PROTEIN (Kjeldahl)

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 procedure is applicable to the determination of protein nitrogen in corn, corn starches, corn syrups, feedstuffs and steepwater when suitable amounts of sample, sulfuric acid and catalyst are employed. Without additional modification, it is not applicable to the estimation of total nitrogen in the presence of 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 in conjunction with 800 mL capacity Kjeldahl flasks and suitable connecting bulbs are recommended.

PROTEIN — continued

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 ($CuSeO_3 \bullet H_2O$); 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 L 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.

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

PROTEIN — continued

PROCEDURE

METHOD PARAMETERS TABLE

Sample Type	Sample Weight (g)	Concentrated Sulfuric Acid mL	0.1 N sulfuric acid solution mL
Corn	2	30	25-35
Corn Starch	10	60	5-15
Corn Syrup	10	60	5-15
Feedstuffs	1	30	30-70
Steepwater	1	30	30-50

For corn and feedstuffs:
Grind about 50 g of sample through a laboratory cutting mill to 20 mesh or finer and mix thoroughly. Determine moisture content of ground sample by an approved method or alternate procedure giving equivalent results.
For weighing liquid samples:
Weigh specified amount of sample into a suitable glazed paper cup or into a 10 mL Pyrex beaker and transfer quantitatively to the digestion flask with the aid of little water.
For weighing solid samples:
Weigh specified amount of sample and transfer quantitatively to the digestion flask.
Add 10 g potassium sulfate (Note 1) and 0.3 g copper selenite (Note 2). Add specified amount of concentrated sulfuric acid. Place flask in 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 sulfuric acid solution as specified from chart into a 500 mL Erlenmeyer flask. Add distilled water and connect flask to distillation assembly so that condenser delivery tube is immersed in absorbing acid (Note 3).

After cooling digest in Kjeldahl flask (Note 4), dilute carefully with about 300 mL of distilled water; mix thoroughly, and add a pinch of granular zinc to prevent

PROTEIN — continued

bumping during distillation. Add sufficient 50% sodium hydroxide solution to make the reaction mixture strongly alkaline (Note 5: 75 mL usually sufficient), pouring it down side of 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 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 6).

Make 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 (Note 7)

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

% Protein = % Nitrogen × 6.25

NOTES AND PRECAUTIONS

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

2. 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 oxide. Changing the catalyst may require changes in the procedure, and a revalidation of the method of analyses of pure compounds containing known levels of nitrogen.

3. 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 distilled 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 normal procedure. Increase the quantity of indicator for a sharper end point.

PROTEIN — continued

4. 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.

5. 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.

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

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.

METHOD HISTORY

Combined the Protein, Kjeldahl methods for Corn (A-18), Corn Starch (Unmodified) (B-48), Corn Syrup (E-52), Corn Sugar (F-44), Feedstuffs (G-22) and Steepwater (J-56) on 11-09-2010.

Corn, Protein, Kjeldahl (A-18), Date of Acceptance 6-03-1957, Revised 2-23-2001.

Corn Starch (Unmodified), Protein, Kjeldahl (B-48), Date of Acceptance 11-26-56, Revised 2-23-2001.

Corn Syrup, Protein, Kjeldahl (E-52), Date of Acceptance 8-25-1952, Revised 10-23-2001.

PROTEIN — continued

Corn Sugar, Protein, Kjeldahl (F-44), Date of Acceptance 10-12-1960, Revised 10-09-2009.

Feedstuffs, Protein, Kjeldahl (G-22), Date of Acceptance 11-12-1962, Revised 10-23-2001.

Steepwater, Protein, Kjeldahl (J-56), Date of Acceptance 9-17-1976, Revised 9-30-1997.