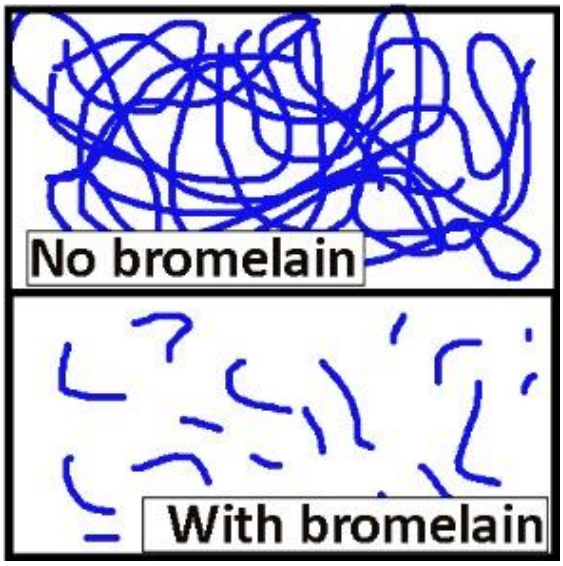
Effect of Temperature on Enzymes – Bromelain

As you recall from reading the section on naming and preparation of enzymes, bromelain is a protease found in pineapples. As a protease, bromelain breaks down peptide bonds in proteins. In the case of gelatin, bromelain breaks down gelatin to produce short peptides and amino acids. These peptides and amino acids can no longer crosslink to form the gelatin matrix. If you haven't read the section on naming and preparing enzymes, please read it now before proceeding with the lab.



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, the gelatin molecules are chopped up and can no longer crosslink or form the matrix or gel (bottom panel).

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 enzyme 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 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 peptides that lead to denaturation.

The activities in today's lab are designed to exemplify the effect of temperature on bromelain activity. You will be using fresh pineapple juice, canned pineapple juice, boiled fresh juice and frozen juice. Write a hypothesis for your experiment here:

_____

Materials

Test tubes – 5
Distilled water
Pipettes
Fresh pineapple juice
Prepared liquid gelatin (warm)
Canned pineapple juice
Ice bath
Hot plate
Beaker (500 mL) with boiling beads
Beaker for storing test tube in freezer
Test tube clamps
Sharpie
Test tube rack
Small beaker - 25 mL
Ruler

Procedure

1. Plug in the hot plate. Fill the 500 mL beaker half full, place it on the hot plate. Turn the hot plate about ¾ of the way on. This is your boiling water bath.

2. Pick up 5 test tubes and a test tube rack.

3. Label the test tubes as follows: water, fresh pineapple, canned pineapple, fresh boiled, fresh frozen. Mark the test tubes 3 cm from the bottom.

4. Pour about 5 mL of fresh pineapple juice into a small beaker and take it back to your bench.

5. Use a pipette to add 20 drops of water to the test tube labeled water. Use the same pipette to add 20 drops of fresh pineapple to the test tube labeled fresh pineapple, add another 20 drops of pineapple juice to the test tube labeled fresh boiled and another 20 drops to the test tube labeled fresh frozen.

6. Rinse the beaker containing pineapple juice. Pour a small amount (~1 mL) of canned pineapple juice into the beaker. Return to your bench.

7. Use a new pipette to pipette 20 drops of canned pineapple juice into the test tube labeled canned pineapple.

8. Place the test tube labeled 'fresh boiled' into the boiling water bath. Boil the tube for at least 5 minutes. After 5 minutes remove the tube using the test tube clamp and place the tube back in the test tube rack.

9. Place the test tube labeled 'fresh frozen' in the freezer for 20 minutes. If a freezer is not available, then place the tube in an ice bath (ice bucket). Make sure your tubes are labeled with your group name. After 20 minutes return the tube to your test tube rack.

10. Fill all of your test tubes to the 3 cm mark with the warm gelatin. The gelatin should be warm and liquid. Swirl the test tube to mix the water, and juices with the gelatin. Replace the tubes in the test tube rack. Allow the tubes to incubate on the benchtop for 20 minutes.

11. Place all of the tubes in the ice bath for 15 minutes. Remove the tubes from the ice bath and tip gently to note if the gelatin has gelled. Record the results below.

Test tube contents	Did the gelatin gel?	Was bromelain active?
water		
Fresh juice		
Canned juice		
Fresh boiled juice		
Fresh frozen juice		

1. How do you explain your results?

2. Was your hypothesis supported?

3. What was your dependent variable?

4. What was/were your independent variables?

5. Name the enzyme.

6. Name the substrate.

7. Name the product/s?

8. Why do manufacturers recommend that fresh pineapple not be added to gelatin desserts?

9. You absolutely want pineapple in your gelatin dessert. How can you do that without ending up with gelatin soup?

10. What would happen if fresh pineapple were added to yogurt?