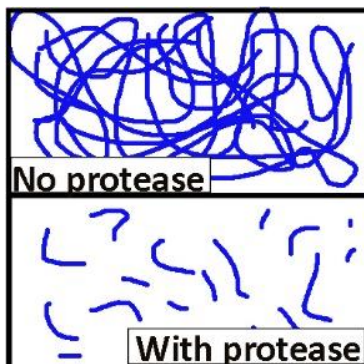


The Effect of Concentration on Enzyme Activity – Papain

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 temperature on enzyme activity using the papain and gelatin. 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.

Enzymes have evolved to function optimally under specific conditions of pH, temperature and concentration. When enzymes are exposed to conditions well outside their optimum, their activity is impacted. The conditions can be such that the enzyme is permanently damaged or denatured and ceases to function. Conversely, the enzymes activity may be decreased only during the time in which the conditions have been altered. For example, we refrigerate food to slow down spoilage. Bacteria cause spoilage. In the refrigerator the cold conditions inhibit microbial enzymes and slow down bacterial replication and metabolism. If you take something out of the refrigerator and leave it on the counter all day, bacteria and fungi in the food product warm-up and start to actively grow and metabolize. Bacteria replicate very quickly and can spoil food quickly. That is why food scientists recommend that food be thawed in the refrigerator, not on the countertop.

Cold temperatures can disrupt internal bonding in proteins and affect the flexibility of the molecule needed for catalysis. However, the decline in enzyme activity is more likely due to kinetic changes caused by temperature. There simply are fewer interactions occurring between the active site and the substrate. High temperatures up to a point can increase the interactions between the active site and the substrate and can actually increase enzyme activity. At a certain point however, temperature increases damage enzymes/proteins by breaking internal bonds or causing different bonding arrangements within the peptide that lead to denaturation.

Materials:

Gelatin – in a hot bath

Test tubes – 3

Marker

Test tube rack

Ice bath

Meat tenderizer

1/8 Tsp measuring spoon

Hot plate

Distilled water

500 mL beaker, boiling beads

1. Pick up 3 test tubes and test tube rack from the supply table.
2. Turn on the hot plate. Place the beaker containing water and boiling beads on the hot plate.
3. Label test tubes, C, 1, and 2. Mark each tube 3 cm from the bottom of the tube. Label each test tube with the group members' names.
4. Add 2/8 Tsp of meat tenderizer to tube 1 and 2. Add enough distilled water to tube 2 to cover the meat tenderizer, swirl the tube to dissolve the meat tenderizer and then immediately place the tube in the boiling water bath. Boil the tube for at least 5 minutes. Boil **ONLY** tube 2.
5. Retrieve tube 2 from the boiling water bath.
6. Add distilled water to cover the meat tenderizer in tube 1. Add water to tube 2 if necessary to replace any water that evaporated during boiling.
7. Add gelatin to tubes C, 1, and 2 to the 3 cm line. Swirl the tubes to mix the contents and allow the contents to incubate at room temperature for 15 minutes.
8. After 10 minutes place all of the tubes in the ice bath.
9. Leave the tubes in the ice bath for 15 minutes, then remove the tubes. Tip the tubes slightly to determine if the gelatin gelled.
10. Record your results below.

	Enzyme quantity	Was the tube boiled? (yes, no)	Did the gelatin gel?
C			
1			
2			

1. What was your independent variable/s?
2. What is your dependent variable?
3. What is the control?
4. What was the effect of boiling tube 2 on enzyme activity?

5. Name the enzyme.

6. Name the enzyme's substrate.

7. Name the enzyme's product.