

# Hydrolysis to Hydrolysate

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Proteins are molecules essential to the structure and function of all living organisms. They are made up of chains of amino acids linked by peptide bonds and folded in a variety of complex structures. Proteins must be broken down into amino acids and peptides by hydrolysis, utilizing any of a number of methods, in order to provide nutrients in forms that cells may easily utilize. Protein hydrolysates, also called peptones, are the result of the hydrolysis process on protein material. For over a century, peptones have been used as the main nutritive components in culture media.

Peptones supply the carbon, nitrogen, minerals and growth factors needed to support microbial and mammalian cell metabolic processes, thereby promoting cell proliferation and production. Additionally, peptones have been used as serum replacements and media supplements in mammalian cell culture media.

Commonly used protein sources, as starting material, include meat, casein and whey (milk proteins), gelatin, soybean.

Enzyme sources include animal organs (pancreatin and pepsin), papaya (papain), fig (ficin), pineapple (bromelain) and microbes.

BD offers many different peptones representing numerous combinations of protein and enzyme combinations in order to satisfy a wide variety of nutritional, regulatory and application needs.

## Protein Hydrolysis

Hydrolysis of the starting material is accomplished by utilizing one of the following methods:

### Acid Hydrolysis

Acid hydrolysis is a fairly harsh process, usually carried out at high temperature. This process attacks all peptide bonds in the protein substrate, destroying some of the individual amino acids.

For example, tryptophan is usually totally lost in an acid hydrolysis. Cystine, serine and threonine are partially broken down and asparagine and glutamine are converted to their acidic forms. Vitamins are mostly destroyed by acid hydrolysis. Salt may be formed during neutralization of an acid hydrolysis, resulting in a product with high salt content.

### Enzymatic Hydrolysis

Proteolytic enzymes hydrolyze proteins more gently than acids, do not require high temperature and usually target specific peptide bonds.

The material that results from a proteolytic digestion is a mixture of amino acids and polypeptides of varying lengths. For example, the enzyme pepsin will cut an amino acid chain where there is a phenylalanine or leucine bond. Papain will cut the chain adjacent to arginine, lysine and phenylalanine. Pancreatin shows activity at arginine, lysine, tyrosine, tryptophan, phenylalanine and leucine bonds.

### Microbial Hydrolysis

Microbial proteases, proteolytic enzymes secreted by microorganisms, are becoming more widely used in peptone production.

Proteases from bacterial, algal, fungal and yeast sources offer a wide variety of enzyme activities, can be produced in large scale, and usually require only simple purification.

### Autolysis

The hydrolysis process used for preparing yeast extract products is different from the process for protein hydrolysis.

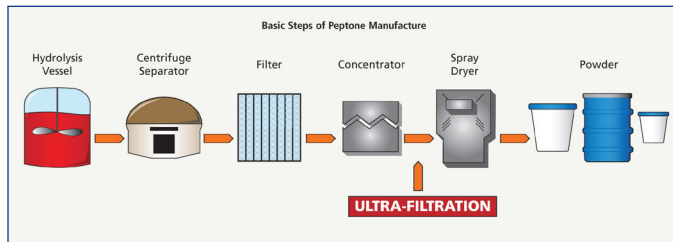
Yeast extracts are autolysates, whereby yeast cells are hydrolyzed by enzymes from within the yeast cell itself.



## Peptone Manufacture

Most peptones are manufactured following the same basic processing steps including:

(Note: Yeast Extract products follow a different manufacturing pathway summarized on the right)



### Hydrolysis/Digestion

Protein and demineralized water are combined to form a thick suspension of protein material in large-capacity digestion vessels, which are stirred continuously throughout the hydrolysis process. The protein suspension is then adjusted to the optimal pH for the specific enzyme chosen for the hydrolysis. Enzyme is added when the pH and temperature are optimal. The amount of enzyme necessary, time for digestion, and control of pH and temperature are dependent on the desired degree of hydrolysis.

### Centrifugation/Concentration

Once the predetermined degree of protein digestion is achieved, the enzymatic activity is halted. The suspension is either heated to inactivate enzymes or neutralized to inactivate acids or bases. The protein slurry is then centrifuged and/or filtered to remove insoluble materials and to clarify and concentrate the product. Vacuum-evaporation may be used for rapid concentration.

### Filtration

The peptone syrup, resulting from the centrifugation step, contains approximately 67% solids, and may then undergo further processing for pH adjustment, pasteurization, and/or filtration. Filtration methods can be utilized to characterize certain attributes of the end-product. For example, ultrafiltration (UF) is a membrane filtration process used to separate or concentrate constituents of protein solutions based on molecular weight. Ultrafiltration is used to create a product that is low in endotoxin, the toxin-containing lipopolysaccharide part of the cell wall shed from viable gram-negative bacteria and released when gram-negative bacteria die. Endotoxins will cause illness in humans, so they are considered contaminants that must be avoided or minimized in the preparation of pharmaceutical products.

### Drying

The final drying step of the process further concentrates the peptone by spray-drying or by pan-drying in vacuum ovens, which prepares the material for final milling, blending and packaging.

## Yeast Extract Manufacture

The manufacturing process for yeast extract products are characterized as autolysis. *Saccharomyces cerevisiae*, is usually grown to a high cell density in a molasses-based medium optimized for the particular yeast strain. The batch culture is exposed to a controlled temperature or osmotic shock that causes the yeast to die without inactivating the yeast's endogenous enzymes and begins the autolysis, where the yeast's own digestive enzymes are responsible for breaking down the yeast protein. Once autolysis is halted, insoluble material is separated out by centrifugation and several filtration steps (including ultrafiltration as required). The final filtration product may then be concentrated and drum or spray dried.



**BD Biosciences**

Advanced Bioprocessing  
800.638.8663  
bdbiosciences.com/advbio