Lilia Sanchez
Laboratory 3- Properties of Enzyme Action
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**PURPOSE: 3-C: Digestion of fat with pancreatic lipase and bile salts-** The purpose of this experiment is to test how the digestion of fat affects the pH of the solution and how bile affects the rate of digestion.

**Procedure:**

1. We added just enough litmus powder to a container of dairy cream to produce a medium blue color. We poured 3 ml of the litmus cream into 4 separate test tubes. Into two additional test tubes we poured 3 ml of 2% pancreatin. Then we preincubated the litmus cream and the pancreatin separately in a 37°C water bath for 5 minutes. Lastly, we prepared four test tubes as follows:

Tube #1: 3 ml cream + 3 ml pancreatin

Tube #2: 3 ml cream + 3 ml distilled water

Tube #3: 3 ml cream + 3 ml pancreatin +pinch of bile salts

Tube #4: 3 ml cream + 3 ml distilled water + pinch bile salts

2. We gently shook each tube for 30 seconds to mix in the bile salts. Then we incubated all four tubes in 37°C water bath for 30 minutes, checking every 10 minutes. We recorded the time and number of the tube. Lastly, we removed the tubes from the water bath.

3. We then tested the pH of each tube using pH paper and noted the odor and color of each tube.

NOTE: Blue litmus will turn pink in an acid environment.

4. We summarized the results in the following table: Tube Color-pH-Odor-Time to change color.

5. We explained how the digestion of fat affects the pH of the solution and how bile affects the rate of digestion.

## RESULTS: 30 MINUTES

| 30 MIN | PH | COLOR | ODOR |
|---|---|---|---|
| TUBE 1 | 6 | LIGHT PURPLE | CHEESY |
| TUBE 2 | 7 | DARK PURPLE | LESS CHEESY |
| TUBE 3 | 5 | DARKER PURPLE | REALLY CHEESY |
| TUBE 4 | 6 | EMULSIFYING DARKER/PURPLE BOTTOM | LITTLE CHEESY |

## Discussion:

In this experiment, I believe the experiment did work. Tube 1, which contained a solution of Pancreatin and Litmus cream only, was noted to have a pH level of 6 which left a light purple color with a distinct cheesy odor. This seemed to be the baseline to the contents of the experiment. In Tube 2, that contained litmus cream and distilled water, had an increased pH level of 7, thus making the solution color change to a dark purple. The odor was not as distinct as Tube 1. I found this to be due to the onset of the hydrolysis. Tube 3, which presented with a pinch of bile salt had a decreased pH of 5 after 30 minutes. In this tubule process, I find that the absence of water tends to decrease the solubility and then decreases the pH level from Tube 2. The color was much darker than Tube 1 and Tube 2 and had a much stronger odor than the other 2 tubes. In Tube 4, that contained litmus cream, distilled water and salt, ended with a pH level of 6, making the color of the solution an even darker purple than Tube 3, but in this tubule, the emulsification process did begin and separated the solution with a 40:60 ratio of light purple/dark purple, which settled at the bottom of the tube. The

odor to Tube 4 had a less “cheesy” distinction. I believe this may have had to do with the decreased level of acidity.

**Conclusion:**

The purpose of this experiment was to analyze the digestion of fat with pancreatic lipase and bile salts.

Major Findings:

- Pancreatic Lipase (Pancreatin) alone has no effect on breaking down fats/sugars due to its water-soluble enzyme being ineffective on large lipid droplets, which are water insoluble.

- Water does play a role in process of the chemical breakdown and solubility.

-Bile salts help overcome the insolubility of pancreatin digestion by acting as emulsifying agents, which break down fats into smaller droplets so that lipase has a larger surface area for its hydrolysis

- The combination of pancreatin, water, and bile salts, created higher level of solubility due to lipase now having a larger surface area for its hydrolysis/emulsification of fats.