4.4.04 - Animal Feed / Nitrogen

AOAC Official Method 971.09
Pepsin Digestibility
of Animal Protein Feeds

Filtration Method
First Action 1971
Final Action 1973

A. Principle

Defatted test portion is digested 16 h with warm solution of pepsin under constant agitation. Insoluble residue is isolated by filtering, washed, dried, and weighed to determine percent residue. Residue is examined microscopically and analyzed for protein. Filtration method is applicable to all animal proteins. Methods are not applicable to vegetable proteins or mixed feeds because of presence of complex carbohydrates and other compounds not digested by pepsin.

B. Apparatus

(**a**) *Agitator.—See* Figure **971.09**. Continuous, slow speed (15 rpm), end-over-end type, to operate inside incubator at 45 ± 2°C and carry 8 oz (250 mL) screw-cap prescription bottles, or equivalent. Agitator and bottles available from Terry Sims, 1901 Laurel Rd, Oceanside, CA 92054, Tel: +1-760-722-4119. Stirring or reciprocating (shaking) type agitator cannot be used because solid particles collect on sides of bottle and do not contact pepsin solution. If heat from agitator motor raises incubator temperature to >45°C, mount motor outside incubator by drilling hole through side of incubator and connecting motor to agitator with extension shaft and coupling (available from agitator supplier).

(**b**) *Settling rack.*—Wood or metal to hold digestion bottles at 45° angle. May be made from 2 boards nailed horizontally into "V" cut into vertical end pieces. Also available from agitator supplier, (**a**).

(**c**) *Filtering device.*—Modified California Buchner, **962.09C**(**d**) (*see* 4.6.01), available from Labconco Corp., 8811 Prospect Ave, Kansas City, MO 64132, No.

55100. (If edge of screen is rough, smooth with small-tip soldering iron.) Use with retainer sleeve, 2 2.75 (7 cm) od stainless steel tube, available from agitator supplier, (**a**).

(**d**) *Glass fiber filter*.—7 cm, Whatman, Inc., 934-AH, or equivalent.

(**e**) *Moisture dishes*.—Al, 78 mm od 20 mm, with outside cover and vertical sides.

C. Reagent

Pepsin solution.—0.2% pepsin (activity 1:10,000) in 0.075M HCl; *do not use pepsin NF or pepsin of activity other than 1:10 000. Prepare just before use by diluting 6.1 mL HCl to 1 L and heating to 42-45°C.* Add pepsin and stir gently until dissolved. Do not heat pepsin solution on hot plate or overheat.

D. Preparation of Sample

Sieve sample, **965.16** (*see* 5.1.01), through No. 20 sieve. Grind portion retained on sieve to pass No. 20 sieve. Combine both portions and blend by stirring and shaking in pint (500 mL) jar. Thorough blending is essential. Because of high fat content of many animal products, grinding without sieving may cause sticking in mill, loss of moisture or fat, or poorly blended sample.

E. Extraction

Prepare extraction thimble from 11 cm Whatman No. 2 paper, or equivalent, as follows: Fold paper in half; straighten paper and refold at right angles to first fold; turn paper over and repeat process with folds at 45° to original fold; while holding creased paper in one hand, place short test tube (6-8 mm smaller in diameter than extractor sample holder or cup in which thimble is to be used) at its center; fold along natural crease lines to form 4-pointed star around tube; and wrap points in same direction around tube to complete thimble.

Weigh 1.000 g ground test portion (0.500 g poultry by-products or hydrolyzed feathers because of gummy nature and amount of residue) into thimble and extract 1 h with ether at condensation rate of 3-4 drops/s. (If Soxhlet is used, top of thimble should extend above siphon tube to avoid loss of solid particles. If paper containing test portion is totally submerged in siphon cup, test portion must be completely wrapped in paper.) Observe ether extract to determine that no solid particles were carried into solvent. For approximate fat content determination, evaporate ether, and dry and weigh residue. Remove paper from container or cup and let dry at room temperature. Unfold, and quantitatively brush defatted test portion into digestion

bottle, avoiding contamination by brush bristles or filter paper fibers. Use of powder funnel is helpful to avoid loss.

F. Pepsin Digestion

To defatted test portion in agitator bottle, add 150 mL freshly prepared pepsin solution prewarmed to 42-45°C. Be sure test portion is completely wetted by pepsin solution. Stopper bottle, clamp in agitator, and incubate with constant agitation 16 h at 45°C.

G. Treatment of Residue

Dry individual sheets of glass fiber filter, **B**(**d**), 30 min at 110°C in moisture dishes with cover open. Cool in desiccator 30 min with cover closed, and weigh (W_1).

Remove bottles from agitator. Place in 45° angle settling rack and loosen caps. Let residue settle 15 min. Place weighed filter in California Buchner, **B**(**c**), apply suction, and moisten with H_2O. Place retainer sleeve on filter and press down gently. Rinse particles of residue on cap onto filter with small amount H_2O. Carry bottle from rack to filter at same angle as settled and slowly pour contents through filter as continuous small stream, avoiding all unnecessary agitation. Liquid passes through paper as rapidly as poured, with residue spreading over surface of filter but not covering it completely until all or practically all of liquid has passed through. If filtration rate becomes slow, it may be accelerated by adding acetone washes described below, but only if no significant amount of digestion mixture remains on funnel when acetone is added. (Filtration [passage of aqueous mixture through filter] should be complete within 1 min with most proteins.) After supernate has passed through filter, quantitatively transfer residue onto filter as follows:

Add 15 mL acetone to bottle. Hold thumb over bottle neck and shake vigorously. Release pressure, replace thumb over bottle neck, and shake bottle in inverted position over filter. Remove thumb, letting acetone and residue discharge onto filter. Repeat rinse with second 15 mL portion acetone, shaking and releasing pressure as above. Inspect bottle, and rinse further with acetone, using policeman, if necessary. If >3 mm liquid remains on paper when acetone washes are started, it may be necessary to use three 15 mL acetone washes instead of 2 to increase filtration rate.

After all liquid passes through funnel, wash residue and inside surface of retainer sleeve with 2 small portions acetone from wash bottle or hypodermic syringe, and suck dry. Remove retainer sleeve from funnel. Transfer filter to original moisture dish. Scrape or brush any residue particles or filter clinging to retainer sleeve or funnel onto filter in moisture dish. Dry in oven, cool, and weigh as before (W_2). Calculate percent indigestible residue = (W_2 - W_1) 100/g test portion.

Determine indigestible protein by transferring filter containing residue directly to Kjeldahl flask. Proceed as in **954.01** (*see* 2.4.14). (*Caution:* Violent reaction may take place when NaOH is mixed with diluted digestion mixture, caused by large excess H_2SO_4 due to small amount organic material from residue and none from glass filter. Avoid by thoroughly mixing and cooling digestion mixture before addition of NaOH or by using 20 mL H_2SO_4 in Kjeldahl digestion instead of 25 mL.) Make blank determination on 1 sheet of glass filter and subtract from each test portion determination, if necessary. Calculate percent protein based on original test portion weight. Result represents percent indigestible protein in test portion. Convert to percent crude protein content of test portion not digested, "protein indigestible" = percent indigestible protein in test portion 100/percent total crude protein in test portion.

References:

J. Agric. Food Chem. **3,** 159(1955).
JAOAC **40,** 606(1957); **41,** 233(1958);
42, 231(1959); **43,** 320(1960); **54,** 669(1971); **55**, 702(1972).