

45.4.07

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 μ m; or Corning No. 36060 Büchner, fritted disk, Pyrex, 60 mL, ASTM 40–60 μ m). Clean thoroughly, heat 1 h at 525°C, and soak and then rinse in H₂O. 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 H₂O 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 H₂O 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 H₂O 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 (Na₂HPO₄) (or 1.753 g dihydrate) and 9.68 g sodium phosphate monobasic monohydrate (NaH₂PO₄ H₂O) (or 10.94 g dihydrate) in ca 700 mL H₂O. Dilute to 1 L with H₂O. 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 μ mole 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 α -glucosidase.—3000 + 300 Ceralpha units/mL (1 unit of enzyme is required to release 1 μ mole *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 μ mole 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 μ mole tyrosine/min at pH 7.5 and 37°C). Color by Folin-Ciocalteu 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 μ mole 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 μ mole 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 μ mole *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 α -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 (pretested) available from a number of companies.

(h) *Sodium hydroxide solution*.—0.275M. Dissolve 11.00 g NaOH ACS in ca 700 mL H₂O in 1 L volumetric flask. Dilute to volume with H₂O.

(i) *Hydrochloric acid solution*.—0.325M. Dilute stock solution of known titer, e.g., 325 mL 1M HCl, to 1 L with H₂O.

(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
-Glucan (barley gum) ^a	-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:

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

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

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