Purpose

Enzymes, as proteins, are subject to denaturing, which disrupts their tertiary molecular structure. Denaturing breaks the weak hydrogen bonds that link adjacent portions of the protein, thus, changing the tertiary structure. Upon denaturing, an enzyme permanently loses its catalytic abilities. Denaturing is caused by extreme changes in temperature and pH. Thus, enzymes function within very narrow limits of temperature and pH**.**

Procedures

3-A: Salivary amylase and the digestion of carbohydrates

1. Fifteen ml of saliva have been provided in a small beaker or graduated cylinder. Place 3 ml of saliva into a test tube and boil the contents for 15 minutes. Place 3 ml of saliva into a second tube and cool it in an ice bath. (Your instructor may have already prepared these two tubes. You may be instructed to use 10 drops = 1 ml.)
2. Prepare and label five test tubes as follows using a 0.5% starch solution:

Tube #1 – 3 ml starch + 3 ml distilled water 37 C water bath
Tube #2 – 3 ml starch + 3 ml saliva 37 C water bath
Tube #3 – 3 ml starch + 3 ml saliva (cooled) ice bath
Tube #4 – 3 ml starch + 3 ml saliva (boiled) 37 C water bath
Tube #5 – 3 ml starch + 3 ml saliva + 5 drops
concentrated hydrochloric acid (HCl) 37 C water bath

3. Incubate the tubes for one hour at the temperatures indicated.
4. After the tubes have incubated, pour half of each tube's contents into a new test tube. Test one set of tubes for starch using Lugol's solution and the other set for maltose using Benedict's solutions as follows:

Lugol's: Add 2 drops of Lugol's solution to 3 ml of incubated starch solution.
Benedict's: Add 2 ml of Benedict's solution to 3 ml of incubated starch solution; heat the starch/Benedict's solution for 5 minutes.

5. Lugol's solution, an amber iodine-containing reagent, will turn dark navy blue in the presence of starch. Benedict's solution is a blue cupric (Cu+2) solution, and when heated will be reduced to form a reddish precipitate of cuprous oxide (Cu_2O) in the presence of sugar. Color tests will be scored as follows:
7. Determine which tube has the most sugar and which has the most starch.
8. Observe the effect of HCl on enzyme activity. Determine an approximate pH range for ptyalin activity. Consider what this range tells you about the effectiveness of ptyalin once it is passed into the stomach.
9. Determine if any tubes have intermediate results between starch and sugar or if any tested positive for both. Consider what would happen if the tubes were left to incubate for a longer period of time.

3-B: Gastric digestion of protein

1. Finely chop egg white (albumin) into very tiny pieces. Weigh out approximately 0.1 g of egg white and add the 0.1 g of egg white to each of the eight test tubes.

2. Prepare two sets of tubes with the following solutions. Label the tubes A and B for each condition. Determine the pH of each tube:
Tube #1: 5 ml 5% pepsin + 5 ml 0.5% HCl
Tube #2: 5 ml 5% pepsin + 5 ml distilled water
Tube #3: 5 ml 0.5% HCl + 5 ml distilled water
Tube #4: 5 ml 5% pepsin + 5 ml 0.5% sodium hydroxide (NaOH)
3. Mix contents thoroughly by shaking or mixing with a glass rod.
4. Incubate all the tubes in a 37 C water bath for 1 hour. Gently stir each tube every 10-15 minutes while incubating. Test the final pH of the solutions and estimate the amount of protein digestion using a scale of (+++) for complete digestion, (++) for most but not complete digestion, (+) for partial digestion, and (-) for no digestion.
5. Summarize the results in the following table:
Tube Initial pH Final pH Estimated digestion
#1 A/B
#2 A/B
#3 A/B
#4 A/B
6. Determine which tube showed the most digestion of fiber and estimate the optimal pH of pepsin.
7. Compare the effects of HCl on protein digestion by pepsin with the effects of HCl on starch digestion by ptyalin (Ex. 3-A). Explain the physiological significance of these Effects.

3-C: Digestion of fat with pancreatic lipase and bile salts
1. Add just enough litmus powder to a container of dairy cream to produce a medium blue color. Pour 3 ml of the litmus cream into 4 separate test tubes. Into two additional test tubes pour 3 ml of 2% pancreatin. Preincubate the litmus cream and the pancreatin separately in a 37 C water bath for 5 minutes. Then prepare four test tubes as follows:
Tube #1: 3 ml cream + 3 ml pancreatin
Tube #2: 3 ml cream + 3 ml distilled water
Tube #3: 3 ml cream + 3 ml pancreatin + pinch of bile salts
Tube #4: 3 ml cream + 3 ml distilled water + pinch bile salts
2. Gently shake each tube for 30 seconds to mix in the bile salts. Incubate all four tubes in a 37 C water bath for 1 hour, checking every minute for the first 5 minutes or until the first tube changes color, then every 15 minutes for the rest of the hour. Record the time and number of the tube. Continue checking for the remainder of the hour.
3. Remove the tubes from the water bath. Test the pH of each tube using pH paper and note the odor and color of each tube.
NOTE: Blue litmus will turn pink in an acid environment.
4. Summarize the results in the following table:
Tube Color pH Odor Time to change color
#1
#2
#3

#4
5. Explain how the digestion of fat affects the pH of the solution and how bile affects the rate of digestion.

Results

Tube	**Color**	**pH**	**Odor**	**Time to Change Color**
#1	pink	5	Spoiled milk	20
#2	blue	6	Nothing	35
#3	lavender	3	Swiss cheese	35
#4	blue	6	Nothing	35

Discussion
Fats affect the pH of the solution by lowering pH and creating a foul smell. Bile affects the rate of digestion by raising pH and causing a color change. I was able to record my data on a table.

Conclusion
The emulsification of fats is easier to digest with bile enzymes.