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| **METHOD OF ANALYSIS OF PROTEIN IN BIORINGA** |
| **Reference - Indian standard IS : 7219 - 1973** |

**PRINCIPLE**

The sample is oxidized in the presence of sulphuric acid and nitrogenous compounds are converted into ammonium sulphate. Mercury is added to the digestion mixture as a catalyst and alkali sulphate as a boiling-point elevator. Ammonia is liberated by adding an excess of alkali and is quantitatively distilled into a measured volume of standard hydrochloric or sulphuric acid. The acid not neutralized by ammonia is back-titrated with standard alkali.

**APPARATUS**

**For Digestion -** Use 500- to 800-ml Kjeldahl flasks. Conduct digestion over a heating device adjusted to bring 250 ml water at 25°C to rolling boil in approximately 5 minutes. if gas, or for 30 minutes if electric. To test heaters, preheat for 10 minutes, to prevent superheating. Add 3 to 4 boiling chips or glass beads to prevent superheating.

**For Distillation-**Fit the flask with a rubber stopper through which passes the lower end of an efficient scrubber trap or bulb to prevent mechanical carryover of alkali during distillation. Connect the upper end of the trap to a condenser by rubber or glass tubing. Immerse the trap outlet of the condenser in such a way as to ensure complete absorption of ammonia distilled over into acid in a 500-ml Erlenmeyer flask.

**REAGENTS**

* **Concentrated Sulphuric Acid -** 93 to 98 percent by mass, nitrogen-free.
* **Mercuric Oxide or Metallic Mercury -** nitrogen-free.
* **Potassium Sulphate or Anhydrous Sodium Sulphate -** nitrogen-free.
* **Zinc Granules**
* **Sulphite or Thiosulphate Solution -** Dissolve 40 g potassium sulphide or 80 g hydrated sodium thiosulphate in 1 litre distilled water.
* **Sodium Hydroxide pellets, flakes or solution-** nitrogen free. For solution, dissolve about 450 g solid sodium hydroxide in distilled water, cool, and dilute to 1 litre. 20°C. The specific gravity should be at least 1.36 at 20°C.
* **Hydrochloric or Sulphuric Acid, Standard Solution -** 0.1 or 0.5 N Standardize against primary standard and against sodium hydroxide standard solution.
* **Sodium Hydroxide Standard Solution -** 0.1 N. Standardize against primary standard and against standard acid solution.
* **Methyl Red Indicator -** Dissolve 1 g methyl red in 200 ml alcohol.

**SAMPLING**

Prepare a representative and homogeneous sample as appropriate for the specific product to be analysed.

**PROCEDURE**

**Digestion -** Accurately weigh 0.7 to 2.2 g of the sample into the digestion flask. Add 0.7 g mercury oxide or 0.65 g mercury and 15 g powdered potassium sulphate or anhydrous sodium sulphate, and 25 ml sulphuric acid. Ratio of salt to acid ( m/v ) should be approximately 1: 1 at the end of digestion for proper temperature control. Digestion may be incomplete at a lower ratio and nitrogen may be lost at a higher ratio.

Each gram of fat consumes 10 ml and each gram of carbohydrate 4 ml sulphuric acid during digestion. Place the flask in an inclined position on a heater and heat gently until foaming ceases. A small amount of paraffin or silicon antifoam may be added to reduce foaming. .Boil vigorously until the solution becomes clear and then continue boiling it for 1 to 2 hours.

**Distillation -** Cool, add about 200 ml distilled water, and in order to avoid complex formation, add 25 ml of the sulphide or thiosulphate solution. Mix to precipitate the mercury. Add a few zinc granules to prevent bumping, incline flask, and add without agitation 25 g of sodium hydroxide as solid or equivalent as solution, to make solution strongly alkaline ( the thiosulphate or sulphide solution may be mixed with the sodium hydroxide solution before addition to the flask ). Immediately connect flask to distillation bulb or trap on condenser, and, with tip of the condenser immersed in a measured quantity standard acid ( usually 50 ml, 0.5 N or an appropriate quantity of 0.1 N ) in the receiver, rotate flask to mix the contents thoroughly; then heat immediately until all ammonia has distilled over ( at least 150 ml distillate ). Lower the receiver before stopping distillation and wash tip of condenser with distilled water. Back-titrate excess acid with standard 0.1 N sodium hydroxide, using methyl red as indicator. Correct for blank determination in reagents.

**Blank -** Conduct determinations using all reagents and 2 g of sugar.

**CALCULATION, EXPRESSION AND INTERPRETATION OF RESULTS**

**Calculation of Nitrogen Content**

 **Nitrogen content ( N) in g= (A–B ) – ( C–D ) x 0.001 4**

where

A=volume in ml O-1 N acid measured for main distillation,

B=volume in ml 0.1 N alkali used for back-titrating A,

C=volume in ml 0.1 N acid measured for blank distillation, and

D=volume in ml 0.1 N alkali used for back-titrating C.

**Calculation of Total Protein Protein**

 **N x 100 x Conversion factor**

 **Percent by mass =**

 **W**

where

N =mass of nitrogen content in g of original sample,

W=mass of sample in g.