

Laboratory 3: Properties of Enzyme Action: 3-C: Digestion of fat with pancreatic lipase and bile salts.

**Purpose:** The purpose of this lab is to examine the characteristics of the action of pancreatic lipase and bile salts on lipids. We will also be learning the what impact the digestion of fat has on pH levels, and how bile influences the rate of digestion.

**Procedure**

3-C: Digestion of fat with pancreatic lipase and bile salts.

Pancreatic lipase has a major role in fat digestion, but by itself, lipase is ineffective because it is a water-soluble enzyme trying to act on large lipid droplets, which are water insoluble. Bile salts help overcome this problem by acting as emulsifying agents, which break the fat into smaller droplets so that lipase has a larger surface area for its hydrolysis of fats. The pancreas also aids digestion of lipids by secreting sodium bicarbonate ( $\text{NaHCO}_3$ ). This compound provides pH of around 7.8 in the small intestine, which is optimal for the action of the pancreatic enzymes. In the following exercise, you will examine some aspects of the action of pancreatic lipase and bile salts on lipids.

1. Add just enough litmus powder to a container of dairy cream to produce a medium blue color. Pour 3 ml of the litmus cream into 4 separate test tubes. Into two additional test tubes pour 3 ml of 2% pancreatin. Pre incubate the litmus cream and the pancreatin separately in a 37°C water bath for 5 minutes. Then prepare 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. Gently shake each tube for 30 seconds to mix in the bile salts. Incubate all four tubes in a 37°C water bath for 1 hour, checking every minute for the first 5 minutes or until the first tube changes color, then every 15 minutes for the rest of the hour. Record the time and number of the tube. Continue checking for the remainder of the hour.
3. Remove the tubes from the water bath. Test the pH of each tube using pH paper and note the odor and color of each tube. \*NOTE: Blue litmus will turn pink in an acid environment.
4. Summarize the results in the following table:

Tube	Color	pH	Odor	Time to change color
#1				
#2				
#3				
#4				
5. Explain how the digestion of fat affects the pH of the solution and how bile affects the rate of digestion.

**Results:**

Tube	Color	pH	Odor	Time to change color
Tube #1: 3 ml cream + 3 ml pancreatin	Lavender	7	light cheese smell	10 Min- Lavender 20 Min- Lavender 30 Min-Lavender
Tube#2: 3 ml cream + 3 ml distilled water	Lavender	8.5	light milk smell	10 Min-Lavender 20 Min- Purple & Bluish 30 Min- Medium blue
Tube #3: 3 ml cream + 3 ml pancreatin + pinch of bile salts	Lavender	7	strong cottage cheese smell	10 Min- Lavender 20 Min-Light Purple 30 Min- Light Purple
Tube #4: 3 ml cream + 3 ml distilled water + pinch bile salts	Lavender	8	livestock maneuver	10 Min- Lavender 20 Bluish & Grey 30 Min- Light Blue

**Discussion:**

While conducting this experiment we noticed that tube #1 did not change color, and the odor was very light. While, tube #2 had a light smell as well, it did change color. On the other hand tubes 3 & 4 changed color and had very strong odors. The strong odor for tubes 3 & 4 may have been caused by the pinch of bile salts giving it that cottage smell. Bile assist with digestion by breaking down fats into fatty acids. The changes in the pH confirmed that that fats are being broken down.

**Conclusion:** In conclusion, in this lab we learned the important role that bile plays in digestion and emulsification. We learned that during emulsification bile helps by breaking down fats into tiny fat droplets. While in digestion it dissolves fats into fatty acids.