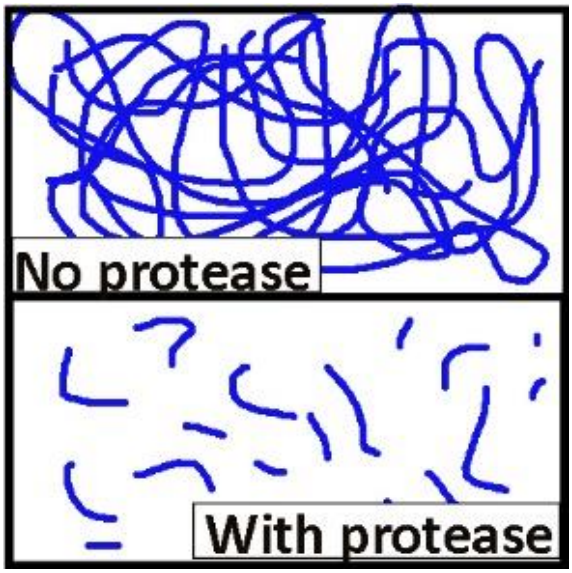
The Effect of pH on Enzyme Activity – Papain

As you recall from reading the section on naming and preparation of enzymes, papain is a protease originally extracted from papaya that breaks down protein to produce smaller molecules called peptides and even smaller monomer units, called amino acids. Papain is the active agent in meat tenderizers like Adolph's Meat Tenderizer. Meat tenderizers work by digesting collagen, the long, tough, fibrous protein that threads through meats and holds them together. Meat tenderizers pre-digest your meat!

In today's activity you will be examining the effect of pH on enzyme activity using pH buffers, the enzyme papain and gelatin. Acid and base functional groups compete with hydrogen bonds and alter the ionization of R-groups that are critical to protein structure. By competing with established bonds within the molecules and altering existing bonds, pH changes can potentially change the shape of the active site and therefore potentially change the activity of the enzyme. Protein structure involves at least three levels of structure and in some cases a fourth level of structure. Primary structure is a linear listing of the amino acids in a peptide. Secondary structure (alpha helix, beta pleated sheet) is created by the interaction of polar entities within the individual amino acids and the formation of hydrogen bonds. Tertiary structure results from the interaction of the R-groups. Quaternary structure results from the interaction of peptides.

Gelatin is a long fibrous protein that gels by forming crosslinks. When gelatin is exposed to the protease, the protease hydrolyzes the protein's peptide bonds. The protein is broken down into small peptides and amino acids. The smaller components cannot crosslink to form the gel matrix, the gelatin cannot gel.



Gelatin a long, fibrous protein will crosslink to itself under normal conditions producing a matrix or gel (top panel). When exposed to a protease such as bromelain or papain, the gelatin molecules are chopped up and can no longer crosslink or form the matrix or gel (bottom panel).

When doing enzyme studies researchers can either look for the presence of the enzyme's product or the absence or disappearance of the enzyme's substrate. In the activity today, you will be looking indirectly for the absence of intact substrate.

Materials:

Gelatin – in a hot bath
Test tubes – 5
Marker
Test tube rack
pH buffers
Meat tenderizer
1/8 Tsp measuring spoon

1. Pick up 5 test tubes and test tube rack from the supply table.
2. Label test tubes, 2, 4, 7, 10 and 12. Mark each tube at 1 and 3 cm from the bottom of the tube. Label each test tube with the group members' names.
3. Add 1/8 Tsp of meat tenderizer to each test tube.
4. Fill each tube to the 1 cm line with the appropriate buffered solution, e.g., pH 2 buffer in the pH 2 test tube. Gently swirl the tube to dissolve the meat tenderizer. Allow the tubes to sit on the benchtop for 10 minutes. Swirl the tubes occasionally.
4. After 10 minutes, fill all 5 tubes up to the 3 cm mark with warm gelatin.
5. Swirl tubes gently. Allow tubes to incubate on the benchtop for 15 minutes.
6. After the 15 minute incubation period, place all of the tubes in the ice bath.
7. After 10-15 minutes in the ice bath, remove the tubes. Tip the tubes slightly to determine if the gelatin jelled.

Record your results below.

	Did the gelatin gel?	Was papain active?
2		
4		
7		
10		
12		

1. What was your independent variable/s?

2. What is your dependent variable?

3. What was the apparent pH optimum? How can you tell?

4. Name the enzyme.

5. Name the substrate.

6. Name the products of the enzyme.