Digestive Enzymes (lab 10.2)

Background information on Digestive Enzymes

The background information for understanding today's exercise is found in lab 10.2 in your lab manual. Although we will use slightly different procedures to demonstrate digestive enzymes than the one described in lab 10.2, you should read lab 10.2 and answer the lab report questions in the lab report section.

a) Digestion of Starch Carbohydrate with Amylase Enzyme

In this activity, you will digest starch with amylase enzyme. Read section A in the lab manual for the background on this activity.

1) Obtain 4 clean test tubes and label them 1a, 2a, 3a, and 4a with a wax pencil.

2) On the front desk, add solutions to the test tubes using the following table:

	Tube 1a	Tube 2a	Tube 3a	Tube 4a
Starch (5 mL)	√	√	√	√
Water (3 mL)	√			
Amylase (3 mL)		√	√	
Boiled amylase (3 mL)				√
HCl acid (3 drops)			√	

3) Put all four tubes in a 37 degree water bath for 15 minutes. During the 15 minutes, do the following:

a) Obtain 4 clean empty test tubes. Label them 1b, 2b, 3b, and 4b.

b) Get a hotplate and a 300 ml metal beaker (these are stored in labeled cabinets under the back counter). Add 200 ml of water (from the sink) to the beaker and set it boiling.

4) After the 15 minute digestion of the starch is finished, pour half of tube 1a into empty tube 1b. Pour half of tube 2a into empty tube 2b. Pour half of tube 3a into empty tube 3b. Pour half of tube 4a into empty tube 4b.

5) To all four of the "b" tubes (1b, 2b, 3b, and 4b), add 20 drops to each of Benedict's reagent (a sky-blue solution in a dropper bottle). Then place all four of the "b" tubes into the boiling water for 2 minutes. After 2 minutes, use the table below to determine the amount of maltose disaccharide in each solution.

Color	**Amount of maltose**
Blue or clear	None
Green	Very little
Yellow	Some
Orange	Much
Red	Very much

Record the results in data table A on the last page of this handout.

6) To all the "a" tubes (tubes 1a, 2a, 3a, and 4a) add 3 drops of Gram's iodine solution. Mix by flicking the tubes at the bottom. Use the following table to determine the amount of starch in each solution.

Color	**Amount of starch**
Yellow or Clear	None
Gray	Some
Black//Dark blue	Very much

Record the results in data table A on the last page of this handout.

7) After having your instructor check your results, all solutions can be disposed of down the drain, except for the tubes with Benedict's reagent, which are poured into a special waste container. Lastly, wash out all test tubes with soapy water and use the test tube brush. The last rinse should be with deionized water.

b) Digestion of Protein (Egg Albumin) with Pepsin Enzyme

In this activity, you will digest boiled egg white (a protein called egg albumin) with an enzyme called pepsin. Read section B in the lab manual for the background on this activity.

1) Obtain 4 clean test tubes and label them 1 – 4 with a wax pencil.

2) On back counter, add solutions to the test tubes using the following table:

	Tube 1	Tube 2	Tube 3	Tube 4
Egg white (2 mL)	√	√	√	√
Pepsin (5 mL)	√	√	√	
Water (5 mL)				√
HCl acid (1 drop)		√	√	√

3) Put tubes 1, 2, and 4 in a 37 degree water bath for 15 minutes. Put tube 3 in an ice bath for 15 minutes.

4) After 15 minutes, inspect the egg white puree in each tube. Use the table below to determine the amount of digestion in each tube.

Egg white	**Amount of digestion**
Milky and opaque (can't see through)	Little or no digestion.
Slightly milky and semi-clear	Some digestion.
Not milky at all and fully clear	Very much digestion.

5) Record the results in data table B on the last page of this handout. After having your instructor check your results, all solutions can be disposed of down the drain. Lastly, wash out all test tubes with soapy water and use the test tube brush. The last rinse should be with deionized water.

c) Digestion of Triglyceride (Vegetable Oil) with Lipase Enzyme

In this activity, you will digest vegetable oil (a triglyceride) with an enzyme called lipase. The lipase enzyme is supplied to you in a pancreatic juice solution called pancreatin. Read section C in the lab manual for the background on this activity.

1) Obtain 3 clean test tubes and label them 1 – 3 with a wax pencil.

2) On the back counter, add solutions to the test tubes using the following table:

	Tube 1	Tube 2	Tube 3
Veg oil (3 mL)	√	√	√
Water (5 mL)	√	√	
Lipase (5 mL)			√
Bile salts (10 drops)		√	√

3) Mix thoroughly by flicking the bottom of all three tubes. Put all tubes in a 37 degree water bath for 30 minutes.

4) After 30 minutes, add 5 drops of phenol red pH indicator to all test tubes. Mix thoroughly. Use the table below to determine the amount of digestion by the pH in each tube.

Color of pH indicator	**pH**	**Amount of digestion**
Red or Pink	Neutral	Little or no digestion.
Orange-Pink	Slightly acidic	Some digestion.
Yellow	Acidic	Very much digestion.

5) Record the results in data table C on the last page of this handout. After having your instructor check your results, all solutions can be disposed of down the drain. Lastly, wash out all test tubes with soapy water and use the test tube brush. The last rinse should be with deionized water.

Data Table A

Tube:	Ingredients and temperature*	Iodine color:	Benedicts color:	Digestion analysis**
1				
2				
3				
4				

Data Table B

Tube:	Ingredients and temperature*	Milky:	Clear:	Digestion analysis**
1				
2				
3				
4				

Data Table C

Tube:	Ingredients and temperature*	Color:	Digestion analysis**
1			
2			
3			

* In this column, list all substances in the tube during the digestion and also list the temperature at which the tube was incubated. You do not need to list the volumes of the substances.

** For digestion analysis, write "Best digestion" for the tube that had the best digestion. For all other tubes, write a short phrase stating exactly **why** the digestion was not the best. In other words, state the specific environmental condition that was not optimum for the digestion. For example, "Temperature too high".