



# Manufacture of casein hydrolysates and its industrial application



- **Protein hydrolysates have assumed a new dimension in food industry.**
- **Manufacture of protein hydrolysates is well-established process for utilization of protein rich food by-products, wastes and non-conventional food proteins.**
- **Soy sauce: First protein hydrolysate.**
- **Fundamental flavour characteristic of protein hydrolysate is suggestive of meat flavour.**
- **Casein: Important substrate for preparation of milk protein hydrolysates.**
- **Extent of protein hydrolysis represents extent of protein breakdown to peptides and amino acids and is expressed either as % amino nitrogen or as degree of hydrolysis (DH: one of important controlling factors, which reflect product quality. ).**
- **Degree of hydrolysis is ratio of number of peptide bonds cleaved and total number of peptide bonds in intact protein.**

# Processes of Casein Hydrolysates Manufacture

**Acid hydrolysis**



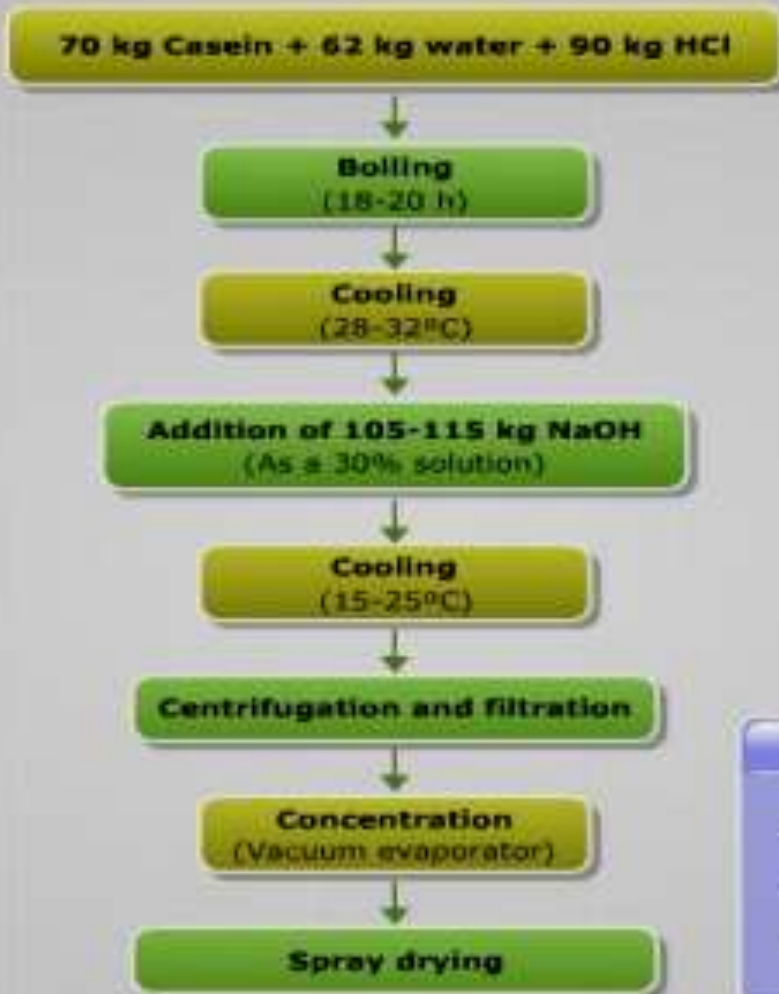
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graph TD; A[Acid hydrolysis] --> B[Alkaline hydrolysis]; B --> C[Enzymatic hydrolysis];
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**Alkaline hydrolysis**

**Enzymatic hydrolysis**

# Acid hydrolysis

- Casein or caseinates are allowed to react with arsenic-free hydrochloric acid (35-45%) or sulphuric acid for a period of 4-18 h.
- Reaction is carried out at higher temperature (80-100°C) either at atmospheric or higher pressure.
- Content is neutralized to pH 6.0-7.0 by an alkali.
- Product is then either concentrated or dried.
- Process details given in a patent comprises of mixing 70 kg casein with 62 kg water and 92 kg purified arsenic-free hydrochloric acid (sp.gr. 1.17 and 34% concentration), and boiling with stirring for approximately 18-20 h until casein is fully broken. Followed by cooling to 28-32°C and gradual addition of 105-115 kg pure sodium hydroxide (as a 30% aqueous solution) to the mixture to bring the pH to 6.6-6.7. Neutralized mixture is then cooled to 15-25°C and then purified by centrifugation and filtration. Clear liquid is spray dried at 95-100°C obtaining a readily water soluble powder. Contains 53.5-53.7% amino acids, 41-41.5% NaCl and 2.0-2.5% other minerals.



Casein hydrolysates	
Amino acids	- 53.5-53.7%
NaCl	- 41.0-41.5%
Other minerals	- 2.0-2.5%

Flow diagram for production of casein hydrolysates by acid hydrolysis



## Limitations

- Entails complete/partial destruction of some of amino acids (tryptophan).
- Process also possess problem of removing residual acid from products of hydrolysis.
- Neutralization of acid employed for hydrolysis result in formation of salts which in turn becomes another limiting factor in food/dietic applications.
- Use of mont morillonite to inhibit discolouration during acid hydrolysis at high temperature, has been successfully tried.
- Hydrolysis of milk protein is carried out at 80-95°C by non oxidizing acids (HCl and/or H<sub>2</sub>SO<sub>4</sub>) in the presence of (i) orthophosphorus acid and/or its salts (ii) acid activated mont morillonite.

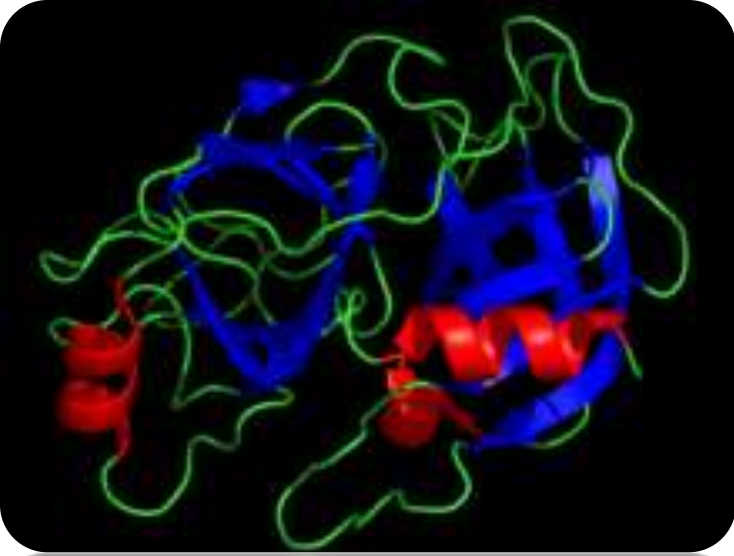
## **Alkaline hydrolysis**

- **This is another process of manufacturing casein hydrolysates.**
- **Not effective method as it leads to the destruction of some and the racemisation of most amino acids.**
- **Also results in partial chemical hydrolysis, oligomerisation and destruction of functional groups of amino acid residues.**

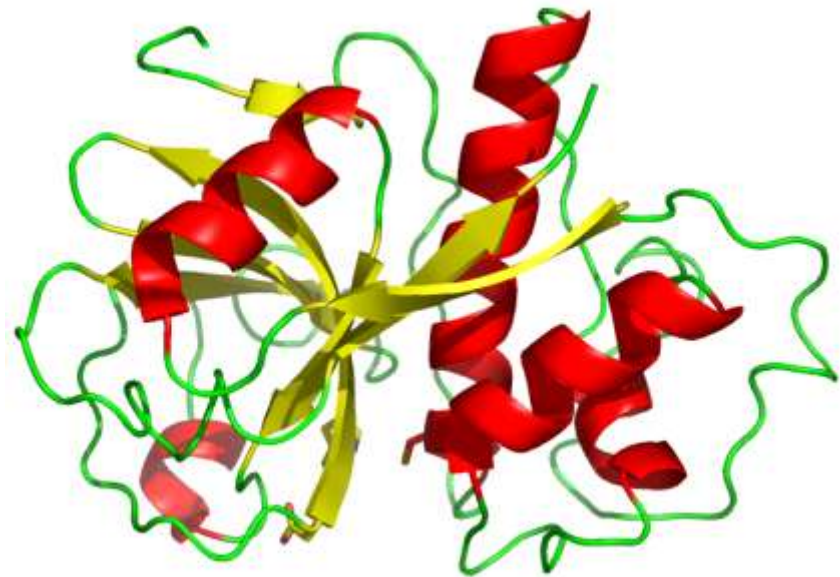
## **Enzymatic hydrolysis**

- **Enzymatic hydrolysis of protein is preferred over acid or alkali hydrolysis as the former causes no destruction or racemization of the amino acids and nutritional quality of the original protein is retained.**
- **This process of hydrolysis of casein results in product with high content of water soluble nitrogen and low salt.**





# Enzymatic Production of Casein Hydrolysates



- **Proteolytic enzymes are used to produce casein hydrolysates.**
- **Enzymes have ability to hydrolyze proteins to peptides and amino acids.**
- **Chain length of peptides formed is dependent upon the extent of hydrolysis, condition of hydrolysis, type, concentration and activity of enzyme, and type of protein to be hydrolysed.**
- **Proteolytic enzymes could be obtained from plant (Papain, Ficin, Bromelain), animal (Pepsin, Trypsin, Rennin), microorganisms (Neutrase, Esperase, Pronase).**

# Classification of Enzymes

## Endopeptidases

These enzymes have preference for certain side chains on amino acids adjoining peptide bond and divided into three groups:

1.

- Pepsin type of protease is characterized by a preference for amino acids with free carboxyl groups

2.

- Trypsin types are characterized by a preference for amino acids with basic group

3.

- Chymotrypsin types are characterized by a preference for amino acids with aromatic or bulky chains

Papainase type enzymes (papain, chymopapain, ficin, bromelain) are endopeptidases

# Exopeptidases

These are mostly microbial enzymes that split terminal amino acids from one end of the chain by hydrolysis of peptide bond. Divided in two groups:

**1.**

- **Carboxy (exo) peptidases that act on the terminus of chain carrying free carboxyl group**

**2.**

- **Amino (exo) peptidases that start from the other end i.e. the terminus of chain carrying free amino group**

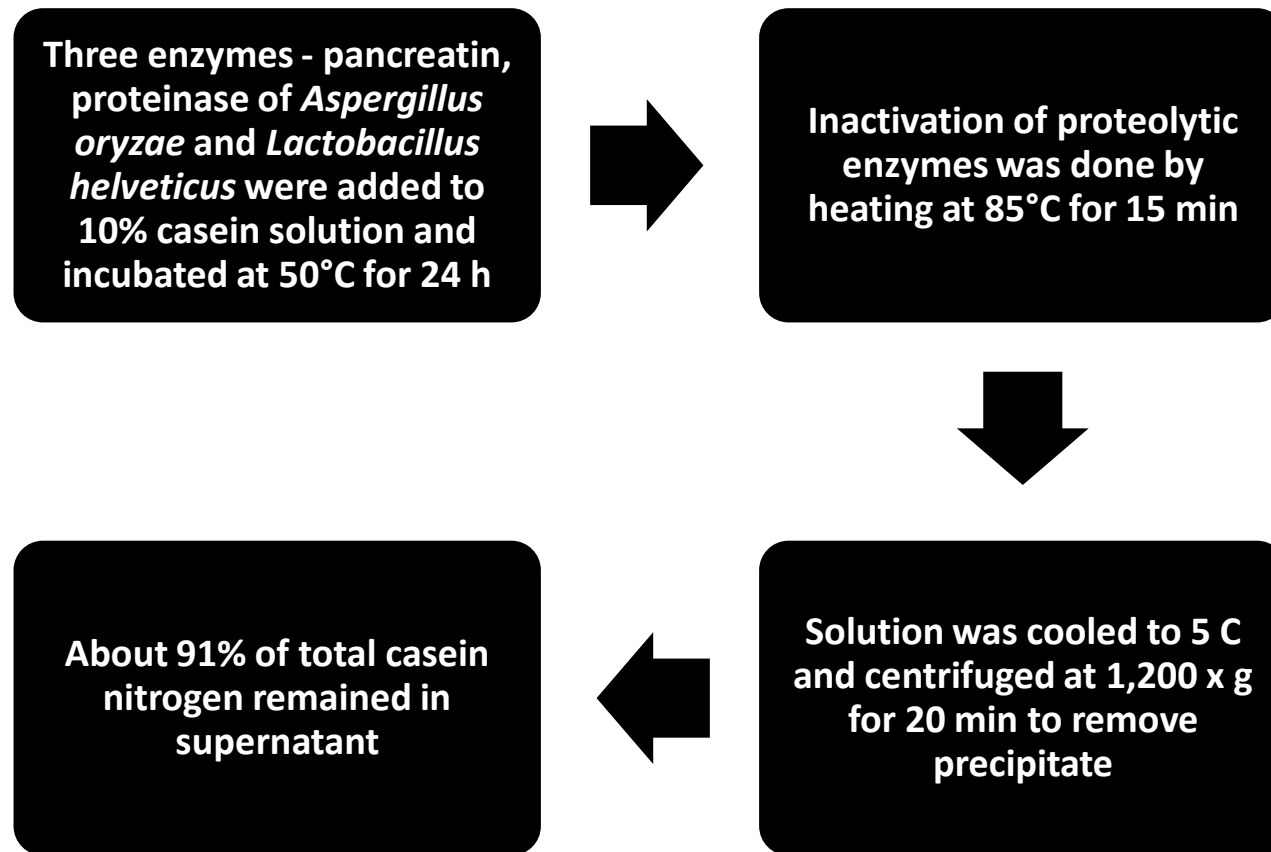
- ❑ Choice of protease for protein hydrolysis depends mainly on its specificity and also on its pH optimum, heat stability and presence of activators/inhibitors.**
- ❑ pH optima of pepsin, papain and pancreatin are 3.0, 7.0 and 8.0 respectively.**
- ❑ Individual endopeptidases do not split all or even majority of peptide bonds in protein system leading to formation of bulky, hydrophobic acid chains, which give bitter taste.**
- ❑ Exopeptidases hydrolyze carboxyl and amino terminal amino acids of such peptides, thus eliminating bitter taste.**

## **Enzymatic Production of Casein Hydrolysates**

- **Hydrolysis of casein has been mostly carried out as a single stage process (where enzyme is added once during hydrolysis period) and a two -stage process (where two or more enzymes are added at subsequent intervals of hydrolysis).**
- **Manufacture of protein hydrolysates include suspension of protein in appropriate amount of water, incubation with enzyme at appropriate pH and temperature in presence of preservative like chloroform, pasteurize to inactivate enzymes, removal of insoluble material by centrifugation, concentration, drying and packing in moisture proof container.**

# Single stage hydrolysis

- **Common enzymes used: Pepsin, papain, trypsin, pancreatin and microbial proteinases, though other proteolytic enzymes: Corolase PS, Corolase L10, Maxatase LS 400,000, Novozym 257 etc. can also be used.**



## Two stage hydrolysis

- In a two stage process, combination of different enzymes have been used.
- **Clegg et al. (1974):** Twelve kg of casein was suspended in 220 l water at pH 6.2 -6.3 and digested with papain at 40°C for 8 h, and then with a pig kidney homogenate (serving as source of exopeptidases) at pH 7.8-8.0 for 24 h. Hydrolysate was then passed through Russel separator to remove insoluble material followed by pasteurization at 83-85°C for 3-5 min. After concentration up to 20-23%, total solids in Wiegand evaporator and holding at 60 C, product was spray dried. Process completed in 60 h. Process was costly, time consuming and results in production of significant amount of free amino acids. First endopeptidase papain (4%) and then an exopeptidase leucine aminopeptidase (0.015%) are used to obtain casein hydrolysates with reduced bitterness and high (46%) free amino acids.



12 kg Casein suspended in 220 l H<sub>2</sub>O

↓ ← (Approx. 700 ml 40% NaOH)

pH 6.2-6.3

↓ ← (480 g papain (1), or 240 g papain (2), 1 l chloroform)

Incubation  
(40°C/8 h)

↓ ← (Approx. 1 l 40 % NaOH)

pH 7.8-8.0

↓ ← (5 l pig kidney homogenate, 1 l 4% MnCl<sub>2</sub>, 500 ml chloroform)

Incubation  
(40°C/24 h)

Pass through Russel separator

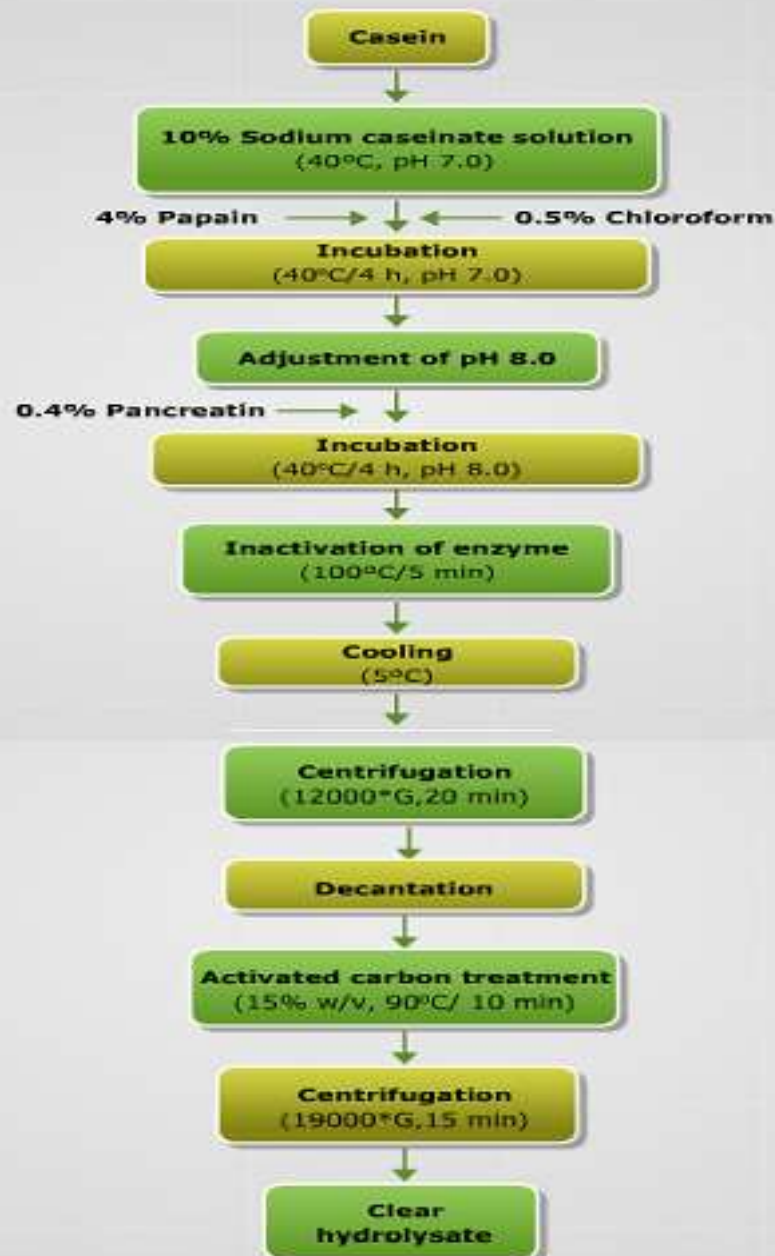
Pasteurisation  
(83-85°C/3-5 min)

Concentration  
(TS 20-23%)

Spray drying

Flow diagram of two stage enzymatic hydrolysis for production of casein hydrolysates

- **Cogan et al. (1981):** Use of papain, pepsin enzymes.
- **Khanna and Gupta (1996):** Enzymatic production of casein hydrolysates in short hydrolysis period of 8 h. Sodium caseinate solution (10% TS) at pH 7.0 was optimum for hydrolysis with papain. 4% papain in first stage and 0.4% pancreatin in the second stage, each stage with 4 h of hydrolysis gave optimum degree of hydrolysis. Casein hydrolysate produced was definitely bitter. Minimum 15% activated carbon treatment was necessary for debittering the casein hydrolysate, though this treatment resulted in 40.90% N loss through adsorption on activated carbon. Yield and recovery of liquid casein hydrolysate were 47.98% and 46.23% respectively. Liquid product had 10.25% TS, 1.93% nitrogen, 1.19% ash.

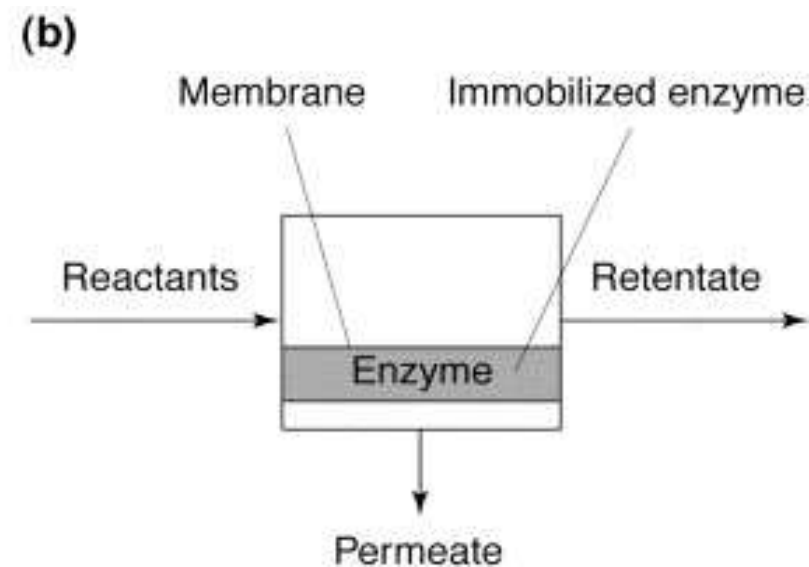
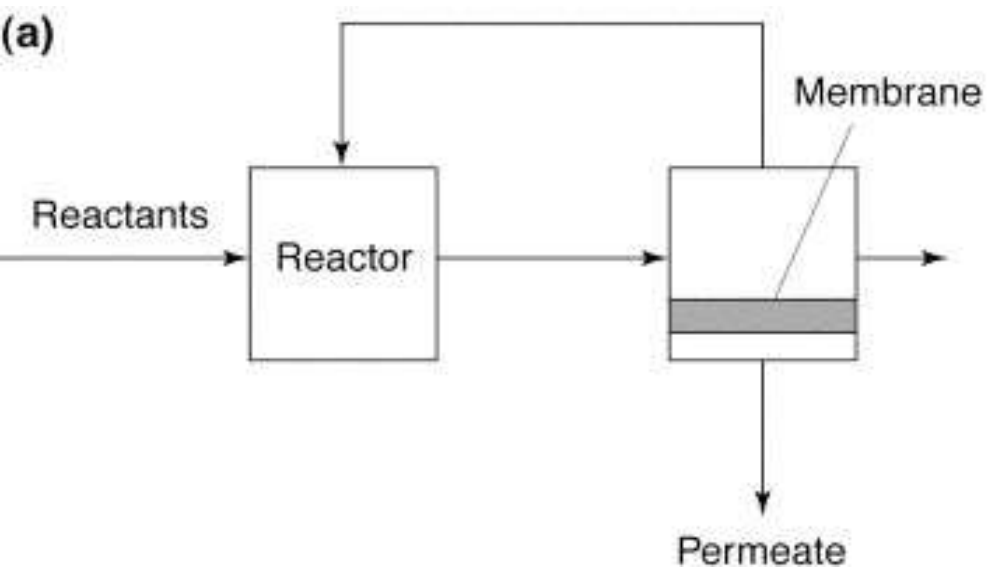


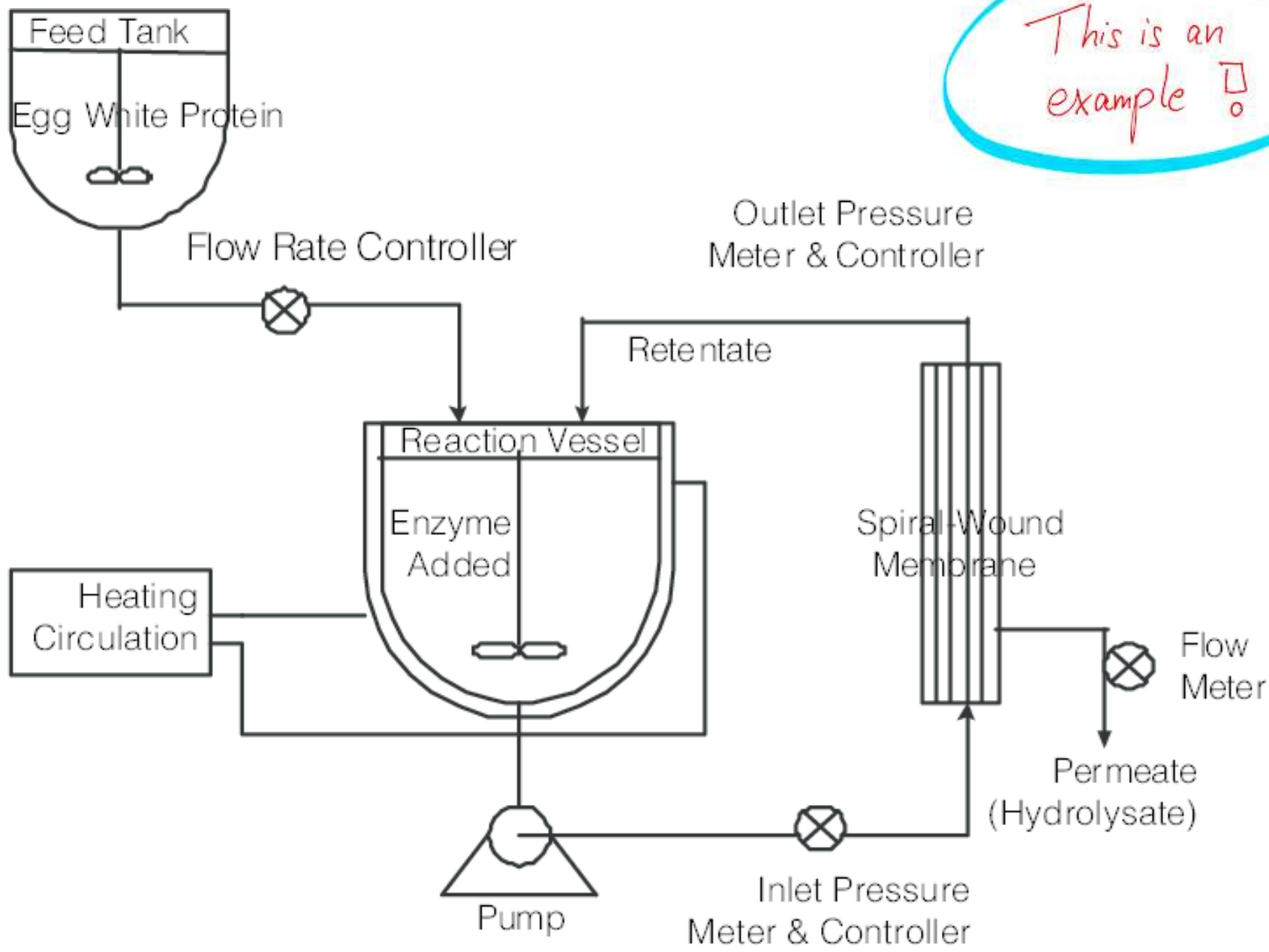
Flow diagram of two stage enzymatic hydrolysis for production of casein hydrolysates

# **Continuous Hydrolysis of Proteins in Membrane Reactor**

- **Membrane technology has led to a new concept: continuous reaction and simultaneous separation of product from the reaction mixture.**
- **A membrane module with appropriate pore size and physicochemical properties is incorporated into the reactor, which contains the enzyme.**
- **Feed is continuously pumped into reaction mixture while product is continuously withdrawn as permeate.**
- **Molecular size of product can be controlled by proper selection of pore size of membrane.**
- **Enzyme is recycled and reused, thus improving enzyme utilization and overall productivity.**
- **Main system components include a vessel coupled in semi-closed-loop configuration to a membrane module via a recirculation pump.**
- **Provisions are made for control of temperature, pH and agitation in reaction vessel.**

- **Casein suspension is pre-adjusted to required pH and temperature (in food vessel) and pumped into reaction vessel at a flow rate equal to permeate flux.**
- **Reaction mixture is continuously recycled with pump inserted in line between reaction vessel and membrane module inlet.**
- **Membrane modules may be polysulfone hollow fibers of 5,000 and 10,000 molecular weight cut-off.**





*This is an example!*