

ENZYME BIO TECHNOLOGY

- Enzymes are proteins which act as biological catalysts.
- Enzymes play an important role in activities of cell & organisms. It shows maximum activity between 35°C to 40°C.
- Inactive at 0°C and above 65°C gets denatured.

The pH of medium has direct effect on enzyme action. Enzymatic activity is reduced by formaldehyde, free iodine, heavy metals & tannins.

- **SOLUBILITY:** Soluble in water & dil. alcohol.
Conc. alcohol precipitates enzymes.

PROPERTIES OF ENZYMES

- Enzyme catalyses only a specific range of reaction --- only one reaction is catalysed by a given enzyme.
- As a group, they effectively catalyse hydrolytic reaction dehydrations, oxidation , reduction reactions, acyl-transfer reactions, aldol condensations, polymerisations and free radical reactions .
- Under optimal conditions, most of the enzymatic reactions proceed 8-10 times more rapidly than the corresponding non-enzymatic reactions.

CLASSIFICATION OF ENZYMES : (H TO LIS)

Hydrolases

Catalysis hydrolytic reactions

Transferases

Transfer of chemical group from one molecule to another

Oxido-Reductases

Catalyse the oxidation reduction reactions.

Lyses

Catalyse the addition of groups to double bonds or vice versa.

Isomerases

Responsible for intra molecular rearrangement.

Synthetases

Catalyse the condensation of 2 molecules coupled with the cleavage of pyrophosphate bond.

BASED ON THE SITE OF ACTION (ENZYMES)

ENDO ENZYMES: (Intra cellular enzymes)

- Enzymes act only inside the cells.
- They involve in the synthesis of cell compounds, food reserves and bio energetic ie liberation of energy from food stuffs.
- Eg: Synthetases, Isomerases, phosphorylases.

EXO ENZYMES: (Extra cellular enzymes)

- Enzymes are secreted outside the cells.
- They are digestive in their function.
- Hydrolyse very complex molecule in to simpler compounds.
- Eg:proteases, lipases, amylases acting on proteins, lipids, starches.

APO ENZYMES

- Enzyme moiety comprises of a protein component and a prosthetic group of non-protein component, which is called as a co-factor or co-enzyme .
- Eg: certain metals and vitamins are co enzymes.

PROTEOLYTIC ENZYMES

1.PAPAIN (VEGETABLE PEPSIN)

Synonyms: Papayotin , veg pepsin , arbutin , nematolyt , caroid, summetrin , tromasin , velardon, vermizym .

Biological source: Dried purified latex obtained from the milky juice of unripe fruits of "Carica papaya" linn.

Family: "Caricaceae"

Description:

- **Odour:** characteristic
- **Colour:** purified papain is white or greyish white or light brown.
- **Nature:** slightly hygroscopic , amorphous powder.
- **Solubility:** completely soluble in water & glycerol . Insoluble in most organic solvents(Best quality of papain digest 300 times its own weight of egg albumin).

Chemical constituents

- Contains enzymes similar to pepsin . It is a mixture of proteolytic enzymes like peptidase I- converts proteins in to polypeptides and d peptides.
- Renin like milk coagulating enzyme.
- Amylolytic enzyme and a clotting enzyme similar to pectase.
- Papain molecule consists of one folded polypeptide chain of 212 amino acids with mol.wt up to 23,400 dalton.

Method of preparation

Method-I

- Carica papaya is a 10-15 m herbaceous tree.
- Fruits are spherical or cylindrical weighing up to 20 lbs .
- Deep incisions on full grown ,un ripe fruit on 4 side.
- Milky juice coagulates(latex).
- Latex is collected and dried under sun or artificial heat.
- Crude papain is purified by dissolving in water and ppt with alcohol. keep it in a well closed container.
- Functions at neutral & alkaline media, Ph—5-best activity
- Total ash value NMT 1%
- Proteolytic activity between the PH 5-6 .

Method-II

- The latex of carica papaya fruits is collected in aluminium trays.
- To the collected latex , potassium meta bisulphite (5g/kg of latex) is added.
- The extraneous matter is cleared out by passing through sieves and latex is dried in vaccum shelf drier at 55°c-60°c.
- It is also processed by spray drying method .The dried latex is called papain.

Uses

- Papain is used as a protein digestant and as an anthelmintic .
- To relieve the symptoms of episiotomy (incision of vulva) and in meat industry for tenderizing meat.
- Papain in combination with other enzymes like amylases used to produce digestive aid .
- Papain (10%)used in ointment for wound (for removal of dead tissue).
- It is used in cheese manufacture as a substitute for rennin.
- Medicinally it is used as an anti inflammatory agent.

2. PEPSIN

Synonym: “Pepsinum”.

It is a substance containing proteolytic enzyme and is present in the gastric juice of animals.

Botanical source: Obtained from the glandular layer (mucous membranes) of fresh stomach of hog, “*Sus scrofa*” or “*Sus domesticus*”. “Domestic pig”

Family: “Suidae”.

Description:

- Colour : light buff or white
- Taste : little acidic or saline
- Odour : slightly meaty
- Nature : amorphous powder
- Solubility : soluble in water, insoluble in alcohol, ether & chloroform.

Properties:

- If pepsin is heated with alkali or pancreatic enzymes, its biological activity is lost.
- Pepsin shows maximum activity at p^H 1.8.
- Pepsin has the capacity to digest 2500 times its weight of coagulated egg albumin.
- Pepsin is also available in other forms which may digest even up to 10,000 times than weight of coagulated egg albumin.
- Pepsin degrades protein into peptones and proteases.
- (Converts insoluble proteins into soluble peptones and proteases. Its action is inhibited by Na chloride and by alcohol and is completely destroyed at a temperature of 70°c).

Uses:

- Employed in dyspepsia(deficiency in gastric secretion).

Method of preparation

Method 1

- Stomach linings of pigs digested with HCl clarified under controlled evaporations
- On dialysis it gives concentrated digested solutions on vacuum evaporation spongy pepsin is obtained

Method 2

- The mucous lining of the stomach is either stripped off and minced [or] it is scraped off and made into pulp

- Pulp is placed in water acidulated with HCl kept at the body temperature 37 °C until autolysis takes place
- After 2 hours a clear liquid containing pepsin and pepton are formed from the mucous tissue
- Filtered and add NaCl [or] ammonium sulphate
- Pepsin precipitates peptones remains in solution

- Ppt pepsin is suspended in water in a dialyser salt formation occur removed by dialysis
- The aq.soln of pepsin precipitated by alcohol. ppt pepsin occur dried at low temp below 45 c
- Pepsin residue obtained is powdered

USES

- Employed in dyspepsial [defciency in gastric secretion]

3 .UROKINASE

Biological source: Protease enzyme isolated from human urine and from human kidney cells by tissue culture or by re combinant DNA technology.

Method of preparation:

Method-1

- Produced by re combinant DNA using genetically manipulated *E.coli* cells.
- pro urokinase is produced first and then converted in to active form by plasmin or kallikrein.

Method-2

- Urokinase used medicinally is purified directly from human urine, adsorbents such as silica gel or kaolin used to concentrate and purify urokinase.
- Further purified by precipitation with sodium chloride and ethanol or chromatography.
- Human urokinase needs sterile filtration, aseptic filling and freeze dryings.

Properties

- White powder, soluble in water.
- Activation of endogenous fibrinolytic system which converts plasminogen to plasmin and degrades fibrinogen, fibrin clots and other plasma proteins.
- Urokinase enzyme occurs in 2 different forms as a single and double polypeptide chain forms.
- It has a half life of 10-15 minutes after I.V administration.

Chemical constituents

- It is an protease enzyme occurs as a single low mol. Weight (33 k Da) and double high mol. Wt(54KDa) poly peptide chain forms.
- A single chain is produced by re combinant DNA technique and is known as SCUPA.

Uses

- Used to dissolve (lyses) fibrin or blood clots in anterior chamber of eye.
- it is generally administered I.V in dose of 4400 units/kg body wt/ hour for 12 hours. It has a half life of 10-16 minutes.

4. SERRATIO PEPTIDASE

- It is a proteolytic enzyme derived from the bacteria belonging to genus serratia present in the gut of silk worm.
- Originally it was discovered in serratia E15 species. it is produced by fermentation bio technology.
- This enzyme is considered as very effective bacterial enzyme and it is found to have better effect than trypsin and chymotrypsin with negligible toxicity and side effects.

- Given orally it enters systemic circulation in un changed form and can penetrate in to all tissues especially inflamed areas.
- It exerts histamine and bradykinin hydrolysing and proteolytic enzymes effects.
- Hence it reduces capillary permeability and also breaks down proteins and exudates and hence supports wound healing.
- It is an bacterial enzyme shows rarely the allergic reactions. It is used in inflammation, sputum liquification, and as an antibiotic.

5.BROMELIN

Biological source: Bromelain is a mixture of proteolytic enzymes from the stem and ripen fruits of pine apple plant *Ananas comosus*.

Family: *Bromeliaceae*

Description: it is odourless to slightly buff coloured powder with irritating taste.

Solubility:slightly soluble in water,in soluble in organic solvents like ether, chloroform, alcohol.

Uses: In inflammation,oedema due to surgery and injury.

6. STREPTOKINASE

- It is an enzyme obtained from culture filtrates of β – hemolytic streptococci group C.
- This enzyme has the property of activating human plasminogen to plasmin.
- It is available as a sterile, friable , solid or white powder.
- It is soluble in water with maximum activity at PH 7.
- The solution at higher concentrations is stable for 6 hours at 4°C, Otherwise dilute solutions are unstable.

- **Uses** : it is used in the treatment of thrombo embolic disorders for the lysis of pulmonary emboli, arterial thrombus, deep vein thrombus and acute coronary artery thrombosis.
- The activity of this enzyme is due to activation of plasminogen to a proteolytic enzyme namely plasmin which degrades fibrin clots, fibrinogen and other plasma proteins.

1. CASEIN (PROTEINS)

Biological source : Casein is a principal phosphor protein in milk and constitutes 3% milk. It comprises about 80% total protein content of milk.

There are 2 types of casein in the market,

- 1. Acid casein :** warm skimmed milk is acidified with dilute acid, curd will form it is washed several times, dried and pulverized.
- 2. Rennet casein:** skimmed milk is treated with an enzyme, rennet extract product is separated and purified.

Description: It is a white, slightly yellow, tasteless, odour less, amorphous, solid hygroscopic, stable when dry but deteriorate rapidly when damp.

Solubility: it is in soluble in water, soluble in dilute alkalies, concentrated acids but precipitates from dilute acid solutions.

Chemistry of Casein: Casein is a phospho protein containing about 0.85%phosphorus and 0.75%sulphur.it contains about 15 aminoacids. Mol.weight 75,000-3,70,000. Iso electric point 4.7. nitrogen content 15-16%.

USES OF CASEIN

- It is useful as dietary supplement.
- Source of protein in pre and post care, as a base.
- In the standardization of proteolytic enzymes and as a emulsifying agent.
- Industrially it is used in sizing of textiles and paper as an adhesive.

2. GELATIN (PROTEIN)

Synonyms: Gelatina, Gel foam, pura gel.

Biological source: Gelatin is a protein extracted by partial hydrolysis of animal collagenous tissue like skins, tendons, ligaments and bones with in boiling water.

Gelatin is obtained from the domestic animals such as ox (***Bos taurus*** linn) and sheep (***ovis aries*** linn).

Family: ***Bovidae***

Description

- This protein product is available in the form of flakes, sheets, strips or a coarse or fine powder.
- It has a characteristic odour and faintly yellow to amber colour. Taste is slightly bland.
- Gelatin should be free from chondrin, the absence of chondrin can be confirmed by its failure to produce a precipitate with lead acetate, alum, ferric chloride and copper sulphate.

- **Solubility:** It is insoluble in cold water but soluble in hot water, in cold water it swells, softens and absorbs about 5-10 times of its weight of water.
- with hot water it forms a jelly on cooling.
- It is soluble in a mixture of water and glycerine but insoluble in fixed and volatile oils, alcohol, chloroform and ether.

- The quality of gelatin is expressed as **bloom strength**.
- Gelatin is used as coating of pills, as vehicle for suppositories and as an emulsifying agent for capsule manufacture and microbial culture media higher jelly strengths are used.
- The jelly strength is designated by bloom gelometer number.

Chemical constituents

- It is a protein, chemically it contains different amino acids like lysine an essential amino acid but does not contain tryptophan. Gelatin is composed of gluten protein.
- **Preparation of Gelatin:** For the manufacture of gelatin the bones are to be defatted and de calcified with organic solvent and mineral acid respectively.
- The material obtained by this treatment is treated with water at 85°C in successive quantities due to which collagen dissolves in to gelatin.

- It is further bleached and concentrated under reduced pressure to specific gelatin content and allowed to set in shallow trays such moulded gelatin is dried in drying room to eliminate moisture.
- **Identification tests:** it evolves ammonia when heated with soda lime.
- It is precipitated by tri nitro phenol and solution of tannic acid but not with alum, lead acetate or acids which indicates that it does not contain chondrin.
- It gives a white precipitate with mercuric nitrate and on warming turns to brick red colour.

- Dissolve 0.5g in 100 ml of water by heating and use this solution for the following tests,
- To a few ml of solution add a few drops of 10% tannic acid solution white precipitate produced which does not dissolve on heating.
- Add millons reagent to a few ml of the solution white precipitate produced which becomes red on heating.
- Add 10% picric acid solution yellow precipitate is produced.

USES

- Gelatin is mainly used in manufacture of hard and flexible capsule shells.
- It is also used in preparing pessaries pastes and suppositories.
- Gelatin in the form of absorbable gelatin sponge is used as haemostatic.

- Gelatin is employed for micro encapsulation of drugs, perfumes, flavours and some industrial materials.
- It is used as a vehicle for certain injections, like heparin in the form of Pitkin's menstrum which contains gelatin, dextrose, acetic acid and water.
- It is also used in the preparation of culture media