PROTEIN DETERMINATION BY KJELDAHL METHOD

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INTRODUCTION

In the presence of sulphuric acid and catalyst, the nitrogen atom in the nitrogenous organic compound is converted to ammonium sulphate. The ammonia is then distilled from an alkaline medium and absorbed in boric acid. The ammonia is then determined by titration with a standard mineral acid.

Taking protein as an example, it is as follows:

$$\text{Protein N} \xrightarrow[\text{Catalyst}]{H_2SO_4} (NH_4)_2SO_4 + CO_2\uparrow + H_2O$$

$$(NH_4)_2SO_4 + NaOH \longrightarrow Na_2SO_4 + 2NH_4OH$$

$$NH_4OH \longrightarrow NH_3 + H_2O$$

$$3NH_3 + H_3BO_3 \longrightarrow NH_{4+} + BO_3 \blacksquare$$

$$BO_3 \blacksquare + 3H^+ \longrightarrow H_3BO_3$$

I APPARATUS

Kjeldahl digestion and assembly ("Tecator" brand)
Kjeldahl digestion tube, 250 ml
Kjeldahl distillation apparatus ("Tecator" brand)
Conical flask 250 ml
Automatic burettes 50 ml with 2000 ml reservoir bottle
Magnetic stirrer

II REAGENTS

a) Sulphuric acid (H_2SO_4), nitrogen free

b) Catalyst

Mix 9 parts of potassium sulphate (K_2SO_4) anhydrous, nitrogen free with 1 part of copper sulphate ($CuSO_4$), anhydrous, nitrogen free.

c) NaOH solution (40% w/v)

Dissolve sodium hydroxide (NaOH), technical grade, mini pearls, in distilled water.

d) Boric acid (4% w/v)

e) Anti-bumping granules

f) Ethanol (95% v/v)

g) Standard 0.1N Sulphuric acid

Break ampoule for preparation of standard solution, empty content into 1 L volumetric flask and dilute with nitrogen free distilled water until the mark. Cap and invert the volumetric flask until solution is well mixed. Transfer the contents to the automatic burette.

h) Indicator

Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol).

III PROCEDURE

1. Accurately weigh the homogenous fish sample (1g) or pipette a suitable quantity of protein fraction solution (20 ml myofibrillar or sarcoplasmic protein fraction or 40 ml non-proteinous nitrogen fraction) and place in digestion tube. Add 7 g catalyst, 3 to 5 anti-bumping granules and 20 ml of conc. H_2SO_4. Also prepare a tube containing the above chemicals except fish sample as blank. Cover tube with exhaust manifold and place tube in the preheated digestor and digest at about 110 - 130°C for 15 mins (ignore this process if non liquid sample is to be digested). Turn the digestor to digestion temperature normally around 420°C and digest the sample until the solution is light green (1 hr for fish sample) and then a further 15 mins. Remove tube and leave to stand until sample is cooled. Add cautiously 60 ml distilled water.

2. Switch on distillation apparatus and pre-wash for 10 mins. Dispense 25 ml 4% boric acid into a 250 ml conical flask and place the flask under the condenser, ensuring that the condenser tip is immersed in the boric acid solution. Connect the digestion tube carefully into the digested sample. Immediately turn on the steam supply valve to initiate the distillation. Heat for 4 mins until all ammonia has passed over into the boric acid. Lower the conical flask ensuring the condenser tip is not immersed in solution and continue heating for a further 1 min. Collect approximately 120 ml distillate. Wash tip of condenser with distilled water.

   Place conical flask containing ammonia distillate on magnetic stirrer. Add 1 ml indicator and titrate the sample with standard 0.1N sulphuric acid until the solution changes from green to pinkish. Read volume of acid used for titration.

IV CALCULATIONS

Calculate the protein nitrogen (mgN/100 g or 100 ml samples) as follows:

a) solid/semi-solid fish sample

$$\text{protein nitrogen} = \frac{(b - a) \times 0.1 \times 14.00}{W_s} \times 100 \qquad (1)$$

where Ws = weight (g) or volume (ml) of sample

a = volume (ml) of 0.1N H_2SO_4 used in blank titration

b = volume (ml) of 0.1N H_2SO_4 used in sample titration

14.00 = atomic weight of nitrogen

b) Calculation of percentage protein

The above protein nitrogen (mgN/100 g or 100 ml sample) can also be presented as percentage protein nitrogen fraction and is expressed as follows:

$$\% \text{ protein} = \frac{(b - a) \times 0.1 \times 14.00}{W} \times 100 \times \frac{6.25}{1000}$$

where $\frac{(b - a) \times 0.1 \times 14.00}{W_s} \times 100$ is similar to formula (1)

1000 : the conversion of mgN/100 g to gN/100 g sample

6.25 : the protein-nitrogen conversion factor for fish and its by-products