

16.5.09

**AOAC Official Method 993.26
Light Filth in Whole Wheat Flour**

**Flotation Method
First Action 1993
Final Action 1996**

See Table 993.26 for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Whole wheat flour is digested without effect on insect exoskeleton or mammalian hair contaminants. These oleophilic filth elements are separated from nonoleophilic food products by attraction to the oil phase of an oil–aqueous mixture. The oil phase is trapped off, filtered, and examined microscopically for filth elements.

B. Apparatus

- (a) Sieve.—(1) No. 230 plain-weave, 945.75B(r) (see 16.1.01); and (2) sieve handle, 945.75B(s) (see 16.1.01).
- (b) Reflux apparatus.—975.49A(e) (see 16.14.05).
- (c) Wildman trap flask.—2 L, 945.75B(h)(4) (see 16.1.01).
- (d) Filter paper.—Ruled, 945.75B(s) (see 16.1.01).

C. Reagents

- (a) HCl solution.—3%. Add 24 mL HCl to 776 mL H₂O.
- (b) Isopropanol solutions.—(1) 100%. (2) 40% aqueous solution.
- (c) Mineral oil.—945.75C(p) (see 16.1.01).
- (d) Tween 80–40% isopropanol solution.—See 945.75C(x) (see 16.1.01).
- (e) Na₄EDTA–40% isopropanol solution.—See 945.75C(z) (see 16.1.01).

D. Isolation

Add 800 mL 3% HCl solution, C(a), to 2 L beaker. Place on preheated hot plate and, magnetically stir so stirring bar is visible in Vortex, 945.75B(n) (see 16.1.01). Accurately weigh 50 g whole wheat flour to nearest 0.5 g into 250 mL beaker. Transfer flour portionwise to 3% HCl solution. Rinse sides of 250 mL beaker with

3% HCl solution from wash bottle and add washings to 2 L beaker. Cover with watch glass and bring to full boil. Remove watch glass and boil gently 15 min while magnetically stirring.

Wet-sieve, 970.66B(a) (see 16.1.02), portionwise on sieve, B(a)(1), with gentle stream of hot (50 –70°C) tap water until rinse is clear. Use of sieve handle, B(a)(2) or equivalent, is recommended. Retain original beaker. Wash residue to side of sieve with hot tap water, and rinse residue with 100% isopropanol, C(b)(1).

Quantitatively transfer residue to original beaker, washing with 100% isopropanol. Add 100% isopropanol to 400 mL mark on beaker and boil gently 5 min, using reflux apparatus, B(b), inserted into beaker top. Remove beaker from reflux apparatus and quantitatively transfer beaker contents to sieve.

Wet-sieve with gentle stream of hot tap water until rinse is clear. Wet residue on sieve with 40% isopropanol, C(b)(2), and quantitatively transfer residue to trap flask, B(c), using 40% isopropanol. Dilute to 600 mL with 40% isopropanol and boil gently 5 min with magnetic stirring. Remove from heat, add 65 mL mineral oil, C(e), and magnetically stir 3 min, 970.66B(c) (see 16.1.02). Let stand 1–2 min after stirring.

Add mixture of 5 mL Tween 80–40% isopropanol solution, C(d), and 5 mL Na₄EDTA–40% isopropanol solution, C(e), slowly, down stirring rod. Hand-stir 30 s with gentle rotary motion. Let stand undisturbed 1–2 min. Fill flask with 40% isopropanol, clamp rod, and let stand 30 min. Stir bottom contents every 5 min for first 20 min and leave undisturbed for final 10 min. Spin stopper (wafer) to remove any trapped residue and trap off, 970.66B(b) (see 16.1.02), into 400 mL beaker, using 40% isopropanol to rinse neck of flask. Add 40 mL mineral oil to flask and hand-stir 15 s with gentle up-and-down motion. Fill flask with 40% isopropanol and let stand for 20 min. Spin stopper and trap off as before, rinsing neck with 100% isopropanol.

Filter beaker contents through filter, B(d), and examine microscopically at 30 .

Reference: *J. AOAC Int.* 77, 1150(1994).

Table 993.26. Interlaboratory study results for determination of light filth in whole wheat flour by flotation method

Spike added/ 50 g flour	Mean	s _r	s _R	RSD _r , %	RSD _R , %
Rat hairs					
5	4.8 (4.4) ^a	1.4 (1.5)	1.4 (1.5)	29.8 (34.3)	29.8 (34.3)
10	9.1 (9.0)	1.2	1.2	13.5 (13.0)	13.5 (13.0)
15	14.4 (14.1)	1.2 (1.0)	1.3 (1.2)	8.3 (6.9)	9.2 (8.5)
Insect fragments					
5	4.7	0.8	0.8	16.0	16.0
15	12.6 (13.8)	1.1 (1.5)	3.0 (1.6)	8.9 (10.8)	23.6 (11.5)
30	25.9	3.6 (3.5)	4.8 (4.6)	14.0 (13.6)	18.4 (17.9)

^a Third-party counts in parentheses if different from collaborator.