Digestive Enzyme Lab

Objectives

1. To describe the function of enzymes
2. To define: reactants, products, activation energy
3. To describe the enzymatic digestion of carbohydrates by salivary amylase
4. To describe the enzymatic digestion of protein by pepsin
5. To describe the emulsification of fat by bile salts
6. To understand the enzymatic digestion of fat by pancreatic lipase

These demonstrations may be performed by your instructor or by a group designated by your instructor.

Demo 1: Pineapple Jell-O

Look at the box of Jell-O. Notice that there is a note on the side of the box which tells you that you cannot use fresh pineapple, papaya, figs or kiwi fruits in Jell-O salads (perhaps you have not yet tried to make Jell-O salad with fresh pineapple, papaya, kiwi fruit or figs, but let's just assume for the moment that this is something you wish to do). Why do these fruits prevent Jell-O from jelling?

In your lab group, list several possible reasons why these fruits prevent Jell-O from jelling, and write them in the lab assignment.

Once you have your possible explanations listed, observe the demonstration. Your instructor will prepare fresh gelatin and fill 6 tubes with the contents as listed below:

Tube 1: gelatin
Tube 2: gelatin + fresh pineapple
Tube 3: gelatin + fresh pineapple that was frozen and thawed
Tube 4: gelatin + canned pineapple
Tube 5: gelatin + fresh pineapple juice
Tube 6: gelatin + juice from canned pineapple

Fill in the data table after all tubes have chilled and answer the questions on the assignment sheet.

Demo 2: Liver Catalase Demonstration

Catalase is an enzyme that converts hydrogen peroxide (produced naturally by metabolic processes in the body) into water and oxygen gas:

$2H_2O_2$ ---> $2H_2O + O_2$

Your instructor will set up three beakers and add approximately 30 ml of hydrogen peroxide to each:

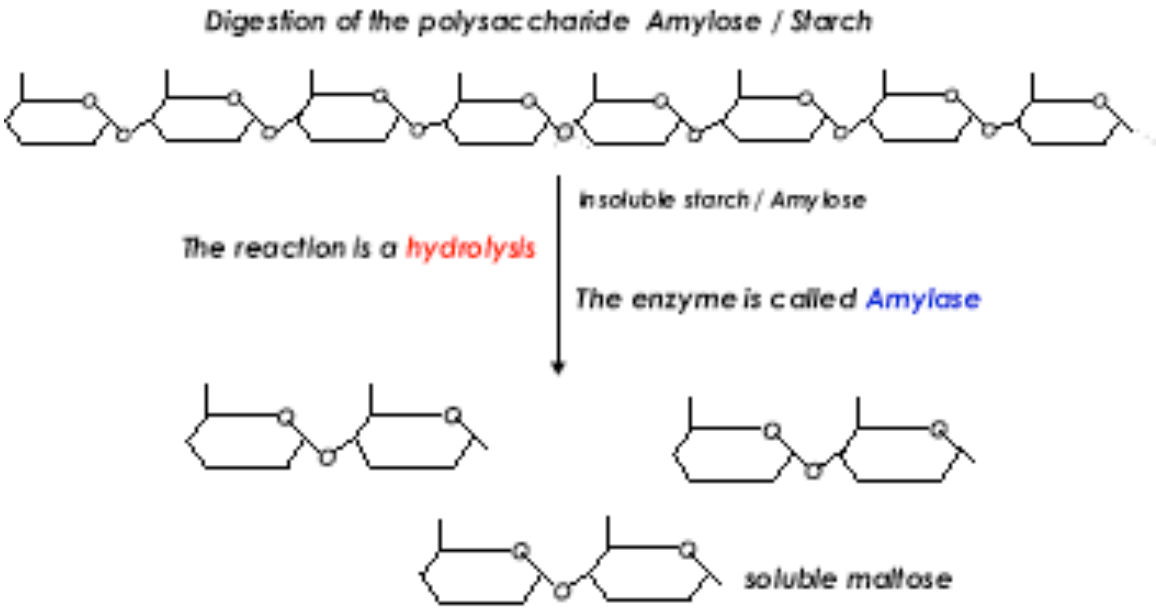
1. Beaker 1 will contain H_2O_2 + a rusty nail
2. Beaker 2 will contain H_2O_2 + fresh chicken liver
3. Beaker 3 will contain H_2O_2 + cooked chicken liver (optional)
4. Beaker 4 will contain H_2O_2

Fill in the data table and answer the questions on the assignment sheet.

NOTE: Work with your group to get all three of the following exercises incubating as quickly as possible. (Assign each person to set up one exercise for example).

Lab Exercise 1: Digestion of Starch by Salivary Amylase

The digestion of a carbohydrate such as starch begins in the mouth, where is it 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).

Exercise 1: Procedure: Use **plastic tubes** except for the one you will boil

1. Label 5 clean plastic 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 (use and keep HCl in the hood)
5. Transfer the remaining saliva in a **glass test tube** and bring it to a boil by passing the tube through the flame of a Bunsen burner (see Figure 1). 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.
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 (see Figure 2). Record your results in the assignment sheet.
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 (see Figure 3).
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)	++++

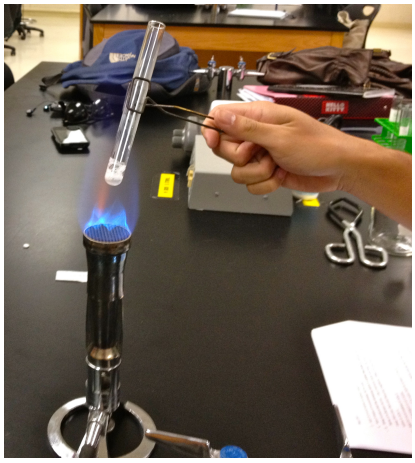


Figure 1.

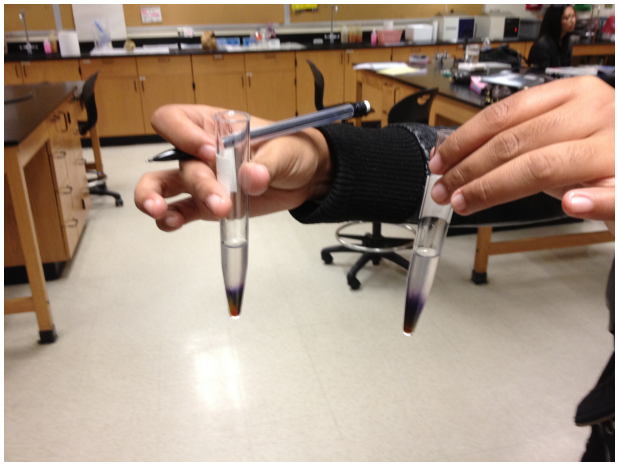


Figure 2.

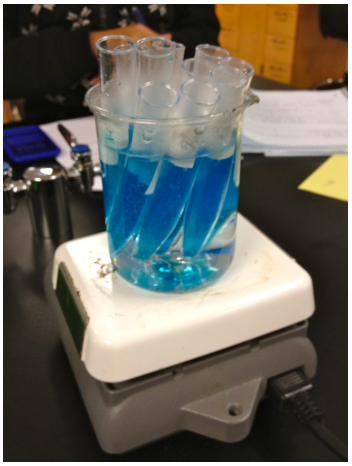
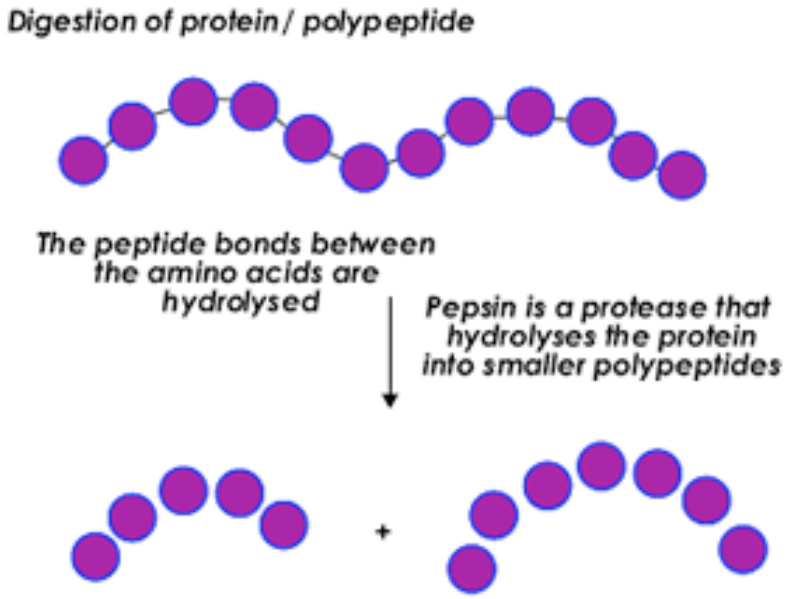


Figure 3.

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.



Exercise 2: Procedure:

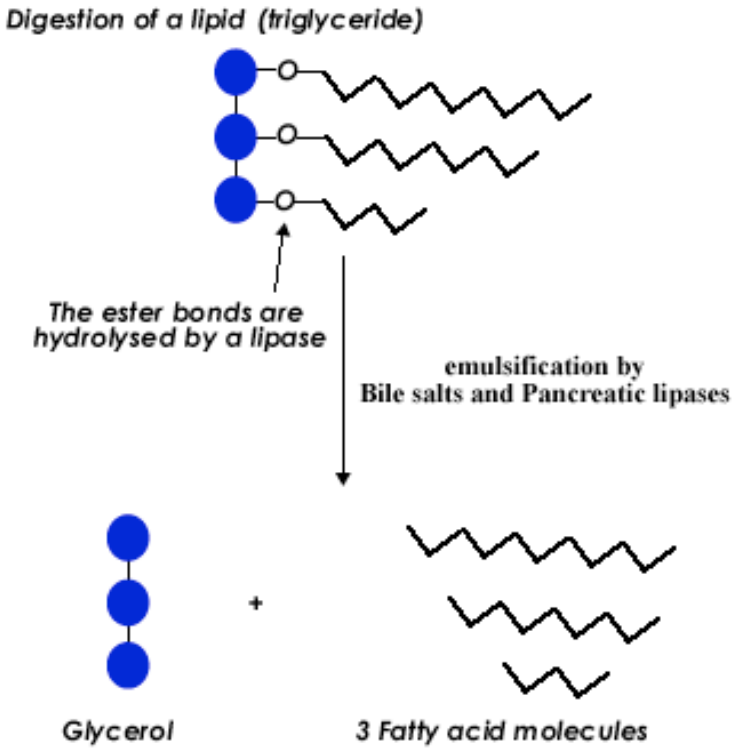
1. Label 5 clean test tubes. Using a razor blade, cut 5 slices of egg white about the size of a fingernail and as thin as possible. It is ESSENTIAL that the slices be very thin and as uniform in size as possible. Place a slice of egg white in each of the five tubes.
2. Add 1 drop of distilled water to tube 1.
3. Add 2 drop of HCl (in the hood) to tubes 2, 3 and 4.
4. Add 1 drop of NaOH (in the hood) to tube 5.
5. Add 5.0 ml of pepsin to tubes 1, 2, 3 and 5.
6. Add 5.0 ml of distilled water to tube 4.
7. Place tubes 1, 2, 4 and 5 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.
8. Record the appearance of the egg white in the data in your assignment sheet.

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 few grains of bile salts

   Tube 2: add 5.0 ml of pancreatin solution

   Tube 3: add 5.0 ml of pancreatin solution AND a few grains of bile salts

3. Check the pH of all tubes and record as 'time 0' by using wide-range pH paper first, then narrow-range pH paper. Please conserve the pH paper - it's expensive!!
4. **Incubate the tubes at 37°C for 100 minutes, checking the pH every 20 minutes,** recording the data in your assignment sheet.

Energy, Enzymes and Digestion – Assignment

Name: _____

Pineapple Demonstration

1. Possible reasons why pineapple, papaya, figs and kiwi fruit prevents Jell-O from jelling:

2. Fill in the data table below:

Tube Contents	Result
Gelatin	
Fresh pineapple + gelatin	
Fresh pineapple that was frozen + gelatin	
Canned pineapple + gelatin	
Fresh pineapple juice + gelatin	
Canned pineapple juice + gelatin	

3. What is the purpose of the tube containing only gelatin?

4. What prevents Jell-O from jelling with fresh pineapple?

Liver Catalase Demonstration

1. Fill in the data table below:

Beaker Contents	Result
1. H_2O_2 + rusty nail	
2. H_2O_2 + raw liver	
3. H_2O_2 + cooked liver	
4. H_2O_2	

2. What is the purpose of tube #4?

3. What did the results demonstrate about the conversion of hydrogen peroxide into water and oxygen? What did you learn about catalase?

Salivary Amylase

1. Fill in the data table below:

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		

2. Which tube(s) contained the most starch following incubation? Which tube(s) contained the most maltose? What conclusions can you draw from these results?

3. What does it mean when tubes tested + for both starch and maltose? What might happen to the tube if you let it incubate for a longer period of time?

4. Reviewing your data, what do you think happens to salivary amylase once you swallow your saliva? Explain.

5. What effect does cooking have on enzyme activity? Why?

Digestion of Albumin by Pepsin

1. Enter your observations in the table below:

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	
5: protein + pepsin + NaOH, 37°C	

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.

Digestion of Fat by Pancreatic Juice and Bile Salts

1. Record your data (pH readings) in the table below:

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

2. Explain why the digestion of fats should affect the pH of the solution.

3. What is the function of bile salts?

4. In which tube did fat digestion occur most rapidly? Explain why.