Crude Protein—Improved Kjeldahl Method, Copper-Titanium Dioxide Catalyst Modification
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Objective
This is a modified Kjeldahl method in which CuSO₄/TiO₂ is used as catalyst. The method determines total nitrogen in nitrate-free material, wheat flour and grain, cereal adjuncts, yeast foods, and animal feeds. The sample is digested in H₂SO₄, and N is converted to NH₃, which is distilled and titrated.

Apparatus
1. Kjeldahl flasks, Pyrex or equivalent, 650–800-ml capacity; used for both digestion and distillation.
2. Digestion heaters, 500–600 W (depending on voltage and on how close flash bulbs are to heating elements). Heater unit should bring 250 ml water at 25° to vigorous boil in 5 min with hot burners.
3. Digestion unit; consists of electric heaters, large lead tube, and plastic fume stack with suction fan capable of exhausting toxic fumes to outside air.
4. Distillation unit; to consist of Iowa State-type connecting bulbs (traps) 36 × 100 mm, Pyrex glass condenser tubes, pure gum-rubber stoppers and tubing, electric heating units (600 W), and condenser tubes capable of being kept cool with adequate amounts of cool water during distillation and with thermo-water control on stills. Upper ends of bulbs connect with high-quality rubber tubing to condenser tubes; lower ends connect with rubber stoppers to 800-ml distillation flask. Lower ends of condenser tubes have rubber-connected glass or polyethylene tubes that lead to:
   5. Receiving bottles or flasks, 300-ml capacity.
   6. Proper burets for dispensing a) concentrated H₂SO₄, b) caustic soda, c) 0.1N H₂SO₄, and d) 0.1N NaOH. See Note 1.

Reagents
1. H₂SO₄, concentrated (95–98%, nitrogen-free; specific gravity 1.84).
2. Catalyst: 16.7 g potassium sulfate, 0.01 g anhydrous CuSO₄, 0.6 g TiO₂, 0.3 g pumice. See Note 2.
3. Antibumping agent: alundum, 8–14 mesh.
4. NaOH, pellets or solution (low N). For solution, dissolve approximately 450 g solid NaOH in water and dilute to 1 liter. Specific gravity of solution should be 1.36 or more.
5. Methyl red indicator. Dissolve 1 g methyl red (Na salt) in 100 ml MeOH (95%). Other indicators may be used satisfactorily. See Note 3.
6. Standard NaOH, 0.1N. Weigh 73 g NaOH per 18 liter water and standardize. May be standardized by titration against pure acid potassium phthalate (NBS SRM for Acidimetry 84 is recommended) dissolved in CO₂-free
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water, using phenolphthalein as indicator; 0.5108 g will neutralize 25 ml 0.1000N NaOH. Other recognized standardization methods may be used. See Note 4.

7. Standard H$_2$SO$_4$, 0.1N. Add 50.4 ml H$_2$SO$_4$ (reagent grade, specific gravity 1.84) to 18 liter water. Titrate against standard NaOH and adjust as necessary, using methyl red as indicator. Other recognized standardization procedures may be used. See Notes 4 and 5.

Procedure

1. Weigh quickly and accurately well-mixed and finely ground sample (bread, 2 g prepared by Method 62-05; yeast foods, 0.5 g; wheat and other grains, feeds and feedstuffs, 1.0 g). Place in digestion flask. (Sample may be placed in nitrogen-free paper to prevent clinging to sides of flask.) Add catalyst (reagent 2), 0.3 g pumice (if not in catalyst), 0.5–1.0 g alundum granules, and 20 ml concentrated H$_2$SO$_4$. (Add additional 1.0 ml H$_2$SO$_4$ for each 0.1 g fat or 0.2 g other organic matter if sample weight is over 1.0 g.)

2. Heat samples at this 5-min boil rate until dense white fumes clear bulb of flask (about 10 min), swirl gently, and continue heating additional 40 min. (Note: reagent proportions, heat input, and digestion time are critical factors—do not change.) Cool, cautiously add about 250 ml water, and cool to room temperature. (Note: Add water as soon as possible to reduce amount of caking. If excessive bumping occurs during distillation, increase dilution water from 250 ml to about 300 ml.)

3. Prepare titration flask by adding appropriate volume of acid standard solution to amount of water such that condenser tip will be sufficiently immersed to trap all NH$_3$ evolved. Add 3–4 drops indicator solution.

4. Add additional 0.5–1.0 g alundum granules to cooled digestion flask. Optionally, 2–3 drops of tributyl citrate may be added to reduce foaming. Slowly, down side of flask, add sufficient NaOH solution such that mixture will be strongly alkaline. Immediately connect flask to distillation apparatus, mix completely, and distill at about 7.5-min boil rate until 150 ml distillate is collected in titration flask. Then set titration flask down so that condenser tube is completely drained.

5. Titrate excess standard acid in distillate with NaOH standard solution. Run blank determination or reagents and correct for blank titer.

Calculation

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\% \text{ Protein} = \frac{(B - S) \times N \times 1.4007 \times f}{\text{sample weight (g)}}
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where \(B\) = ml alkali back-titration of blank; \(S\) = ml alkali back-titration of
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sample; \( N \) = normality of alkali; and \( f \) = 5.7 for bread, wheat, and wheat flour, \( f \) = 6.25 for other grains, \( f \) = 6.38 for milk products, and \( f \) = 6.25 for samples of unknown source.

Notes and Precautions
1. In routine testing of a large number of samples, use large dispensing burets for concentrated acid and alkali. For receiver acid (which may contain an indicator) and 0.1\( N \) titrating NaOH, use automatic zero burets at the titrating table.
2. As a catalyst, copper sulfate-titanium dioxide is recommended as less hazardous than either mercury or selenium or their compounds. Specific parameters of time, heat input, and salt-acid ratio are important. Adequate exhaust ventilation must be provided in digestion-distillation area.
3. Mixed indicator consisting of 0.75 g methyl red and 0.625 g Guinea green per liter or 0.75 g methyl red and 0.5 g methylene blue (Ref. 4) dissolved in 300 ml alcohol may be used. Any indicator used should have sharp end point and distinct color change.
4. Rodkey (Ref. 4) has successfully applied tris (hydroxymethyl) aminomethane as a convenient primary standard for direct standardization of acid solutions.
5. Reeder and Patton (Ref. 3) suggested use of reagent-grade sodium acid sulfate (NaHSO\(_4\)\( \cdot \)H\(_2\)O) in water to make a standard solution equivalent to standard H\(_2\)SO\(_4\); 13.81 g/liter will give 0.1000\( N \) solution.
6. To check the entire protein method, digest 0.1 g pure ammonium oxalate (monohydrate) with 1 g pure sugar using the regular procedure. The resulting protein should be 11.24% calculated as follows: \((\text{NH}_4\)\(_2\)C\(_2\)O\(_4\)\( \cdot \)H\(_2\)O\) with molecular weight of 142.12 contains 28.016 g nitrogen or 19.713%; 19.713% \( \times \) factor 5.7 \( \div \) 10 (0.1-g sample) = 11.24% protein.

References