

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 remaining acid.

SCOPE

The method applies to the determination of protein nitrogen in corn grain 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

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 capacity Kjeldahl flasks, suitable connecting bulbs and a running water scrubber for corrosive fumes are recommended.

REAGENTS

1. Sulfuric Acid, Concentrated: Reagent grade (96% H₂SO₃, sp g 1.84

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2. Potassium Sulfate: Reagent grade potassium sulfate (K_2SO_4), free from nitrogen
3. Copper Selenite: Reagent grade copper selenite ($CuSeO_3 \cdot 2H_2O$); available from VWR or from WACO (Wilkins Anderson Co.) chemical distributor catalogues. In either case, the selenium based catalysts are EM Science (an affiliate of Merck KgaA of Germany) Kelmate N Kjeldahl Digestion Mixtures. Mixtures #200/201 include elemental Se in combination with a copper salt that produce required copper selenite during digestion. Mixture #600 is copper selenite dihydrate and mixture #601 contains added pumice to aid smooth boiling during digestion, so that granular zinc (item 8 below) may be omitted.
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 about 50 g of sample through a laboratory cutting mill to 20 mesh or finer and mix thoroughly (Note 1). Determine moisture content of ground sample by an approved method or alternate procedure giving equivalent results.

Weigh about 2 g of ground sample to the nearest 0.1 g and transfer quantitatively to the digestion flask. Add 10 g of potassium sulfate (Note 2) and 0.3 g of copper selenite (Note 3). Add 30 mL of concentrated sulfuric acid. Place flask in

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inclined position on digestion unit and heat below boiling until frothing has ceased. Increase heat until acid boils briskly and digest for 90 minutes after the reaction mixture clears.

Measure accurately an excess of standard 0.1 *N* sulfuric acid solution (usually 25 to 35 mL, depending on nitrogen content of sample) into a 500-mL Erlenmeyer flask (Note 4). Connect flask to distillation assembly so that the condenser delivery tube is immersed in the absorbing acid (add purified water if necessary).

Cool the digest in the Kjeldahl flask (Note 5), 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 6: 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 the 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 and titrate excess acid with standard 0.1 *N* sodium hydroxide solution using about 0.25 mL of methyl red-bromcresol green mixed indicator (Note 7).

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

CALCULATION

% Nitrogen (dry basis) =

$$= \frac{(\text{mL } 0.1N \text{ H}_2\text{SO}_4 - \text{Blank} - \text{mL } 0.1N \text{ NaOH}) \times 0.0014 \times 100 \pm \times 100}{\text{Sample Wt. (g)} \times (100 - \text{Sample Moisture, \%})}$$

% Protein = % Nitrogen \times 6.25 (Note 9)

NOTES AND PRECAUTIONS

1. If the moisture content is above 20%, it is advisable to predry sample prior to grinding. Place sample to be ground in open dish (but protected from

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dust or other contamination) in a warm, well-ventilated place so that the grain will dry reasonably fast and reach an approximate air-dried condition in from 14 to 24 hours. Moisture loss need not be recorded since moisture content of the ground sample will be determined.

2. Potassium sulfate serves to increase the reaction boiling point, thereby hastening the oxidation. It may be replaced by anhydrous sodium sulfate.
3. Catalysts other than copper selenite have been used with success in modifications of the Kjeldahl method. These include copper metal, copper sulfate, mercuric oxide, metallic mercury and titanium dioxide. Changing the catalyst may require changes in the procedure, and a revalidation of the method by analyses of pure compounds containing known nitrogen contents is advised.
4. Fifty mL of 4% aqueous boric acid may be used alternatively for absorption of ammonia. In this case, distillate volumes should be adjusted to a constant value by addition of purified water, if necessary. The boric acid solution containing ammonia is back-titrated with standard sulfuric acid eliminating the use of standard alkali as in the standard procedure. Increase the quantity of indicator for a sharper end point.
5. If the reaction mixture crystallizes, 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.
6. It is essential that the digestion mixture be made strongly alkaline prior to distillation of ammonia. This can be checked periodically by addition of phenolphthalein indicator to the diluted digest prior to alkali addition and shaking. If sufficient alkali has been added, the contents of the flask will turn pink when shaken.
7. Methyl red indicator or methyl red-methylene blue mixed indicator may be used.

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8. 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.
9. In the analysis of grains other than corn, or materials not derived from corn, use the appropriate factor for conversion of nitrogen to protein. For example, the conversion factor is 5.7 for wheat products.