

Digestive Enzyme Lab

Objectives

1. To describe the enzymatic digestion of carbohydrates by salivary amylase
2. To describe the enzymatic digestion of protein by pepsin
3. To describe the emulsification of fat by bile salts
4. To understand the enzymatic digestion of fat by pancreatic lipase

Background Information:

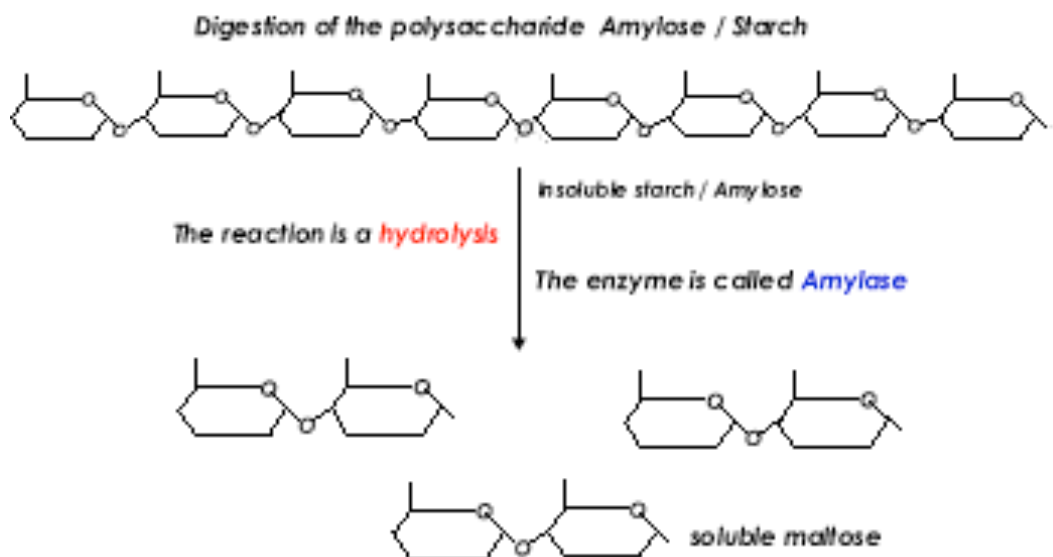
The digestive system breaks down food (complex polymers) into monomers through enzymatic digestion. Only very small molecules, such as monosaccharides or amino acids can be absorbed across the gut epithelia. This lab will examine the optima for 3 important digestive enzymes.

GENERAL NOTES:

This lab requires a lot of work at the beginning and the end of the lab period. BE ON TIME and work as a team to get ALL tubes incubating at once in order to get the best results. Use plastic tubes unless otherwise indicated.

Lab Exercise 1: Digestion of Starch by Salivary Amylase

The digestion of a carbohydrate such as starch begins in the mouth, where it is mixed with saliva containing the enzyme salivary amylase. Starch, a long chain of repeating glucose molecules, is hydrolyzed (cut) by amylase into shorter polysaccharide chains and eventually into the disaccharide maltose (known as a reducing sugar), which consists of two glucose subunits:

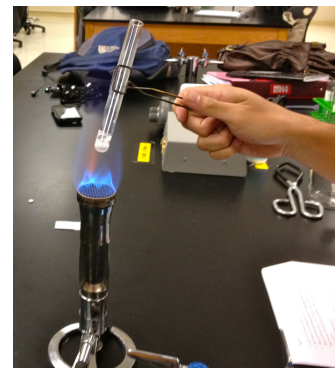


In this experiment, you will examine the effects of pH and temperature on the activity of salivary amylase. You will measure the activity of salivary amylase by measuring the amount of product formed using Benedict's reagent, which consists of an alkaline solution of cupric ions (Cu^{++}). Cupric ions will be reduced to cuprous ions (Cu^+) in the presence of maltose, forming a visible yellow-colored precipitate of cuprous oxide (Cu_2O).

Procedure: Use **plastic tubes** except for the one you will boil

1. Label 5 clean test tubes 1 – 5.
2. Obtain 10 ml of saliva (use a cup and then transfer using a disposable 1.0 ml transfer pipette.) Think about chocolate chip cookies if necessary. If this doesn't work, chew a piece of paraffin. **ONLY 1 PERSON PROVIDES SALIVA. NO MIXING!!!**
2. Add 3.0 ml of distilled water to tube 1.
3. Add 3.0 ml of saliva to tubes 2 and 3.
4. Add 3 drops of concentrated HCl to tube 3.
5. Bring to just a boil the remaining saliva in a **glass test tube** by passing the tube through the flame of a Bunsen burner. Use a test-tube clamp and keep the tube at an angle, pointed away from your face and from your neighbors. When it is cool, add 3.0 ml of the boiled saliva to tube 4.
6. Add 3.0 ml of maltose to tube 5 (tube 5 is a positive control for both starch and maltose).
7. Add 5.0 ml of starch to all 5 tubes.
8. Incubate all tubes in a 37°C water bath for *at least 1.5 hours*.
9. Label 5 new test tubes 1 – 5.
10. Divide the contents from the first set of 5 tubes in half by pouring half of the contents from each into the newly labeled tubes.
11. Test one set of 5 tubes for starch by adding a few drops of Lugol's reagent (containing iodine) to each tube. A positive result is indicated by the development of a purplish black color. Record your results.
12. Test the other set of 5 tubes for the presence of maltose by adding 5.0 ml of Benedict's reagent to each of the tubes and then immersing them in a rapidly boiling water bath for **2 minutes**. (Don't worry - the plastic tubes won't melt unless you leave them in the boiling water for 5 minutes).
13. Remove the tubes from the water bath and rate the result using the following scale:

Blue (no maltose)	-
Green	+
Yellow	++
Orange	+++
Red (most maltose)	++++

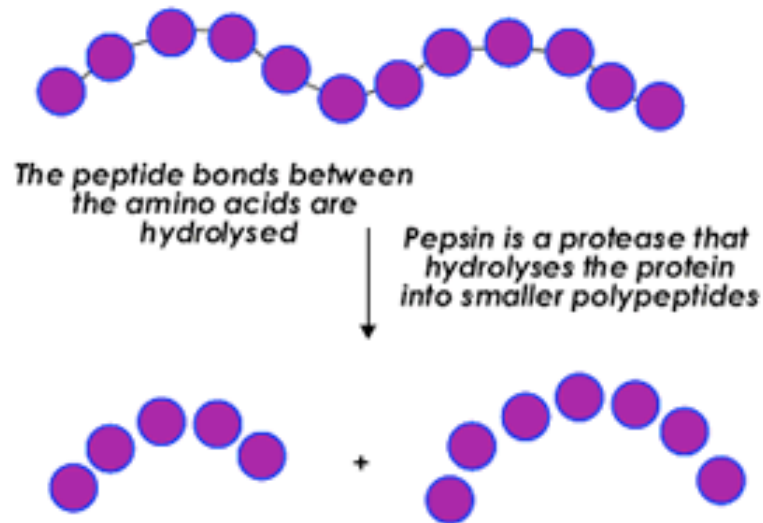


Record the results in your lab notebook:

Tube Contents	Starch After Incubation (Lugol's)	Maltose After Incubation (Benedict's)
Tube 1: starch + distilled water		
Tube 2: Starch + saliva		
Tube 3: Starch + saliva + HCl		
Tube 4: Starch + boiled saliva		
Tube 5: Maltose		

Lab Exercise 2: Digestion of Protein (Egg Albumin) By Pepsin

Pepsin is an enzyme that is secreted by the chief cells in the stomach which digests proteins. In this exercise, you will digest albumin, the major protein in egg whites.

Digestion of protein / polypeptide**Exercise 2 Procedure:**

1. Label 4 clean test tubes. Using a razor blade, cut 4 slices of egg white about the size of a fingernail and as thin as possible. It is ESSENTIAL that the slices be very thin and as uniform in size as possible. Place a slice of egg white in each of the five tubes.
2. Add 1 drop of distilled water to tube 1.
3. Add 2 drop of HCl to tubes 2 and 3 and 4.
4. Add 5.0 ml of pepsin to tubes 1, 2 and 3.
5. Add 5.0 ml of distilled water to tube 4.
6. Place tubes 1, 2, and 4 in a 37°C water bath. Place tube 3 in an ice bath or freezer. Incubate all tubes for at least 1.5 hours. Thaw the frozen tube after the incubation.

7. Record the appearance of the egg white in a data table in your lab notebook.

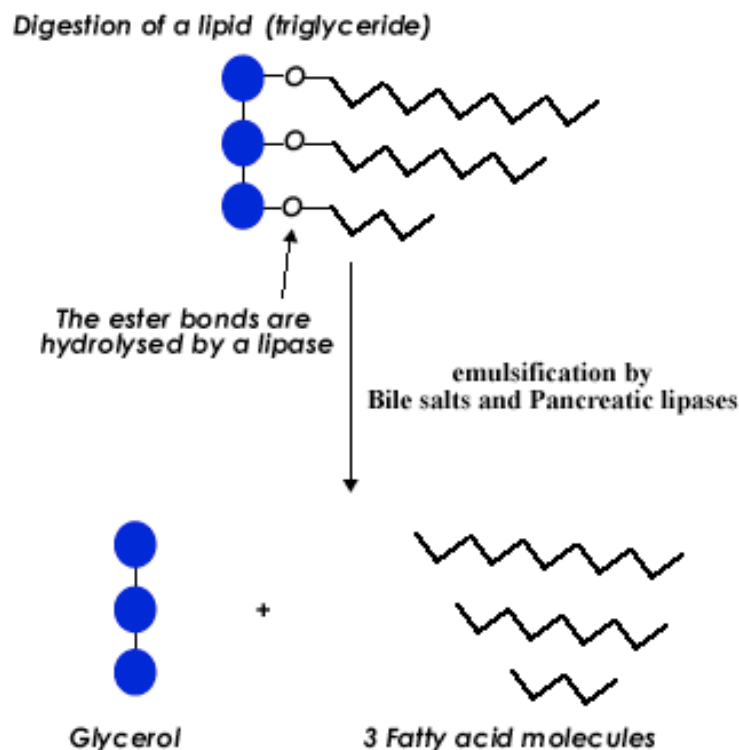
Tube Contents / Incubation Condition	Appearance of Egg White After Incubation (describe)
1: protein + pepsin, 37°C	
2: protein + pepsin + HCl, 37°C	
3: protein + pepsin + HCl, 0°C	
4: protein + HCl, 37°C	

Lab Exercise 3: Digestion of Fat (cream) by Pancreatic Juice and Bile Salts

Since fat is not soluble in water, dietary fat enters the duodenum in the form of large fat droplets which must be broken down into much smaller pieces before digestive enzymes can act upon them. There are two processes required for fat digestion:

Emulsification refers to the breakdown of large droplets into smaller droplets, (just as dishwashing detergents act on grease). Bile salts are responsible for this.

Digestion of fat into monoglycerides and fatty acids (accomplished by lipases, such as pancreatic lipase, which you will use today). You can measure the digestion of fats by lipases because as the fatty acids are produced by enzymatic breakdown, the pH of the solution drops.



Exercise 3 Procedure:

1. Add 3.0 ml of cream to three test tubes, numbered 1-3.
2. Add the following:
 - Tube 1: add 5.0 ml of water and a pinch of bile salts
 - Tube 2: add 5.0 ml of pancreatin solution
 - Tube 3: add 5.0 ml of pancreatin solution AND a pinch of bile salts
3. Check the pH of all tubes and record as 'time 0'

4. Incubate the tubes at 37°C for 1 hour, checking the pH every 20 minutes, recording the data in your lab notebook.

Time	Tube 1: Cream + Bile Salts	Tube 2: Cream + Pancreatin	Tube 3: Cream + Bile Salts + Pancreatin
0 minutes			
20 minutes			
40 minutes			
60 minutes			
80 minutes			
100 minutes			

Post-Lab Questions:**Salivary Amylase**

1. Which tube(s) contained the most starch following incubation? Which tube(s) contained the most maltose? What conclusions can you draw from these results?
2. Did you have any tubes which tested + for both starch and maltose? What does this mean? What might happen to the tube if you let it incubate for a longer period of time?
3. Reviewing your data, what do you think happens to salivary amylase once you swallow your saliva? Explain.
4. What effect does cooking have on enzyme activity? Why?

Digestion of Albumin by Pepsin

1. What can you conclude about the pH optimum for pepsin? Where in the body might you find this pH?
2. Compare the effects of HCl on protein digestion by pepsin with the effects of HCl on starch digestion by salivary amylase. Explain the physiological significance of these effects.
3. Why does freezing food preserve it?

Digestion of Fat by Pancreatic Juice and Bile Salts

1. Explain why the digestion of fats should affect the pH of the solution.
2. What is the function of bile salts?
3. In which tube did fat digestion occur most rapidly? Explain why.