EXPERIMENT 5 CRUDE PROTEIN (TOTAL PROTEIN)

Structure

5.1 INTRODUCTION

Proteins are fundamental food components, both functionally and nutritionally. Dietary protein is supplied from plant and animal sources. Proteins are needed to build and repair body tissue and for the metabolic functions of our bodies. The crude protein is determined by estimating total nitrogen in any food material.

Objective

After studying this experiment, you should be able to:

- determine the total protein content of foods.

5.2 EXPERIMENT

5.2.1 Principle

Total nitrogen in all the samples is determined by the Kjeldahl method. It is a three step experiment as given below;

1. **Digestion:** It results in complete hydrolysis of the sample converting all protein and other nitrogenous compounds into ammonia.

2. **Distillation:** Distillation of the digested sample is the process during which the ammonia is released which is trapped in boric acid solution to yield ammonium borate.

3. **Titration:** The solution containing ammonium borate is titrated against 0.1 or 0.01 N HCI.

The protein content is estimated by multiplying % Nitrogen by a '**Protein factor'** as given below. In case factor is not known, 6.25 is commonly used.

Protein/product type	Protein factor
Egg	6.25
Milk	6.38
Meat	6.25
Rice	5.95
Barley	5.83
Wheat (whole)	5.83
Wheat (flour)	5.70
Maize	6.25

5.2.2 Requirements (Equipment/Machinery/Instrument and Chemicals/ Material)

- Conc. sulphuric acid
- Catalyst powder: Contains Cupric sulphate (penta-hydrate) and potassium sulphate (1:5 w/w).
- Sodium hydroxide solution (50%)
- Boric acid – indicator solution.

Solution A: It is prepared by dissolving 40g boric acid in 1.95 litres hot distilled water.

Solution B: It is prepared by dissolving 0.01 of bromo cresol green in 10ml of 95% ethyl alcohol.

Solution C: It is prepared by dissolving 0.05 g methyl red in 50 ml of 95% ethyl alcohol.

Finally solutions B and C are mixed and 50ml of this solution is made up to 2 litres with boric acid solution.

- Digestion assembly including Kjeldahl flasks / tubes.
- Distillation assembly
- Burette, pipette, conical flasks etc.

5.2.3 Procedure

1. Digestion of sample

- Accurately weigh 50 to 100mg sample (in duplicate) and transfer into two different Kjeldahl digestion tubes and label them.
- Add 4 ml conc. sulphuric acid and 100mg of catalyst powder to each digestion tube.
- Place the tubes on a heater to allow digestion at slow heat (100^{o}C) for 30 min. and gradually increase the temperature to 200^{o}C in about 1hour and finally to 420^{o}C until the colour of the content changes from dark brown to bluish green.
- The digested samples are then removed from heater and allowed to cool.

2. Distillation of sample

- Thoroughly clean the distillation unit and allow preheating.
- Now add 10 to 15 ml distilled water to each Kjeldahl tube/ flask.
- Close the stopcock and fill the reservoir with water to 2/3 its volume.
- Now transfer the diluted digested sample into the sample funnel and open the stopcock to allow the sample to drop into the mixing chamber.
- Rinse the Kjeldahl tubes with 10-15ml of distilled water and add the wash water to mixing chamber.
- Close the stopcock of the sample addition funnel and add sodium hydroxide (50%) solution to the sample funnel.
- Place the receiver conical flask containing 10 ml of boric acid with indicator with the outlet tube properly submerged into the solution.
- Now allow the sodium hydroxide solution to drop slowly into the mixing chamber by gently opening the valve of the sample addition funnel. Add 15-20ml of distilled water to the sample addition funnel and allow it to drop into the mixing funnel. Now close the sample addition funnel leaving some residual water in the funnel to work as water seal.
- Start heating of the content of the mixing chamber and continue for 20-30min, or until the colour of the indicator solution is changed from bluish purple to bluish green. Collected 15-20 ml of distillate.
- Finally slow down the heating intensity and gently remove the receiver flask while rinsing the outlet tube.

3. Titration

- Now titrate the distillate against 0.01 N HCl till the bluish green colour changes to pink.
- Run a blank preparation which has been identically prepared except that it does not contain the sample.

5.2.4 Observations

Parameters			
Sample titration value, ml			
Blank titration value, ml			

% Nitrogen is calculated as follows:

$$\%\text{Nitrogen} = \frac{(\text{Sample titre} - \text{Blank titre}) \times \text{N} \times 14 \times 100}{\text{mg of sample}}$$

N = Normality of HCl

% Crude protein = %Nitrogen × Protein factor.

5.2.5 Results

Calculate crude protein using above formula. Take the average of three values and report the crude protein content in percent.

5.3 PRECAUTIONS

- The digestion should be done in a closed cabinet so as to avoid inhalation of the fumes.
- During distillation, the outlet tube must be submerged into boric acid.