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Bio 2312 Lab

Tuesday 2:30-5:00

Professor Niloufar Haque

Chemical Breakdown of Foodstuff

For the body to maintain homeostasis, we need nutrients. Before we receive the necessary nutrients, the food is mechanically broken down into small particles. Then the particles are digested into the molecules that can now be absorbed into the blood, making it possible for the nutrients to reach the cells.

Enzymes are large protein molecules that are produced by body cells. They are biological catalysts, giving them the ability to increase the rate of a chemical reaction and not be a part of the product (*Pearson*, 2018). The digestive enzymes are hydrolyses. The molecules they act on, substrates, are organic food molecules that break down when adding water to the molecular bonds. In this lab, you'll learn about three chemical reactions, and the first one is starch digestion by salivary amylase. Amylase is an enzyme that catalyzes the hydrolysis of starch into sugars. Amylase is in the saliva and starts the chemical process for digestion. The next chemical reaction is protein digestion by trypsin. Trypsin is an enzyme produced by pancreas proteins into small peptides. BAPNA is a synthetic trypsin substrate that binds dye to amino acids. Trypsin hydrolysis of BAPNA separates the dye molecule from the amino acid, leading the solution to change from colorless to bright yellow. The color change proves the hydrolysis by trypsin. The final chemical reaction demonstrates the emulsification action of bile and accessing fat digestion by lipase. Lipase is the enzyme the body uses to break down the fat in food for the intestines to absorb.

Activity 1: Salivary Amylase Digestion of Starch

| Tube no. | 1A | 2A | 3A | 4A | 5A | 6A |
|-----------------------------------|--------------------|--------------------|--------------------|------------------------------------|--------------------|--------------------|
| Addictives (3gtt ea): | Amylase, Water | Starch, Water | Maltose, Water | Amylase, Boiled Amylase, Starch | Amylase, Starch | Amylase, Starch |
| Incubation condition: | 37°C | 37°C | 37°C | 37°C | 37°C | 0°C |
| IKI test (color change): | No Color Change | Blue/Black | No Color Change | Blue/Black | No Color Change | Blue/Black |
| Result: Negative or Postive | Negative | Positive | Negative | Positive | Negative | Postive |
| Benedict's test (color change): | No Color Change | No Color Change | Orange | No Color Change | Orange | No Color Change |
| Result: Negative or Postive | Negative | Negative | Positive | Negative | Postive | Negative |

Materials and Method:

Before starting the activity, each tube was labeled 1A-6A to be easily identified when preparing the solution. Each tube represents a specific treatment, including three controls and three tests. The tubes had three drops of the following, tube 1A had amylase and water, tube 2A had starch and water, tube 3A had maltose and water, tube 4A contained amylase that was boiled for 4 minutes with starch added after it, tube 5A had amylase and starch, tube 6A had amylase and starch. Test tubes 1A-5A were incubated in 37°C water for an hour, while tube 6A was incubated at 0°C.

A drop of Lugol iodine was added to each sample; starch was detected if the iodine turned blue or black. For the Benedicts test, we added three drops to each tube; this will serve as an indicator for the presence of maltose if a red precipitate forms when heating the solution. Tube 1A was a

control containing amylase only, so it showed negative for both tests. Tube 2A only contained starch, so it tested negative for maltose and turned black when testing for the presence of starch. Test 3A was a control containing maltose, so it showed positive for the presence of maltose but negative for starch. In tube 4A, the amylase was boiled, so the enzyme could not fully digest the starch, resulting in a positive IKI test and a negative test for maltose. Tube 5A had a positive test for maltose and a negative test for starch. Lastly, tube 6A showed a positive test for starch and negative for maltose.

Activity 2: Trypsin Digestion of Protein

| Tube no. | 1T | 2T | 3T | 4T | 5T |
|-----------------------------|-------------------|-----------------|-------------------------------------|----------------|-------------------|
| Addictives (3gtt ea): | Trypsin, Water | BAPNA, Water | Trypsin, Boiled trysin, BAPNA | Trypsin, BAPNA | Trypsin, BAPNA |
| Incubation condition: | 37°C | 37°C | 37°C | 37°C | 0°C |
| Color Change: | No color change | No color change | No Color Change | Yellow | Yellow-ish |
| Result: Negative or Postive | Negative | Negative | Negative | Positive | Positive |

Material and Method:

In this activity, each tube represents a specific treatment, including two controls and three tests. Tube 1 had three drops of both trypsin and water. Tube 2T had three drops of both BAPNA and water. Tube 3T had three drops of trypsin, then the tube was put in boiling water and sat there for 5 minutes. Tube 4T had three drops of both trypsin and BAPNA. Tube 5T had three drops of both trypsin and BAPNA. Then test tubes 1T-4T were set in the water bath for an hour, excluding

5T since it needed to be set at 0°C. Test tube 1T was a control containing trypsin, leading it to have no color change. Test tube 2T was also controlled and only contained BAPNA. In the absence of an enzyme, it could not release a yellow solution, and the solution remained transparent. In tube 3T, the trypsin was boiled, resulting in the enzyme being denatured, which was therefore not capable of digesting the BAPNA that was later added, so it remained colorless. Test tube 4T had a positive test, while 5T had a faint yellow solution leading the results to be positive.

Activity 3: Pancreatic Lipase Digestion of Fats

| Tube no. | 1L | 2L | 3L | 4L | 5L | 4B | 5B |
|-----------------------------------|----------------------|---------------------------|--|-----------------------------|-----------------------------|---|---|
| Addictives (5 gtt ea): | Pancreatin, water | Litmus cream, water | Pancreatin, boiled pancreatin, litmus cream | Pancreatin, litmus cream | Pancreatin, litmus cream | Pancreatin, litmus cream, bile salts | Pancreatin, litmus cream, bile salts |
| Incubation condition: | 37°C | 37°C | 37°C | 37°C | 0°C | 37°C | 0°C |
| Color Change: | No color change | No color change | No color change | Pink | No color change | Light pink | Light Purple |
| Result: Negative or Postive | Negative | Negative | Negative | Positive | Negative | Positive | Positive |

Material and Methods:

In activity 3, we assessed the digestion of fats by lipase. Also in this lab, we checked the digestion by testing the pH. If the sample changes to a pink color, it confirms the acid and digestion is present. The test tubes were labeled 1L-5L and two additional test tubes labeled 4B and 5B. In tube 1L, there were five drops of both pancreatin and water. In 2L, there were five drops of both litmus cream and water. Tube 3L contained five drops of pancreatin that boiled for four minutes with litmus cream added after. In tubes 4L and 5L, there were five drops of both

pancreatin and litmus cream. Tube 4B and 5B had five drops of both pancreatin, litmus cream, and bile salts. After the addictive was added to tubes 1L-4L and tube 4B, the tubes were incubated in 37°C water. At the same time, tubes 5L and 5B were incubated in 0°C water. After an hour passed, litmus powder was added to create the litmus cream. Again, a color change will indicate whether there was a change in PH.

Conclusion:

After completing the experiments, it's clear why enzymes are an essential part of our daily lives. Enzymes help speed up chemical reactions in the human body. They are necessary for respiration, muscle, and nerve function and are essential for healthy digestion.

References:

Marieb,E (2019). Human Anatomy & Physiology Laboratory Manuel (Fetal Pig), 13th edition, 2019. Pearson Education, Inc