The Digestive System Processes: Chemical and Physical Digestion

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Objective

The main function of the digestive system is to break down food, absorb nutrients used by body cells, and eliminate waste (Martini et al. 2018). The digestive system first breaks down ingested materials (food etc.) and then disassembles the molecules into smaller fragments by hydrolysis. Ingestion occurs when food enters the oral cavity. During chemical digestion, we observe the enzyme-mediated hydrolysis of food into nutrients absorbed and used by the body. After molecules get disassembled into smaller fragments, the cells absorb molecules that get released into the bloodstream. This occurs either by breaking them down to provide energy for the synthesis of ATP or the molecules are used to synthesize carbohydrates, lipids, and proteins (Martini et al. 2018).

Digestion of carbohydrates occurs in two steps. The first step involves carbohydrases produced by the salivary glands and pancreas. The second step uses brush border enzymes. Carbohydrate digestion begins in the mouth during mastication through salivary amylase. Salivary amylase breaks down starches into a mixture composed of disaccharides and trisaccharides. Brush border enzymes of the intestinal microvilli break disaccharides and trisaccharides into monosaccharides before absorption occurs.

Lipid digestion involves lingual lipase from glands of the tongue and pancreatic lipase from the pancreas. Lipases are water-soluble enzymes and lipids tend to form large drops excluding the water molecules. Due to this lipase only interacts with surfaces of the lipid drops. Lingual lipases start with the breakdown of triglycerides in the mouth and continue to the next location: the stomach. The bile salt used in the lab is known to improve chemical digestion by

emulsifying lipid drops into tiny emulsion droplets(Martini et al. 2018). This provides better access for the pancreatic lipase.

Protein digestion is known to be extremely time-consuming. First, the three-dimensional organization of food has to be disrupted by proteases accessing individual proteins. The step involves mechanical digestion in the oral cavity and chemical digestion in the stomach. The strong acid in the stomach damages the secondary and tertiary structure of the protein. This exposes peptide bonds to enzymatic action (Martini et al. 2018).

The food we eat in our body has to be broken down so that the nutrients from it can reach cells of our body. This occurs in different stages. First, the food gets broken into small particles and the particles are chemically (enzymatically) digested into the molecules that can be absorbed (Marieb et al. 2019). The digestion of food occurs through enzymatic action. Enzymes are large protein molecules produced by body cells; it increases the rate of a chemical reaction. Digestive enzymes are called hydrolytic enzymes (Marieb et al. 2019). The substrates on which they attach to are organic food molecules that they disintegrate through the addition of water to the molecular bonds.

Digestive enzymes can function outside body cells in the digestive tract hence their activity can be studied in a test tube. The objective of the experiment is to observe how the digestive system works through the usage of different enzymes. The result of the enzymes in the different digestion environments will then be observed.

Material and Methods

In Activity 1 to measure the hydrolysis of starch to maltose by salivary amylase a test tube rack, 10 test tubes, a wax marking pencil, a dropper bottle of distilled water and dropper bottles of maltose, amylase and starch solutions were used. Before the start of the experiment each tube was marked with a pencil. Two students prepared the controls (tubes 1A to 3A) and the other two prepared experimental (tubes 4A to 6A). All tubes were placed in a rack in the 37 degree water bath for approximately an hour. As time went on we took turns as a group shaking the rack. After the hour ended we proceeded to perform the amylase assay. A spot plate and dropper bottles of Lugol's iodine solution and Benedict's solution were used. The boiling water bath was set up.

While the water heated, six different test tubes from the original ones were marked (1A-6A). Beaker 1A was filled with amylase and water, Beaker 2A was filled with starch and water, Beaker 3A was filled with maltose and water, beaker 4A was filled with amylase, beaker 5A was filled with amylase and starch and lastly beaker 6A was filled with amylase and starch. A pipet was then used to drop samples from tubes 1A-6A into the tubes numbered at the beginning. In each tube a sample and drop of Lugol's iodine (IKI) solution was dropped to check for the presence of starch.After, 3 drops of Benedict's solution was then dropped into the tubes. Each tube was placed in a beaker of boiling water for 5 minutes. This was to test for the presence of sugar.

In Activity 2 to test protein digestion by trypsin five test tubes, a tube rack, a dropper bottle of trypsin and BAPNA were used. Once again students in the group were split into two. Two students prepared the controls (1T and 2T) and the other two the experimental tube (3T

-5T). Each tube was marked with a pencil and three drops of the mentioned additives were added inside the tube. The tubes placed in the rank were then placed in bath water for approximately 1 hour. Once again at the end of the hour like Activity 1, the tubes were examined for trypsin assay to check for the hydrolysis test.

During Activity 3 we obtained nine test tubes, test tube rack and one dropper bottle of each of the solutions indicated. This was to demonstrate the emulsification action of bile and assess fat digestion by lipase. To demonstrate the action of bile on fats two test tubes were prepared and marked 1E and 2E. In tube 1E 20 drops of water and 4 drops of vegetable oil were added. In tube 2E 20 drops of water, 4 drops of vegetable oil and a pinch of bile salts were added. A parafilm was used to cover and shake the tubes. The tubes were then left at room temperature. The tubes were checked 10-15 minutes later to check for emulsification. After figuring out which tubes emulsification occurred in, other tubes were then prepared. Two students prepared the controls (1L and 2L) and the other two set the experimental tubes (3L to 5L, 4B and 5B). Each tube was then marked with a pencil and 5 drops of the additives indicated were dropped into the tube. In tubes 4B and 5B a pinch of bile salts were added in the tube. To make sure the contents of the tube were properly mixed a parafilm was used to mix it. All the tubes labeled were then placed in a water bath for approximately an hour. After the hour was completed a lipase assay was performed to check for pH change detected by the litmus powder indicator.

Results

In activity 1 after placing the drop and drop of Lugol's iodine solution we checked for the presence of starch and maltose. A blue-black color in the tube indicated that there was a presence

of starch. This is referred to as a positive starch test (Table 1). If the mixture does not turn blue this is referred to as a negative starch test. The same goes for the presence of maltose. When a green to orange precipitation forms it means that maltose is present. This indicates a positive sugar test. A negative sugar test indicates that there is no color change (Table 1).

Table 1 Salivary Amylase Digestion of Starch

Tube no.	**1A**	**2A**	**3A**	**4A**	**5A**	**6A**
Additives (3 gtt ea)	**Amylase, water**	**Starch, water**	**Maltose, water**	**Boil amylase 4 min, then add starch**	**Amylase, starch**	**Amylase, starch**
Incubation condition	**37°C**	**37°C**	**37°C**	**37°C**	**37°C**	**0°C**
IKI test (color change)	**Yellow**	**Dark purple/ black**	**yellow**	**black/ dark purple**	**yellow**	**black**
Result: (+) or (-)	**-**	+	**-**	+	**-**	+
Benedict's test (color change)	**blue**	**blue**	**orange**	**blue**	**red**	**red**
Result (+) or (-)	**-**	**-**	+	+	+	+

In activity 2 while assessing protein digestion the presence of yellow indicated a positive hydrolysis test. A clear mixture indicates that a negative hydrolysis test occurred (Table 2).

Table 2 Trypsin Digestion of Protein

Tube no.	**IT**	**2T**	**3T**	**4T**	**5T**
Additives (5 gtt ea)					
Incubation condition	**37°C**	**37°C**	**37°C**	**37°C**	**37°C**
Color change	**No color (clear)**	**No color (clear)**	**No color (clear)**	**Yellow**	**Lighter yellow**
Result: (+) or (-)	**-**	**-**	**-**	+	+

In Activity 3 the tubes prepared at the beginning with the vegetable oil, water, and salts were used to check for emulsification. If emulsification has not occurred the oil will be floating on the surface of the water. If emulsification does occur the fat droplets will be suspended through the water. In the activity, we check for the emulsification action of bile and assess fat digestion by lipase. If digestion occurs, the fatty acids produced will turn the litmus cream from blue to pink.

Table 3 Pancreatic Lipase Digestion of Fats

Tube no.	**1L**	**2L**	**3L**	**4L**	**5L**	**4B**	**5B**
Additives (5 gtt ea)	**Pancreatin, water**	**Litmus cream, water**	**Boil pancreatin 4 min then add 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	**Bluish purple**	**Bluish purple**	**Bluish purple**	**pink**	**purple**	**Bright pink**	**Bright purple**

Result: (+) or (-)	-	-	-	+	+	+	+

Conclusion

The objective of the lab was to observe how the digestive system works specifically during chemical digestion. Chemical digestion is the enzyme-mediated hydrolysis of food. The breakdown of the foods can be absorbed and used by the body. The three activities in the lab explain/create an image as to how enzymes work during digestion. The data from the lab supports the hydrolysis of the products by the enzymes. The results of activity 1 confirm the hydrolysis of starch to maltose by the salivary enzyme. We observed the color change following the presence of starch and maltose. This was confirmed by the colors blue-black and green-orange.

In Activity 2 we observed protein digestion by trypsin. Trypsin is an enzyme that is produced by the pancreas (Marieb et al.2019). It hydrolyzes proteins to small peptides. According to the lab manual BAPNA, one of the additives used in Activity 2 is a synthetic color-producing substrate (Marieb et al.2019). We were able to confirm hydrolysis occurred through the presence of yellow color after BAPNA was added. If it was clear no hydrolysis occurred. We were able to see some confirmation of the enzyme at work. In Activity 3 we assess the digestion of fat by lipase and the emulsification of bile. Fatty acids acidify solutions (Marieb et al.2019) which decreases pH. In the lab, we confirmed that digestion was going on by testing pH. A pH change from blue to pink confirms the acidity and digestion going on.

References

Marieb E and Smith L. 2019. Human Anatomy and Physiology Laboratory Manual. 13: 589-594.

Martini FH, Nath JL, and Bartholomew EF. 2018. Fundamentals of Anatomy and Physiology. 11: 885-901