

CHAPTER 6	Investigation 6.B: Optimum pH for Two Protease Enzymes	BLM 6.2.9
HANDOUT		
Question: How can you use pH to determine which protease, pepsin or trypsin, is secreted into the stomach and which protease is secreted into the small intestine?		

Hypothesis

State a suitable hypothesis for this investigation.

Safety Precautions



- Hydrochloric acid is a strong acid, and sodium hydroxide is a strong base. Both are very corrosive and must not be mixed together.
- Other chemicals used in this investigation may be toxic. Take extra care to avoid getting them in your eyes, on your skin, or on your clothes. Flush spills immediately with plenty of cool water, and inform your teacher.

Materials

- 18 cubes of boiled egg white (protein samples)
- 10 mL distilled water
- 10 mL 2% pepsin solution
- 10 mL 5% trypsin solution
- 15 mL dilute hydrochloric acid (0.01 mol/L)
- 15 mL dilute sodium hydroxide (0.01 mol/L)
- wax pencil
- 6 test tubes
- test-tube rack
- water bath or incubator at 37 °C
- metric ruler
- 10-mL graduated cylinder
- test-tube holder

Procedure

1. Use a wax pencil to label the test tubes as follows: C-2, C-8, P-2, P-8, T-2, and T-8. The test tubes labelled C are your controls. The test tubes labelled P will contain pepsin, and the test tubes labelled T will contain trypsin. The numerals indicate the pH of the contents of the test tubes.
2. Put three cubes of boiled egg white into each test tube. Observe the size and appearance of the cubes in each test tube. Record your observations in the data table below.

Size and Appearance of Egg White Before and After Digestion by Pepsin and Trypsin

Test tube	Before digestion		After digestion	
	Size of cubes	Appearance of cubes	Size of cubes	Appearance of cubes
C-2				
C-8				
P-2				
P-8				
T-2				
T-8				

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3. Add 5 mL of distilled water to each test tube labelled C. Add 5 mL of pepsin solution to each test tube labelled P. Add 5 mL of trypsin solution to each test tube labelled T.
4. Add 5 mL of dilute hydrochloric acid to each test tube labelled 2. Add 5 mL of dilute sodium hydroxide solution to each test tube labelled 8.
5. Place the test tubes in a water bath or incubator at 37 °C, and leave them overnight. The temperature must be maintained between 35 °C and 39 °C during this time.
6. Observe the contents of the heated test tubes. Note any changes in the size and appearance of the cubes of egg white in each test tube. Record your observations in your data table.
7. Dispose of the contents of the test tubes as directed by your teacher, and clean up your work area.

Analysis

1. How did the contents of the test tubes with protease differ from the control test tubes?

2. a) At what pH did pepsin break down protein more completely?

- b) At what pH did trypsin break down protein more completely?

Conclusions

3. Based on your results, which protease would break down protein in the small intestine? Justify your answer.

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4. Based on your results, which protease would break down protein in the stomach? Justify your answer.

Extensions

5. a) What is the significance of maintaining the temperature of the test tubes between 35 °C and 39 °C?
- b) Based on the experimental design, describe the degree of certainty you have in the conclusions you stated in questions 3 and 4. (**Hint:** Think carefully about all the conditions and variables you tested in this investigation. Identify any possible sources of error, both in the experimental design and the way you conducted it.)
6. What, if any, difference do you think there would be if you manipulated a different variable, rather than pH? Design a procedure to investigate the possible impact of manipulating a different variable. With your teacher's permission, and with careful attention to safety, carry out your procedure.