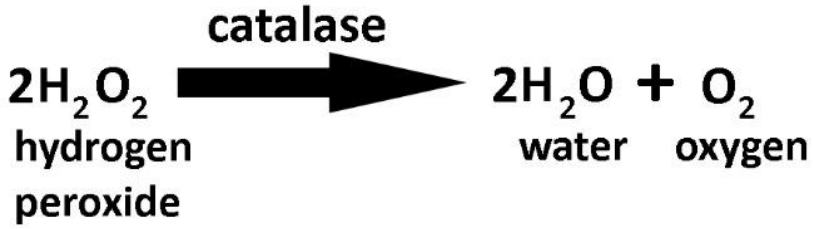
A little bit about the Enzymes used in these activities……..

Enzymes studies have been conducted for many years. Historically many enzymes were given names by the researchers studying them. Some of those names are still commonly used today, for example trypsin, pepsin, and bromelain. The name itself doesn't tell you very much about the enzyme or its function. However, more recently scientists have standardized the naming of enzymes based on their classification (function). Additionally, as part of the standardization all enzyme names end in –ase, for example catal**ase**, with the added suffix it is easier now to identify enzymes.

Depending on the activities selected by your instructor you may use one or more of these enzyme extracts in your experiments.

Catalase

Catalase is an enzyme found in every cell in your body and in nearly every organism that lives in an oxygen containing environment. Catalase catalyzes the breakdown of hydrogen peroxide to oxygen and water. Hydrogen peroxide is a by-product of metabolism.



You have seen catalase in action if you have every poured hydrogen peroxide on a cut. Bubbles of oxygen immediately form in the wound. Oxygen is being produced by catalase in your cells breaking down hydrogen peroxide.

While we normally think of oxygen in a favorable way, oxygen and its related compounds are actually strong oxidizing agents and can cause tremendous damage to biological molecules. Think about how oxygen in the atmosphere reacts with the exposed metal of cars. Oxygen interacting with metal leads to rust. Oxidation is analogous to rusting. Biological molecules, especially those containing iron or metals don't work well when oxygen species react with them. There is actually very little free oxygen in your body. Most of the oxygen in your body is bound to carrier molecules, like hemoglobin and is released where it is needed and in the amounts it is needed to prevent random oxidation of biological molecules. Catalase is one of several enzymes that helps decrease these reactive oxygen species within cells.

Enzyme Preparation

Catalase is in every tissue in most organisms but the highest levels of activity are observed in liver. Liver after all is the organ tasked with detoxifying the blood. If you choose not to use liver, then potato or turnip can be used.

Liver

Beef or chicken livers can be used. The advantages of using liver is that activity is very high and liver can be frozen and the enzyme activity is not affected. The enzyme suspension should be made immediately before use. It does not store for long periods of time.

1. Add 2 chicken livers or the equivalent amount of beef liver (1/4 cup) to a blender.
2. Add 100 mL of cold water to the blender. Blend till homogenized.
3. Cover a beaker loosely with 3 layers of cheesecloth or alternatively line a funnel with 3 layers of cheesecloth.
4. Pour the liver extract through the cheesecloth. Gently gather and squeeze the cheesecloth to extract more liquid.

The enzyme extract is ready to use. Use immediately.

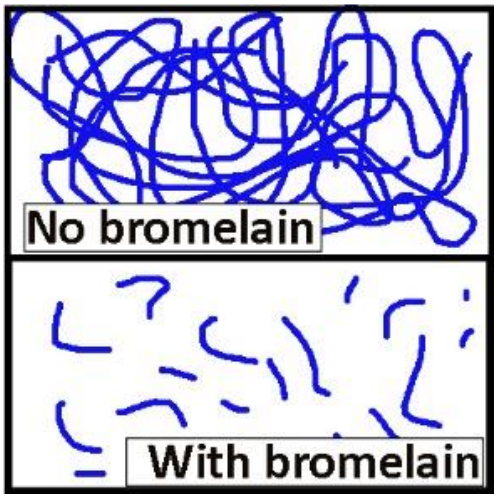
Potato or Turnip

1. Weigh out 100 gm of peeled potato or turnip.
2. Add potato or turnip to blender. Add 100 mL of cold water.
3. Blend till homogenized.
4. Cover beaker loosely with 3 layers of cheesecloth or alternatively line a funnel with 3 layers of cheesecloth.
5. Pour the extract through the cheesecloth. Gently gather and squeeze the cheesecloth to extract more liquid.

The enzyme extract is ready to use. Use immediately.

Bromelain

Bromelain is an example of one of the enzymes that has been known and researched for many years. Bromelain is found in pineapple (a bromeliad) and pineapple stems. It is classified as a protease, an enzyme that breaks down proteins. Bromelain will breakdown large proteins to smaller peptides and amino acids. The next time you are in the supermarket read the label on a gelatin box. It will specifically warn the purchaser not to add fresh pineapple. Gelatin is a large protein that crosslinks to form the gel (see below). If you add fresh pineapple, the enzymes in the pineapple chop up the gelatin molecules preventing crosslinking and jelling; you end up with gelatin soup!



Gelatin a long, fibrous protein will crosslink to itself under normal conditions producing a matrix or gel (top panel). When exposed to a protease such as bromelain or papain, the gelatin molecules are chopped up and can no longer crosslink or form the matrix or gel (bottom panel).

What would happen if you used canned or frozen pineapple?

Interestingly, bromelain is getting attention today as an anti-inflammatory drug and pain medication for use in osteoarthritis. Although the jury is still out on it efficacy, its use is an active area of research.

Enzyme Preparation

1. Peel a fresh pineapple. Slice into 1 cm rounds. Slice each round in half.
2. Add 1 half round pineapple piece to blender. Add 100 mL of cold water.
3. Blend till homogenized.
4. Cover beaker loosely with 3 layers of cheesecloth or alternatively line a funnel with 3 layers of cheesecloth.
5. Pour the extract through the cheesecloth. Gently gather and squeeze the cheesecloth to extract more liquid.

Use extract immediately. Proteases will digest themselves, so the juice should be stored on ice and used immediately.

Other fruit extracts, for example kiwi, papaya, and fresh fig all possess proteolytic activity.

Papain

Papain is another protease. It is an enzyme, originally extracted from papaya fruit, which breaks down proteins to smaller peptides and amino acids. Papain is the enzyme commonly used in meat tenderizers and in some contact lens cleaners. Meat tenderizers work by digesting collagen fibers. Collagen fibers give tissue strength and make meat tough.

Enzyme Preparation

Papain can be prepared from fresh papaya or used directly from a bottle of Adolf's Meat Tenderizer.

Papaya

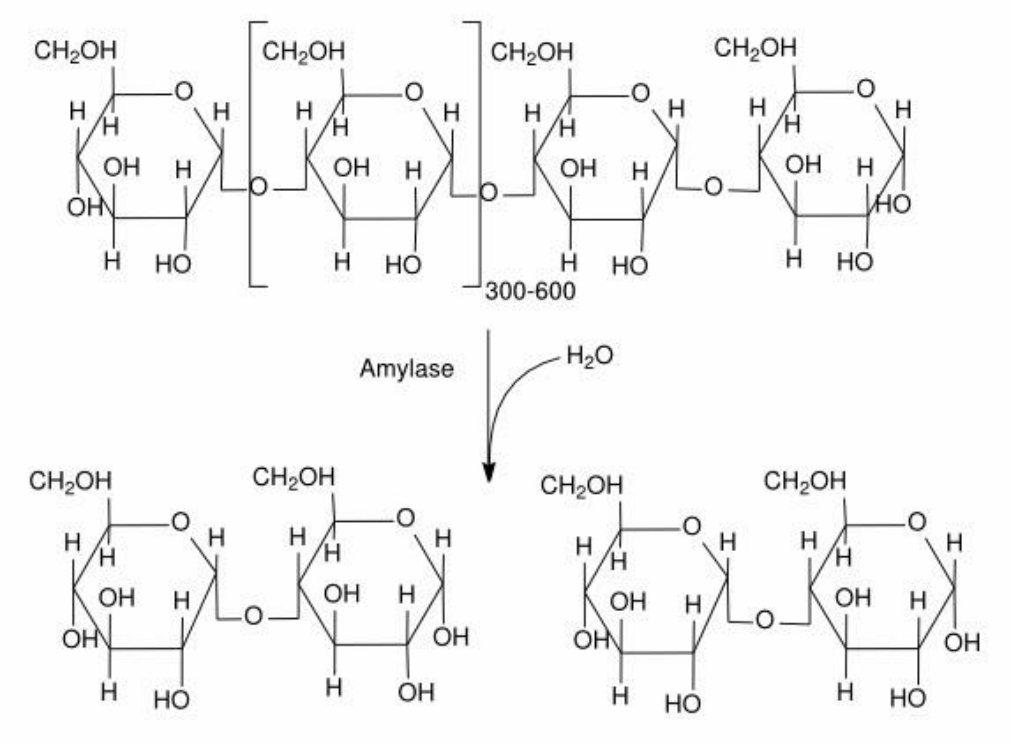
1. Peel a ripe papaya. Remove the seeds.
2. Slice the papaya into 1 cm rounds.
3. Chop 1 round into smaller pieces and add to blender.
4. Add 100 mL of water. Blend till homogenized.
5. Cover beaker loosely with 3 layers of cheesecloth or alternatively line a funnel with 3 layers of cheesecloth.
6. Pour the extract through the cheesecloth. Gently gather and squeeze the cheesecloth to extract more liquid.
7. Use extract immediately. Proteases will digest themselves, so the juice should be stored on ice and used immediately.

Adolf's Meat Tenderizer can be used directly and suspended according to the protocol provided.

Amylase

Amylase breaks down starch to a smaller form called dextrin and ultimately down to the disaccharide maltose, a reducing sugar. Starch is a large macromolecule (carbohydrate). Starch is also a major component of most diets. Starch cannot pass through the intestine to serve as nourishment; it is too

large. It must be broken down, hydrolyzed to smaller molecules before organisms can derive any nutrition from it. Salivary amylase, produced in the mouth and secreted in saliva, is the first step in the digestion of starch. Digestion begins in your mouth.



(https://commons.wikimedia.org/wiki/File:Amylase_reaction.png)

In humans amylase is secreted into saliva and into the digestive juices of the intestine by the pancreas.

Enzyme Preparation

Human Salivary Amylase

1. Spit into a test tube. Chewing sugarless gum or wax cubes may stimulate salivation.
2. When using human saliva, measure only the level of liquid, not the bubbles.

At the end of lab take precautions to dispose of all human fluids appropriately.

Amylase solution

1. Dissolve .1 gm of amylase in 100 mL of water.
2. Use immediately.