PROTEIN

PRINCIPLE

Many modifications of the Kjeldahl method have been accepted for the estimation of protein in organic materials. It comprises sample oxidation and conversion of nitrogen to ammonia which reacts with excess sulfuric acid, forming ammonium sulfate. The solution is made alkaline and the ammonia is determined by distilling into an excess of standard acid, followed by titrating the excess acid.

SCOPE

The method applies to the determination of protein nitrogen in feedstuffs, syrups, sugars, starches and other protein-bearing materials when suitable amounts of sample, sulfuric acid and catalyst are employed. Without additional modification, it is not applicable to estimation of nitrogen in mixtures containing nitrates and nitrites.

SAFETY NOTE

Person(s) performing this method should be trained in the handling and disposal of concentrated acids and alkalis, with emphasis on preparation of aqueous solutions, and in coping with potential spills. Accordingly, they should wear appropriate protective equipment and prepare samples and solutions under a fume hood. They should also understand the performance limits and exhaust (scrubber) requirements of the Kjeldahl apparatus available to them. Glassware should be carefully inspected for defects before use. Dispose of spent copper and selenium catalyst according to Good Laboratory Practice and existing regulations.

SPECIAL APPARATUS

Standard Kjeldahl digestion and distillation equipment together with 800-mL Kjeldahl flasks and suitable connecting bulbs are recommended.

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REAGENTS

1. Sulfuric Acid, Concentrated: Reagent grade (96% H_2SO_4, sp g 1.84)

2. Potassium Sulfate: Reagent grade potassium sulfate (K_2SO_4), free from nitrogen

3. Copper Selenite: Reagent grade copper selenite ($CuSeO_3 \bullet 2H_2O$); available from Bodman Chemicals, Media, Pennsylvania 19063. Also, a complete digestion mixture (SEL-DAHL) containing potassium sulfate, copper selenite, and pumice is available from American Scientific Products.

4. Sodium Hydroxide Solution, 50%

5. Sodium Hydroxide Solution, 0.1 *N*: Standard

6. Sulfuric Acid Solution, 0.1 *N*: Standard

7. Methyl Red-Bromcresol Green Indicator: Dissolve 0.33 g bromcresol green and 0.66 g methyl red dyes in 1 liter of 95% ethyl alcohol. Add sufficient 0.1 *N* sodium hydroxide solution to produce a green color; add dropwise just sufficient 0.1 *N* hydrochloric acid solution to produce a deep wine-red color.

8. Zinc Metal: Granular, 20 mesh, C.P. grade

PROCEDURE

Grind sample completely through a laboratory cutting mill to 20 mesh or finer, taking precautions to prevent significant change in moisture, and mix thoroughly (Note 1).

Weigh accurately about 1 g of ground and blended sample and transfer to a Kjeldahl flask (Note 2). Add 10 g of potassium sulfate (Note 3), 0.3 g of copper selenite (Note 4), and 30 mL of concentrated sulfuric acid. Place flask in inclined position on digestion unit and heat below boiling until frothing has ceased.

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Increase heat until acid boils briskly and digest for 90 mins. after reaction mixture clears.

Measure accurately an excess of standard 0.1 *N* sulfuric acid solution (usually 30 to 70 mL, depending on nitrogen content of sample) into a 500 mL Erlenmeyer flask. Connect flask to distillation assembly so that condenser delivery tube is immersed in absorbing acid (Note 5).

Cool the digest in the Kjeldahl flask (Note 6), dilute carefully with about 300 mL of purified water, *mix thoroughly*, and add a pinch of granular zinc to prevent bumping during distillation. Add sufficient 50% sodium hydroxide solution to make the mixture strongly alkaline (Note 7; 75 mL usually sufficient), pouring it down the side of the flask to avoid mixing immediately with the acid solution. Connect flask to condenser by means of connecting bulb, turn on heater, and mix contents of flask gently by swirling. Distill at a moderate rate until all ammonia has passed into the absorbing solution (250 mL of distillate collected normally).

Remove receiving flask, add about 0.25 mL of methyl red-bromcresol green mixed indicator solution (Note 8), and titrate the excess acid with 0.1 *N* standard sodium hydroxide solution to a permanent green end point.

Conduct a blank determination on all reagents substituting pure sucrose or dextrose for the sample, and determine the 0.1 *N* sulfuric acid equivalence (blank).

CALCULATION (Note 9)

$$\% \text{ Nitrogen (as is)} = \frac{(\text{mL } 0.1\ N\ H_2SO_4 - \text{Blank} - \text{mL } 0.1\ N \text{ NaOH}) \times 0.0014 \times 100}{\text{Sample Wt. (g)}}$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25$$

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NOTES AND PRECAUTIONS

1. To prevent significant moisture loss when grinding a series of samples, allow the mill to cool between samples.

2. Most feedstuff samples contain about 20 to 60% protein and a 1 g sample is appropriate. If the sample contains significantly more or less protein, a proportionately smaller or larger sample may be used.

3. Potassium sulfate serves to increase the reaction boiling point thereby hastening the oxidation. It may be replaced with anhydrous sodium sulfate.

4. Catalysts other than copper selenite are used with success in modifications of the Kjeldahl method. These include copper sulfate, mercuric oxide, metallic mercury and electrolytic copper. Changing the catalyst may require changes in the procedure.

5. Fifty milliliters of 4% aqueous boric acid solution may be used alternatively for absorption of ammonia. In this case however, the titration is dilution-sensitive and distillate volumes (samples and blanks) should be adjusted to constant value by dilution with purified water prior to titration. The ammonia-containing boric acid solutions is titrated directly with standard alkali as in the normal procedure. When the boric acid absorbing solution is employed, increase the quantity of indicator for a sharper end point.

6. If the reaction mixture crystallizes to solid form, the test must be discarded because ammonia or nitrogen recovery will be low. The phenomenon can be avoided by increasing the volume of concentrated sulfuric acid used for sample digestion. A proportionate increase in the volume of concentrated sodium hydroxide solution used for neutralization may be required.

7. It is essential that the digestion mixture be made strongly alkaline prior to distillation of ammonia. This can be checked by addition of an indicator such as phenolphthalein to diluted digest prior to alkali addition and

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shaking. If sufficient alkali has been added, the indicator color change will be noted when contents of the flask are shaken.

8. Methyl red indicator or methyl red-methylene blue mixed indicator may be used if preferred.

9. The normalities of the standard acid and standard alkali must be known, and the equivalent volumes of 0.100 *N* reagents must be calculated for use in the equation.