Crude Protein—Improved Kjeldahl Method

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Objective

This modified Kjeldahl method determines total nitrogen in nitrate-containing materials and animal feeds. The sample is digested in sulfuric acid; ammonia is distilled; and excess acid is titrated. A conversion factor of 6.25 is used for feedstuffs.

Apparatus

1. Kjeldahl flasks for digestion, total capacity about 500–800 ml, made of hard, moderately thick, well-annealed glass.
2. Digestion heaters, 600-W. Heater unit should bring 250 ml water at 25° to vigorous boil in 5 min with hot burners.
3. Distillation flask. Use Kjeldahl or other suitable flask of 500- to 800-ml capacity, fitted with rubber stopper through which passes lower end of efficient bulb or trap to prevent NaOH being carried over mechanically during distillation. Connect upper end of bulb tube to condenser tube by means of rubber tubing.

Reagents

1. H_2SO_4, 93–98% H_2SO_4, nitrogen-free. *Caution*: always add acid to water. Wear face shield and heavy rubber gloves to protect against splashes.
2. Mercuric oxide or metallic mercury (HgO or Hg), reagent grade, nitrogen-free. *Caution:* see Notes 1 and 2.
3. Potassium sulfate (or anhydrous Na_2SO_4), reagent grade, nitrogen-free.
4. Salicylic acid, reagent grade, nitrogen-free.
5. Sulfide or thiosulfate solution. Dissolve 40 g commercial K_2S in 1 liter water. (Solution of 40 g Na_2S or 80 g $Na_2S_2O_3 \cdot 5H_2O$ in 1 liter may be used.)
6. NaOH, pellets or solution, nitrate-free. For solution, dissolve approximately 450 g solid NaOH in 1 liter water. (Specific gravity of solution should be 1.36 or higher.) *Caution:* extremely caustic; can cause severe burns. Protect skin and eyes.
7. Zinc granules, reagent grade.
8. Zinc dust, an impalpable powder.
9. Methyl red indicator. Dissolve 1 g methyl red in 200 ml alcohol.
10. Standard HC1 or H_2SO_4, 0.1*N* (Methods **70-20.02**, **70-80.01**). Other recognized standardization methods may be used. See Note 2.
11. Standard alkali solution, 0.1*N* (Method **70-70.01**).

Check standard solutions, each standardized with primary standard, one against the other.

Test reagents before using, by blank determination with 2 g sugar, which ensures partial reduction of any nitrates present.

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Crude Protein—Improved Kjeldahl Method (continued)

Procedure

General

1. Place weighed sample (0.7–2.2 g) in digestion flask. Add 40 ml H_2SO_4 containing 2 g salicylic acid. Shake until thoroughly mixed and let stand, with occasional shaking, for 30 min or more; then add 5 g $Na_2S_2O_3 \cdot 5H_2O$ or 2 g zinc dust. Shake and let stand 5 min; then heat over low flame until frothing ceases. Turn off heat.

2. Add 0.7 g HgO or 0.65 g metallic Hg, 15 g powdered K_2SO_4 or anhydrous Na_2SO_4, and 25 ml H_2SO_4. If sample larger than 2.2 g is used, increase H_2SO_4 to 10 ml for each g of sample. Place flask in inclined position and heat gently until frothing ceases (if necessary, add small amount of paraffin to reduce frothing); boil briskly until solution clears and then for at least 30 min longer.

3. Cool, add approximately 200 ml water, cool below 25°, add 25 ml sulfide or thiosulfate solution, and mix to precipitate mercury. Add a few zinc granules to prevent bumping, tilt flask, and add layer of NaOH (25 g solid reagent or 50 ml concentrated solution to make contents strongly alkaline) without agitation. (Thiosulfate or sulfide solution may be mixed with NaOH solution before it is added to flask.)

4. Immediately connect flask to distilling bulb on condenser and, with tip of condenser immersed in 25 ml standard acid in receiver, rotate flask to mix contents thoroughly; then heat until all ammonia has distilled (at least 150 ml distillate).

5. Titrate excess standard acid in distillate with standard alkali solution, using methyl red indicator.

6. Correct for blank determinations on reagents.

Feeds and feedstuffs

Use 1.0-g sample (Method **64-50.01**). Crude protein = N × 6.25 (see Table **46-18**).

Calculations

$$\% \text{ Nitrogen} = \frac{(B - S) \times N \times 1.4007}{\text{sample weight (g)}}$$

where B = ml alkali back-titration of blank, S = ml alkali back-titration of sample, N = normality of alkali.

Notes

1. Mercury is hazardous in contact with ammonia, halogens, and alkali. Vapors are extremely toxic and cumulative. To avoid environmental contamination, dilute liquid remaining in Kjeldahl distillation flask to ~300 ml

Crude Protein—Improved Kjeldahl Method (continued)

with water, cool to room temperature, and add 50 ml 30% hydrogen peroxide. Warm gently to initiate reaction. Let reaction go to completion in warm flask, and separate precipitated HgS.

2. As a catalyst, copper sulfate is recommended as less hazardous than either mercury or selenium or their compounds. See Ref. 2. The specific parameters of time, heat input, and salt/acid ratio are important.

3. Rodkey (Ref. 4) has successfully applied tris (hydroxymethyl) aminomethane as a convenient primary standard for direct standardization of acid solutions.

References

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