

33.2.11

AOAC Official Method 991.20 Nitrogen (Total) in Milk

Kjeldahl Methods
First Action 1991
Final Action 1994

IDF-ISO-AOAC Method

Results of the interlaboratory study supporting acceptance of the method [expressed on a protein basis ($N = 6.38$)]:

$$s_r = 0.014; s_R = 0.017; RSD_r = 0.385\%; RSD_R = 0.504\%$$

A. Principle

Milk is digested in H_2SO_4 , using $CuSO_4 \cdot 5H_2O$ as catalyst with K_2SO_4 as boiling point elevator, to release nitrogen from protein and retain nitrogen as ammonium salt. Concentrated NaOH is added to release NH_3 , which is distilled, collected in H_3BO_3 solution, and titrated.

Traditional Method

B. Apparatus

(a) *Digestion flasks*.—Kjeldahl. Hard, moderately thick, well-annealed glass. Total capacity ca 500 or 800 mL.

(b) *Distillation flasks*.—Same Kjeldahl flask as in (a), fitted with rubber stopper through which passes lower end of efficient rubber bulb or trap to prevent mechanical carryover of NaOH during distillation. Connect upper end of bulb to condenser tube by rubber tubing. Use graduated 500 mL Erlenmeyer titration flask to collect distillate. Trap outlet of condenser in manner to ensure complete absorption of NH_3 distilled into boric acid solution.

(c) *Digestion/distillation system*.—Traditional apparatus with adjustable controls for individual flasks.

(d) *Titration buret*.—50 mL. Class A or equivalent.

C. Reagents

(a) *Sulfuric acid*.—95–98% H_2SO_4 . Nitrogen free.

(b) *Copper catalyst solution*.— $CuSO_4 \cdot 5H_2O$. Nitrogen free. Prepare solution 0.05 g/mL H_2O .

(c) *Potassium sulfate*.— K_2SO_4 . Nitrogen free.

(d) *Sodium hydroxide solution*.—50% (w/w) nitrate-free NaOH.

(e) *Boiling chips*.—Mesh size 10 suggested. High purity, amphoteric aluminum granules, plain.

(f) *Methyl red/bromocresol green indicator solution*.—Dissolve 0.2 g methyl red and dilute to 100 mL in 95% ethanol. Dissolve 1.0 g bromocresol green and dilute to 500 mL in 95% ethanol. Mix 1 part methyl red solution with 5 parts bromocresol green solution (combine all of both solutions).

(g) *Boric acid solution*.—4%, with indicator. Dissolve 40 g H_3BO_3 and dilute to 1 L in water and add 3 mL methyl red/bromocresol green indicator solution, (f). Solution will be light orange color.

(h) *Hydrochloric acid standard solution*.—0.1000M. Prepare as in 936.15 (see A.1.06) or use premade solution of certified specification range 0.0995–0.1005M and use 0.1000M for calculation.

(i) *Ammonium sulfate*.—99.9% $(NH_4)_2SO_4$.

(j) *Tryptophan or lysine hydrochloride*.—99% $C_{11}H_{12}N_2O_2$ or $C_6H_{15}ClN_2O_2$.

(k) *Sucrose*.—Nitrogen free.

D. Preparation of Test Solution

Add 15.00 g K_2SO_4 , 1 mL $CuSO_4 \cdot 5H_2O$ catalyst solution, and 8–10 boiling chips to digestion flask. Warm milk to 38 °C. Mix milk as in 925.21 (see 33.2.02). Weigh warm sample (5 ± 0.1 mL) and immediately place in digestion flask. (Note: Weights must be recorded to nearest 0.0001 g.) Add 25 mL H_2SO_4 , rinsing any milk on neck of flask into bulb. Flask may be stoppered and held for digestion at later time. Digest and distill a blank (all reagents and no test product) each day.

E. Determination

(a) *Digestion burner setting*.—Conduct digestion over heating device that can be adjusted to bring 250 mL H_2O at 25°C to rolling boil in ca 5–6 min. To determine maximum heater setting to be used during digestion, preheat 10 min (gas) or 30 min (electric) at burner setting to be evaluated. Add 3 or 4 boiling chips to 250 mL H_2O at 25°C and place flask on preheated burner. Determine heater setting that brings water from 25°C to rolling boil in 5–6 min on each burner. This is maximum burner setting to be used during digestion.

(b) *Digestion*.—Place flask in inclined position with fume ejection system on. Start on setting low enough so that test portion does not foam up neck of Kjeldahl flask. Digest at least 20 min or until white fumes appear in flask. Next, increase burner setting half way to maximum burner setting determined in (a) and heat for 15 min. Next, increase heat to maximum setting determined in (a). When digest clears (clear with light blue–green color), continue to boil 1–1.5 h at maximum setting (total time ca 1.8–2.25 h).

To determine specific boil time needed for analysis conditions in laboratory, select a high protein, high fat milk test sample and determine protein content using different boil times (1–1.5 h) after clearing. Mean protein test increases with increasing (0–1.5 h) boil time, becomes constant, and then decreases when boil time is too long. Select boil time that yields maximum protein test.

At end of digestion, digest should be clear and free of undigested material. Cool acid digest to room temperature (ca 25 min). Cooled digest should be liquid or liquid with few small crystals. (Large amount of crystallization before addition of water indicates too little residual H_2SO_4 at end of digestion and can result in low test values.) After digest is cooled to room temperature, add 300 mL H_2O to flask and swirl to mix (for 800 mL flasks add 400 mL H_2O). When room temperature water is added some crystals may form and then go into solution; this is normal. Let mixture cool to room temperature before distillation. Flasks can be stoppered for distillation at later time.

(c) *Distillation*.—Turn on condenser water. Add 50 mL H_3BO_3 solution with indicator to graduated 500 mL Erlenmeyer titration flask and place flask under condenser tip so that tip is well below H_3BO_3 solution surface. To room temperature diluted digest, carefully add 75 mL 50% NaOH down sidewall of Kjeldahl flask with no agitation. NaOH forms clear layer under the diluted digest. Immediately connect flask to distillation bulb on condenser. Vigorously swirl flask to mix contents thoroughly; heat until all NH_3 has been distilled (150 mL distillate; 200 mL total volume). Do not leave distillation unattended. Flasks (500 mL) may bump at this point. Lower receiving flask and let liquid drain from condenser tip. Turn off distillation heater. Titrate H_3BO_3 receiving solution with standard 0.1000M HCl solution to first trace of pink. Lighted stir plate may aid visualization of end point. Record mL HCl to at least nearest 0.05 mL.