32.1.17 - Cereal Foods / Wheat Flour

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 -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 Buchner, 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, sensitivity ±0.1 mg.

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

(**g**) *Oven.*—Capable of 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.*—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**) *-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°C and 28°C, adjust by interpolation.)

(**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 Sample

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 test samples, 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 solution is completely dispersed (to prevent lump formation, which would make test material inaccessible to enzymes).

Add 50 µL heat-stable -amylase solution, stirring at low speed. Cover beakers with Al foil, and incubate in 95-100°C H_2O 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 test 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°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, by method **960.52** (*see* 12.1.07), using *N* 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:

$$B = \qquad - P_B - A_B$$

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:

$$DF =$$

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).

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

Table 991.43B: Standards for testing enzyme activity