EXPERIMENT 2 DETERMINATION OF CRUDE PROTEIN

Structure

2.0 OBJECTIVES

After going through this experiment, you will be able to:

- determine the amount of crude protein in any meat sample; and
- evaluate the nutritional quality of meat and meat products.

2.1 INTRODUCTION

The concentration of crude protein in a meat sample gives the idea about the quality of the meat. The **Kjeldhal method** is most frequently used procedure for measuring nitrogen and protein concentration in biological materials. There are two types of Kjeldhal methods- **Macro-Kjeldhal** and **Micro-Kjeldhal** method. Generally, Macro-Kjeldhal method is used for the analysis of food samples and Micro-Kjeldhal method for serum analysis.

Kjeldhal method does not distinguish the nitrogen contribution from true protein and non-protein nitrogenous substances such as urea, uric acid, ammonium salt etc. The insoluble protein left by precipitation with a suitable precipitating agent after filtering Non-Protein Nitrogen (NPN) is termed as true protein. The NPN is calculated from the difference between the total crude protein nitrogen and the value of precipitated true protein nitrogen. The different precipitating agents commonly used are trichloroacetic acid, copper hydroxide, zinc-barium hydroxide etc. Most commonly used precipitating agent is ten per cent trichloroacetic acid.

2.2 EXPERIMENT

Determination of crude protein by Macro-Kjeldahl method.

2.2.1 Principle

The protein content of food stuff (meat tissue) is obtained by estimating the nitrogen and multiplying the nitrogen value by 6.25. The tissue is digested with sulphuric acid in presence of a catalyst (selenium oxide, mercury or copper sulphate) which converts organic nitrogen into ammonium sulphate. Ammonia is then liberated by adding alkali and is distilled into a known volume of a standard acid which is then back titrated.

2.2.2 Requirements

a) Apparatus

1. Digestion bench placed in digestion chamber
2. Kjeldahl Distillation Unit
3. Kjeldhal flask (500-800ml)
4. Burette
5. Conical flask
6. Volumetric flask
7. Measuring cylinder
8. Weighing Balance
9. Pipettes(2ml,10ml)

b) Chemicals

1. Potassium sulphate
2. Copper sulphate
3. Sodium hydroxide (NaOH)
4. Commercial sulphuric acid (H_2SO_4)
5. Methyl red Indicator

c) Reagents: (Required for the analysis of one sample)

1. Digestion mixture: Mix 49g potassium sulphate and 1g copper sulphate powder.
2. 40 per cent sodium hydroxide: Dissolve 40g sodium hydroxide in 100ml distilled water.
3. N/10 sodium hydroxide: Dissolve 4g sodium hydroxide in 100ml distilled water.
4. N/10 sulphuric acid: Dilute 28ml of concentrated sulphuric acid to 100ml with distilled water.
5. Methyl red indicator: 0.1 gram of the indicator dissolved in 60 ml of alcohol and distilled water added to 100 ml.

Beside these glass beads are also required.

2.2.3 Procedure

The method of estimation of nitrogen by Kjeldhal method includes three steps;

(a) Digestion

(b) Distillation and

(c) Titration

a) Digestion of meat sample

(i) Weigh accurately 5g of meat samples (muscle) and transfer to Kjeldhal flask.

(ii) Add 50ml of commercial sulphuric acid.

(iii) Add 5g of digestion mixture.

(v) Boil the contents for 3 to 5 hours in a digestion chamber or till the solution is clear without leaving any undigested black particles. Adhering material inside walls of the flask needs one or two washings in between after cooling. A few glass beads may be placed inside the Kjeldhal flask to avoid bumping.

(v) Transfer the digested material after cooling by dissolving with distilled water followed by 5 to 6 repeated washings. Make up the final volume to 100ml with distilled water (Y ml).

(vi) Similarly run a blank without sample.

b) Distillation

(i) Pipette 10ml (Xml) of aliquot of digested sample in a volumetric flask from Kjeldhal flask of distillation unit.

(ii) Add 20ml of 40% NaOH to make the contents alkaline (i.e. add more NaOH till the contents turn blue or black). Wash with a small quantity of distilled water and close the end immediately with a pinch cock. Seal the funnel with a little amount of distilled water to avoid escape of ammonia.

(iii) Steam distills the contents of the distillation unit by boiling with water in a round bottom flask connected to distillation unit. Add glass beads to avoid bumping.

(iv) Collect released ammonia in 25ml N/10 sulfuric acid in a conical flask to ensure all nitrogen in the form of ammonia is distilled.

(v) Remove the conical flask with distillate (25ml N/10 sulphuric acid) after washing the tip of the condenser with a few ml of distilled water.

(vi) Wash the distillation unit 2 to 3 times with distilled water with the help of back suction developed by vacuum due to displacement of boiling flask from heater, to make the apparatus ready for distillation of next sample.

c) Titration

(i) Titrate the distillate (from step b–v above) in the conical flask against N/10 sodium hydroxide taken in a burette till the red colour developed on adding 2 drops of methyl red as indicator changes to yellow colour (B ml).

(ii) Similarly distill and titrate the blank without sample (B_1 ml) i.e. 25 ml N/10 H_2SO_4.

2.2.4 Observations

Weight of the meat sample = W g

Volume (ml) made out of digested sample = Y ml

Volume (ml) of aliquot taken for distillation = X ml

Volume of N/10 sodium hydroxide utilized to titrate N/10 H_2SO_4 distillate of blank =B_1

Volume of N/10 sodium hydroxide utilized to titrate N/10 H_2SO_4 distillate of sample =B

Volume of N/10 H_2SO_4 utilized for absorption of ammonia = Volume of N/10 sodium hydroxide utilized =B_1-B

2.2.5 Calculation

$$\text{The crude protein content (g \%)} = \frac{Y \times (B1\text{-}B) \times 14\,d \times 6.25}{W \times X} \times 100$$

Here Normality of

NaOH(d) = N/10=0.1N

(g %) = g protein/100g sample

Derivation factor

Usually percentage nitrogen in protein is around 16. It means 1 gm nitrogen will be present in 100/16 = 6.25 gm of protein. Therefore derivation factor is 6.25.

(14= Atomic weight of nitrogen)

2.2.6 Result

The amount of crude protein in the given sample = ... g/100g.

Result Table

Sl. No.	Weight W(g)	Vol. of digest Y (ml)	Vol. of aliquot taken for distillation X(ml)	Vol. of NaOH used with 25 ml N/10 H_2SO_4 B_1(ml)	Vol. of NaOH used with sample B(ml)	Vol. of H_2SO_4 used B_1-B (ml)	% Crude Protein
Sample 1							
Sample 2							
Sample 3							
Sample 4							
Sample 5							

2.3 PRECAUTIONS

- Handle sulfuric acid carefully and add slowly to sample.
- Boiling of sample should be carried out in open area otherwise fumes of H_2SO_4 create problem in working environment. While boiling the flask should be protected from any extraneous entry.
- There should not be any carbon/black particles left after digestion.
- Add distill water to digested sample only after cooling and should be done slowly.
- To avoid any chance of escape of Nitrogen, nozzle tip should be always in touch of bottom and inside liquid distillate.
- Titration should be carried carefully and better to keep flask on white paper while mixing so that a colour difference could be detected easily.