12	12	10	12	10
Mean (%)	30.05	10.35	25.52	11.20	20.93	15.58	23.52	18.05
s_r	1.05	0.59	1.52	0.85	0.96	0.85	0.77	0.60
s_R	1.95	1.13	2.08	1.71	1.97	1.27	2.61	1.55
RSD_r	3.49	5.67	5.95	7.57	4.61	5.49	3.26	3.32
RSD_R	6.47	10.90	8.13	15.26	9.41	8.18	11.08	8.61
HORRAT	2.70	3.87	3.31	5.49	3.72	3.09	4.46	3.33

[a] Samples: A = cabbage; B = mixed grains with apple flakes; C = chocolate with fructooligosaccharides; D = biscuits containing fructooligosaccharides; E = defatted cookies with oat graham, polydextrose, and RS2 starch; F = peanuts; G = oat bran; and H = whole-wheat bread with 2% α-cyclodextrin. s_r: within laboratory variability; s_R: between laboratory variability; RSD_r: within laboratory relative variability; and RSD_R: between laboratory relative variability.

Institute, Kellogg, Kraft Foods, Macroanilitca Neotron Laboratories, Matsutani, Medallion Laboratories/General Mills, Megazyme, MRI Laboratories, Roquette, Silliker US Laboratories, Tate and Lyle, University of Maine, and VWA.

References

1. AACC International. *Approved Methods of Analysis*, 11th ed. AACC International, St. Paul, MN, 2010.
2. AACC International. *Approved Methods of Analysis*, 11th ed. rev. AACC International, St. Paul, MN, 2011.
3. Codex Alimentarius Commission. ALINORM 09/32/26. Published online at www.codexalimentarius.net/web/archives.jsp. FAO/Who, Rome, Italy, 2009.
4. Codex Alimentarius Commission. ALINORM 09/32/REP Published online at www.codexalimentarius.net/web/archives.jsp. FAO/Who, Rome, Italy, 2009.
5. Codex Alimentarius Commission. ALINORM 10/33/26 Published online at www.codexalimentarius.net/web/archives.jsp. FAO/Who, Rome, Italy, 2010.
6. Codex Alimentarius Commission. ALINORM 10/33/REP Published online at www.codexalimentarius.net/web/archives.jsp. FAO/Who, Rome, Italy, 2010.
7. DeVries, J. W., and Rader, J. I. Historical perspective as a guide for identifying and developing applicable methods for dietary fiber. J. AOAC Int. 88:1349, 2005.
8. McCleary, B. V. An integrated procedure for the measurement of total dietary fibre (including resistant starch), non-digestible oligosaccharides and available carbohydrates. Anal. Bioanal. Chem. 389:291, 2007.
9. McCleary, B. V., DeVries, J. W., Rader, J. I., Cohen, G., Prosky, L., Mugford, D. C., Champ, M., and Okuma, K. Determination of total dietary fiber (Codex definition) by enzymatic-gravimetric method and liquid chromatography: Collaborative study. J. AOAC Int. 93:221, 2010.
10. Post, B. E., Marshak, M. R., and DeVries, J. W. Simultaneous ion removal and quantitation of low-molecular-weight dietary fiber from high-molecular-weight dietary fiber filtrates using liquid chromatography. J. AOAC Int. 93:234, 2010.
11. Prosky, L., Asp, N. G., Furda, I., DeVries, J. W., Schweizer, T. F., and Harland, B. F. Determination of total dietary fiber in foods and food products: Collaborative study. J. Assoc. Off. Anal. Chem. 68:677, 1985.
12. Trowell, H. Crude fibre, dietary fibre and atherosclerosis. Atherosclerosis 16:138, 1972.
13. Trowell, H. Ischemic heart disease and dietary fibre. Am. J. Clin. Nutr. 25:926, 1972.
14. Trowell, H. C. Definitions of fibre. Lancet 1:503, 1974.
15. Trowell, H. C., Southgate, D. A. T., Wolever, T. M. S., Leeds, A. R., Gassull, M. A., and Jenkins, D. J. A. Dietary fiber redefined. Lancet 1:967, 1976.
16. U.S. Code of Federal Regulations. Health claims: Soluble fiber from certain foods and risk of coronary heart disease (CHD). 21 CFR § 101.81. Published online at http://cfr.vlex.com/source/code-federal-regulations-food-drus-1070. Food and Drug Administration, Washington, DC, 2011.