Enzymes (Plants, Fungi, and Animals)

SC Academic Standards: 6.L.4; 7.L.3A; H.B.2A; H.B.3A;

NGSS DCI: PS3.D; HS-LS1.A; HS-LS1.C; HS-LS1-7

Science and Engineering Practices: S.1A.1; S.1A.2; S.1A.3; S.1A.4; S.1A.5; S.1A.6; S.1A.7

Crosscutting Concepts: Patterns; Cause and Effect: Mechanism and Explanation; Structure and Function; and Stability and Change.

Focus Question(s): How do enzymes work? What affects the rate of an enzyme-mediated reaction?

Conceptual Understanding: The essential functions of a cell involve chemical reactions that take place between many different types of molecules (including carbohydrates, lipids, proteins and nucleic acids) and are catalyzed by enzymes.

Background: Enzymes are important organic molecules in all living things – they are proteins, composed of amino acid chains, and they catalyze (speed up) exergonic (spontaneous) reactions, allowing them to occur at temperatures within the range tolerated by living cells. Millions of metabolic reactions occur in living bodies (plants and animals and bacteria!) and these reactions could not occur without enzymes. Once such reaction is aerobic cellular respiration; another is digestion of milk products, a third is the conversion (hydrolysis) of starch to sugar, which happens in seeds as they germinate, and a fourth is the decomposition of hydrogen peroxide in the cells of all living things. There are millions of enzymes, and each enzyme has its own specific shape; this three – dimensional shape is critical to the enzyme's function. An active site is found somewhere on the surface of each enzyme and the substrate, or reactants, that fit (bind) into the active site can then be converted to product without the change or destruction of the enzyme itself (in fact, after the product is formed, the enzyme can be reused to convert more substrate to product). The active site often changes shape to mold around the substrate in a way called an "induced fit" (previously thought of as a more rigid lock and key model).

Enzymes are abundant in the aerobic cellular respiration process. During **aerobic** cell respiration, **oxygen** is needed to release the stored energy of glucose, and CO_2 and H_2O are produced. The reaction for aerobic cell respiration is shown here:

$C_6H_{12}O_6 + O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + 36 or 38 ATP chemical energy + heat Energy

However, this isn't a simple one step process - there are 4 parts to aerobic cellular respiration (glycolysis, pyruvate oxidation, the Krebs (or citric acid) cycle, and chemiosmosis / oxidative phosphorylation / electron transport chain) and each part consists of multiple enzyme-mediated steps. For example, the first step in glycolysis is the phosphorylation of the glucose ring – a reaction that occurs with the help of the enzyme hexokinase, resulting in glucose-6-phosphate (G6P). In the second step of glycolysis, you see the conversion of glucose-6-phosphate to fructose-6-phosphate (F6P). This reaction occurs with the help of the enzyme phosphoglucose isomerase (PI). And so on.

Since plants and animals both undergo aerobic cellular respiration in their mitochondria (indeed, both plants and animals have mitochondria), you would expect to see the enzymes needed to mediate the cellular respiration reactions in both types of organisms. Often it is easiest to assume the enzymes are present if the substrate does get converted to product (which would not occur if the enzymes were lacking).

In plants, you can test for the presence of hydrolysis enzymes (such as amylase) with a glucose indicator and you can test for the enzymes required by aerobic cellular respiration (needed to make ATP to power the cell) using an indicator for the product CO_2. Here, the complex carbohydrate starch (a good energy rich storage form found in seeds) is converted (hydrolyzed, using the enzyme amylase) to glucose sugar (which is a smaller carbohydrate and which is an easier starting point for aerobic cellular respiration). Then, aerobic cellular respiration occurs and the glucose (plus oxygen) is converted into water and CO_2 and ATP using a variety of enzymes. And because carbon dioxide is a product of aerobic cellular respiration of we can infer the presence of enzymes if CO_2 is produced. Seeds in a dormant state (dry) are not undergoing hydrolyzing starch in order to begin aerobic cellular respiration, but seeds that have water and good temperatures will (note: light is not a requirement for germination you can test this too by trying to germinate seeds in the dark (mimicking being under soil!)).

The increasing concentration of CO_2 in a closed environment can be observed using a pH indicator solution, as when the CO_2 dissolves in a water solution it is converted to carbonic acid, which makes the solution more acidic (lower in pH). To observe the formation of CO_2 you can use an indicator solution (bromothymol blue – which turns yellow in the presence of CO_2, or when pH lowers). It may be good to start this inquiry by discussing indicator solutions (like the yellow-brown iodine, which, when placed on a starchy potato, turns blue-black to indicate the presence if starch). Using a straw and a test tube or cup filled halfway with water and bromothymol blue, blow into the blue solution until it turns, first green, then yellow. Be careful not to blow to hard – BB stains clothing!

In animals, the enzyme lactase (note that many enzymes, though not all, end in the suffix –ase) breaks down the disaccharide milk sugar lactose into the monosaccharides glucose and galactose. People who are lactose – intolerant don't

make this enzyme, and so drinking or eating milk products gives them stomach aches (unless they take a pill containing lactase). Since glucose can be tested for with urine test strips, we can test milk that has the enzyme lactase added to it to see if the lactose has been converted to glucose.

Yeasts, as well as all living things, also make an enzyme called catalase. This enzyme will help to break down (decompose) hydrogen peroxide, a waste product of cellular metabolic processes (including the electron transport chain in aerobic cellular respiration and in photosynthesis). Hydrogen Peroxide, H_2O_2, happens to be toxic, so having catalase is a good and necessary thing. Catalase converts hydrogen peroxide into water and oxygen:
$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$. We can detect the presence of catalase by soaking filter paper discs with catalase (produced by yeast cells), and dropping the soaked discs into a hydrogen peroxide solution. The filter paper discs initially sink, but, as decomposition of hydrogen peroxide occurs, oxygen gas production causes the filter paper to float.

In this lab we will investigate how enzymes work by first, testing that enzymes are present in animal and plant systems and second by studying factors that affect the rate of enzyme-mediated reactions.

Materials:
Part 1: each group needs 60 dry pea seeds (take 30 of these and soak in wet paper towels for 2-3 days prior to the experiment), 3 closed containers (old mayonnaise or peanut butter jars), water, 3 test tubes (that will fit in closed container), paper towel, water, Bromothymol Blue solution, benedicts solution, 2 test tubes (and test tube rack, and holders), mortar and pestle, hot plate, 500 ml beaker, paper towels.
Part 2: liquid lactaid (1 oz, $20 on Amazon.com), toothpicks, spot plates, droppers, whole milk, glucose solution, glucose test strips (urine test strips for diabetic sugar testing)
Part 3: 3% hydrogen peroxide (brown bottle at drugstore), baker's yeast, filter paper, hole punch, 1-liter flask, 500 ml beaker, 6-9 oz plastic cups, tweezers, timer, and for student choice experiments some of the following: salt, pH paper, HCl / NaOH, graduated cylinders, ice, hot plates, thermometers
Part 4: fresh and frozen pineapple, Jell-O (plain), beakers, ice, boiling water, test tubes / racks, spoons, stirring rods, knife for cutting pineapple, meat tenderizer.

Procedure: Part 1 (Plant Enzymes – Amylase and Starch; CO_2 production)

To test for amylase enzyme converting starch to sugar:
1. Take 5 dry seeds and crush with mortar / pestle.
2. Add crushed seeds to a test tube containing 5 ml of water and 10 drops of benedict's solution.
3. Repeat steps 1 and 2 with 5 germinated (soaked) seeds.

4. Put both test tubes in a hot (slow boil) water bath (beaker on a hot plate will do, but add paper towel to inside of beaker so boiling water doesn't shake test tubes to the point of cracking).
5. Leave tubes in the water bath for ten minutes. A positive benedict's reaction, indicating presence of glucose sugar, is a greenish –to brownish orange color (from the original sky blue).

   Test tube 1: Initial color _____ Color after boiling 10 minutes: _____

   Test tube 2: Initial color _____ Color after boiling 10 minutes: _____

   (**note**: talk to your kids about qualitative versus quantitative measurements).

Next, to test for production of CO2 as a result of aerobic cellular respiration (glucose + O_2 → CO_2 + water)

1. Take about 50 pea seeds – 25 are dry and 25 have been soaked for 4 days in wet paper towels, and are germinating,
2. Put the 25 dry seeds in a closed container (like a cleaned peanut butter jar).
3. Place a test tube with water and bromothymol blue in the container as well, and then seal with the top.
4. Take the 25 germinating seeds and repeat, in a new container, with a second test tube of BB/water, and seal that top.
5. Last, make a container that has no seeds, add the test tube of BB/water, and seal it.
6. Talk about controlled variables, like same size / type container in all three set-ups, and talk about control experiments. This is also a good time to talk about qualitative (color, or feel, or sound for example) versus quantitative data (good hard numbers).
7. Observe the test tubes over the next few hours, and days. In the container with germinating seeds, the color of the liquid in the test tube should turn yellow as CO_2 from the germinating seeds is released. You can make a data table like this, recording the color of the test tube each day:

Date	**Jar with dry beans**	**Jar with germinating beans**	**Jar with no beans**
Day zero			
Day 1			
Day 2			
Day 3			
Day 4			

Table 1. Carbon dioxide production by germinating seeds as seen by color change.

Procedure: Part 2 (Animal Enzymes – Lactase and Milk)

1. Get a spot plate and label 4 wells: a) treated milk b) untreated milk c) liquid lactase solution and d) glucose solution.
2. Place 2 drops of whole milk in the wells labeled a) treated milk and b) untreated milk
3. Place 1 drop of the liquid lactase solution in the wells labeled a) treated milk c) liquid lactase solution. Use a toothpick to stir well a).
4. Place one drop of glucose solution in the well labeled d) glucose solution.
5. Test all 4 solutions for the presence of glucose using Clinistix glucose test strips. Put a clean, unused, strip into each well for one second, then remove - after ten seconds compare the color on the test strip to the bottle to get a reading.

Solution	**Result of Glucose Test Strip**
a) treated milk	
b) untreated milk	
c) liquid lactase solution	
d) glucose solution	

Table 2. Glucose test for milk products.

Procedure: Part 3 (Yeast and Catalase Enzymes)

1. Activate the yeast by dumping the contents of one packet in about 400 ml of warm water – it will be ready in 30 minutes. Prepare this in advance!
2. Prepare a Hydrogen Peroxide solution by diluting store-bought 3% H_2O_2 with tap water: about 10 ml of 3% peroxide and 1000 ml of water. You can adjust this – sometimes if the peroxide isn't fresh, you may need to add more if it takes a long time for the reaction to work, add more peroxide.
3. Use a hole punch to create 30 discs from your filter paper.
4. Soak 10-15 discs in the yeast solution. Yeast, like most living cells, make the enzyme catalase in order to decompose the toxic hydrogen peroxide into water and oxygen. It is just easier for us to get it from the yeast solution, rather than from our bodies!
5. Add the filter paper discs, one at a time, to a cup containing about 50 ml of dilute hydrogen peroxide. It should sink. Time how long it takes to float to the surface. When it is at the surface, add the next catalase – soaked filter paper disc and time it – thus getting replicates. Do this ten times. (If it takes longer than 2 minutes to float, add some more peroxide to your solution).

6. Plan an investigation: what factors will affect the rate of the enzymatic reaction (how long it takes catalase to decompose the peroxide into water and oxygen, as denoted by the time it takes oxygen bubbles to build up and cause the disc to float – a faster reaction time means a shorter time until the disc floats). Will you test the effects of different pH levels, temperature, salt, concentration of peroxide, or concentration of enzyme (by diluting the bakers yeast into 800 ml of water?
7. Perform your experiment.
8. Graph: you can make a bar graph of the average time for the reaction to complete (discs to float) in the control catalase experiment versus the experimental variable (temperature of water? Amount of catalase (yeast)? pH?

Disc #	**Time to float (sec)**
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
AVERAGE time to float (sec):	

Table 3. Time it takes for Catalase – mediated reaction of Hydrogen Peroxide → Water + Oxygen to occur.

Disc #	**Time to float (sec)**
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
AVERAGE time to float (sec):	

Table 4. Experiment on how _____ affects the rate of catalase – mediated reaction.

Procedure: Part 4 (How temperature affects pineapple enzyme) After doing the previous enzyme experiments, you can set the students loose on this challenge: design and perform a controlled experiment that shows the effects of pineapple on gelatin to answer the **question: at which temperature will the pineapple enzyme denature?** Make sure your experiment includes the following:

- A hypothesis
- A detailed experimental design which includes
    - Effects of fresh pineapple on gelatin
    - Effects of frozen pineapple on gelatin

    - Effects of freshly cooked pineapple on gelatin
    - A test that determines how gelatin behaves without any additives
- A data table
- A list of independent, dependent, and controlled variables.
- Number of trials
- How you will measure the dependent variable.
- An explanation of the enzyme, substrate and product(s) of your experiment.

Background for Pineapple and Jell-O experiment: Boxed Jell-O instructions say not to add fresh or frozen pineapple to the gelatin mix – if you do, the gelatin won't set (or gel). But why not? Gelatin is made out of the discarded, tougher parts of animals raised for meat products (like bone, hooves, and skin). These tough parts are made primarily of proteins. Jell-O and other gelatins get their structure from links formed between chains of collagen, which is a protein. Gelatin can be extracted from any kind of animal, but cows are most common. After grinding the bones of the animal, the pulverized bones are soaked in a strong base to soften them, and then passed through progressively stronger acid solutions (solutions with lower pH's). This smelly "skin and bone soup" gets boiled and the gelatin layer is skimmed off the top of the liquid in the pot, then dried into a powder. For dessert Jello-O, sugar, flavorings, and artificial colors are added before packaging.

The story behind pineapples is a bit more appetizing: pineapples (*Ananas comosus*) are monocotyledons, (one cotyledon), are South American in origin, and belong to the bromeliad family. While pineapple used to be the United State's second most important fruit (most were grown in Hawaii), today, most canned pineapple comes from the Phillipines. The pineapple fruit, like many tropical fruits (think: papaya, kiwi), contains an enzyme in its fruits, leaves, and stems, called ***bromelain*** that breaks down protein (making it a *protease*). When you add pineapple to the Jell-O, you break the links as fast as they form, so the gelatin never sets up – it stays as single amino acids, not a chain of amino acids, which would be a protein. Bromelain can be a useful thing though: products such as meat tenderizers contain this or other proteases.

In this lab, you will be asked to design your own experiment to test the effect of pineapple on gelatin. We'd like to know 1) whether all pineapple (fresh, frozen, or boiled (as when you process canned pineapple) reduces the gelling capability of Jell-O and 2) is there an optimum temperature for the bromelain enzyme?

Data Analysis: We do a lot of **qualitative** data analysis here –looking at color changes to see if the reaction occurred, as in using bromothymol blue to see if CO2 was produced from germinating seeds. **Quantitative** means you are taking data

points – time it takes for the catalase reaction to occur, for example, with the yeast and filter paper discs. With numbers, you can graph!

Plants: If starch is being converted into glucose using amylase enzyme (so it can then be broken down into ATP via aerobic cellular respiration), then glucose should be present. Benedict's solution tests for glucose sugar: was glucose present or not in dry seeds? In germinating (soaked) seeds?

If aerobic cellular respiration is occurring to provide ATP for seedling growth, then CO_2 should be produced ($C_6H_{12}O_6 + O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + 36 or 38 ATP chemical energy + heat Energy). Which test tubes showed the bromothymol blue turn to a green then yellow color? The yellow color indicates the presences of CO_2, which means that enzymes are also present, the enzymes that break down glucose and oxygen into water and CO_2.

Animals: Animals that can digest milk products produce lactase enzyme, to turn milk sugar (lactose) into glucose and galactose. Using a synthetic liquid lactase, which solutions showed the breakdown of lactose into glucose?

Yeast: All cells produce catalase which is used to convert the toxic waste product H_2O_2 into oxygen and water. If filter paper soaked in catalase is added to peroxide, the resulting oxygen bubbles should cause it to float. How long did it take the filter paper discs to float? What factors affected the rate of reaction, and how did the affect the rate? You can create a bar graph with the control (average time for disc to float – control) versus your treatment (average time for disc to float – treatment X).

How Temperature affects pineapple enzymes: When students are finished exploring enzymes, set them up with a challenge to design their own experiment: how does fresh pineapple affect gelatin - specifically, we want to answer the **question 'how does temperature affect the rate of the bromelain / gelatin reaction?' or - at which temperature will the pineapple enzyme denature?** You will end up graphing temperature of pineapple on the X and formation of gelatin on the Y (yes = 1 ; no = 0). When the pineapple enzymes are working, no Jell-O will gel.

Extensions: For a briefer demonstration of the enzymatic effect, chew and hold a saltine cracker in your mouth for 60 seconds. The amylase in your saliva will break down the starch and the cracker will start to taste sweet due to the sugar produced. You can also test for amylase enzyme by taking some starch solution (Make a 0.5% starch solution by mixing 1g of cornstarch with 200 mL of distilled water until dissolved. Shake well before each use) and adding saliva (which contains salivary amylase): 1. Fill a test tube with 3 mL of 0.5% starch solution. 2. Add a drop of iodine to the starch solution (taking care to keep the iodine off the opening of the tube) 3. Carefully "spit" between 1 and 2 mL of saliva into the tube, cap and mix gently by inverting the tube 3 times. 4. Record your observation at this "initial

time". 5. Place the tubes in warm (37 C) water. 6. Check the tube and record your results every two minutes for 16 minutes. If the starch is still present, the iodine turns blue black – but when the salivary amylase has completely broken down the starch into maltose sugar, the iodine remains its yellowish brown color. Graph the time it takes for the solution to remain yellow-brown (and not turn blue-black, indicating starch is still present).

For another experiment on what types of fruits keep Jell-O from gelling, try chopping various types of fruit into small pieces. I've used apple, orange, kiwi, tinned pineapple and fresh pineapple. Mix up some Jell-O according to the instructions. Add the Jell-O to each fruit in a separate container, and have a further container with just Jell-O as a control. Leave in a fridge for a couple of hours to set. Does all the jelly set properly? What happens if you use tinned fruit (heated in the process of canning)?

Reflection Questions:

- **What was produced in the germinating seeds that was not produced from the ungerminated seeds? What does this tell us? (**Seeds that have been germinated show the presence of glucose (as tested for using glucose urine test strips, from the pharmacy, as used by diabetics) but seeds that are dry, and not germinating yet, will not).

- **How is milk sugar broken down?** (the enzyme lactase breaks lactose (or milk sugar) into glucose and galactose).

- **How does Temperature affect the rate of enzymatic reactions? (**Most enzymes denature with heat and acidity, so will have a long (perhaps forever) reaction time – but enzymes will also have a temperature and pH at which they work "best" Usually enzymes found in our bodies, like amylase, work best at body temperature (37C) and are denatured (their 3-D shape unravels so the active site isn't present) at high temperatures. At low temperatures, below the optimal temperature, the reaction takes longer.

  What is meant by an 'optimum', and what would a graph showing an optimum look like? (an optimum is the point at which the reaction is occurring the fastest – or has the shortest reaction time. Most enzymes denature with heat and acid, so will have a long (perhaps forever) reaction time – but enzymes will also have a temperature and pH at which they work "best" Usually enzymes

- found in our bodies, like amylase, work best at body temperature (37C) and body pH (7) – though some enzymes found in the stomach work best at lower (more acidic) pH's. Usually a graph showing an optimum looks like a U

when graphing rate of reaction or reaction time. The lowest point in the U is the optimal point.

- **Hypothesize how pH and how enzyme concentration would affect the rate of an enzymatic reaction** (low, acidic pH's would stop the reaction from occurring, as would heat).

- **How does gelatin "gel"?** (Gelatin is a protein derived from collagen, the major component of the connective tissue of animals. The large, stringlike protein molecules of the gelatin (processed collagen) wiggle around in the hot water solution. As the gelatin mixture begins to cool, the protein strands have less and less energy to wiggle, until eventually they eventually bond together. If everything happens correctly, bonding occurs at points along the strands, forming pockets that trap the surrounding liquid. When the process is done, the collagen forms a three-dimensional structure or matrix, known as a semisolid colloidal gel. It is this matrix that gives Jell-O its structural integrity).

  From Scientific American, October 21, 1999
  http://www.scientificamerican.com/article/what-is-jell-o-how-does-i/

  > "In its natural state, collagen exists as fibers that contain three polypeptide chains entwined into a helical structure. Collagen is converted to gelatin by heating it in the presence of water. This procedure breaks down the relatively weak (noncovalent) bonds holding the three polypeptides together, as well as some of the stronger, covalent bonds, and produces a solution in which the polypeptides are arranged into a predominantly amorphous structure.
  >
  > When the solution of gelatin cools below a certain temperature, the molecules tend to associate with one another in order to regain some of their original helical structure. In this way, junction zones are formed. The junction zones mark a local return of the original form: three polypeptide chains in a helical formation. If there is enough gelatin present, a gel will form. The gel consists of a three-dimensional network of gelatin molecules linked by these junction zones, which is capable of entraining large amounts of water through capillary forces. This gel has solid-like characteristics, although it is really a viscoelastic material.
  >
  > Gelatin is a thermoreversible, cold-setting polymer: if the gel is reheated, it will convert back to a liquid because the forces favoring the amorphous state (mainly configurational entropy) outweigh those favoring the aggregated state (mainly hydrogen bonds). For this

reason, gelatin cannot be used in 'cook and serve' products such as puddings that are supposed to form gels when heated. These products must incorporate a heat-setting polymer, such as starch."

- **How does the pineapple enzyme bromelian keep Jell-O from forming? (**Pineapple contains a chemical called **bromelain**, which contains two enzymes capable of digesting proteins, which are called proteases. Jell-O and other gelatins get their structure from links formed between chains of collagen, which is a protein. When you add pineapple to the Jell-O, you break the links as fast as they form, so the gelatin never sets up. The enzymes in bromelain are inactivated once they have been heated to about 158° F (70° Celsius), so while fresh pineapple prevents Jell-O from gelling, gelatin made using canned pineapple (which was heated during the canning process) won't ruin the dessert.

  When you dissolve the gelatin powder in hot water, you break the weak bonds that hold the collagen protein chains together. Each chain is a triple-helix that will float around in the bowl until the gelatin cools and new bonds form between the amino acids in the protein. Flavored and colored water fills in the spaces between the polymer chains, becoming trapped as the bonds become more secure. Jell-O is mostly water, but the liquid is trapped in the chains so Jell-O jiggles when you shake it. If you heat the Jell-O, you will break the bonds that hold the protein chains together, liquefying the gelatin again).

Models and Explanations: In this lab we explored ways to use indicator solutions to test for the presence of different molecules such as CO_2 or glucose sugar. **A student who demonstrates understanding** of these concepts can explain how an indicator solution alerts us to the presence of a product that we might otherwise not see - and the presence of the product indicates a reaction occurred. For example, the changing of bromothymol blue from a blue to yellow color indicated that aerobic cellular respiration had occurred, converting glucose to CO_2 and water. **Further, this student will understand** what enzymes are, what they do, and what factors affect rate of enzymatic reactions. **The student should be able to explain the resulting graph** from the pineapple enzyme experiment to test the effects of temperature on rate of enzymatic activity, pointing out the optimal temperature for the pineapple protein (bromelian), **and the student should be able to describe how** to set up an experiment to test the effect of pH and enzyme concentration on the rate of enzymatic reactions.

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Student Worksheet:

Enzymes are important organic molecules in all living things – they are proteins, composed of amino acid chains, and they catalyze (speed up) exergonic (spontaneous) reactions, allowing them to occur at temperatures within the range tolerated by living cells. You also need enzymes for endergonic reactions (and energy too!). Usually, the same enzyme catalyzes the forward and reverse reaction – but different reactions require different enzymes, and many reactions are in fact pathways, with multiple steps, each requiring it own specific enzyme. Millions of metabolic reactions occur in living bodies (plants and animals and bacteria!) and these reactions could not occur without enzymes. Once such reaction is aerobic cellular respiration; another is digestion of milk products, a third is the conversion (hydrolysis) of starch to sugar, which happens in seeds as they germinate, and a fourth is the decomposition of hydrogen peroxide in the cells of all living things.

We will study all of these things then set you loose on a challenge: design your own experiment to test the effect of pineapple on Jell-O gelatin. We'd like to know 1) whether all pineapple (fresh, frozen, or boiled (as when you process canned pineapple)) reduces the gelling capability of Jell-O and 2) is there an **optimum** temperature for the enzyme (bromelain) found in pineapple?

Part 1 (Plant Enzymes – Amylase and Starch; CO_2 production)
To test for amylase enzyme converting starch to sugar:

Test tube 1: Initial color _____ Color after boiling 10 minutes: _____

Test tube 2: Initial color _____ Color after boiling 10 minutes: _____

- **Which seeds showed evidence of starch being broken down into glucose?**
- **Why would seeds break their starch reserves down into glucose?**

To test for production of CO_2 as a result of aerobic cellular respiration (glucose + O_2 → CO_2 + water)

Date	Jar with dry beans	Jar with germinating beans	Jar with no beans
Day zero			
Day 1			
Day 2			
Day 3			
Day 4			

Table 1. Carbon dioxide production by germinating seeds as seen by color change, using bromothymol blue indicator for carbon dioxide.

- **Which jars showed evidence of germination?**
- **Why / How is CO_2 produced during seed germination?**

Part 2 (Animal Enzymes – Lactase and Milk)

Solution	Result of Glucose Test Strip
a) treated milk	
b) untreated milk	
c) liquid lactase solution	
d) glucose solution	

Table 2. Glucose test for milk products.

- **Which situation(s) led to the milk sugar (lactose) being broken down into glucose and galactose?**
- **What caused the milk sugar (lactose) to break down into simpler sugars?**

Part 3 (Yeast and Catalase Enzyme)

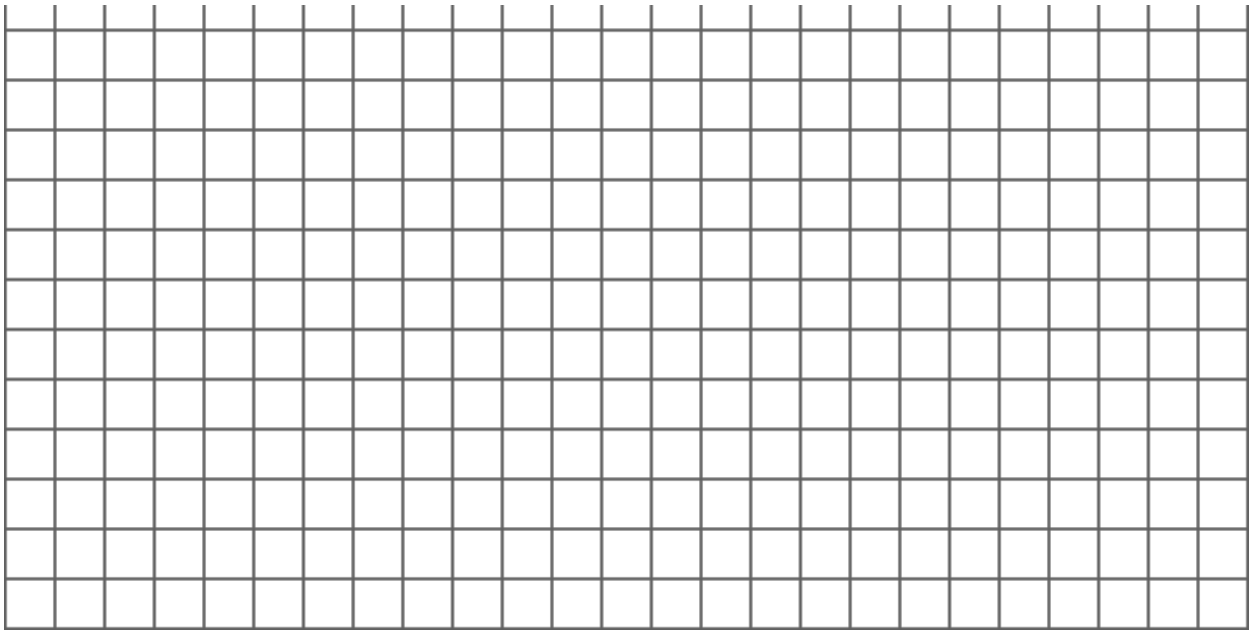
Disc #	Time to float (sec)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
AVERAGE time to float (sec):	

Table 3. Time it takes for Catalase – mediated reaction of Hydrogen Peroxide → Water + Oxygen to occur (control).

Disc #	Time to float (sec)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
AVERAGE time to float (sec):	

Table 4. Experiment on how _____ affects the rate of catalase – mediated reaction.

- **What was the reaction in this experiment?**
- **What enzyme was produced by the yeast?**
- **How did this enzyme cause the filter paper to float?**
- **How did your experimental variable affect the rate of your catalase-mediated reaction?**



Part 4 (How temperature affects the pineapple enzyme *bromelain*):

Boxed Jell-O instructions say not to add fresh or frozen pineapple to the gelatin mix – if you do, the gelatin won't set (or gel). But why not? The pineapple fruit, like many tropical fruits (think: papaya, kiwi), contains an enzyme in its fruits, leaves, and stems, called ***bromelain*** that breaks down protein (making it a *protease*). When you add fresh pineapple to the Jell-O, the enzyme break the links (peptide bonds) as fast as they form, so the gelatin never sets up – it stays as single amino acids, not a chain of amino acids, which would be a protein.

We'd like to know 1) whether all pineapple (fresh, frozen, or boiled (as when you process canned pineapple) reduces the gelling capability of Jell-O and 2) is there an optimum temperature for the enzyme found in pineapple (bromelian)?

Experimental Design: Title _____

Hypothesis:

Independent Variable:

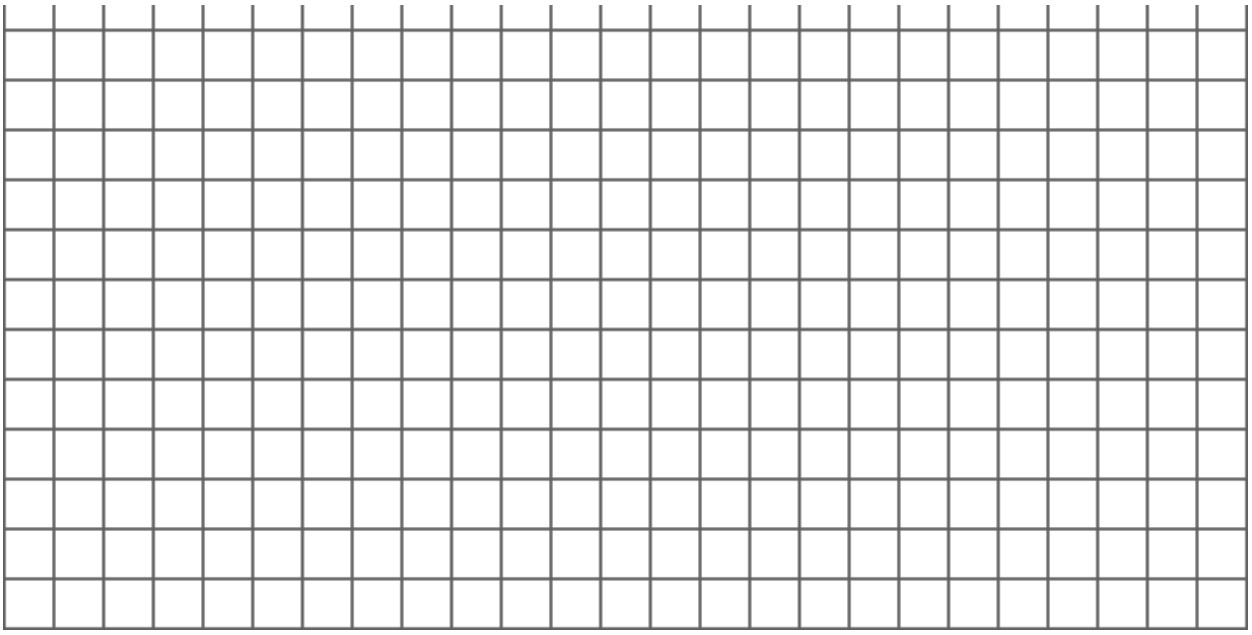
Dependent Variable:

Controlled Variables:

 # Trials:

Control Experiment:

Data Table:



1. Clearly describe the results of your experiment, in respect to 1) if canned, fresh, and frozen pineapple all had the same effects on Jell-O and 2) how temperature affects the rate of the enzymatic reaction

2. What is the enzyme in your experiment?

3. What is the substrate in your experiment?

4. What type of organic molecule is gelatin?

5. What type of organic molecule is bromelain?

6. Why were the results of the canned (cooked) pineapple different than the results of the fresh, raw pineapple? Be specific (how does boiling affect enzymes?)!

7. What is meat tenderizer and what does it do?

8. How did the temperature of the pineapple affect whether or not the Jell-O formed a gelatin?

9. Was there an optimum temperature for the bromelain enzyme?

10. What evolutionary advantage would pineapples have for having the enzyme bromelain?