5) Protein

* Introduction:-

- protein are an abundant component in all cells, and almost all except storage proteins are important for biological fun^n and cell stru.
- They are composed of elements including Hydrogen, carbon, nitrogen, oxygen and sulfur.
- Twenty α-amino acids are the building blocks of proteins, the amino acid residues in an protein are linked by peptide bonds.
- For example, simple proteins, contain only amino acids upon Hydrolysis, but conjugated proteins also contain non-amino-acid components.
- Proteins have unique conformation that could be altered by denaturants such as heat, acid, alkali, 8M Urea, 6M guanidine-HCl, organic solvents and detergents.

* Importance of Analysis :-

1) Nutrition labeling :-
2) Pricing :- It is measured by nitrogen content (e.g. cereal grains; milk for making certain dairy product, e.g. cheese).
3) Functional property investigation:- Proteins in various types of food have unique food functional properties.
4) Biological activity determination:- Some protein, including enzymes or enzyme inhibitors, are relevant to food science and nutrition.

* Content of protein in Foods :-

The protein contents of selected food items are listed in below Table.

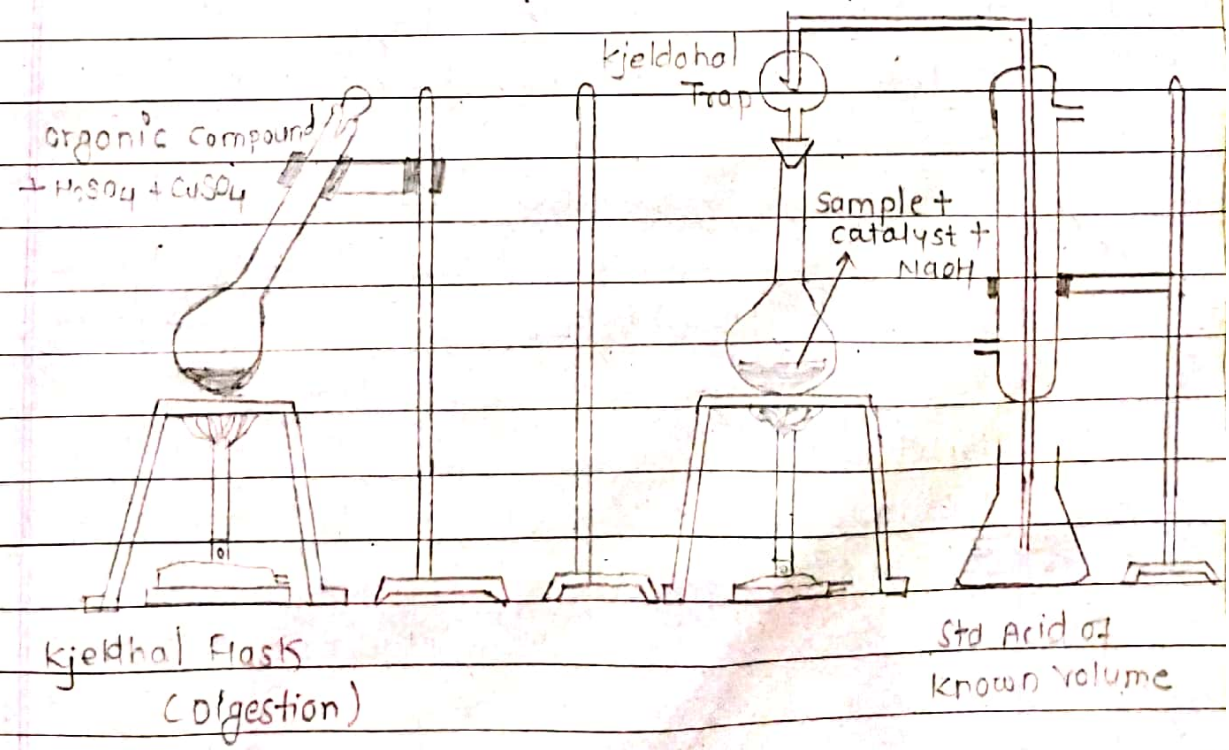
Food item	Percent Protein (wt%)
• Cereals and pasta	
Rice, brown, long-grain raw	7.9
Rice, white, long-grain, regular	7.1
White flour, whole-grain, ~~yellow~~	13.7
Corn-Flour, whole-grain, yellow	6.9
Cornstarch	0.3
• Dairy product	
milk, reduced fat, Fluid, 2%	3.2
milk, nonfat, dry, regular, vit-A	36.2
Cheese, cheddar	24.9
• Fruits and Vegetable	
Apple, raw, with skin	0.3
Asparagus, raw	2.2
Potato, whole, flesh and skin	2.0
• Legumes	
Soybean, mature seeds, raw	36.5
Beans, kidney, all type	23.6
Tofu, raw, Firm	15.8
Tofu, raw, regular	8.1
• Meat, poultry, Fish	
Beef, chuck, arm pot roast	21.4
Beef, cured, dired beef	31.1
Chicken, raw	23.1
Egg, raw, whole, Fresh	12.6

* Methods :-

1) Kjeldahl Method
2) Dumas (N combustion) Method
3) Infrared spectroscopy method

1) Kjeldahal Methods :-

- In the Kjeldahal procedure, protein and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts.
- The total organic nitrogen is converted to ammonium Salfate.
- The digest is neutralized with alkali and distilled into a boric acid solution.
- The borate ~~ions~~ anions formed are titrated with standardized acid, which is converted to nitrogen in the sample.
- The result of the analysis represents the crude protein content of the food since nitrogen also comes from nonprotein components.



Procedure :-

Sample Preparation :-

solid foods are ground to pass a 20-mesh screen sample for analysis should be homogenous. No other special preparation are required.

Digestion :-

- place sample (accurately weighed) in a kjeldahal flask.
- Add acid and catalyst; digest until clear to get complete breakdown all of organic matter.
- Nonvolatile ammonium sulfate is formed from the reaction of nitrogen and sulfuric acid.

$$\text{Protein} \xrightarrow{H_2SO_4, \text{Heat}} (NH_4)_2SO_4$$

Neutralization and Distillation

- The digest is diluted with water.
- Alkali-containing sodium thiosulfate is added to neutralize the sulfuric acid
- The ammonia formed is distilled into a boric acid solution containing the indicators methylene blue and methyl red.

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$$

$$NH_3 + H_3BO_3 \text{ (Boric acid)} \rightarrow NH_4^+ + H_2BO_3^- \text{ (Borate ion)}$$

Titration :-

Borate anion (proportional to the amount of nitrogen) is titrated with standardized HCl.

$$H_2BO_3^- + H^+ \rightarrow H_3BO_3$$

calculations:-

Mole of HCl = moles of NH_3 = moles of N in the sample

A reagent blank should be run to substract reagent nitrogen from the sample nitrogen.

$$\%N = N\,HCl \times \frac{\text{corrected Acid Volume}}{\text{g of sample}} \times \frac{14\,g\,N}{mol} \times 100$$

Where, N HCl = normality of HCl, in mol / 1000ml.

corrected acid vol. = (ml std. acid sample) − (ml. std. acid for blank)

14 = atomic weight of nitrogen

A factor is used to convert percent N to percent crude protein.

most protein contain 16% N, so the conversion factor is 6.25 (100/16 = 6.25)

$$\frac{\%N}{0.16} = \%\text{ protein}$$

or

$$\%N \times 6.25 = \%\text{ protein}$$

Applications:-/Advantage:-

1. Applicable to all types of Foods.
2. Inexpensive (if not using an automated system)
3. Accurate; an official method for crude protein content.
4. Has been modified (micro kjeldhal method) to measure microgram quantities of proteins.

Disadvantage :-

1 Measures total organic nitrogen, not just protein nitrogen
2 Time consuming (at least 2h to complete)
3 Corrosive reagent

2] Dumas Method :-

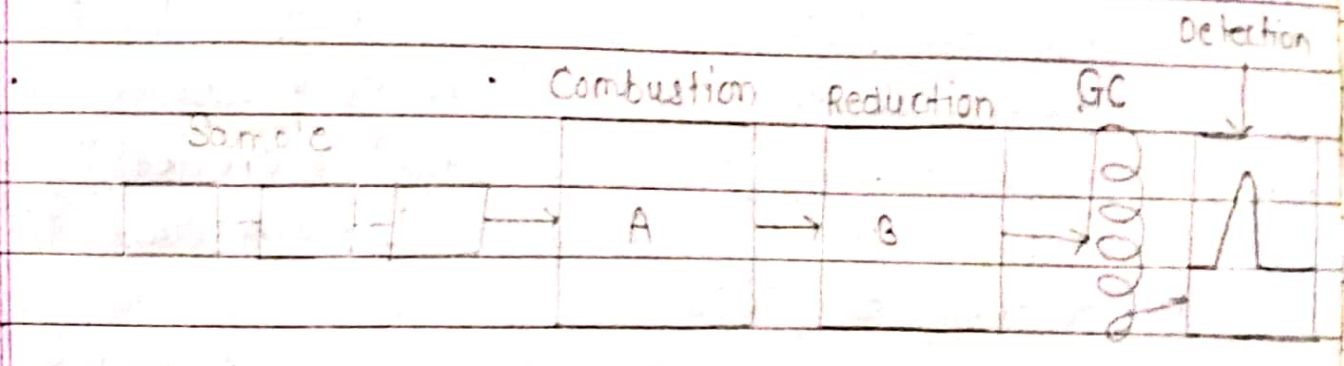
Principle :-

- The combustion method was introduced in 1831 by Jean-Baptiste Dumas.
- Sample are combusted at high temp. (700-1000°C) with a flow of pure oxygen.
- Nitrogen contaning component produced include N_2 and Nitrogen oxide.
- The Nitrogen oxide are reduced to nitrogen in copper reduction column are a high temp (600°C).
- The total nitrogen (including inorganic Fraction) i.e inculding nitrate and nitrite) is quantitated by gas chromatography using a thermal conductivity detector (TCD).

Procedure :-

- Sample (approximetely 100-500mg) are weighed into a tin capsule & introduced to a combustion reactor in automated equipment.
- The nitrogen released is measured by a built-in gas chromatograph.

Figure:- Flow diagram of the component of Dumas Nitrogen analyzer.



General component of a Dumas nitrogen analyzer.
A is incierator, B copper reduction of unit for converting nitrogen oxides to nitrogen, and GC gas chromatography column.

* Application:-

Advantage:-
1. Require no Hazardous chemicals.
2. Can be accomplished in 3min.
3. Recent automated instrument can analyze upto 150 samples without attention.

Disadvantage:-
1. Expensive equipment is required.
2. Measure total organic Nitrogen, not just protein Nitrogen.