Heading

Title

**Introduction**

**Purpose** To investigate enzymes and factors that affect them.

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**Discussion**

An Enzyme is very selective in the reaction it catalyzes and has a shape that determines the enzyme’s specificity. The specific reactant that an enzyme acts on is called the enzyme’s substrate and a substrate fits into a region of the enzyme called the active site. Enzymes are specific because only specific substrate molecules fit into their active site.

Enzymes are proteins that catalyze reactions, meaning the speed up the chemical reaction, but are not part of the reaction. This occurs because enzymes lower the activation energy required for a chemical reaction to take place. Proteins are composed of amino acids that connect and fold into specific shapes to do a specific job. The specific shape of the protein is sensitive to many different factors such as temperature, pH, and other environmental conditions. This lab will test the action of an enzyme and the effects or temperature and pH on the enzyme.

**Pineapple**

Pineapple’s lush, tropical sweetness is reason enough to enjoy it any way you can, but this fruit also contains bromelain, a natural enzyme found in both the fruit and the stem. Fresh pineapple is much more flavorful than canned and despite its tough bristly shell, is easy to prepare. It also contains 2-3 times more bromelain than canned pineapple.

**Bromelain**

The pineapple plant contains protein-digesting enzymes called, as a group, bromelain. In the health world, these enzymes are regarded as useful in reducing muscle and tissue inflammation (hence the joint pain and wound-healing possibilities), as well as acting as a digestive aid. In the cooking world, on the other hand, bromelain is regarded as the enemy of the gelatin dessert. If you use fresh pineapple in gelatin, the enzyme eats the protein and the gelatin will not gel. The classic kitchen trick for getting around this pineapple-gelatin incompatibility is to cook the pineapple, thus reducing the power of the bromelain. Bromelain can be found in meat tenderizers as an alternative to fresh pineapple.

**Gelatin**

Gelatin contains protein called collagen (a primary component of joints, cartilage, and nails), and various amino acids (histidine, lysine, leucine, tryptophan, and valine, to name a few). Remember: amino acids are the building blocks of proteins.

Gelatin has long been a key ingredient for providing support for “jelled” deserts, salads, frozen drinks, and soft candies such as Gummi Bears. (In fact, the word gelatin is derived from the Latin “gelatus”, meaning stiff or frozen.)

In addition to its famous “jiggly” food uses, gelatin with its flexible, dissolvable structure is also used to manufacture capsules (both hard and “soft-gel”) to hold medications, vitamins, and other dietary supplements. Gelatin is an ingredient in film coatings, medical devices such as artificial heart valves, and in specialized meshes used to repair wounds, to name a few.

The enzyme found in pineapple, Bromelain, breaks down proteins. Gelatin (Jello) contains structural proteins. When the Bromelain in pineapple interacts with the proteins in gelatin (Jello), the gelatin proteins are denatured or degraded, and the gelatin liquifies from a solid.

**Part 1: How do Enzymes Affect a Reaction?**

**Materials:**

1 envelope Knox (unflavored) Marking pen Masking Tape

Gelatin 3 test tubes 1 test tube rack

Metric measuring cup 3 – 1 ml disposable pipettes 1 spoon

Fresh Pineapple Juice Apple Juice Hot plate to heat water

100 ml graduated cylinder 10 ml graduated cylinder

**Procedures**

1. Number and label the test tubes “1 – 3” using masking tape.
2. Prepare a gelatin solution by adding 1 package of gelatin to 90 ml of boiling water. Stir well with a spoon until the gelatin is dissolved and add 30 ml of cold water.
3. Place 3 ml of the designated substance into each test tube (see data table below). **Use a separate pipette for each type of juice**. *Failure to do so may result in mixing of the juice types and inaccurate results*.
	* Tube 1: water only
	* Tube 2: fresh pineapple juice
	* Tube 3: apple juice
4. Add 10 ml of gelatin mixture to each test tube. Shake well to ensure proper mixing and place your samples in the refrigerator overnight using a test tube rack.
5. On Day 2, check the contents of each test tube in terms of whether it is solid or liquid, and record your observations.

**Hypothesis** *(If/then/because)*

|  |  |  |
| --- | --- | --- |
| **Test Tube** | **Juice** | **State of test tube contents on Day 2 (liquid or solid)** |
| 1 | Water |  |
| 2 | Fresh Pineapple Juice |  |
| 3 | Apple Juice |  |

Was your hypothesis supported by the data? Why or why not?

**Part 2:** **What Effect does Temperature have on an Enzyme?**

**Materials:**

1 envelope Knox (unflavored) Marking pen Water

Gelatin 4 test tubes 1 test tube rack

Metric measuring cup 1 – 1ml disposable pipettes 1 spoon

Fresh Pineapple Juice Hot plate to heat water Thermometer

100 ml graduated cylinder 10 ml graduated cylinder

**Procedures**

1. Prepare a gelatin solution by adding 1 package of gelatin to 90 ml of boiling water. Stir well with a spoon until the gelatin is dissolved and add 30 ml of cold water.
2. Number and label the test tubes from 1 – 4 using masking tape.
3. Record the test tube numbers and the initial temperatures for each test tube (they should all be at the same temperature). This will be considered at room temperature.
4. Next, add 3 ml of the fresh pineapple juice to each test tube.
5. You will leave test tube 1 as is … do NOT heat it.
6. Heat each test tubes 2 – 4 to the appropriate temperature as given in the data table. Leave test tube 1 at room temperature.
	1. Start with all the test tubes in cool water in a water bath.
	2. Gradually increase the temperature of test tubes 2 – 4 in 2⁰ C increments, withdrawing the numbered test tubes in order as the appropriate temperature level in the bath is reached.)
7. After the test tubes have been pulled from the water bath, add 10 ml of Knox gelatin (prepared in step 1) to each test tube and mix well.
8. Finally, place the test tubes in the refrigerator overnight.
9. On day 2, check the contents of each test tube in terms of whether it is solid or liquid, and record your observations.

**Hypothesis** *(If/then/because)*

|  |  |  |
| --- | --- | --- |
| **Test Tube** | **Temperature** | **State of test tube contents on Day 2 (liquid or solid)** |
| 1 | Room Temperature |  |
| 2 | 40° C |  |
| 3 | 60° C |  |
| 4 | 100° C |  |

Was your hypothesis supported by the data? Why or why not?

**Part 3: What Effect does pH have on Enzyme Activity?**

**Materials:**

1 envelope Knox (unflavored) Marking pen Water

Goggles 3 test tubes 1 test tube rack

Metric measuring cup 4 – 1ml disposable pipettes 1 spoon

Fresh Pineapple Juice Vinegar (acid) Bleach (base)

Hot plate to heat water 100 ml graduated cylinder 10 ml graduated cylinder

**Procedures**

1. Prepare a gelatin solution by adding 1 package of gelatin to 90 ml of boiling water. Stir well with a spoon until the gelatin is dissolved and add 30 ml of cold water.
2. Label 1 test tube “A” for acid, 1 test tube “B” for base, and the last test tube “C” for control (water).
3. Place 3 ml of fresh pineapple juice into each test tubes.
4. Add 1 ml of acid to test tube “A”; add 1 ml of base to test tube “B”; and add 1ml of water to test tube “C”. **Be sure to use a different pipette for each test tube to avoid contamination.**
5. Add 10 ml of gelatin mixture to each test tube. Mix well, being careful to not get any of the acid or base on your skin.
6. Refrigerate the test tubes overnight and on day 2 check the contents of each test tube in terms of whether it is solid or liquid, and record your observations.

**Hypothesis** *(If/then/because)*

|  |  |  |
| --- | --- | --- |
| **Test Tube** | **Contents** | **State of test tube contents on Day 2 (liquid or solid)** |
| A | Acid |  |
| B | Base |  |
| C | Control (water) |  |

Was your hypothesis supported by the data? Why or why not?

**Conclusions**

**Address Hypothesis** Be sure that you wrote a hypothesis and addressed it for each part (1-3).

**Analysis** *(fill in the blanks)*

An Enzyme is very selective in the reaction it \_\_\_\_\_ and has a shape that determines the enzyme’s \_\_\_\_\_. The specific reactant that an enzyme acts on is called the enzyme’s substrate and a substrate fits into a region of the enzyme called the \_\_\_\_\_ site. Enzymes are specific because only specific \_\_\_\_\_ molecules fit into their active site.

Enzymes are \_\_\_\_\_ that catalyze reactions, meaning the \_\_\_\_\_ \_\_\_\_\_ the chemical reaction, but are not part of the reaction. This occurs because enzymes \_\_\_\_\_ the \_\_\_\_\_ energy required for a chemical reaction to take place. Proteins are composed of amino acids that connect and fold into specific shapes to do a specific job. The specific shape of the protein is sensitive to many different factors such as \_\_\_\_\_, \_\_\_\_\_, and other environmental conditions.

**Questions**

1. Using the three items: (1) gelatin, (2) substance gelatin is mixed with, and (3) water, state the independent variable, the dependent variable, and the control in this experiment.

2. In this lab, what evidence took place that showed the action of the enzyme?

3. What two factors in this lab denatured the enzymes? Give specific data for each factor.

**Errors**

* Using the same pipette for different test tubes, contaminating the results.
* Not mixing the gelatin properly.
* Not mixing the test tube ingredients properly.
* Not being consistent or accurate in measurements.

**Bibliography**

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