Determination of crude protein

**Crude Protein (CP):** is amount of nitrogen in feed staff multiplier in 6.25

**Crude protein include:**

1- **True protein (TP):** Involve groups of AAs associated with each peptide bond.
2- **Non-Protein N (NPN):** This include all compounds contain N, for example:
   
   **A:** Free AAs.
   **B:** Urea and uric acids.
   **C:** Ammonia. and all nitrogenous compounds.

**Crude protein** content is calculated from the N content of the feed, using Kjeldahl method.

**Crude P can be estimated in 2 methods:**

- **Macro Kjeldahl:** For feed containing large amounts of N.
- **Micro Kjeldahl:** For feed materials containing small amounts of N.

**Scientific Principle of determination (CP):**

* The principle of Kjeldahl method is to split NH2 groups from the compounds by using strong acid (H2SO4).
* Convert it to salt (NH4)2SO4, then
* Turn it to ammonia which liberated by using NaOH to NH3 OH.
* Distilled in a given volume of acid (0.1 N) with four drops of detector, then
* Titrate with 0.1 N NaOH until change the color to calculate amount of ammonia

**The scientific principles of the experiment:**

Sample + sulfuric acid → ammonium sulfate + NH4OH → hydroxide ammonia

liberate ammonia + H2SO4 (0.1N) → titration with Na2S2O3 (0.1N) → find out the amount of ammonia.

**Procedure**

**A- Macro Kjeldahl**

Crude protein can be estimated in this way for the feed containing large amounts of N (10-45% CP).

To calculate N content in feed, there are three steps to follow:

1- **Digestion**

   - Weight 2gm of feed (conc.) in Kjeldahl flask + 2 pellets as assistant factors.

   **The pellets contain:**

   18 gm Na2SO4 or K2SO4 + 1gm Cu SO4.5H2O (crystals).

   OR

   Cu SO4 + K2SO4 + Se O3.  (1 : 4 : 0.75)

   These pellets are used to help in the process of digestion (to raise the boiling point) in order to speed up the reaction.

2- Add 20 ml of H2SO4 conc. acid (98%) as a strong oxidant.

3- Boiling for 4-6 hr.

4- Stop boiling, cooling, then add 200ml of dist. water.

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Transfer all the content to a 250ml volumetric flask (quantitative transfer), then complete the volume to 250ml with distilled water.

**Reactions**

\[
\text{Protein N-H-O-C + H}_2\text{SO}_4 \xrightarrow{\text{Boiling}} \text{NH}_4^+ + \text{SO}_2 + \text{CO}_2 + \text{H}_2\text{O}
\]

\[
\text{NH}_4^+ + \text{H}_2\text{SO}_4 \rightarrow (\text{NH}_4)_2\text{SO}_4
\]

### 2- Distillation

- **a**- Take 10ml of the sample to the distilatory + 10ml Na OH (40%).
- **b**- For distillation use Markham device.
- **c**- Use receptor flask contain 25ml( 0.1N ) H\textsubscript{2}SO\textsubscript{4} + 2-4 drops of Methyl Red (Indicator).
- **d**- Start the reaction in the distillatory (by heating) to evaporate NH\textsubscript{3} and then condense it and returned to the receptor flask for 15 min or 50ml.

**Reactions:**

\[
(\text{NH}_4)_2\text{SO}_4 + \text{NaOH} \rightarrow \text{NH}_4\text{OH} + \text{Na}_2\text{SO}_4
\]

\[
\text{NH}_4\text{OH} \rightarrow \text{NH}_3↑ + \text{H}_2\text{O}
\]

NH\textsubscript{3} will condensed and absorbed by H\textsubscript{2}SO\textsubscript{4} or Hcl 0.1N.

Mitigating factor 250 / 10 = 25 (used in the calculation).

### 3- Titration

To know the volume of evaporated NH\textsubscript{3} (which absorbed by 0.1N H\textsubscript{2}SO\textsubscript{4}), titrate with 0.1N NaOH. Example:

Suppose that NaOH used = 15ml

So the size of the remaining acid is neutralized with NH\textsubscript{3} in the flask.

NH\textsubscript{3} volume will be 25 - 15 = 10ml

Each 1 ml of NH\textsubscript{3} contains 0.0014 gm N.

**Calculation:**

\[
\% \text{CP} = \frac{\text{NH}_3 \text{ Vol.} \times 0.0014 \times 6.25 \times \text{dilution factor}}{\text{Weight of sample}} \times 100
\]

0.0014 = Atomic weight of N diluted in 1000ml dist. water.

6.25 = Constant coefficient of any protein that contain 16% N.

100 / 16 = 6.25