AOAC Official Method 985.29
Total Dietary Fiber in Foods

Enzymatic–Gravimetric Method
First Action 1985
Final Action 1986

AOAC–AACC Method
Codex-Adopted–AOAC Method*

A. Principle

Duplicate test portions of dried foods, fat-extracted if containing >10% fat, are gelatinized with Termamyl (heat-stable α-amylase), and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. (When analyzing mixed diets, always extract fat prior to determining total dietary fiber.) Four volumes of ethyl alcohol are added to precipitate soluble dietary fiber. Total residue is filtered, washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying, residue is weighed. One duplicate is analyzed for protein, and other is incinerated at 525°C and ash is determined. Total dietary fiber = weight residue – weight (protein + ash).

B. Apparatus

(**a**) *Fritted crucible.*—Porosity No. 2 (Pyrex No. 32940, coarse, ASTM 40-60 μm; or Corning No. 36060 Büchner, fritted disk, Pyrex, 60 mL, ASTM 40-60 μm). Clean thoroughly, heat 1 h at 525°C, and soak and then rinse in H_2O. Add ca 0.5 g Celite to air-dried crucibles and dry at 130°C to constant weight (≥ 1 h). Cool and store in desiccator until used.

(**b**) *Vacuum source.*—Vacuum pump or aspirator equipped with in-line double vacuum flask to prevent contamination in case of H_2O backup.

(**c**) *Vacuum oven.*—70°C. Alternatively, 105°C air oven can be used.

(**d**) *Desiccator.*

(**e**) *Muffle furnace*.

(**f**) *Water baths.*—(*1*) *Boiling*. (*2*) *Constant temperature.*—Adjustable to 60°C, with either multistation shaker or multistation magnetic stirrer to provide constant agitation of digestion flasks during enzymatic hydrolysis.

(**g**) *Beakers.*—Tall-form, 400 or 600 mL.

(**h**) *Balance.*—Analytical, readability to 0.1 mg.

(**i**) *pH meter.*—Standardized with pH 7 and pH 4 buffers.

C. Reagents

(**a**) *95% Ethanol.*—v/v. Technical grade.

(**b**) *78% Ethanol.*—Place 207 mL H_2O into 1 L volumetric flask. Dilute to volume with 95% ethyl alcohol. Mix and dilute to volume again with 95% ethyl alcohol if necessary. Mix. One volume H_2O mixed with 4 volumes 95% ethyl alcohol will also give 78% ethyl alcohol final concentration.

(**c**) *Acetone.*

(**d**) *Phosphate buffer.*—0.08M, pH 6.0. Dissolve 1.400 g sodium phosphate dibasic, anhydrous (Na_2HPO_4) (or 1.753 g dihydrate) and 9.68 g sodium phosphate monobasic monohydrate (NaH_2PO_4 H_2O) (or 10.94 g dihydrate) in ca 700 mL H_2O. Dilute to 1 L with H_2O. Check pH with pH meter.

(**e**) *Alpha-amylase (heat stable).*—Termamyl. (*1*) Store in refrigerator. *Based on Nelson/Somogyi reducing sugar with soluble starch as substrate.*—10 000 + 1000 units/mL (1 unit is defined as the amount of enzyme required to release 1 μmole reducing sugar equivalents/min at pH 6.5 and 40°C). (*2*) *Based on Ceralpha method using p-nitrophenyl-maltosaccharide as substrate in the presence of a thermostable alpha-glucosidase.*—3000 + 300 Ceralpha units/mL (1 unit of enzyme is required to release 1 μmole *p*-nitrophenyl/min at pH 6.5 and 40°C).

(**f**) *Protease.*—Keep refrigerated. (*1*) *Casein assay.*—300–400 Units/mL. (1 protease unit is defined as the amount of enzyme required to hydrolyze (and solubilize in TCA) 1 μmole tyrosine equivalents/min from soluble casein at pH 8.0 and 40°C); 7–15 units/mg (1 unit will hydrolyze casein to produce color equivalent to 1.0 μmole tyrosine/min at pH 7.5 and 37°C). Color by Folin-Ciocalteau reagent. (*2*) *Azo-casein assay.*—300–400 Units/mL [1 unit endo-peptidase activity is defined as the amount of enzyme required to hydrolyze (and solubilize in TCA) 1 μmole tyrosine equivalents/min from soluble casein at pH 8.0 and 40°C].

(**g**) *Amyloglucosidase.*—Keep refrigerated. (*1*) *Starch/glucose oxidase–peroxidase method.*—2000–3300 Units/mL (1 unit enzyme activity is defined as the amount of enzyme required to release 1 μmole glucose/min at pH 4.5 and 40°C). (*2*) *PNPBM (p-nitrophenyl beta-maltosidase) method.*—130–200 Units/mL (1 unit enzyme activity [PNP unit] is the amount of enzyme, which in the presence of excess levels of beta-glucosidase, will release 1 μmole *p*-nitrophenyl from *p*-nitrophenyl beta-maltosidase/min at 40°C).

The only enzyme which has been found to be significantly contaminated with interfering activities is amyloglucosidase. Thermostable alpha-amylase and protease from commercial sources have been found to be generally free of interfering enzymes. Low levels of beta-glucanase have been detected in protease preparations, but at levels well below that which would interfere with total dietary fiber analysis. The major contaminant in amyloglucosidase preparation was shown to be an endo-cellulase and resulted in endo-depolymerization of mixed-linkage beta-glucan from barley and oats, with resultant underestimation of this dietary fiber component. The contamination of amylogucosidase with endo-cellulase (beta-glucanase) can be easily detected.

Alternatively, there are kits containing all 3 enzymes (pretested) available from a number of companies.

(**h**) *Sodium hydroxide solution.*—0.275M. Dissolve 11.00 g NaOH ACS in ca 700 mL H_2O in 1 L volumetric flask. Dilute to volume with H_2O.

(**i**) *Hydrochloric acid solution.*—0.325M. Dilute stock solution of known titer, e.g., 325 mL 1M HCl, to 1 L with H_2O.

(**j**) *Celite.*—Acid-washed.

Table 985.29. Test samples for enzyme purity

Test sample	Activity tested	Test portion weight, g	Expected recovery, %
Citrus pectin	Pectinase	0.1	95–100
Stractan (larch gum)	Hemicellulase	0.1	95–100
Wheat starch	Amylase	1.0	0–1
Corn starch	Amylase	1.0	0–2
Casein	Protease	0.3	0–2
β-Glucan (barley gum)[a]	β-Glucanase	0.1	95–100

[a] Sigma Chemical Co. or Megazyme International Ireland, Ltd.

D. Enzyme Purity

To ensure absence of undesirable enzymatic activity in enzymes used in this procedure, run materials listed in Table **985.29** through entire procedure each time lot of enzymes is changed, or at maximum interval of 6 months to ensure that enzymes have not degraded.

E. Test Portion Preparation

Determine total dietary fiber on dried test sample. Homogenize test sample and dry overnight in 70°C vacuum oven, cool in desiccator, and dry-mill test sample to 0.3–0.5 mm mesh. If test sample cannot be heated, freeze-dry before milling. If high fat content (>10%) prevents proper milling, defat with petroleum ether (3 times with 25 mL portions/g test sample) before milling. Record loss of weight due to fat removal and make appropriate correction to final % dietary fiber found in determination. Store dry-milled test sample in capped jar in desiccator until analysis is carried out.

F. Determination

Run blank through entire procedure along with test portions to measure any contribution from reagents to residue.

Weigh duplicate 1 g test portions, accurate to 0.1 mg, into 400 mL tall-form beakers. Test portion weights should not differ >20 mg. Add 50 mL pH 6.0 phosphate buffer to each beaker. Check pH and adjust to pH 6.0 ± 0.2 if necessary. Add 0.1 mL Termamyl solution. Cover beaker with Al foil and place in boiling water bath 15 min. Shake gently at 5 min intervals. Increase incubation time when number of beakers in boiling water bath makes it difficult for beaker contents to reach internal temperature of 95°–100°C. Use thermometer to indicate that 15 min at 95°–100°C is attained. Total of 30 min in water bath should be sufficient.

Cool solutions to room temperature. Adjust to pH 7.5 ± 0.2 by adding 10 mL 0.275M NaOH solution.

Add 5 mg protease. (Protease sticks to spatula, so it may be preferable to prepare enzyme solution (50 mg in 1 mL phosphate buffer) and pipet 0.1 mL to each sample just before use.

Cover beaker with Al foil. Incubate 30 min at 60°C with continuous agitation. Cool. Add 10 mL 0.325M HCl solution. Measure pH and dropwise add acid if necessary. Final pH should be 4.0–4.6. Add 0.3 mL amyloglucosidase, cover with Al foil, and incubate 30 min at 60°C with continuous agitation. Add 280 mL 95% ethyl alcohol preheated to 60°C (measure volume before heating). Let precipitate form at room temperature for 60 min.

Weigh crucible containing Celite to nearest 0.1 mg, then wet and redistribute bed of Celite in crucible by using stream of 78% ethyl alcohol from wash bottle. Apply suction to draw Celite onto fritted glass as even mat. Maintain suction and quantitatively transfer precipitate from enzyme digest to crucible.

Wash residue successively with three 20 mL portions of 78% ethyl alcohol, two 10 mL portions of 95% ethyl alcohol, and two 10 mL portions of acetone. Gum may form with some products, trapping liquid. If so, break surface film with spatula to improve filtration. Time for filtration and washing will vary from 0.1 to 6 h, averaging 0.5 h per sample. Long filtration times can be avoided by careful intermittent suction throughout filtration.

Dry crucible containing residue overnight in 70°C vacuum oven or 105°C air oven. Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine weight of residue.

Analyze residue from 1 test portion of set of duplicates for protein by 960.52 (*see* 12.1.07), using N ×6.25 as conversion factor, except in cases where N content in protein is known.

Incinerate second test portion of duplicate 5 h at 525°C. Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine ash.

G. Calculations

Determination of blank:

$$B = \text{blank, mg} = \text{weight residue} - P_B - A_B$$

where weight residue = average of residue weights (mg) for duplicate blank determinations; and P_B and A_B = weights (mg) of protein and ash, respectively, determined in first and second blank residues.

Calculate TDF as follows:

$$\text{TDF, \%} = [(\text{weight residue} - P - A - B) / \text{weight test portion}] \times 100$$

where weight residue = average of weights (mg) for duplicate blank determinations; and P and A = weights (mg) of protein and ash, respectively, in first and second test portion residues; and weight test portion = average of 2 test portion weights (mg) taken.

References: *JAOAC* **68,** 677(1985); **69,** 259(1986).

Revised: June 2003

* Adopted as a Codex Defining Method for gravimetry/enzymatic digest of total dietary fibre in special foods.

AOAC Official Method 991.42
Insoluble Dietary Fiber in Foods and Food Products

Enzymatic–Gravimetric Method, Phosphate Buffer
First Action 1991
Final Action 1994

(Applicable to determination of insoluble dietary fiber in vegetables, fruits, and cereal grains.)

See Table **991.42** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Duplicate test portions of dried foods, fat-extracted if they contain >10% fat, are gelatinized with Termamyl (heat-stable alpha-amylase) and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Soluble dietary fiber is removed by filtering and washing residue with water. Remaining residue, insoluble dietary fiber (IDF), is washed with 95% ethanol and acetone, dried, and weighed. One duplicate is analyzed for protein, and the other is incinerated at 525°C to determine ash. IDF is weight of residue less weight of protein and ash.

B. Apparatus

See 985.29B (*see* 45.4.07).

C. Reagents

See 985.29C (*see* 45.4.07). [*Note:* Reagent (**e**), α-amylase, is available from a number of sources.]

(**a**) *85% Methanol.*—Place 150 mL H_2O into 1 L volumetric flask and dilute to volume with methanol.

D. Enzyme Purity

See 985.29D (*see* 45.4.07).

E. Preparation of Test Sample

Analyze dry foods without pretreatment whenever possible. Mill dry products to 0.3–0.5 mm mesh. Homogenize and freeze-dry wet foods before milling. If high fat content (>10%) prevents proper milling, defat with petroleum ether (3 times with 25 mL portions/g test portion) before milling. Determine residual moisture in milled foods by drying overnight in 70°C vacuum oven, or 5 h in 105°C air oven. Record weight loss due to fat and/or water, and make appropriate correction to final percent total dietary fiber. (*Note:* For foods high in sugars that cannot be dried by lyophilization, extract 3 times each with 10 volumes of 85% methanol to remove sugars, which may interfere in the determination.)

F. Determination

Run blank with test portions to measure any contribution from reagents to residue.

Weigh duplicate 1 g test portions, accurate to 0.1 mg, into 400 mL tall-form beakers. Duplicate test portion weights should not differ >20 mg. Add 50 mL phosphate buffer to each beaker. Check pH and adjust to pH 6.0 ± 0.2, by adding 0.275M NaOH or 0.325M HCl. Add 0.1 mL alpha-amylase to each beaker. Cover beakers with Al foil and place in boiling water bath. Shake beakers gently at 5 min

Table 991.42. Interlaboratory study results for insoluble dietary fiber in foods and food products, enzymatic–gravimetric method, phosphate buffer

Food/food product	No. labs	IDF, average %	s_r	s_R	RSD_r, %	RSD_R, %	HorRat
Beans, butter	10	17.36	0.41	1.96	2.34	11.31	4.35
Beans, French	10	25.64	0.83	1.51	3.23	5.87	2.39
Beans, kidney	13	16.33	0.74	1.04	4.53	6.39	2.43
Brussels sprouts	15	30.23	0.69	2.39	2.27	7.89	3.30
Cabbage	9	21.60	0.86	1.68	4.00	7.79	3.10
Carrots	12	32.29	1.74	3.68	5.38	11.39	4.81
Chick peas	12	16.69	1.73	2.80	10.38	16.80	6.42
Okra	14	24.15	1.55	3.28	6.43	13.57	5.48
Onions	12	13.32	0.87	1.57	6.51	11.79	4.36
Parsley	12	34.39	1.22	4.69	3.56	13.64	5.81
Turnips	12	21.38	1.41	3.55	6.60	16.61	6.59
Apples	4	55.57	0.51	2.53	0.92	4.55	2.08
Apricots	5	44.92	0.39	3.69	0.86	8.22	3.65
Figs, Calimyrna	5	43.07	2.41	7.92	5.59	18.40	8.11
Figs, Mission	6	33.61	0.93	4.06	2.76	12.09	5.13
Peaches	6	39.53	0.86	2.44	2.17	6.16	2.68
Prunes	6	46.18	2.82	8.98	6.11	19.44	8.66
Raisins	8	49.18	2.71	9.49	5.51	19.30	8.68
Barley	12	4.30	0.43	0.62	9.92	14.33	4.47
Rye flour	15	11.81	0.58	1.02	4.87	8.62	3.13
Soy bran	13	65.24	0.91	2.40	1.40	3.68	1.73
Wheat germ	9	15.67	0.71	0.96	4.54	6.13	2.32

intervals throughout incubation. When thermometer indicates beaker contents have reached 100°C, continue incubation 15 min. Total of 30 min in bath is usually sufficient. Cool solutions to room temperature. Adjust to pH 7.5 ± 0.1 by adding ca 10 mL NaOH solution.

Add 5 mg protease to each solution. Protease sticks to spatula, so it may be preferable to prepare enzyme solution (50 mg in 1 mL phosphate buffer) just before use, and pipet 0.1 mL to each test mixture.

Cover beakers with Al foil. Incubate 30 min at 60°C with continuous agitation. Cool. Check pH and adjust to pH 4.0–4.6 with ca 10 mL HCl solution. Add 0.3 mL amyloglucosidase, cover with Al foil, and incubate 30 min at 60°C with continuous agitation.

Weigh crucible containing Celite to nearest 0.1 mg, then wet and redistribute bed of Celite in crucible using stream of water from wash bottle. Apply suction to draw Celite onto fritted glass as even mat. Apply enzyme-digested mixture from beaker to crucible, filtering into suction flask. Wash residue 2 times with 10 mL water (removing soluble dietary fiber), 2 times with 10 mL 95% ethanol, and 2 times with 10 mL acetone. Break surface film that develops after addition of digest to Celite with spatula, to improve filtration. Careful intermittent suction throughout filtration and back-bubbling with air, if available, will speed up filtrations. Normal suction can be applied at washing.

Dry crucible containing residue overnight in 70°C vacuum oven or 5 h in 105°C air oven. Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weights to determine residue weight.

Using one of duplicates, scrape residue, Celite, and fiber mat onto filter paper which can be folded shut, and analyze for protein by 960.52 (*see* 12.1.07). Use $N \times 6.25$ as conversion factor.

Incinerate second of duplicates 5 h at 525°C. Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine ash.

G. Calculations

See 985.29G (*see* 45.4.07), calculating IDF as described for TDF.

Reference: *J. AOAC Int.* **75**, 360(1992).

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AOAC Official Method 991.43
Total, Soluble, and Insoluble Dietary Fiber in Foods

Enzymatic–Gravimetric Method, MES–TRIS Buffer
First Action 1991
Final Action 1994

(Applicable to processed foods, grain and cereal products, fruits, and vegetables.)

See Table **991.43A** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Duplicate test portions of dried foods, fat-extracted if containing >10% fat, undergo sequential enzymatic digestion by heat stable α-amylase, protease, and amyloglycosidase to remove starch and protein. For total dietary fiber (TDF), enzyme digestate is treated with alcohol to precipitate soluble dietary fiber before filtering, and TDF residue is washed with alcohol and acetone, dried, and weighed. For insoluble and soluble dietary fiber (IDF and SDF), enzyme digestate is filtered, and residue (IDF) is washed with warm water, dried and weighed. For SDF, combined filtrate and washes are precipitated with alcohol, filtered, dried, and weighed. TDF, IDF, and SDF residue values are corrected for protein, ash, and blank.

B. Apparatus

(**a**) *Beakers.*—400 or 600 mL tall-form.

(**b**) *Filtering crucible.*—With fritted disk, coarse, ASTM 40–60 μm pore size, Pyrex 60 mL (Corning No. 36060 Büchner; Corning, Inc., Science Products, Corning, NY 14831 USA, or equivalent). Prepare as follows: Ash overnight at 525°C in muffle furnace. Let furnace temperature fall below 130°C before removing crucibles. Soak crucibles 1 h in 2% cleaning solution at room temperature. Rinse crucibles with H_2O and then deionized H_2O; for final rinse, use 15 mL acetone and then air-dry. Add ca 1.0 g Celite to dry crucibles, and dry at 130°C to constant weight. Cool crucible ca 1 h in desiccator, and record weight, to nearest 0.1 mg, of crucible plus Celite.

(**c**) *Vacuum system.*—Vacuum pump or aspirator with regulating device. Heavy walled filtering flask, 1 L, with side arm. Rubber ring adaptors, for use with filtering flasks.

(**d**) *Shaking water baths.*—(*1*) Capable of maintaining 98° ± 2°C, with automatic on-and-off timer. (*2*) Constant temperature, adjustable to 60°C.

(**e**) *Balance.*—Analytical, readability ±0.1 mg.

(**f**) *Muffle furnace.*—Maintaining 525° ± 5°C.

(**g**) *Oven.*—Maintaining 105°C and 130° ± 3°C.

(**h**) *Desiccator.*—With SiO_2 or equivalent desiccant. Biweekly, dry desiccant overnight at 130°C.

(**i**) *pH meter.*—Temperature compensated, standardized with pH 4.0, 7.0, and 10.0 buffer solutions.

(**j**) *Pipetters.*—With disposable tips, 100–300 μL and 5 mL capacity.

(**k**) *Dispensers.*—Dispensing 15 ± 0.5 mL for 78% ethanol, 95% ethanol, and acetone; 40 ± 0.5 mL for buffer.

(**l**) *Magnetic stirrers and stir bars.*

C. Reagents

Use deionized water throughout.

(**a**) *Ethanol solutions.*—(*1*) *85%.*—Place 895 mL 95% ethanol into 1 L volumetric flask, dilute to volume with H_2O. (*2*) *78%.*— Place 821 mL 95% ethanol into 1 L volumetric flask, dilute to volume with H_2O.

(**b**) α-*Amylase solution (heat stable).*—Store at 0°–5°C. (*1*) *Based on Nelson/Somogyi reducing sugar with soluble starch as substrate.*—10 000 ± 1000 units/mL (1 unit is defined as the amount of enzyme required to release 1 μmole reducing sugar equivalents per minute at pH 6.5 and 40°C). (*2*) *Based on Ceralpha method using p-nitrophenyl-maltosaccharide as substrate in the presence of a thermostable alpha-glucosidase.*—3000 ± 300 Ceralpha units/mL (1 unit of enzyme is required to release 1 μmole *p*-nitrophenyl per minute at pH 6.5 and 40°C).

(**c**) *Protease.*—Prepare 50 mg/mL enzyme solution in MES–TRIS buffer fresh daily. Store at 0°–5°C. (*1*) *Casein assay.*—300–400 units/mL [1 protease unit is defined as the amount of enzyme required to hydrolyze (and solubilize in TCA) 1 μmole tyrosine equivalents per minute from soluble casein at pH 8.0 and 40°C]; 7–15 units/mg (1 unit will hydrolyze casein to produce color equivalent to 1.0 μmole tyrosine per minute at pH 7.5 and 37°C). (Color by Folin–Ciocalteau reagent.) (*2*) *Azo-casein assay.*—300–400 units/mL [1 unit of endo-peptidase activity is defined as the amount of enzyme required to hydrolyze (and solubilize in TCA) 1 μmole tyrosine equivalents per minute from soluble casein at pH 8.0 and 40°C].

(**d**) *Amyloglucosidase.*—Store at 0°–5°C. (*1*) *Starch/glucose oxidase–peroxidase method.*—2000–3300 units/mL (1 unit of enzyme activity is defined as the amount of enzyme required to release 1 μmole glucose per minute at pH 4.5 and 40°C). (*2*) *PNPBM (p-nitrophenyl beta-maltosidase) method.*—130–200 units/mL [1 unit of enzyme activity (PNP unit) is the amount of enzyme, which in the presence of excess levels of beta-glucosidase, will release 1 μmole *p*-nitrophenyl from *p*-nitrophenyl beta-maltosidase per minute at 40°C].

The only enzyme which has been found to be significantly contaminated with interfering activities is amyloglucosidase. Thermostable alpha-amylase and protease from commercial sources have been found to be generally free of interfering enzymes. Low levels of beta-glucanase have been detected in protease preparations, but at levels well below that which would interfere with total dietary fiber analysis. The major contaminant in amyloglucosidase preparation was shown to be an endo-cellulase and resulted in endo-depolymerization of mixed-linkage beta-glucan from barley and oats, with resultant underestimation of this dietary fiber component. The contamination of amyloglucosidase with endo-cellulase (beta-glucanase) can be easily detected. Alternatively, there are kits containing all 3 enzymes (pre-tested) available from a number of companies.

(**e**) *Diatomaceous earth.*—Acid washed Celite.

(**f**) *Cleaning solution.*—Liquid surfactant-type laboratory cleaner, designed for critical cleaning. Prepare 2% solution in H_2O.

(**g**) *MES.*—2-(*N*-Morpholino)ethanesulfonic acid.

(**h**) *TRIS.*—Tris(hydroxymethyl)aminomethane.

(**i**) *MES–TRIS buffer solution.*—0.05M MES, 0.05M TRIS, pH 8.2 at 24°C. Dissolve 19.52 g MES and 12.2 g TRIS in 1.7 L H_2O. Adjust pH to 8.2 with 6M NaOH, and dilute to 2 L with H_2O. (*Note:* It is important to adjust pH to 8.2 at 24°C. However, if buffer temperature is 20°C, adjust pH to 8.3; if temperature is 28°C, adjust pH to 8.1. For deviations between 20° and 28°C, adjust by interpolation.)

Table 991.43A. Interlaboratory study results for total, soluble, and insoluble dietary fiber in foods (fresh weight basis), enzymatic–gravimetric method, MES–TRIS buffer

Food	Mean, g/100 g	s_r	s_R	RSD_r, %	RSD_R, %	HorRat
Total dietary fiber (TDF)						
Barley	12.25	0.36	0.85	2.88	6.89	2.51
High-fiber cereal	33.73	0.70	0.94	2.08	2.79	1.19
Oat bran	16.92	1.06	2.06	6.26	12.17	4.66
Soy bran	67.14	1.01	1.06	1.50	1.58	0.74
Apricots	1.12	0.01	0.01	0.89	0.89	0.23
Prunes	9.29	0.13	0.40	1.40	4.31	1.51
Raisins	3.13	0.09	0.15	2.88	4.79	1.42
Carrots	3.93	0.13	0.13	3.31	3.31	1.02
Green beans	2.89	0.07	0.07	2.42	2.42	0.71
Parsley	2.66	0.07	0.14	2.63	5.26	1.53
Soluble dietary fiber (SDF)						
Barley	5.02	0.40	0.62	8.01	12.29	3.92
High-fiber cereal	2.78	0.44	0.56	15.83	20.14	5.88
Oat bran	7.17	0.72	1.14	10.04	15.90	5.35
Soy bran	6.90	0.30	0.60	4.35	8.70	2.91
Apricots	0.53	0.02	0.02	3.77	3.77	0.86
Prunes	5.07	0.11	0.31	2.17	6.11	1.95
Raisins	0.73	0.05	0.16	6.85	21.92	5.24
Carrots	1.10	0.07	0.18	6.36	16.36	4.16
Green beans	1.02	0.08	0.11	7.84	10.78	2.71
Parsley	0.64	0.03	0.10	4.69	15.63	3.66
Insoluble dietary fiber (IDF)						
Barley	7.05	0.61	0.61	8.62	8.62	2.90
High-fiber cereal	30.52	0.44	0.71	1.44	2.33	0.98
Oat bran	9.73	0.85	1.17	8.74	12.02	4.24
Soy bran	60.53	0.70	0.70	1.16	1.16	0.54
Apricots	0.59	0.02	0.02	3.39	3.39	0.78
Prunes	4.17	0.07	0.09	1.68	2.16	0.67
Raisins	2.37	0.04	0.07	1.69	2.95	0.84
Carrots	2.81	0.09	0.16	3.20	5.69	1.66
Green beans	2.01	0.08	0.08	3.98	3.98	1.11
Parsley	2.37	0.12	0.24	5.06	10.13	2.89
Total dietary fiber (SDF + IDF)						
Barley	12.14	0.39	0.70	3.21	5.77	2.10
High-fiber cereal	33.30	0.63	0.90	1.89	2.70	1.14
Oat bran	16.90	0.99	1.49	5.86	8.82	3.38
Soy bran	67.56	0.56	0.94	0.83	1.39	0.66
Apricots	1.12	0.02	0.02	1.79	1.79	0.46
Prunes	9.37	0.12	0.30	1.28	3.20	1.12
Raisins	3.10	0.05	0.18	1.61	5.81	1.73
Carrots	3.92	0.11	0.13	2.81	3.32	1.02
Green beans	3.03	0.09	0.12	2.97	3.96	1.17
Parsley	3.01	0.12	0.23	3.99	7.64	2.26

Table 991.43B. Standards for testing enzyme activity

Standard	Activity tested	Weight of standard, g	Expected recovery, %
Citrus pectin	Pectinase	0.1–0.2	95–100
Arabinogalactan	Hemicellulase	0.1–0.2	95–100
β-Glucan	β-Glucanase	0.1–0.2	95–100
Wheat starch	α-Amylase + AMG	1.0	0–1
Corn starch	α-Amylase + AMG	1.0	0–1
Casein	Protease	0.3	0–1

(**j**) *Hydrochloric acid solution.*—0.561M. Add 93.5 mL 6M HCl to ca 700 mL H_2O in 1 L volumetric flask. Dilute to 1 L with H_2O.

D. Enzyme Purity

To ensure absence of undesirable enzymatic activities and presence of desirable enzymatic activities, run standards listed in Table **991.43B** each time enzyme lot changes or at maximum interval of 6 months.

E. Preparation of Test Suspension

Prepare test portions as in 985.29E (*see* 45.4.07) (if fat content of test sample is unknown, defat before determining dietary fiber). For high sugar products, desugar before determining dietary fiber by extracting 2–3 times with 85% ethanol, 10 mL/g, decanting, and then drying overnight at 40°C.

Run 2 blanks/assay with test portions to measure any contribution from reagents to residue.

Weigh duplicate 1.000 ± 0.005 g test portions (M_1 and M_2), accurate to 0.1 mg, into 400 mL (or 600 mL) tall-form beakers. Add 40 mL MES–TRIS buffer solution, pH 8.2, to each. Stir on magnetic stirrer until test portion is completely dispersed (to prevent lump formation, which would make test material inaccessible to enzymes).

Add 50 µL heat-stable α-amylase solution, stirring at low speed. Cover beakers with Al foil, and incubate in 95°–100°C water bath 15 min with continuous agitation. Start timing once bath temperature reaches 95°C (total of 35 min is normally sufficient).

Remove all beakers from bath, and cool to 60°C. Remove foil. Scrape any ring from inside of beaker and disperse any gels in bottom of beaker with spatula. Rinse beaker walls and spatula with 10 mL H_2O.

Add 100 µL protease solution to each beaker. Cover with Al foil, and incubate 30 min at 60° ± 1°C with continuous agitation. Start timing when bath temperature reaches 60°C.

Remove foil. Dispense 5 mL 0.561M HCl into beakers while stirring. Adjust pH to 4.0–4.7 at 60°C, by adding 1M NaOH solution or 1M HCl solution. (*Note:* It is important to check and adjust pH while solutions are 60°C because pH will increase at lower temperatures.) (Most cereal, grain, and vegetable products do not require pH adjustment. Once verified for each laboratory, pH checking procedure can be omitted. As a precaution, check pH of blank routinely; if outside desirable range, check test solutions also.)

Add 300 µL amyloglucosidase solution while stirring. Cover with Al foil, and incubate 30 min at 60° ± 1°C with constant agitation. Start timing once bath reaches 60°C.

F. Determination of Total Dietary Fiber

To each digested test solution, add 225 mL (measured after heating) 95% ethanol at 60°C. Ratio of ethanol to test solution volume should be 4:1. Remove from bath, and cover beakers with large sheets of Al foil. Let precipitate form 1 h at room temperature.

Wet and redistribute Celite bed in previously tared crucible **B(b)**, using 15 mL 78% ethanol from wash bottle. Apply suction to crucible to draw Celite onto fritted glass as even mat.

Filter alcohol-treated enzyme digestate through crucible. Using wash bottle with 78% ethanol and rubber spatula, quantitatively transfer all remaining particles to crucible. (*Note*: If some products form a gum, trapping the liquid, break film with spatula.)

Using vacuum, wash residue 2 times each with 15 mL portions of 78% ethanol, 95% ethanol, and acetone. Dry crucible containing residue overnight in 105°C oven. Cool crucible in desiccator ca 1 h. Weigh crucible, containing dietary fiber residue and Celite, to nearest 0.1 mg, and calculate residue weight by subtracting weight of dry crucible with Celite, **B(b)**.

Use one duplicate from each test portion to determine protein, 960.52 (*see* 12.1.07), using $N \times 6.25$ as conversion factor. For ash analysis, incinerate second duplicate 5 h at 525°C. Cool in desiccator, and weigh to nearest 0.1 mg. Subtract weight of crucible and Celite, **B(b)**, to determine ash weight.

G. Determination of Insoluble Dietary Fiber

Wet and redistribute Celite bed in previously tared crucible, **B(b)**, using ca 3 mL H_2O. Apply suction to crucible to draw Celite into even mat.

Filter enzyme digestate, from **E,** through crucible into filtration flask. Rinse beaker, and then wash residue 2 times with 10 mL 70°C H_2O. Combine filtrate and water washings, transfer to pretared 600 mL tall-form beaker, and reserve for determination of soluble dietary fiber, **H**.

Using vacuum, wash residue 2 times each with 15 mL portions of 78% ethanol, 95% ethanol, and acetone. (*Note*: Delay in washing IDF residues with 78% ethanol, 95% ethanol, and acetone may cause inflated IDF values.)

Use duplicates to determine protein and ash as in **F**.

H. Determination of Soluble Dietary Fiber

Proceed as for insoluble dietary fiber determination through instruction to combine the filtrate and water washings in pretared 600 mL tall-form beakers. Weigh beakers with combined solution of filtrate and water washings, and estimate volumes.

Add 4 volumes of 95% ethanol preheated to 60°C. Use portion of 60°C ethanol to rinse filtering flask from IDF determination. Alternatively, adjust weight of combined solution of filtrate and water washings to 80 g by addition of H_2O, and add 320 mL 60°C 95% ethanol. Let precipitate form at room temperature 1 h.

Follow TDF determination, **F**, from "Wet and redistribute Celite bed . . . ".

I. Calculations

Blank (B, mg) determination:

$$B = \frac{BR_1 + BR_2}{2} - P_B - A_B$$

where BR_1 and BR_2 = residue weights (mg) for duplicate blank determinations; and P_B and A_B = weights (mg) of protein and ash, respectively, determined on first and second blank residues.

Dietary fiber (DF, g/100 g) determination:

$$DF = \frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} \times 100$$

where R_1 and R_2 = residue weights (mg) for duplicate test portions; P and A = weights (mg) of protein and ash, respectively, determined on first and second residues; B = blank weight (mg); and M_1 and M_2 = weights (mg) for test portions.

Total dietary fiber determination: Determine either by independent analysis, as in **F**, or by summing IDF and SDF, as in **G** and **H**.

Reference: *J. AOAC Int.* **75**, 395(1992).

Revised: June 2000

AOAC Official Method 993.19
Soluble Dietary Fiber
in Food and Food Products

Enzymatic–Gravimetric Method (Phosphate Buffer)
First Action 1993
Final Action 1996

(Applicable to determination of soluble dietary fiber [SDF] in vegetables, fruit, and cereal grains; and to determination of total dietary fiber [TDF] in conjunction with 991.42 [*see* 32.1.16], Insoluble Dietary Fiber [IDF] in Food and Food Products.)

See Tables **993.19A** and **B** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Duplicate test portions of dried foods, fat-extracted if >10% fat, are gelatinized with heat-stable -amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. IDF is removed by filtering and washing residue with water. SDF in filtrate is precipitated by adding 95% ethanol to filtrate. Precipitate is filtered and washed with 78% ethanol, 95% ethanol, and acetone, dried, and weighed. One duplicate is analyzed for protein, and second is incinerated at 525 C to determine ash. SDF is weight of residue minus weight of protein and ash.

B. Apparatus

See 991.42B (*see* 32.1.16).

C. Reagents

See 991.42C (*see* 32.1.16) with following change:

(**j**) *Celite*.—Medium grade (acid-washed).

D. Enzyme Purity

See 991.42D (*see* 32.1.16).

E. Preparation of Test Portions

Analyze dry foods without pretreatment whenever possible. Dry-mill to 0.3–0.5 mm mesh. Homogenize and freeze-dry wet foods before milling. If high fat content (>10%) prevents proper milling, defat with three 25 mL portions of petroleum ether/g food before milling. Determine residual moisture in milled foods by drying overnight in 70 C vacuum oven or 5 h in 105 C air oven. Record weight loss due to fat and/or water, and make appropriate correction to final % TDF and SDF. (*Note*: For foods high in sugars that cannot be dried by lyophilization, extract test portion 3 each with 10 volumes 85% methanol to remove sugars before milling or lyophilization, which may interfere in determination.)

F. SDF Determination

Proceed as in 991.42F (*see* 32.1.16), from beginning up through "Wash residue … 2 times with 10 mL acetone" in paragraph 5.

Adjust weight of combined filtrate and water washings to 100 g with H_2O. Add 4 volumes (400 mL) 95% ethanol, preheated to 60°C. Let precipitate form at room temperature 60 min.

Tare crucible containing Celite to nearest 0.1 mg; then wet and redistribute Celite bed in crucible, using stream of 78% ethyl alcohol from wash bottle. Apply suction to crucible to draw Celite onto frittered glass as even mat.

Filter precipitate mixture and wash residue successively with three 20 mL portions of 78% ethyl alcohol, two 10 mL portions of 95% ethyl alcohol, and two 10 mL portions of acetone.

Proceed as in 991.42F (*see* 32.1.16), starting with "Break surface film . . ." in paragraph 5 through end of 991.42F (*see* 32.1.16).

G. Calculations

Determination of blank:

$$B = \text{blank, mg} = \text{weight residue} - P_B - A_B$$

where weight residue = average of residue weights (mg) for duplicate blank determinations; and P_B and A_B = weights (mg) of

Table 993.19A. Interlaboratory study results for soluble dietary fiber in foods by enzymatic-gravimetric method (phosphate buffer)

Food	No. of labs	Average SDF, %	s_r	s_R	RSD_r, %	RSD_R, %	HorRat
Apricots	8	11.20	0.42	0.91	3.78	8.11	2.9
Carrots	9	11.53	0.53	1.11	4.59	9.61	3.5
Chick peas	10	1.21	0.23	0.34	19.52	28.28	7.3
Onions	8	4.13	0.91	1.00	21.93	24.12	7.5
Raisins	8	7.95	0.67	0.67	8.41	8.41	2.9
Sugar beet fiber	10	20.65	0.80	1.35	3.88	6.52	2.6

Table 993.19B. Interlaboratory study results for total dietary fiber in foods by enzymatic-gravimetric method (phosphate buffer)

Food	No. of labs	Average TDF, %	s_r	s_R	RSD_r, %	RSD_R, %
Apricots	10	24.63	0.74	0.97	3.03	3.95
Carrots	9	23.25	0.49	0.79	2.10	3.38
Chick peas	10	14.33	0.85	1.00	5.93	7.01
Onions	9	16.13	0.95	1.02	5.88	6.33
Raisins	10	30.28	0.81	2.05	2.69	6.78
Sugar beet fiber	10	66.07	1.15	1.59	1.74	2.41

protein and ash, respectively, determined in first and second blank residues.

Calculate SDF as follows:

$$\text{SDF, \%} = \frac{\text{weight residue} \quad P \quad A \quad B}{\text{weight test portion}} \quad 100$$

where weight residue average of weights (mg) for duplicate test portion determinations; *P* and *A* = weights (mg) of protein and ash, respectively, in first and second sample residues; and weight test portion = average of 2 test portion weights (mg) taken.

Calculate TDF as follows:

$$\text{TDF, \%} = \text{SDF} + \text{IDF [from 991.42 (}\textit{see}\text{ 32.1.16)]}$$

Reference: J. AOAC Int. 77, 690(1994).

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