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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