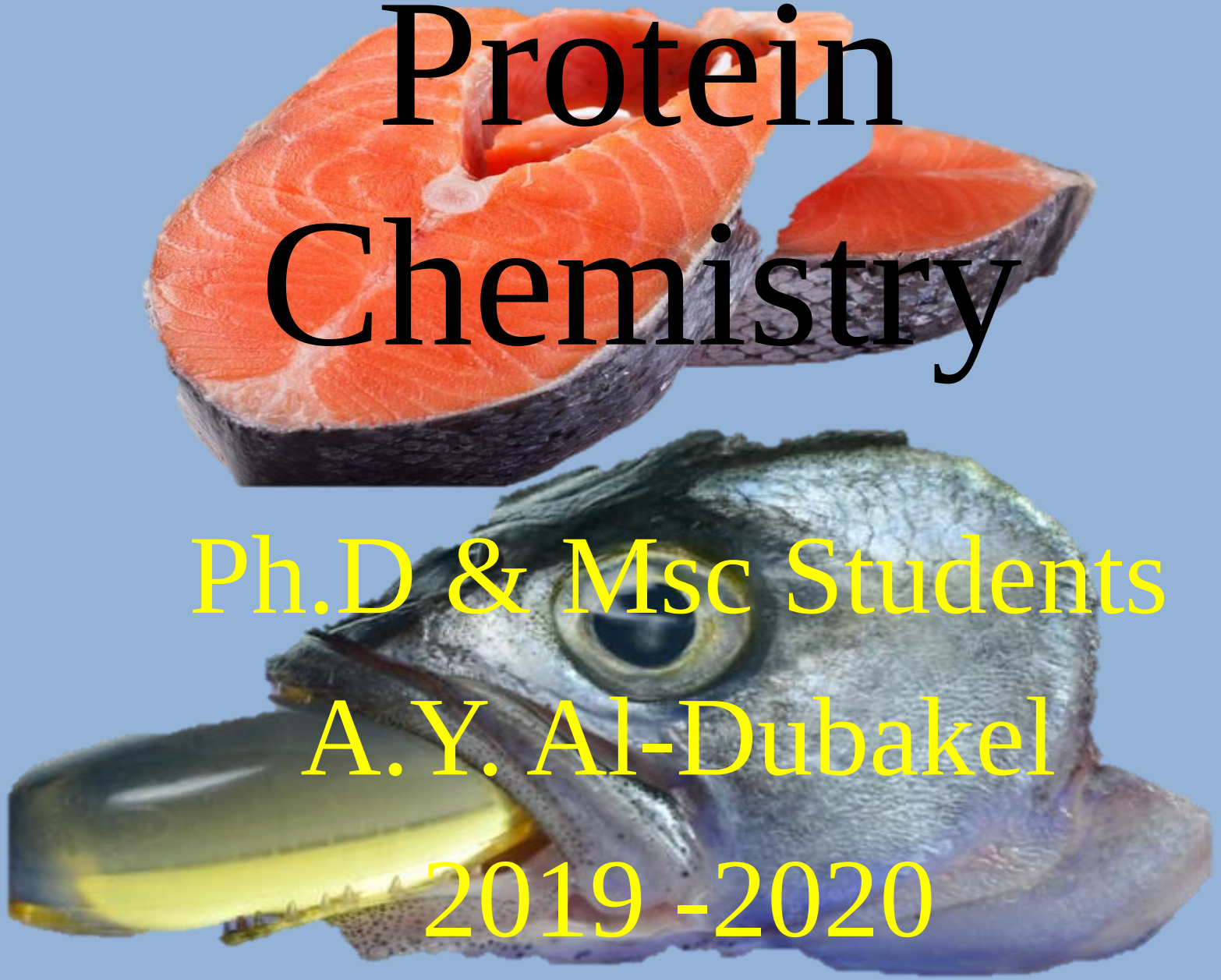
Protein Chemistry



Ph.D & Msc Students

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Lecture 5

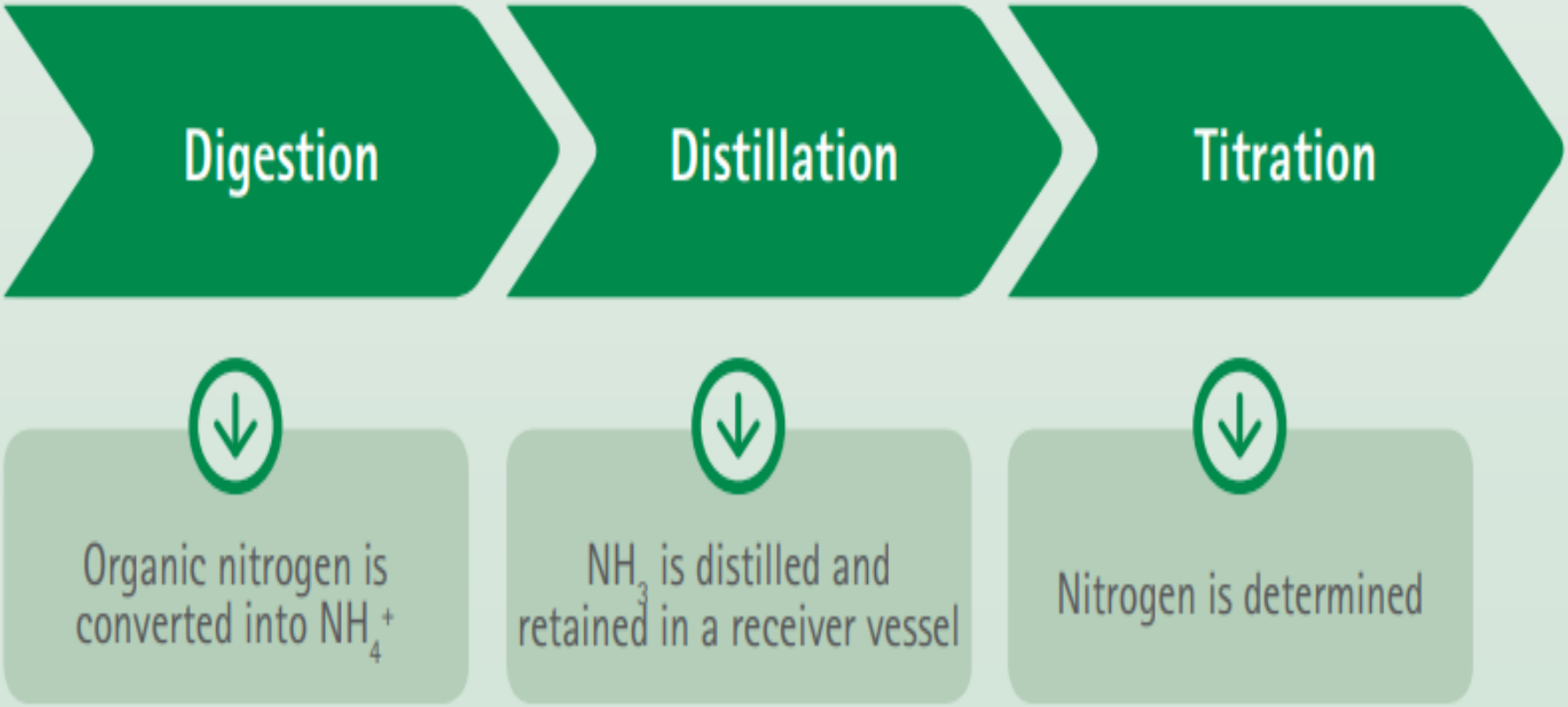
Analysis of Proteins

Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures, nutritional attributes and

ination of Overall Protein Concentration

Kjeldahl method

The Kjeldahl method was developed in 1883 by a Danish chemist called Johann Kjeldahl. A food is digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. It is usually considered to be the standard method of determining protein concentration. The Kjeldahl


Digestion
Distillation
Titration
Organic nitrogen is converted into NH_4^+
NH_3 is distilled and retained in a receiver vessel
Nitrogen is determined

Principles

Digestion

The food sample to be analyzed is weighed into a digestion flask and then digested by heating it in the presence of sulfuric acid (an oxidizing agent which digests the food), anhydrous sodium sulfate (to speed up the reaction by raising the boiling point) and a catalyst, such as copper, selenium, titanium, or mercury (to speed up the reaction)

Distillation (neutralization)

After the digestion has been completed the digestion flask is connected to a receiving flask by a tube. The solution in the digestion flask is then made alkaline by addition of sodium hydroxide, which converts the ammonium sulfate into ammonia gas:

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

The ammonia gas that is formed is liberated from the solution and moves out of the digestion flask and into the receiving flask - which contains an excess of boric acid. The low pH of the solution in the receiving flask converts the ammonia gas into the ammonium ion, and

Titration

The nitrogen content is then estimated by titration of the ammonium borate formed with standard sulfuric or hydrochloric acid, using a suitable indicator to determine the end-point of the reaction.

$$H_2BO_3^- + H^+ \rightarrow H_3BO_3 \quad (4)$$

The concentration of hydrogen ions (in moles) required to reach the end-point

- When boric acid is used as the receiving solution the equation is:

$$\% \text{ Nitrogen} = \frac{(\text{ml standard acid} - \text{ml blank}) \times \text{N of acid} \times 1.4007}{\text{weight of sample in grams}}$$

- When standard acid is used as the receiving solution, the equation is:

$$\% \text{ Nitrogen} = \frac{[(\text{ml standard acid} \times \text{N of acid}) - (\text{ml blank} \times \text{N of base})] - (\text{ml standard base} \times \text{N of base}) \times 1.4007}{\text{weight of sample in grams}}$$

If it is desired to determine % protein instead of % nitrogen, the calculated % N is multiplied by a factor, the magnitude of the factor depending on the sample matrix. Many protein factors have been developed for use with various types of samples. Here you can see the % Nitrogen, the Protein factor and the % Prote

https://www.itwreagents.com/uploads/2018011

https://info.gbiosciences.com/blog/topic/protein-estimation

Food	% Nitrogen	Factor	% Protein
Wheat flour	2.4	5.7	13.7
Red beans	3.4	6.25	21.2
Milk	0.5	6.38	3.3
Egg	2	6.25	12.5
Fish	2.6	6.25	16

Advantages and Disadvantages

Advantages. The Kjeldahl method is widely used internationally and is still the standard method for comparison against all other methods. Its universality, high precision and good reproducibility have made it the major method for the estimation of protein in foods.

Disadvantages. It does not give a measure of the true protein, since all nitrogen in foods is not in the form of protein. Different proteins need different correction factors because they have different amino acid sequences. The technique is