CLASS 4:

Extraction of enzymes

Sources of enzymes

Enzymes are usually obtained from three major sources-

- Plants
- Animals
- Micro-organisms (Bacteria, fungi and yeast)

The advent of fermentation technology led to the preparation of purer form of enzymes from microbes. Today, most of the industrially used enzymes are from microbial source.

- Enzymes are the catalysts responsible for cell metabolism.

- Cells from different sources have been, are, and will continue to be the main source of enzymes.

- Enzymes can be produced from any living organism, either by extracting them from their cells or by recovering them from cell exudates.

- Plant tissues and animal organs were the most important sources of enzymes

- Tens of thousands of different kinds of enzymes are believed to exist in the human body, each with a specific purpose.

- There are three general categories of enzymes: digestive enzymes, metabolic enzymes, and food or plant enzymes.

- The **digestive enzymes** category consists of the enzymes produced within your own body to help break down food into its basic components for digestion.

- **Metabolic enzymes** are found throughout our entire body – in our organs, bones, blood, and even within the cells that produce them.

- They function in support of our heart, lungs, kidneys and brain. Food and plant enzymes are naturally present in raw food.

- They generally serve the same function as digestive enzymes, but these are the enzymes that we may take in through our diets, as opposed to the ones our bodies produce.

- We can obtain these enzymes through eating fresh, raw and uncooked foods like fruits, vegetables, eggs, unpasteurized dairy, meat and fish.

- The modern diet generally revolves around processed and cooked food, but these processes destroy the naturally occurring enzymes contained in the food.

- This places a heavy burden on our bodies to subsidize the enzyme requirement for breaking down that food.

- Raw food contains the necessary proportion and types of enzymes required to digest itself. This remains one of the biggest benefits of a diet centered around raw food.

- The major components of the food (sugar, protein, starch, fat) and their respective caloric amounts determine what type and quantity of enzymes are also present.
- For example, the enzyme amylase is found in high carbohydrate fruits like apples and peaches. Fruits that are high in fat, such as avocados, contain the enzyme lipase.

- Below, we will focus on enzymes we obtain from food sources (animal, plant and fungal) and their respective usefulness.

Animal-Enzymes

Although the source of animal-based enzymes we consume don’t originate from a human body, it is thought they may be similar enough that the human body might do a better job recognizing and utilizing them. However, it is important to note that this is merely a theory.

Reliance on obtaining digestive enzymes from animal sources is challenging, because a majority of the meat and other animal byproducts we consume are processed, pasteurized and/or cooked, which destroys the natural enzymes.

For vegetarians or vegans, animal enzymes are hardly an option, and consuming raw meat or eggs is a dangerous endeavor, due to the risk of bacterial contamination.

From a digestive perspective, there are several important disadvantages associated with animal-based enzyme sources. Temperature sensitivity is one of these.

The human body does not generally have the same temperature as the animal host of these enzymes, which can be destructive to the enzyme upon entering the gastrointestinal tract.

Animal-based enzymes also function exclusively within a limited pH level range, which renders them fairly ineffective in the gut.

They become unstable in a low pH level (acidic) environment, resulting in the enzyme being destroyed before it can perform its function. This pretty much eliminates the stomach as an operational environment.

- As a result, to take in animal enzymes, they are better delivered into the body within a protective enteric (polymer) coating capable of withstanding the stomach’s acidity. This means that the enzymes don’t become available to the body until they reach the small intestines.

- The most common type of animal enzymes used for dietary supplementation are pancreatic enzymes. However, for the reasons outlined above, the general consensus is the best sources of enzymes are plant and fungal.

Plant Enzymes

- Fruits and vegetables are commonly consumed in their raw, natural form.
- This alleviates the overarching issue with animal-based enzymes by preserving the integrity of the enzymes themselves.
- Additionally, plant-based digestive enzymes are effective over a broad scope of pH levels.
- This range is generally believed to be between 3.0 and 9.0, which is highly compatible with the human gastrointestinal environment.
- As a result, plant-based enzymes are well-suited for supporting comprehensive digestive health.

- Four important enzymes often found in plants are protease, amylase, lipase and cellulase. Protease breaks down protein that can be present in meat, fish, poultry, eggs, cheese and nuts.

- Amylase assists your body with the breakdown and subsequent absorption of carbohydrates and starches.

- Lipase aids the digestion of fat. When your diet includes lipase-rich foods, it eases the production burden on the gall bladder, liver and pancreas.

- Cellulase is present in many fruits and vegetables, and it breaks down food fibers, which increases their nutritional value to our bodies.

- The presence of cellulase in plant-based sources is important, because it is not naturally present in the human body.

- Fruits and vegetables are an ideal source for enzymes.

- They are enzyme-rich and easily consumed without needing to be cooked or processed, ultimately preserving the full functionality of the enzymes.

Fungal Enzymes

- Fungal Enzymes have numerous uses. They are critical in the production and preparation of many food products, like beer, soy sauce, miso, baked goods, dairy and processed fruit. One of the oldest known applications is the role of yeast in alcohol fermentation.

- Fungal enzymes are commonly produced from a fungal source called *Aspergillus*. For example, *Aspergillus oryzae* is used in the preparation of sake and soy sauce, while *Aspergillus sojae* is also used in soy sauce preparation, as well as in miso soup.

- One of the most popular and well known culinary fungi is the mushroom.

- Some mushroom species produce enzymes, including hydrolases, esterases, and phenol oxidases. Fungi and their enzymes can also be found in yeast spreads and certain types of cheeses, such as Camembert and blue cheeses.

Fungi can contain a variety of enzymes, such as protease, amylase, lipase, cellulase and tilactase (supports lactose absorption).

Like plant enzymes, fungal enzymes are acid stable and can survive within the pH range of the stomach. They are also suitable for a vegetarian diet, unlike animal-sourced enzymes.

In summary, if you’re interested in increasing your enzyme intake efficiently, the usefulness of plant-sourced and fungal-sourced enzymes outweighs that of animal-sourced enzymes.

Advantage of microbes over other sources of enzymes:

Microbes have a short generation time and hence the times required for enzyme production in large quantities is less compared to plants and animals.

Enzymes can be easily extracted from microbes. If the enzymes are extracted from microbes. If the enzymes are extracellular, they will be secreted easily.

Micro-organisms produce enzymes throughout the year and no seasonal variation is found which is common in case of plants and animals.

- Production of enzymes takes place in the following steps : Isolation of Microorganisms, Strain Development and Preparation of Inoculum Microorganisms are isolated on culture media following the microbiological techniques.

Aim for isolating a suitable microorganism lies in

(a) production of enzyme in high amount and other metabolites in low amount,

(b) completion of fermentation process in short time, and

(c) utilization by the microorganisms of low cost culture medium.

The production process can be divided into four stages:

– Enzyme synthesis: it represents the propagation stage of the producing cells.

– Enzyme recovery: it represents the extraction of the enzyme from the producing cell system and involves solid–liquid separations, cell extraction and/or concentration.

– Enzyme purification: it represents a series of operations after enzyme recovery aiming to remove unwanted contaminants (mainly accompanying proteins).

– Enzyme product formulation: it consists in different operations aimed to give the enzyme product its final presentation; it includes final polishing operations, stabilization and standardization.

- Once a suitable microorganism is obtained its enzyme producing ability is optimized by improving strains and formulating culture medium (pH and temperature).
- An ideal medium must have a cheap source of carbon, nitrogen, amino acids, growth promoters, trace elements and little amount of salts.
- Care must be taken to maintain pH during fermentation. For a specific microbe pH, temperature and formulation of culture medium is optimized prior to inoculation.
- Production of enzymes increases with the concentration of culture medium

- Sterilization and Inoculation of Medium, Maintenance of Culture and Fluid Filteration

- Medium is sterilized batch-wise in a large size fermenter. For this purpose, continuous sterilization method is now becoming popular. And medium is sterilized, inoculation with sucfffficient amount of inoculum is done to start fermentation process.

- Traditional method of enzyme production has been the surface culture technique where inoculum remains on upper surface of broth.
- Now-a-days submerged culture method is most widely practiced because of less chances for infection and possibility for more yield of enzymes.
- Former technique is still in use for production of some of the fungal enzymes, for example, amylase (from *Aspergillus* sp.), protease (from *Mucor* sp. and *Aspergillus* sp.) and pectinase (from *Penicillium* sp. and *Aspergillus* sp.).

- Growth conditions e.g. pH, temperature and oxygen are maintained in fermenter at optimum level.
- These factors differ microbe to microbe and even in the same species of a microbe.
- A little amount of oil is added to fermenter to control foaming as it happens during fermentation. Ar 30-150 h incubation, extracellular enzymes are produced by the inoculated microbe in culture medium.
- Most of enzymes are produced when exponential phase of growth completes but in a few cases, they are produced during exponential phase.
- Besides extracellular enzymes, other metabolites (10-15 per cent) are also produced in the fermented broth. These metabolites are removed used for enzyme purification.

- ***ISOLATION OR EXTACTION.***
- Various methods; Mechanical, Enzymatic and chemical.
- Techniques used for enzyme isolation As mentioned above, generally enzymes are isolated in the cold condition (at 0 to 4o C).
- For the purpose, homogenizing medium as well as container should be in the chilled condition.
- It is preferable to homogenize the tissue in a cold room. The following are the commonly used techniques for enzyme homogenization.

- **Pestle and mortar**
- Pestle and mortar is a moderate technique for tissue homogenization. Mechanical breakdown occurs during the process.
- Sometimes, grinding is done in the presence of purified sand or glass beads for aberration.
- Pestle and mortar is considered to be a moderate grinding technique and rupturing of the cell organelles does not occur if isotonic grinding medium without detergent is used.

Blenders

Waring blender (commonly called as mixie) is comparatively harsh technique of grinding the tissue compared to pestle and mortar and is mostly used for homogenizing the harder tissues (generally the plant tissues).

If the worker is interested in isolating intact cell organelles, then Waring blender is not a preferred technique.

- **Ultra- Sonicator**
- This technique of rupturing the cells is generally used for microbial/ bacterial cells.
- Ultrasonicator generates low as well as high wavelength ultrasonic waves.
- For the purpose, a suitable probe depending on the volume of the homogenizing medium is selected and connected with the ultra-sonicator.
- The container having cells and homogenizing (isolating) medium is put in chilled condition by covering the container with ice.
- There is much generation of heat during ultra-sonication, therefore, ultrasonic waves are thrown in the sample after few seconds interval, every 10 to 15 seconds ultrasonication.

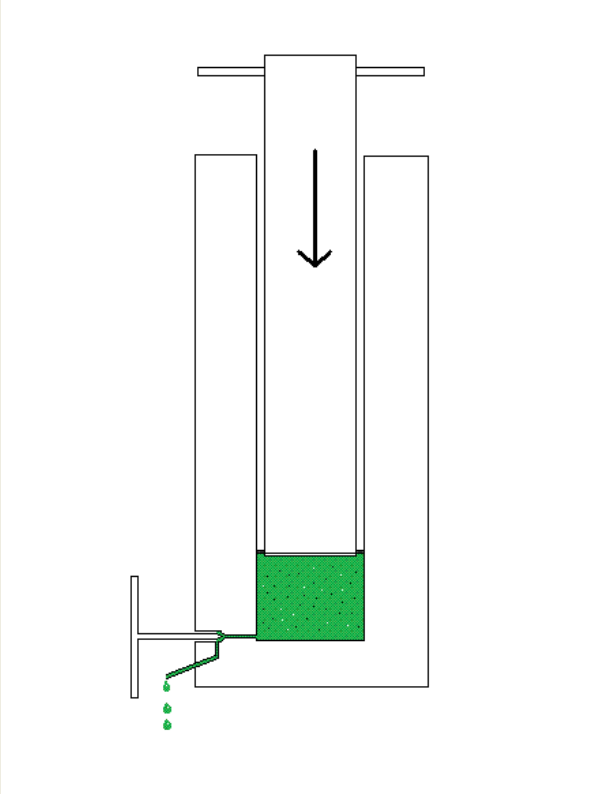
- **Vir-Tis homogenizer**
- This is considered to be a mild technique and generally used for homogenization of soft tissues such as animal tissues. Here a motorized pestle with teeth like aberrations is used. With Vir-Tis homogenizer, generally no rupturing of cell organelles occurs during grinding provided isotonic medium with no detergent is used.

- **Potter Elvejm homogenizer**
- This is also a mild technique and is used for homogenization of soft animal tissues.
- Potter Elvejm Homogenizer is a simple equipment having a pestle like glass rod with teeth like aberrations on its tip.
- There are down aberrations in the tube too on which teeth of the rod are fitted during up and down process of the rod. Up and down process of the pestle is done manually by hand or by mechanical device.

- **Razor blade**
- It is comparatively very mild technique. It is generally used only for isolation of intact cell organelles for the purpose of studying the intracellular localization of the enzyme proteins.
- In the technique, razor blade is used for chopping the tissue in the presence of isolating medium. Although the technique is good for the isolation of intact organelles, but it is unable to rupture all the cells.
- Therefore, there is low recovery of the enzyme due to left out of unruptured or partially ruptured cells. These un-ruptured or partially ruptured cells are removed as cell debris after centrifugation.

Extrusion method

This method relies on the principle that forcing a cell suspension at high pressure through a narrow orifice will provide a rapid pressure drop. This is a powerful mean of disrupting cells especially from bacteria.



- **Lytic enzymes**
- Cell wall and cell membrane lytic enzymes like cellulase, pectinase, xylanase, pectin methyl esterase, lysozyme etc. can be used for rupturing the cells.
- Enzymes being costly are not commonly used for making cell free preparation for isolation of enzymes. In plant tissue culture, lytic enzymes are used to prepare protoplast.

- **Freeze-Thaw**
- With certain susceptible microbes and eukaryotic cells, repeated freezing and thawing results in extensive membrane lesions with release of periplasmic and intracellular proteins.

- **Acetone**

- Drying with acetone is a good method for rupturing the cell membrane.

- Using acetone, powder of the tissue may be prepared which may be stored in a Deep freezer for a long time. It forms a convenient starting material from which the enzyme may be extracted with the isolating medium, whenever required.

- However, one has to take much precautions of low temperature (generally –200C), otherwise, acetone may denature the enzyme protein. Isolation of enzymes from sub-cellular organelles requires rupturing of the organelle.

- Generally for the purpose, organelle is isolated in intact form thus removing the contaminating proteins of the cytoplasm and other cell organelles. Afterwards, cell organelle is ruptured in the presence of a suitable detergent like tween, teepol, digitonin etc.