

16.9.10

AOAC Official Method 973.60 Light Filth in Pork Sausage (Uncooked) and Ground Beef or Hamburger

Enzyme Digestion Method

First Action 1973

Final Action 1988

A. Determination

Grind sausage, using meat grinder with end plate having $\frac{3}{16}$ in. (4.8 mm) holes. Weigh 225 g test portion into 1.5–2 L beaker. Add 980 mL warm water and 20 mL emulsifier, **945.75C(j)(I)** (see 16.1.01), and stir 5 min. Add 20 mL HCl and stir 1 min. Proceed with overnight digestion, **(a)**, or rapid digestion, **(b)**.

With ground beef, use only overnight digestion with 5.0 g 1:10 000 or 10 g NF pepsin. In flotation, **(c)**, omit addition of 50 mL HCl and subsequent boiling. After addition of light mineral oil, let stand 20 min instead of 10 min.

(a) Overnight digestion.—Add 0.5 g 1:10 000 pepsin (BD Biosciences Codified Cat. No. 215110, or equivalent) or 2.0 g NF pepsin and stir 1 min. Digest in 50°C water bath or incubator 18 h. Add 5 mL Triton X-114, **945.75C(bb)(2)** (see 16.1.01), and stir 1 min. Keep all suspensions at digestion temperature in bath until ready to sieve. Sieve portionwise on No. 230 sieve with hot water spray. Transfer to ruled filter paper if small amount residue remains on sieve or proceed with flotation, **(c)**.

(b) Rapid digestion.—Add 2.0 g 1:10 000 pepsin or 10 g NF pepsin and stir 1 min. Digest in 62°C water bath 2 h. Add 5 mL Triton X-114, **945.75C(bb)(2)** (see 16.1.01), and stir 1 min. Keep all suspensions at digestion temperature until ready to sieve. Sieve portionwise on No. 230 sieve. Proceed with flotation, **(c)**.

(c) Flotation.—Wet residue on sieve with 40% isopropanol and immediately transfer quantitatively to 2 L trap flask, using 40% isopropanol. Bring volume to 1 L with 40% isopropanol and add 50 mL HCl. Add magnetic stirring bar, **945.75B(n)** (see 16.1.01), and, with gentle stirring, boil 10 min on magnetic stirrer-hot plate. Cool to room temperature in cold water bath and add 40 mL flotation liquid, **945.75C(k)** (see 16.1.01). Stir magnetically 3 min, **970.66B(c)** (see 16.1.02). Let oil phase separate 1 min, and slowly fill flask with 40% isopropanol by letting liquid

flow down stoppered rod while top of stopper is maintained just above flask contents. After filling flask, gently stir settled plant material with stoppered rod 5–10 s. Let stand undisturbed 5 min and immediately trap off. Add 25 mL light mineral oil, **945.75C(p)** (see 16.1.01), stir gently by hand 30 s, and let stand 10 min. Repeat trapping. Wash flask neck thoroughly with isopropanol and transfer washings to beaker containing trappings. Filter onto ruled paper and examine microscopically.

References: *JAOAC* **56**, 631(1973); **59**, 51(1976).

B. Alternative Method for Sausages

(a) Bulk or link sausages that are easily teased apart.—Weigh 225 g test portion into 1.5–2 L beaker. Add 1 L 10% Tergitol solution, **945.75C(bb)(I)** (see 16.1.01), and 75 12 mm stirring bar; stir magnetically, 5 min, or until thoroughly dispersed. Sieve portionwise on No. 230 sieve, **945.75B(r)** (see 16.1.01). Form filter paper around 1 L beaker, **945.75B(j)** (see 16.1.01), moistening with H₂O to make paper pliable. Insert paper into Büchner, 91 mm id plate, wash with isopropanol, and aspirate to near dryness.

Wet residue on sieve with isopropanol and quantitatively transfer to filter paper cup with isopropanol. Add enough isopropanol to cover residue and, after 1 min, apply vacuum until dripping ceases. Transfer paper cup to 1 L beaker and add 300 mL CHCl₃. Boil on steam bath 5 min. Let solution cool few min, lift paper, drain, and transfer to 300 mL fresh CHCl₃. Repeat 5 min boil and drain. Return paper cup to Büchner and apply vacuum until dripping ceases. Cover residue with isopropanol for 1 min, reapply vacuum, and continue to aspirate 5 min. Quantitatively transfer residue to 2 L trap flask, using 40% isopropanol, and proceed as in **A(c)**, second sentence, "Bring volume to 1 L".

(b) Link sausages compressed into casings so that product is not easily teased apart.—Remove casing and weigh 225 g test portion into 2 L beaker. Add 1 L 10% Tergitol solution, **945.75C(bb)(I)** (see 16.1.01), and stir with mechanical stirrer, **945.75B(e)** (see 16.1.01), at maximum speed at which no splashing occurs, 15 min or until thoroughly dispersed. Proceed as in **(a)**, beginning "Sieve portionwise on No. 230 sieve".

Reference: *JAOAC* **55**, 66(1972).