ANNEX G

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

32.1.17

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.)

Method Performance:

See Table **991.43A** for method performance data.

A. Principle

Duplicate samples 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 Bchner, Corning, Inc., Science Products, Corning, NY 14831, USA, or equivalent). Prepare as follows. Ash overnight at 525° in muffle furnace. Let furnace temperature fall below 130° before removing crucibles. Soak crucibles 1 h in 2% cleaning solution at room temperature.

Table 991.43A Method Performance 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 %
Total dietary fiber (TDF)					
Barley	12.25	0.36	0.85	2.88	6.89
High-fiber cereal	33.73	0.70	0.94	2.08	2.79
Oat bran	16.92	1.06	2.06	6.26	12.17
Soy bran	67.14	1.01	1.06	1.50	1.58
Apricots	1.12	0.01	0.01	0.89	0.89
Prunes	9.29	0.13	0.40	1.40	4.31
Raisins	3.13	0.09	0.15	2.88	4.79
Carrots	3.93	0.13	0.13	3.31	3.31
Green beans	2.89	0.07	0.07	2.42	2.42
Parsley	2.66	0.07	0.14	2.63	5.26
Soluble dietary fiber (SDF)					
Barley	5.02	0.40	0.62	8.01	12.29
High-fiber cereal	2.78	0.44	0.56	15.83	20.14
Oat bran	7.17	0.72	1.14	10.04	15.90
Soy bran	6.90	0.30	0.60	4.35	8.70
Apricots	0.53	0.02	0.02	3.77	3.77
Prunes	5.07	0.11	0.31	2.17	6.11
Raisins	0.73	0.05	0.16	6.85	21.92
Carrots	1.10	0.07	0.18	6.36	16.36
Green beans	1.02	0.08	0.11	7.84	10.78
Parsley	0.64	0.03	0.10	4.69	15.63
Insoluble dietary fiber (IDF)					
Barley	7.05	0.61	0.61	8.62	8.62
High-fiber cereal	30.52	0.44	0.71	1.44	2.33
Oat bran	9.73	0.85	1.17	8.74	12.02
Soy bran	60.53	0.70	0.70	1.16	1.16
Apricots	0.59	0.02	0.02	3.39	3.39
Prunes	4.17	0.07	0.09	1.68	2.16
Raisins	2.37	0.04	0.07	1.69	2.95
Carrots	2.81	0.09	0.16	3.20	5.69
Green beans	2.01	0.08	0.08	3.98	3.98
Parsley	2.37	0.12	0.24	5.06	10.13
Total dietary fiber (SDF + IDF)					
Barley	12.14	0.39	0.70	3.21	5.77
High-fiber cereal	33.30	0.63	0.90	1.89	2.70
Oat bran	16.90	0.99	1.49	5.86	8.82
Soy bran	67.56	0.56	0.94	0.83	1.39
Apricots	1.12	0.02	0.02	1.79	1.79
Prunes	9.37	0.12	0.30	1.28	3.20
Raisins	3.10	0.05	0.18	1.61	5.81
Carrots	3.92	0.11	0.13	2.81	3.32
Green beans	3.03	0.09	0.12	2.97	3.96
Parsley	3.01	0.12	0.23	3.99	7.64

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° 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°, with automatic on-and-off timer. (*2*) Constant temperature, adjustable to 60°.

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

(**f**) *Muffle furnace.*—Capable of maintaining 525 ± 5°.

(**g**) *Oven.*—Capable of maintaining 105 and 130 ± 3°.

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

(**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.*—Capable of 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**) *Heat-stable α-amylase solution.*—Catalog Number A 3306, Sigma Chemical Co., St. Louis, MO 63178, USA, or Termamyl 300L, Catalog Number 361-6282, Novo-Nordisk, Bagsvaerd, Denmark, or equivalent.

(**c**) *Protease.*—Catalog Number P 3910, Sigma Chemical Co, or equivalent. Prepare 50 mg/mL enzyme solution in MES/TRIS buffer fresh daily.

(**d**) *Amyloglucosidase solution.*—Catalog Number AMG A9913, Sigma Chemical Co, or equivalent. Store at 0–5°.

(**e**) *Diatomaceous earth.*—Acid washed (Celite 545 AW, No. C8656, Sigma Chemical Co. or equivalent).

(**f**) *Cleaning solution.*—Liquid surfactant-type laboratory cleaner, designed for critical cleaning (Micro®, International Products Corp., Burlington, NJ 08601, USA, or equivalent). Prepare 2% solution in H_2O.

(**g**) *MES.*—2-(*N*-Morpholino)ethanesulfonic acid (No. M-8250, Sigma Chemical Co., or equivalent.)

(**h**) *TRIS.*—Tris(hydroxymethyl)aminomethane (No. T-1503, Sigma Chemical Co., or equivalent).

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

(**j**) *Hydrochloric acid solution.*—0.561*N*. Add 93.5 mL 6*N* 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. Sample Preparation and Digestion

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

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

Weigh duplicate 1.000 ± 0.005 g samples (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 sample 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° H_2O bath 15 min with continuous agitation. Start timing once bath temperature reaches 95° (total of 35 min is normally sufficient).

Remove all beakers from bath, and cool to 60°. 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° with continuous agitation. Start timing when bath temperature reaches 60°.

Remove foil. Dispense 5 mL 0.561*N* HCl into beakers while stirring. Adjust pH to 4.0–4.7 at 60°, by adding 1*N* NaOH solution or 1*N* HCl solution. (*Note:* It is important to check and adjust pH while solutions are 60° 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 samples also.)

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

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

F. Determination of Total Dietary Fiber

To each digested sample, add 225 mL (measured after heating) 95% ethanol at 60°. Ratio of ethanol to sample 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 samples 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° 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 sample to determine protein, by method **960.52** (*see* 12.1.07), using $N \times 6.25$ as conversion factor. For ash analysis, incinerate second duplicate 5 h at 525°. 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° 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°. Use portion of 60° 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° 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:

$$B = [(BR_1 + BR_2)/2] - P_B - A_B$$

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:

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

where R_1 and R_2 = residue weights (mg) for duplicate samples; 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 samples.

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).

32.1.18

AOAC Official Method 992.16
Total Dietary Fiber
Enzymatic-Gravimetric Method
First Action 1992

(Applicable to determination of total dietary fiber in cereals, beans, vegetables, and fruits.)

Method Performance (dry weight basis):

Turnip, 20.71%
$s_r = 1.01$; $s_R = 1.37$; $RSD_r = 4.85\%$; $RSD_R = 6.60\%$
Wheat bran, 46.30%
$s_r = 0.69$; $s_R = 1.91$; $RSD_r = 1.48\%$; $RSD_R = 4.13\%$
Bean, 18.19%
$s_r = 0.90$; $s_R = 2.06$; $RSD_r = 4.93\%$; $RSD_R = 11.30\%$
Rice, 1.21%
$s_r = 0.18$; $s_R = 0.22$; $RSD_r = 14.73\%$; $RSD_R = 17.94\%$
Whole wheat bread, 10.29%
$s_r = 0.74$; $s_R = 0.80$; $RSD_r = 7.18\%$; $RSD_R = 7.81\%$

A. Principle

Food samples, dried and ground, are fat extracted if containing >5% fat. A portion of sample is treated in autoclave with heat stable amylase, amyloglucosidase, and protease to remove starch and protein. Enzymatically undigested fiber is precipitated by ethanol and filtered. Residue is dried, weighed, ashed, and reweighed. A second portion of sample is refluxed with neutral detergent and treated with α-amylase from porcine pancreas to remove water solube carbohydrates and protein. Residue is dried, weighed, ashed, and reweighed. Total dietary fiber is calculated as sum of the 2 residues.

B. Apparatus

(**a**) *Autoclave or pressure cooker.*—Capable of 15 psi.

(**b**) *Tubes.*—50 mL, heavy duty, with screw caps (Pyrex, Fisher No. 05–558–5B, Fisher Scientific, Pittsburgh, PA 15219, USA or Corning No. 8422, Corning, Inc., Corning, NY 14831, USA are suitable).

(**c**) *Ovens.*—*(1)* Forced draft, capable of maintaining 105 ± 1°. *(2)* Capable of maintaining 55 ± 0.5°.

(**d**) *Water baths.*—*(1)* Boiling. *(2)* Capable of maintaining 60 ± 0.5°.

(**e**) *Balance.*—Analytical, sensitive to 0.1 mg.

(**f**) *Muffle furnace.*—With temperature regulator capable of 525 ± 1° (Fisher Isotemp, Model 497, or Thermoline equipped with Furnatrol controller are suitable).

(**g**) *Neutral detergent fiber extraction system.*—Extraction apparatus with *(1)* condenser to fit 600 mL tall-form beaker without spout, *(2)* hot plate capable of bringing 100 mL neutral detergent to boiling in 5–10 min, and *(3)* filtering device equipped with suitable