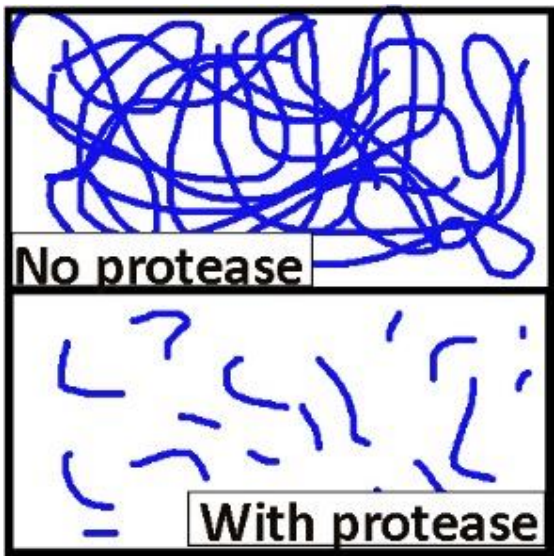
The Effect of Concentration on Enzyme Activity – Papain

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 such as 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 enzyme concentration on enzyme activity using the enzyme papain and gelatin. Gelatin is a long fibrous protein that gels or produces a matrix by forming crosslinks.




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 typically look for the presence of the enzyme's product or the absence or disappearance of the enzyme's substrate. When gelatin is exposed to a 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. In the activity today, you will be looking indirectly for the products of the enzyme.

Materials:

Gelatin – in a hot bath
Test tubes – 4
Marker
Test tube rack
Ice bath
Meat tenderizer
1/8 Tsp measuring spoon

1. Pick up 4 test tubes and test tube rack from the supply table.
2. Label test tubes, C, 1, 2, and 3. Mark each tube 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 tube 1. Add 2/8 Tsp meat tenderizer to tube 2. Add 3/8 meat tenderizer to tube 3.

4. Fill all 4 tubes up to the 3 cm mark with warm gelatin.
5. Swirl tubes gently to dissolve meat tenderizer. Allow tubes to incubate on the benchtop. If there are multiple groups in the classroom your instructor may assign each group an incubation time length.
6. After incubating the test tubes on the bench top for 15 minutes place all of the tubes in the ice baths.
7. After 15 minutes in the ice bath, remove the tubes. Tip the tubes slightly to determine if the gelatin gelled.
8. Record your results below.

Record the results below.

| | Enzyme quantity | Time of Incubation | Did the gelatin gel? |
|---|---|---|---|
| C | | | |
| 1 | | | |
| 2 | | | |
| 3 | | | |

1. What was your independent variable/s?

2. What is your dependent variable?

3. What is the control?

4. What was the effect of increased concentration?

5. Name the enzyme.

6. Name the substrate.

7. Name the products of the reaction.