

5.6. ENZYMES

5.6.1. Introduction

Enzymes are proteins that catalyse or increase the rate of chemical reactions. The enzymes are highly specific and effective protein molecules (except RNAs) that catalyse almost all biochemical reactions. Sometimes non-protein coenzymes or cofactor are also required for the catalytic functions of enzymes. Some **properties of enzymes** are briefed below:

- 1) All enzymes are proteins, except group of catalytic RNAs.
- 2) The molecular weight of enzyme ranges from 12,000 to 1 million or more like other protein molecules.
- 3) The catalytic activity of enzymes is mainly due to their own primary, secondary, tertiary, and quaternary structures of protein and their specific conformation. The catalytic activity of enzyme gets destroyed in case of denaturation or dissociation into sub-units.

- 4) Most of the enzymes require only amino acid residues to show their effect, whereas in some enzymes **cofactor** or **coenzyme** is needed; sometimes both metal ion and coenzyme are needed for enzymatic activity. Cofactors are inorganic ions such as Fe^{2+} , Mg^{2+} , Mn^{2+} , or Zn^{2+} , whereas coenzymes are vitamins (organic essential nutrient required in a small amount in the human body) derived metallo-organic or complex organic molecules; temporarily acting as specific functional group carrier. **Prosthetic groups** are the metal ions or coenzymes which are bonded covalently, evenly, and tightly to the enzyme protein.
- 5) **Holoenzymes** are the catalytically active enzyme made up of coenzyme or metal ions (non-protein part) and **apoenzyme** or **apoprotein** (protein part).
- 6) Enzyme activity can be regulated by some processes such as phosphorylation, glycosylation, etc., these processes alter the structure of a protein found in the enzyme.
- 7) Enzymes are required in very minute quantity. They are not consumed in the overall enzymatic reaction rather obtained whole after completion of reaction. Enzymes do not alter the equilibrium constant (K) of a reaction but only increase the rate of reaction.

5.6.2. Papaya

Papain is an enzyme found in and extracted from papaya. It is a powerful digestive enzyme, thus, is important in digestive processes, involving the breakdown of tough protein fibres.

Synonyms

Papayotin, Vegetable pepsin, Arbuz, Nematolyt, Caroid, Summetrin, Tromasin, Velardon, and Vermizym.

Biological Source

Papain is the dried and purified latex of the green fruits and leaves of *Carica papaya* L. Papain can also be obtained from the juice of stems, leaves, and petioles (the activity is nearly the same as that obtained from the fruit latex).

Family

Caricaceae

5.6.2.1. Occurrence and Distribution

The papaya plant (figure 5.5) is cultivated in Sri Lanka, Tanzania, Hawaii, and Florida.

5.6.2.2. Organoleptic Evaluation

The organoleptic features of papain are:

- 1) It is a white or greyish white coloured powder.
- 2) It is slightly hygroscopic in nature.
- 3) Its solubility in water and glycerol is partial.
- 4) It is a protein made up of 212 amino acids and has a molecular weight of about 23,000 daltons.
- 5) It is resistant to heat.
- 6) It may digest about 35 times of its weight of lean meat.



Figure 5.5: Plant of *Carica Papaya*

- 7) Its best-grades render digestion of 200-300 times their weight of coagulated egg albumin in alkaline media.
- 8) The digestive process occurs at a temperature of 60-90°C, however, 65°C is considered to be optimum.
- 9) The optimum pH for its functioning is 5.0, but it also functions in neutral or alkaline media.
- 10) In crystalline form, it is most stable in pH 5-7 and gets destroyed at 30°C below pH 2.5 and above pH 12.
- 11) It is activated by reduction (**examples** of reducing agents are HCN, H₂S, etc.) and inactivated by oxidation (**examples** of oxidising agents are H₂O₂, iodoacetate, etc.).
- 12) It is a protein digestive enzyme with isoelectric point.

5.6.2.3. Chemical Constituents

Papain contains **proteolytic enzymes** (peptidase I) which convert the proteins into dipeptides and polypeptides. It also contains rennin-like enzymes, clotting enzymes similar to pectase, and an enzyme weakly active on fats.

Papain, papaya proteinase, and chymopapain are the enzymes isolated in crystalline form from the latex. Papain contains nitrogen (15.5%) and sulphur (1.2%). The leaves possess dehydrocarpaines I and II, fatty acids, carpaine, pseudocarpaine, and carotenoids. The fruits contain lauric, myristoleic, palmitoleic and arachidic acids, malonated benzyl-*p-o*-glucoside, 2-phenyl ethyl glucoside, and 4-hydroxy-phenyl-2-ethyl glucoside.

5.6.2.4. Chemical Tests

Papain gives the following chemical tests:

- 1) It decolourises aqueous potassium permanganate solutions.
- 2) It causes curdling of milk (prototypic activity).

5.6.2.5. Therapeutic Efficacy

Papain has the following therapeutic purposes:

- 1) It is used in preventing adhesions, in sloughing and infected wounds, in relieving the symptoms of episiotomy (incision of vulva), internally as a protein digestant, anthelmintic (nematode), and an anti-inflammatory agent.
- 2) It is used in treating dyspepsia, intestinal and gastric disorders, and diphtheria by dissolving the diphtheria membrane.
- 3) It is used in surgery for reducing blood clots (in cases when thromboplasma is undesirable), and for local treatment of buccal, pharyngeal, and laryngeal disorders.
- 4) It is used as an additive in digestive mixtures and liver tonics.
- 5) It reduces enlarged tonsils, and prevents post-operative adhesions, carbuncles, and eschar burns.
- 6) It is an allergic agent which causes severe paroxysmal cough, vasomotor rhinitis, and dyspnoea.

5.6.3. Diastase

Diastase is an enzyme found in malt and produced when the seeds germinate. The enzyme converts starch into maltose and then into glucose. Diastase occurs as a yellowish white, amorphous powder, with a faint characteristic odour.

Synonyms

Maltin, Diastase of malt, Amylase, and malt extract.

Biological Source

Malt extract is obtained from the dried barley grains of one or more varieties of *Hordeum vulgare* Linne.

Family

Poaceae

5.6.3.1. Occurrence and Distribution

Barley is grown abundantly throughout the world; U.S.A., Russia, Canada, India, Turkey, highlands of China, and Tibet are the major producers.

5.6.3.2. Organoleptic Evaluation

Diastase is thermolabile and sensitive to more than 45°C temperature. It shows activity between 35-40°C. It is active in solution of pH 6-7; while it deactivates in acidic solution having pH upto 4.

Diastase catalyses the hydrolysis of 1,4-glucosidic linkages of polysaccharides (such as starch and glycogen). It can be convert 50 times its weight of potato starch into sugars.

Diastase in water forms a colloidal solution, while it gets precipitated in alcohol.

5.6.3.3. Chemical Constituents

Diastase contains dextrin, maltose, traces of glucose, and amylolytic enzyme diastase (8%).

5.6.3.4. Chemical Tests

Diastase gives the following chemical tests:

- 1) **Arsenic:** 0.25gm diastase is taken in a platinum, quartz, or porcelain crucible, to which 10ml of magnesium nitrate in ethyl alcohol (1→50) is added, followed by ignition of alcohol. It is then reduced to ash by heating at 450-550°C. If a carbonaceous substance is left behind, it is wetted with a small amount of nitric acid, and then again heated at 450-550°C. After cooling, the residue is dissolved in 3ml of hydrochloric acid by heating in a water bath. On conducting the test for arsenic using this test solution, the arsenic content should not be more than 4ppm.
- 2) **Heavy Metals:** 0.5gm diastase is carbonised by heating mildly in a quartz or porcelain crucible. After it gets cooled, 2ml nitric acid and 5 drops of sulphuric acid are added and heated till the white smoke disappears. On further heating at 450-550°C, the content reduces to ash. After it gets cooled, 2ml hydrochloric acid is added and evaporated to dryness in a water bath. The resulting residue is added with 3 drops of hydrochloric acid and 10ml of hot water, and is again heated for 2 minutes. After cooling, a drop of phenolphthalein indicator solution is added, followed by ammonia solution till the solution becomes pale red in colour.

The resulting solution is taken in a Nessler cylinder rinsed with water. 50ml test solution is prepared by adding 2ml dilute acetic acid (1→20) and water. When this solution is tested for heavy metals, the content should not be more than 40ppm. For preparing coloured standard solution, 2ml nitric acid, 5 drops of sulphuric acid, and 2ml hydrochloric acid are added and evaporated to dryness in a crucible (made up of

the same material used for test solution preparation). The residue obtained is added with 3 drops of hydrochloric acid, and transferred to another Nessler cylinder. In this cylinder, 2ml lead standard solution, 2ml dilute acetic acid (1→20), and water is added to make up the total volume up to 50ml.

- 3) **Lead:** 0.8gm diastase (if in liquid form is concentrated by evaporation in a water bath) is slowly carbonised by heating, and then reduced to ash by further heating at below 500°C temperature. The obtained ash is added with 20ml dilute nitric acid, gently boiled for 5 minutes, and then filtered (if required). The residue is washed with water and added to the filtrate. Water is also added to make up the total volume up to 50ml. This test solution is tested for lead, whose content should not be more than 10ppm.

5.6.3.5. Therapeutic Efficacy

Diastase has the following therapeutic properties:

- 1) Malt extract and purified diastase are used as amylolytic enzymes.
- 2) They serve as an aid in digestion of starch.
- 3) They are used as bulk producing laxatives.

5.6.4. Yeast

Yeast is a fungus which has a vegetative body of simple individual cell under normal growth conditions. It is a unicellular fungus which spreads by forming mycelia containing small and definite nuclei and reproducing by budding and ascospore foramen. Around 350 species of yeast are known so far. Yeasts are classified based on their uses and morphological characters. Some common **examples** of different types of yeast are wine yeast, baker's yeast, brewer's yeast, and distiller's yeast. Either the brewer's yeast or the baker's yeast is employed in pharmaceutical industries. Yeasts are also classified based on ascospore formation, formation of true or pseudomycelium, and reproduction by budding or fission (**figure 5.6**). Yeast which forms ascospores is termed as **ascomycetes** and that which does not form ascospores is the **fungi imperfecti**.

Synonyms

Brewer's yeast and Baker's yeasts.

Biological Source

Yeast is the unicellular fungal microorganism, *Saccharomyces cerevisiae*.

Family

Saccharomycetaceae

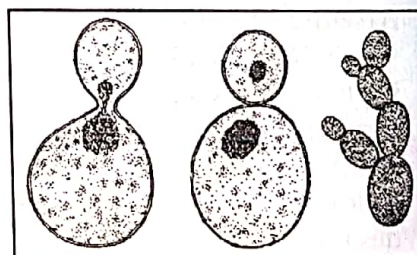


Figure 5.6: Budding Yeast

5.6.4.1. Occurrence and Distribution

Yeast grows naturally in food items, juices, bread, and several fermented or fermenting media. On a large scale, yeast is produced by using citrus, molasses, molasses wort, grain wort, etc.

5.6.4.2. Organoleptic Evaluation

The organoleptic features of yeast are:

- 1) **Colour:** Whitish powder.
- 2) **Odour:** Characteristic.
- 3) **Size:** Unicellular microorganism measuring less than 1.5 μ .

5.6.4.3. Chemical Constituents

Yeast contains moisture (65-85%), nitrogenous compounds (proteins), glycogen, fat, vitamins (thiamine, riboflavin, nicotinic acid, pantothenic acid, folic acid, biotin, etc.), ash, and enzymes (invertase, diastase, zymase and maltase).

5.6.4.4. Therapeutic Efficacy

Yeast has the following therapeutic properties:

- 1) It is used for manufacturing alcohol, beer, and various wines.
- 2) It is used in the bread industry to raise dough.
- 3) Irradiated yeast has been used as a source of vitamin D.
- 4) It is also used for manufacturing glutathione and invertase.
- 5) It is a good source of protein.

19