

33.2.12

AOAC Official Method 991.21 Nonprotein Nitrogen in Whole Milk

Kjeldahl Method
First Action 1991
Final Action 1994

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

$s_r = 0.006$; $s_R = 0.012$; $RSD_r = 2.817\%$; $RSD_R = 5.707\%$

A. Principle

Protein is precipitated from milk by addition of trichloroacetic acid (TCA) solution. Final concentration of TCA in the mixture is about 12%. Precipitated milk protein is removed by filtration. Filtrate contains nonprotein nitrogen components of milk. Nitrogen content of filtrate is determined as in **991.20** (see 33.2.11).

B. Apparatus

See **991.20B** or **991.20I** (see 33.2.11).

C. Reagents

See **991.20C** or **991.20J** (see 33.2.11) and in addition:

(a) *Trichloroacetic acid solution*.—15% (w/v) AR grade CCl_3COOH . TCA is soft, white, deliquescent crystal, which should be stored in container protected from light and moisture.

(b) *Hydrochloric acid standard solution*.—0.010M HCl. Prepare as in **936.15** (see A.1.06). Alternatively, use premade solution of certified specification range 0.0101–0.0099M and use 0.010 for calculation.

D. Preparation of Test Sample

Warm milk to $38 \pm 1^\circ\text{C}$. Mix milk as in **925.21** (see 33.2.02). Immediately pipet milk (10 ± 0.1 mL) into preweighed 125 mL Erlenmeyer flask and weigh. Record all weights to nearest 0.0001 g. Add 40 ± 0.5 mL 15% TCA solution to flask. Weigh flask and contents. Swirl to mix. Let precipitate settle (ca 5 min). Filter (Whatman No. 1 paper, 15 cm, N_2 -free; or equivalent) and collect entire filtrate. Filtrate should be clear and free of particulate matter; if it

is not, repeat sample preparation. Swirl filtrate to mix. Pipet 20 ± 0.2 mL filtrate into a 50 mL beaker and weigh. Pour filtrate from beaker into Kjeldahl digestion flask that contains boiling chips, K_2SO_4 , and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ catalyst solution as in **991.20D** or **991.20K** (see 33.2.11). Immediately reweigh empty beaker. Add H_2SO_4 as in **991.20D** or **991.20K** (see 33.2.11). Flask may be stoppered and held for digestion at later time. Digest and distill a blank solution (16 ± 0.5 mL 15% TCA and no test sample) each day test samples are analyzed. Keep record of blank values. If blank values change, identify cause.

E. Determination

Proceed as in **991.20E** or **991.20L** (see 33.2.11), substituting 0.010M HCl solution for 0.100M HCl solution as titrant in **991.20E(c)** or **991.20L(b)** (see 33.2.11).

F. Calculation

Calculate results as follows:

$$\text{Nitrogen, \%} = \frac{1.4007 \times (V_s - V_b) \times M}{(W_f \times W_m) / [W_t - (W_m \times 0.065)]}$$

where V_s and V_b = mL titrant used for test portion and blank, respectively; M = molarity of HCl solution; W_f = weight, g, of 20 mL filtrate; W_m = weight, g, of milk; and W_t = weight, g, of milk plus 40 mL 15% TCA solution. [Note: Factor 0.065 in denominator assumes that milk contains about 3.5% fat and 3.0% true protein (i.e., $0.035 + 0.030$). Factor may need to be adjusted if liquid dairy products of different composition are analyzed (i.e., concentrated or fractionated skim or whole milk products, etc.).]

$$\text{“Protein equivalent,” \%} = \text{nitrogen, \%} \times 6.38$$

which is nonprotein nitrogen expressed as protein equivalent.

G. Repeatability and Reproducibility

For results of interlaboratory study parameters obtained in study of this method, r value = 0.016 and R value = 0.033 (expressed on protein basis [$N \times 6.38$]).

Reference: *JAOAC* **74**, 281 (1991).

Revised: March 1996