METHODS OF PURIFICATION OF PROTEIN HYDROLYSATES

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ABSTRACT: in this article analyzed methods of protein hydrolyzate cleaning. hydrolysis are presented in this review, focusing on enzymatic and acid hydrolysis techniques. Advantages, disadvantages and optimal conditions are also briefly summarized.

KEY WORDS: Protein extraction; Hydrolysis; Seafood Processing; Nutrient.

Protein hydrolysate is a complex mixture of peptide and amino acids that can be produced from various biomass sources including insects, such as black soldier fly larvae (Hermetia illicens) due to its relatively high protein content.

The most basic function of protein hydrolysates in the applications of biotechnology is to provide a nitrogen source for bacteriological, industrial and specialized media for microbial, plant, animal and insect cell cultures on both a laboratory and industrial scale. However, in many instances protein hydrolysates also provide vitamins, minerals and unknown growth factors resulting in higher yields and productivities.

Solvent, physic, enzymatic and acid hydrolysis are methods, which are widely being applied for proteins extraction with different qualities and efficiencies. The structure of recovered protein molecules can be intact with the solvent and physical methods. Hydrolysis indicates a sustainable method for proteins extraction from various sources (plant, animal, algae) in food processing, resulting in proteins hydrolysates [1].

Protease enzymes cleave long-chain protein at special chemical bonds and generate a mixture of free amino acids and oligopeptides that own better biological, digestible and functional properties for health.

Proteins structure can be destroyed under strong acid (HCl, H2SO4) to produce lactic acid as the final product instead of intermediates (dipeptides or amino acid) from the enzymatic hydrolysis. Following neutralization, the hydrolysates contain a large amount of salt, resulting in suitable utilization of the obtained hydrolysate for flavor and taste enhancement [2].

Protein Purification Methods. Concentration. First, some of the tricks that are often used when working with protein solutions. For example, the concentration of protein solutions. It can be carried out by precipitation of the protein, followed by dissolution of the precipitate in a smaller volume. Usually ammonium sulfate or acetone is used for this. Protein concentration in the initial solution should be at least 1 mg / ml. You can use adsorption proteins from very dilute solutions on an ion exchanger, followed by elution a small amount of saline. For fast concentration of small volumes of protein solutions, dry gel-filtering media can be used (e.g. Sephadex), polyethylene glycol or highly substituted KM-cellulose as water-removing agents. The sample is placed in a dialysis bag, which is immersed into powder that absorbs water. The most productive method of concentration is ultrafiltration. The method is based on the use of semi-permeable membranes with certain pore sizes, while water and small molecules pass through membrane, and on the other side of the membrane remains a concentrated protein solution. This is achieved in special cells (up to 1) with mixing of the solution and with using compressed inert gas. The same principle underlies ultrafiltration on hollow fibers used to concentrate large volumes of solutions.

Dialysis. Concentration and dialysis are closely related. For dialysis low molecular weight substances are removed from the sample and replaced with a buffer. The traditional method is dialysis through cellophane film. On sale dialysis tubes are available in different diameters, usually they do not allow molecules to pass through larger than 15,000-20,000 Yes. For a more complete removal of low molecular weight substances in during dialysis, it is necessary to change the buffer solution in the dialysate. Dialysis is long procedure, they usually put it on at night. Salt removal and buffer replacement it is preferable to use the faster gel filtration method.

Thermal denaturation. At the initial stage of purification, thermal separation is sometimes used to separate proteins. processing. It is effective if the protein is relatively stable under heating conditions, in while the accompanying proteins are denatured. This changes the pH of the solution, processing time and temperature. To select the optimal conditions preliminarily conduct a series of small experiments.

After the first stages of purification, the proteins are far from a homogeneous state. AT the resulting mixture of proteins differ from each other in solubility, molecular mass, total charge of the molecule, relative stability, etc. These differences can be used as the basis for methods for further separation of proteins. Protein purification is a multi-stage process and at each stage we get a fraction richer in secreted protein than in the previous stage. This process is often called fractionation**.**

Thus, after this stage, a plate was obtained, the pores of which contain separated proteins, and the space between them is filled with a nonspecific protein. Now it is necessary to identify whether among the proteins the desired one is responsible for some disease. For detection using antibody treatment. Under primary antibodies understand antibodies to the desired protein. By secondary antibodies is meant antibodies to primary antibodies. An additional special label (the so-called molecular probe) is added to the composition of secondary antibodies, so that later the results can be visualized. Radioactive phosphate or an enzyme tightly bound to the secondary antibody is used as a label. Binding first to primary and then to secondary antibodies has two goals: standardization of the method and improvement of results.

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