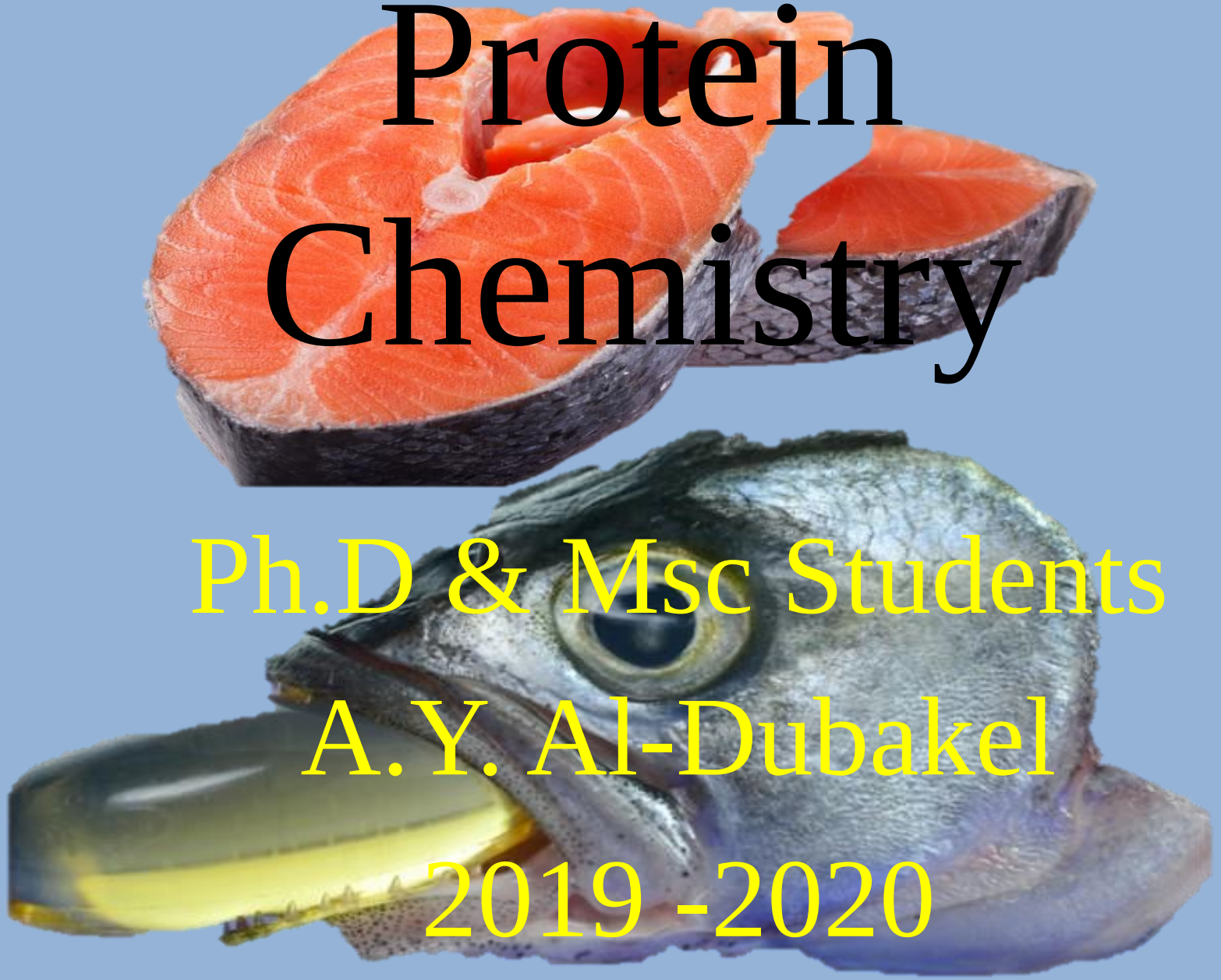
Protein Chemistry



Ph.D & Msc Students

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Enhanced Dumas method

Recently, an automated instrumental technique has been developed which is capable of rapidly measuring the protein concentration of food samples. This technique is based on a method first described by a scientist called Dumas over a century and a half ago. It is beginning to compete with the Kjeldahl method as the standard method of

General Principles

A sample of known mass is combusted in a high temperature (about 900 ^{o}C) chamber in the presence of oxygen. This leads to the release of CO_2, H_2O and N_2. The CO_2 and H_2O are removed by passing the gasses over special columns that absorb them. The nitrogen content is then measured by passing the remaining gasses through a column that has a thermal conductivity detector at the end. The column helps separate the nitrogen from any residual CO_2 and H_2O that may have remained in the gas stream. The instrument is calibrated by analyzing a material that is pure and has a known nitrogen concentration, such as EDTA (= 9.59%N). Thus the signal from the thermal conductivity detector can be converted into a nitrogen content. As with the Kjeldahl method it is necessary to convert the concentration of nitrogen

Advantages and Disadvantages

Advantages: It is much faster than the Kjeldahl method (under 4 minutes per measurement, compared to 1-2 hours for Kjeldahl). It doesn't need toxic chemicals or catalysts. Many samples can be measured automatically. It is easy to use.

Disadvantages: High initial cost. 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

Biuret Method

A violet-purplish color is produced when cupric ions (Cu^{2+}) interact with peptide bonds under alkaline conditions. The biuret reagent, which contains all the chemicals required to carry out the analysis, can be purchased commercially. It is mixed with a protein solution and then allowed to stand for 15-30 minutes before the absorbance is read at 540 nm. The major advantage of this technique is that there is no interference from materials that adsorb at lower wavelengths, and the technique is less sensitive to protein type because it utilizes absorption involving peptide bonds that are

Lowry Method

The Lowry method combines the biuret reagent with another reagent (the Folin-Ciocalteau phenol reagent) which reacts with tyrosine and tryptophan residues in proteins. This gives a bluish color which can be read somewhere between 500 - 750 nm depending on the sensitivity required. There is a small peak around 500 nm that can be used to determine high protein concentrations and a large peak around 750 nm that can be used to determine low protein concentrations. This method is more sensitive to low concentrations of proteins than the biuret method.

Other Instrumental Techniques

Dye binding methods

Turbimetric method

BCA assay

Bradford assay

There are a wide variety of different instrumental methods for determining the total protein content of food materials. These can be divided into three different categories according to their physicochemical principles: (i) measurement of bulk physical properties, (ii) measurement of adsorption of radiation,(iii) measurement of scattering of radiation. Each instrumental methods has its own advantages and

PROTEIN ANALYSIS TECHNIQUES

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

There are three major protein analysis techniques: protein separation, western

1. PROTEIN SEPARATION

Protein electrophoresis is the process of separating or purifying proteins by placing them in a gel matrix and then observing protein mobility in the presence of an electrical field. It's an important approach to studying protein function and the effect of a particular protein on development or a physical function by introducing it into an organism.

The most commonly used technique for protein separation is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins can be separated according to solubility, size, charge and binding affinity. SDS-PAGE

2. WESTERN BLOTTING

This technique uses three elements to identify specific proteins from a complex mixture of proteins extracted from cells: separation by size, transfer to a solid support, and marking target protein using a proper primary and secondary antibody.

The sample of proteins is first electrophoresed by SDS-PAGE to separate the proteins based on molecular