Digestive System Processes: Chemical and Physical

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Introduction

Digestion is one of the fundamental processes of the body in which food is mechanically broken down into small particles and then chemically digested into molecules that can be absorbed (Marieb, pg 589). These nutrients such a protein, carbohydrates, fats, vitamins, etc are all processed and used as energy for the body to function, repair cells, and stay healthy. Proteins break into amino acids and peptides, fats break into fatty acids and glycerol, and carbohydrates break into simple sugars like glucose. Without counting the other organs included in the digestive process, the digestive system is essentially a continuous tube beginning from the mouth and ending at the rectum. It is separated into the upper and lower gastrointestinal tracts with two quadrants each, all of which have different roles and functions in the body. The stages of digestion include ingestion, digestion, absorption, and elimination. After food has entered the mouth, it moves through the pharynx, esophagus, stomach, small intestine, large intestine, and is then eliminated through the rectum. While mechanical digestion is relatively simple in the physical breakdown of food, chemical digestion is a much more complex process in which the chemical makeup is altered in order for the body to absorb all nutrients from the food. All of which would not be possible without one key ingredient: enzymes.

Enzyme proteins are catalysts that speed up a chemical reaction by lowering the activation energy needed in order for the reaction to occur. They are specific to substrates, organic food molecules on which enzymes act on. Each enzyme has its own specific 3D shape consisting of an ‘active site’, which can attract other suitably shaped molecules to bind to the site. A common way to describe this is a “lock and key” mechanism. The enzyme is the lock and the substrate is the key. In the presence of a small amount of the enzyme sucrase, the rate of breakdown is millions of times faster. The enzymes involved in the digestive process are called

hydrolases and their substrates break down the food molecules by adding water. This is called hydrolysis (Marieb, pg 590). Enzymes can be found in pancreatic juice from the pancreas, gastric juice, bile from the liver, intestinal juice from the small intestines, and saliva. Since enzymes are specific to their substrates, their hydrolytic activity can be studied in a test tube. In the following activities, we will be looking at the effects of enzymes on their substrates such as amylase on starch, trypsin on protein, and lipase on lipids as well as the function of bile in the chemical digestion process.

Materials

For all three activities in this experiment, a general supply area of materials are as follows; hot plates, 250ml beakers, boiling chips, tests tubes and test tube rack, wax markers, water bath set at 37°C, ice water bath, and a chart for recording results. For the starch digestion activity, dropper bottles of distilled water, 1% alpha-amylase solution, 1% boiled starch solution, 1% maltose solution, Lugol's iodine solution (IKI), and Benedict's solution are used. For the protein digestion activity, dropper bottles of 1% trypsin and 0.01% BAPNA solution were used. For the bile action and fat digestion activity, dropper bottles of 1% pancreatin solution, litmus cream, 0.1 N HCl, and vegetable oils were prepared along with bile salts and parafilm.

Methods

Activity 1: Salivary Amylase Digestion of Starch

In activity 1, we investigated the hydrolysis of starch to maltose by salivary amylase. The goal is to identify the presence of starch and maltose, the breakdown product of starch, and under which condition the enzyme activity best occurs. 6 test tubes marked with wax markers were labeled 1A-6A, the first 3 tubes serving as controls for the experiment. 3 gtt of distilled water was added to the control test tubes. 3 gtt of amylase were added into test tubes 1A, 5A, and 6A. 3

gtt of starch were added into 2A, 4A, 5A, and 6A. 3 gtt of maltose were added to test tube 3A. For tubes 1A-5A, the incubation condition was set to 37°C for 1 hr and tube 6A's incubation condition was 0°C. The tubes were shaken occasionally to keep the contents evenly mixed. A boiling water bath was set up using the hot plate, boiling chips, and a 250ml beaker. For test tube 4A, 3 gtt of amylase was boiled for 4 minutes, and then 3 gtt of starch was added to the tube. On the spot plate, 6 depressions were marked 1A-6A for sampling. Using a pipet, a sample drop was taken from each tube and placed on their matching place on the spot plate. The first test performed on each sample was the IKI test for color change, in which a blue-black color change would indicate a starch presence and would be regarded as a positive result. Blue results would indicate no starch presence and a negative result. The next test performed was the Benedict's or iodine test, and 3 gtt of Benedict's solution were placed directly into the remaining mixtures of each test tube on the rack. Each tube is placed in the boiling water bath for 5 minutes and monitored for color change. A green to orange precipitate indicates the presence of maltose and a positive sugar result. No color change would indicate a negative sugar result.

Activity 2: Trypsin Digestion of Proteins

In activity 2, we investigated the hydrolysis of proteins to small peptides by trypsin. We prepared dropper bottles of 1% trypsin and 0.01% BAPNA solution. 5 test tubes were labeled 1T-6T, with 1T and 2T serving as the controls for the experiment. 3 gtt of distilled water was added to the control tubes, 3 gtt of trypsin was added to tubes 1T, 4T, and 5T, and 3 gtt of BAPNA solution was added to tubes 2T, 4T, and 5T. For tube 3T, 3 gtt of trypsin was boiled for 3 minutes and then 3 gtt of BAPNA was added. The incubation conditions for tubes 1T-4T were set to 37°C and 5T was set to 0°C, and all tubes were left to incubate in their water baths for approximately 1 hour. The tubes were shaken occasionally to keep the contents evenly mixed. At

the end of the hour, the tubes were examined for color changes. Positive hydrolysis tests were indicated by a yellow color and negative hydrolysis tests were indicated by no color change or a clear mixture.

Activity 3: Pancreatic Lipase Digestion of Fats

For activity 3, we investigated 2 reactions; the hydrolysis of fats and oils by lipase and how bile emulsification aids this process. For the bile emulsification portion, 2 test tubes labeled 1E and 2E are prepared with 20 gtt of distilled water each. 4 gtt of vegetable oil were added to both tubes, with the addition of a pinch of bile salts to tube 2E. Each tube was covered with a square of Parafilm, shaken vigorously, and left to stand at room temperature. Observations were done after 10-15 minutes for emulsification. Emulsification was indicated by droplets of the oil suspending throughout the water, as opposed to the oil floating on the surface of the water. For the fat digestion portion, 5 test tubes were labeled 1L-5L and 2 test tubes were labeled 4B and 5B. Test tubes 1L and 2L served as the controls for the experiment with 5 gtt of distilled water added to each. 5 gtt of pancreatin was added to tubes 1L, 3L, 4L, 5L, 4B, and 5B. 5 gtt of litmus cream was added to tubes 2L, 4L, 5L, 4B, and 5B. A pinch of bile salts was added to tubes 4B and 5B. For tube 3L, 5 gtt of pancreatin was boiled and then 5 gtt of litmus cream was added to the solution. The incubation conditions for tubes 1L-4L were set to 37°C and tubes 5L and 5B were set to 0°C. All tubes were covered with a square of Parafilm, shaken vigorously, and left to incubation in their water baths for approximately 1 hour. The tubes were shaken occasionally to keep the contents evenly mixed. At the end of the hour, fresh litmus cream is made by adding litmus powder in order to perform the lipase assay. The goal is to identify if digestion has occurred by testing the pH of the solutions. If digestion occurs, the fatty acids produced will turn the litmus cream from blue to pink (Marieb).

Results

Activity 1: Salivary Amylase Digestion of Starch

Test tube no.	**1A**	**2A**	**3A**	**4A**	**5A**	**6A**
Additives (3 gtt ea)	Amylase, water	Starch, water	Maltose, water	Boil amylase for 4 mins, 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)	No color change	Color change	No color change	Color change	Color change	Color change
Result: (+) or (-)	_	+	_	+	_	+
Benedict's test (color change)	No color change	No color change	Color change	No color change	Color change	No color change
Result: (+) or (-)	_	_	+	_	+	_

Activity 2: Trypsin Digestion of Protein

Test tube no.	**1T**	**2T**	**3T**	**4T**	**5T**
Additives (3 gtt ea)	Trypsin, water	BAPNA, water	Boil trypsin for 4 mins, then add 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	Color change	No color change
Result: (+) or (-)	–	–	–	+	–

Activity 3: Pancreatic Lipase Digestion of Fats

Test 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	No color change	No color change	No color change	Color change	No color change	Color change	Some color change
Result: (+) or (-)	-	-	-	+	-	+	-

Discussions and Conclusions

For activity 1, Benedict's and IKI tests were performed to detect the hydrolysis of starch to maltose. For the Lugol's test, starch was detected in tubes 2A, 4A, and 6A as indicated by the color change. For the Benedict's test, maltose was expected to be detected in tubes 3A, 5A, and 6A. In the case of tube 4A, the boiling of amylase resulted in the denaturation of the enzyme so no color change occurred. The tubes yielding positive results can also be attributed to the higher temperatures increasing the reaction rate, which is proven by tube 6A yielding a negative result in its 0°C incubation condition. For activity 2, BAPNA solution was used to detect protein digestion by trypsin. All predicted outcomes occurred. Tube 4T yielded a positive result as it had

all the ingredients needed for a reaction to occur which was trypsin and an increased incubation condition of 37°C. For activity 3, two different reactions were observed; fat digestion and bile emulsification. Bile emulsification was first observed with tubes 1E and 2E, showing that bile salts break down fats into smaller molecules that aid the process of fat digestion. Successful fat digestion was detected in tubes 4L and 4B with some positive results yielding in 5B. Tube 4B yielded the most positive result as it had an optimal temperature of 37°C and the addition of bile salts. 5B would have yielded a stronger result in these incubation conditions but did not as it was set to 0°C.

Chemical digestion is a complex process in the body that is essential for obtaining the energy we need to stay alive. In this lab, we observed just a few out of the many enzymes involved in the chemical digestion process and successfully learned how they work with their specific substrates to break down our foods for nutrient absorption. Chemical digestion starts in our mouth with the enzyme amylase breaking down carbohydrates into simple sugars, followed by trypsin breaking down proteins into peptides and amino acids in our small intestine. We also observed how important bile is in the digestion of fat since, without it, the insoluble fat would clump together in our bodies and would be much harder to digest. While this lab was observed virtually, successful results were still yielded and it greatly complimented the lecture on the digestive process.

References

Marieb, E. Smith, L. (2018) Human Anatomy & Physiology Laboratory Manual: Fetal Pig Version. 13th Edition.