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Review Article

Natural Proteins: Sources, Properties, Extraction Techniques and its Future Aspects

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Abstract

Across the world, plant proteins are extensively incorporated as food source because it contains all the essential amino acids. On the other hand, animal proteins possess large protein content but do not have all the essential amino acids. In today's era, micro-organisms are altered for production of recombinant proteins by using recombinant DNA technology. Hence, plants, animals and microbial cells are used as good source of proteins. These proteins are extracted by using various techniques based on their physiochemical properties. Each protein has their own specific properties which make them unique from others. These properties are isoelectric point, solubility, optical activity, colloidal nature and many more. Based on these properties, different extraction methods were discovered like physical, chemical and enzymatic methods. Ammonium sulphate precipitation is popularly applied as protein precipitation procedure. Proteins are essential part of mostly all the industries like textile, paper and pulp, pharmaceutical, therapeutical, detergent, food and nutritional industries.

Keywords: Ammonium Sulphate; CHAPSO(3-[(3 cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate); Extraction; Precipitation; Protein; Zwitter Ion

Introduction

Life is possible on the earth because of some macromolecules and protein is one of them. Amino acids perform crucial role in controlling multiple processes related to gene expression, including modulation of the function of the protein that mediate messenger RNA translation. Biochemical reactions in metabolism are catalysed by enzymes which are mostly protein in nature. Antibodies are proteinaceous part of an adaptive immune system whose predominant work is to bind antigens or foreign substances in the body and target them for destruction. Protein is also alternative source of energy production in the human body when carbohydrates are not present in appropriate amount. Animals cannot synthesize all of the amino acids, but plants can, that's why they are included in daily diet. If amino acids are deficient, then protein synthesis does not occur, as a result protein deficiency related disease may occur. For instance, Kwashiorkor, Marasmus, Cachexia, so it is imperative to stabilize diet containing all essential amino acids. Proteins are not only beneficial as a nutritional aspect but are also extensively use in the industry. Significant industrial protein utilization include food, therapeutic, textile and detergent industry [8,24,25].

Sources of protein

Bacterial as protein source

Protein content of bacteria is relatively high compared to yeast or algae. Several pathogenic species of bacteria are known to have a higher than 80% protein content in their source [2]. *Lactobacillus fermentans*, 87%; *Alcaligenes viscosus,* 84%; *Escherichia coli,* 82%.

Algae as protein source

In terms of productivity and nutrition value, using algae for protein synthesis has various advantages over using traditional high

Organisms	Substrate	Protein Content (%)
Bacillus licheniformis [21]	Potato starch processing waste	68
Haloarcula sp. IRU1[34]	Petrochemical wastewater	76
Methylomonas sp.	Methane salt broth	69
Cupriavidus necator [11]	Synthetic growth medium	40 - 46
Rhizospheric diazotrophs [20]	Brewery wastewater	> 55

Table 1: Some bacterial sources and their protein content.

protein sources [7]. As an example, *Arthrospira maxima* (60-71%), *Arthrospira plantensis* (46-63%), *Euglina gracilis* (50-70%) and *Chlorella vulgaris* (42-55%) [9,10,27,37].

Fungi as protein source

Mycoprotein is high in fibre and protein but low in fat, making it a good choice for people who want to restrict their fat intake while still eating high protein diet. Various fungi are isolated from different substrates which possess high protein content (%w/w dry weight). For instance, *Aspergillus oryzae* (48%), *Neurospora intermedia* (56%) and *Rhizopus oligosporus* (43%) are isolated from thin stillage, vinasse and corn ethanol stillage, respectively [18].

Plant as protein source

Milk, milk products, meat, egg, animal products, fish are contained high amount of protein [3,19].

Part of plant contain protein source	Example
Fruits	Orange, Apple, Grapes, Banana, Peach, Pineapple, Strawberry, Watermelon.
Vegetables	Wheat, Buckwheat, Barley, Rye, Oatmeal, Millet
Legume	Soyabean, Kidney bean, lentils, Navy beans
Seeds and nuts	Almonds, Walnuts, Sunflower seeds, sesame seeds, cashew
Grain	Rye, Barley, Wheat, Brown rice, Wild rice, Oatmeal, millet

Table 2: Parts of plants which contain proteins and their examples.

Animals as protein source

Meat, milk, milk products, egg, chicken and fish are all good sources of protein with excellent balance of amino acids [33-36].

Source	Protein content (Per 100g)	Calories (Energy in 100g)
Milk	3.3g	47 calories
Cheese	26g	316 kcals
Eggs	13g	149 kcals
Yogurt	4.1g	54 kcals
Chicken	32.8g	148 kcals
Beef	26.4g	163 kcals

Table 3: Animal sources with high protein content and calories.

Properties of proteins

Physical properties

Size and Shape: Proteins molecular weight depend on number of amino acids present in particular protein. The proteins range in shape from simple crystalloid spherical structure to long fibrillar structures [4]. On the basis of shape protein can be classified into two categories:

Globular proteins: They are compact spherical molecules that are usually water soluble. These proteins often contain several types of secondary structures. The main driving force for folding water-soluble globular protein molecules is to pack hydrophobic side chains into the interior of the molecule, thus creating a hydrophobic core and hydrophilic surface. Globular proteins are mainly present in seeds and leaf cells of plants. For example, Pepsin, insulin, ribonuclease.

Fibrous protein: They are long ellipsoid and rod-shaped molecules that are insoluble in water and physically tough. These proteins usually consist largely of a single type of secondary structure. For example, silk fibres consist largely of the fibrous protein fibroin, made up of β-sheets whereas fibres of wool consist mainly keratin protein made up of α-helix.

- **Colloidal nature**: Diffusion rate of protein is extremely low and may produce considerable light scattering in solution which results in Tyndall effect because of their colloidal nature and large size.

- **Solubility:** The solubility of protein highly depends upon isoelectric point and ph. Solubility is lowest at isoelectric point and escalates with increasing acidity or alkalinity. This is because when the protein exists as either cations or anions, repulsive forces between ions are high [32].
- **Optical activity:** All amino acids except glycine have chiral carbon and hence they are able to rotate the plane polarised light. In nature, all the proteins are laevorotatory. Optical rotation of an optically active compound depends on the concentration of compound, temperature, wavelength of light use, solvent use to dissolve the sample and the light pathlength.
- **Amphoteric nature:** In aqueous solution, the carboxy group of amino acid in protein may lose a proton and the amino group may accept a proton to give it a dipolar ion which is known as zwitter ion. In this form protein can behave in both ways as an acid and as a base. This can be defined as amphoteric behavior of protein.
- **Denaturation:** Weak linkages or bonds that are present in native structure of protein are broken down during denaturation process. Sometimes denatured protein molecule form large aggregate and precipitate out from the solution which is referred as coagulation.
- **Ion binding capacity:** Based on their net charge, protein can bind with cations and anions and form salts.

Chemical properties

- **Hydrolysis:** Hydrolysis of protein are carried out by acidic agents like HCl(hydrochloric acid), alkaline agents like NaOH(sodium hydroxide) and proteolytic enzyme like trypsin and pepsin. (Figure 1)

Figure 1: Hydrolysis of proteins.

- **Colour reaction:** Biuret reagent is mostly used both as a qualitative test for the detection of the proteins and also as the quantitative test for the estimation of protein in biological material. When a protein solution is treated with alkaline $CuSO_4$ (copper sulfate) reagent, peptide bonds present in the protein that react with copper ions and forms violet coloured complex. The colour deepens which depend on the number of peptide bonds present in the protein. All the proteins except dipeptides react with biuret reagent because a minimum of two peptide linkage is involve in this reaction. The structure of the violet complex is

Figure 2: Example of colour reaction.

Other protein colour reactions are also there like Xanthoproteic test, Millon's test, Sulphur test, Hopkins Cole test and Ninhydrin test.

- **Chemical reaction of the NH_2 group:** NH_2 group of protein reacts with mineral acids, benzaldehyde, FDNB(1-fluoro-2,4-dinitrobenzine) and dansyl chloride. Reaction with FDNB or Sanger's reagent is mentioned here. 1-fluoro-2,4-dinitrobenzine (FDNB) reacts in alkaline solution (ph-9.5) with the free amino group of the N- Terminal amino acid residue of peptide to form a characteristics yellow dinitrophenyl derivative. It can be release from the peptide by either acid or enzymic hydrolysis of the peptide bond and subsequently identified. Sanger first used to this reaction to determine primary structure of the polypeptide hormone insulin. Thus, FDNB is often referred to as Sanger's reagent.

Protein extraction

The extraction of protein is carried out by the lysis of the cell which contains the protein material. Extraction of protein is depending on the physical and chemical properties of the proteins.

It is also depending on the sample sources from where it collected. It helps in the study of mechanisms and action of any enzyme, to identify the gene which encode the particular protein and also important for the study of diseases [12,13,15,20,30].

There are different methods use for the extraction purpose, three types of extraction methods are given below.

- Physical method
- Chemical method
- Enzymatic method

Physical method

Physical and mechanical lysis in which the cell wall and cell membrane are disrupting by applying force. To physically lyse cell to extract protein there are different technique manual.

- **Freeze-thaw:** This method used to lyse the bacterial and mammalian cell. The cell suspension is freeze in dry ice or the freezer can also be used. The frozen cell suspension is later thawed at 37°C. By freezing the cell swell and break and again contrast during thawing it. This process is quite lengthy as to efficiently lyse the cell multiple trials applied. It is seen that the release of recombinant proteins is extracted from the cytoplasm of bacteria by this method.
- **Sonication:** It is a method of physically disrupting open cells. High-frequency sound waves were used to lyse cells, spores, bacteria, and finely sliced tissue in this approach. To provide sound waves, an instrument is employed with a vibrating probe that is immersed in the liquid suspension. Mechanical energy is used to start the creation of bubble vapour. These bubbles burst. Shock waves are emitted through the sample as a result of this. The ultrasonic treatment takes precedence in order to avoid overheating After sonication, the lysate contains DNA, RNA, proteins, and other molecules. plasmid. It's an excellent method for a sample with a volume of less than 100ml.

Figure 3: Sonication procedure.

- **Homogenization: -** Homogenizers are devices that are used to disrupt cells. By pressurising the material and immediately releasing the pressure, the homogenizer pushes the lyse cells. With 6000-10,000 pressure, the French press is an older homogenizer. To attain the needed level of multiple trials are required. The lysate of cells produced using the extraction procedures is separate various components by centrifuging at a precise pressure that has been determined organelles and proteins are both removed from the cells.

Figure 4: Homogenization procedure.

https://www.researchgate.net/publication/352761483

- **Liquid nitrogen grinding (Mortar and pestle):** The mortar and pestle method is often used for disrupting plant cells. The tissue is frozen in liquid nitrogen during this operation. After that, the frozen tissue is pulverised with a mortar and pestle. It the most effective and time-saving method for extracting plant proteins and DNA. The cell walls of plant cells, which are made up of polysaccharides and cellulose, make this approach effective extraction.

Chemical method

The chemical methods used in the lysis of the cell to extract proteins use lysis buffers. The lysis buffer changes the pH which results in the eventually breakdown of the cell membrane [1]. The chemical methods are given below.

- **Alkaline lysis:** Hydroxyl ions (OH^-) are the main constituent of alkaline lysis to lyse the cell membrane. The hydroxyl ions break the fatty acid-glycerol ester bonds, the constituent of the cell membrane. The lysis buffer consists of SDS (sodium dodecyl sulphate) and sodium hydroxide which solubilize the protein and the membrane. The drawback of this method is that this is a slow process.

- **Detergent lysis:** The cell membrane is made up of hydrophilic and hydrophobic molecules in a lipid bilayer. As a result, detergents are utilised to disrupt or break down the cell membrane. Due to the fact that detergents are surfactants, they can destroy both hydrophilic and hydrophobic connections. The cationic, anionic, and non-ionic detergents are classified according to their charge capacity. Both ionic and non-ionic Detergents are typically employed to disrupt mammalian cells, however they can also be used to disrupt bacterial cells. To break down the cell wall, lysozymes and detergents are utilised. Non-ionic compounds are the most common. Because detergents cause less harm to enzymes and proteins, they are used. Constantly used Tween and Triton-X are non-ionic detergents, while CHAPSO(3-[(3-cholamidopropyl) dimethylammonio]-2-hydroxy-1-propanesulfonate) is a zwitterion detergent. SDS, an ionic detergent, is widely employed because of its high affinity for binding to protein. It also rapidly denatures them. The carboxylic or hydrophilic part of ionic detergents is hydrophilic. The hydrophilic portion of cationic detergent is the carboxylic and sulphate group. The hydrophilic portion of cationic detergent is the ammonium group. There is also a chaotropic agent used for the chemical lysis of cells other than the ionic, cationic, and non-ionic detergents. The chaotropic agent includes EDTA (ethylenediaminetetraacetic acid), guanidine, and urea. These can break the structure of water and make it less hydrophilic in nature. Also weakens the hydrophobic interactions.
- **Organic solvent:** Some proteins and enzymes are insoluble in water, dilute salt solution, dilute acids or dilute bases because they are more strongly bound to lipids or have more non-polar side chains in the molecule. Organic solvents like Ethanol, acetone and butanol use for extraction. It is the optimal lipoprotein extract since it contains some hydrophilicity and a more of lipoprotein. However, it must be used at a low temperature. The butanol isolation approach is very useful for recovering proteins and enzymes that are strongly associated with lipids. Butanol has great lipophilicity, notably the ability to dissolve phospholipid; second, in the realm of solubility, butanol has both hydrophilicity and solubility.
- **Enzymatic method:** In cell lysis process many different enzymes are used like lysozyme, cellulose, chitinase, glycanase, pectinase, zymolase. Lysozyme is commonly involved in the extraction of bacterial proteins. By hydrolysing the peptidoglycan contained in bacterial cell wall, lysozyme is frequently employed to lyse them. Gram- positive cells are particularly vulnerable to this hydrolysis due to high amount of peptidoglycan in their cell wall. Due to the presence of an outer membrane and smaller amount of peptidoglycan, Gram- negative bacteria are less vulnerable [23,28].

Precipitation method for protein extraction

Protein precipitation occurs in step-by-step manner. The addition of a precipitating agent and constant mixing destabilises the protein solution by inducing the precipitant and target to colloid. For molecules to diffuse through fluid eddies, enough mixing time is required. Sub microscopic -sized particles are formed during the nucleation phase, and the growth of these particles is controlled by Brownian diffusion. By diffusive addition of individual protein molecules, the growing particles attain a crucial size (0.1-10μm for high and low shear fields, respectively) and continue to expand by colliding into each other and sticking or flocculating. This phase takes longer to complete than mixing the precipitant. During the final stage, ageing in a shear field, the precipitate particles continuously collide and stick, then break apart, until they attain a stable mean particle size, which is dependent on individual proteins. The product of the mean shear rate and the ageing time corresponds with the mechanical strength of protein particles. Aging particles allows them to sustain fluid shear stresses in pumps and centrifuge feed zones without decreasing in size [29].

Precipitation reactions includes salting out, isoelectric precipitation, precipitation by organic solvents, precipitation by heavy metal ions, precipitation by alkaloidal reagent [12,13,15,20,30]. In salting out reaction, ammonium sulphate salt is mainly used. Ammonium sulphate precipitation is done by mixing increasing amounts of ammonium sulphate and extracting the various components of the precipitate protein. When the hydrophobic groups on proteins are exposed to the environment, they attract other hydrophobic groups on other proteins, causing them to aggregate. The protein that forms will be large enough to see. One advantage of

this technology is that it is not much costly and also implement for large quantities. Water-soluble proteins are the primary proteins to be cleared.

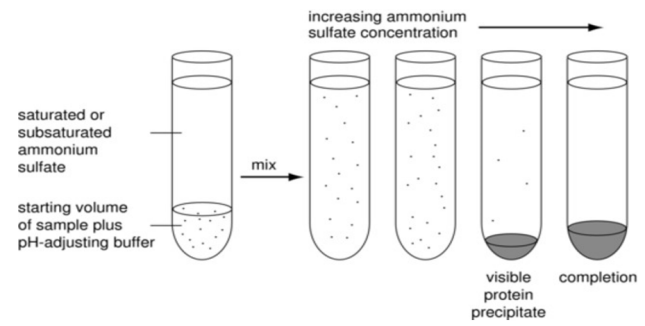

Figure 5: Ammonium sulphate precipitation.

Applications

Proteins are included in a wide range of industrial products. Their uses include the paper industry, detergents, medications, waste degradation, textiles, food, pharmaceuticals, leather, degumming of silk goods, liquid glue production, cosmetics, meat tenderization, cheese production, growth stimulants, and so on. Some of the applications are mentioned below:

- Proteases are employed in the food business for a variety of purposes, including reduced allergic new-born formulae, milk clotting, meat tenderization, and flavouring [17]. They are also crucial in the detergent industry as they remove protein stains.
- Casein as a source of bioactive peptide act on the cardiovascular system, immune system, nervous system and nutrition system [31].
- Some enzymes are incorporated in biosensors for detection of specific substrate. It is widely used as diagnostic tool [5].
- Actin and myosin are interacted with each other to help in muscle movement in human body.
- Protein based nanocarriers are used in drug and delivery system [25].

Future aspects

More study is needed to figure out how to raise consumer knowledge about the need of getting enough protein to be healthy as you get older. High-protein foods are currently marketed mostly to athletes and those looking to lose weight. The number of actual food options on the market is still limited, with the majority of high-protein foods including protein obtained from dairy. Advances in recombinant protein production can solve many disease related problems and health issues. Protein engineering, the isolation and research of new extremophilic microbes, and genetic engineering improvements are all potential advances in the development of protein associated industries [14,16,22].

Conclusion

Thus, being an extraordinary complex molecule, proteins have the most diverse functions and tremendous properties. Humans use plants and animal sources as their daily diet globally. However, applications of bacterial originated proteins and recombinant bacterial proteins are emerging in pharmaceutical and therapeutical industries. Even, due to development of newer technologies and instruments, extraction and purification of proteins become easier, reliable, cheap and less time consuming. In future, may be use of recombinant proteins turn out to be more common.

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Conflict of Interest

Authors have no conflict of interest.

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