PEPSIN-PANCREATIN INSOLUBLE NITROGEN
Updated September 2013

I. Reference: Akeson & Stahman. 1964. A Pepsin Pancreatin Digest Index of Protein Quality Evaluation. J. Nutr. 83:257.

II. Personal Protective Equipment:

A. Lab coat
B. Safety glasses/goggles
C. Latex gloves

III. Reagents:

A. Pepsin-HCl Solution
1. Add 8.25 ml concentrated HCl to ~ 800 ml distilled H_2O
2. Add 100 mg Pepsin and stir gently to dissolve.
3. q.s. to 1 liter.

B. 0.2 N NaOH.
1. Add 10.3 g Sodium Hydroxide (NaOH) pellets to 1000 ml volumetric.
2. Add a small amount of water to dissolve.
3. q.s. to 1 liter.

C. Pancreatin Solution in 0.5 M Phosphate Buffer, pH 8.0.
1. Add 3.66 g Sodium Phosphate (NaH_2PO_4) (monobasic)
2. Add126.94 g Sodium Phosphate ($NaH_2PO_4 \cdot 7H_2O$) (dibasic)
3. Add ~ 800 ml distilled H_2O.
4. Stir until dissolved.
5. Add 0.5335 g pancreatin and stir gently to dissolve
6. q.s. to 1 liter.

IV. Procedure:

A. In 50 ml in vitro tubes weigh out sample containing 15 mg N in triplicate
B. Per tube add 15 ml of pepsin-HCl solution.
C. Incubate 3 hours at 30°C.
D. Neutralize with 7.5 ml 0.2 N NaOH per tube.
E. Add 7.5 ml pancreatin solution.
F. Incubate additional 24 hours at 39°C.
G. Filter through Whatman #541 filter paper as soon as possible.
H. Dry filter paper and run Kjeldahls on them.

V. Calculations:

A. Calculate % pepsin-pancreatin insoluble N or as % of total sample N.