Exp.16 Digestive Enzyme

Introduction:

Digestion is the process to chemically and mechanically convert the food into smaller components to facilitate the absorption by the body.

When food enters the mouth, digestion starts by the action of mastication and the contact of saliva, contains salivary amylase (or ptyalin), which starts the digestion of starch (carbohydrate) in the food. Then the food will be a small round soft mass, called a bolus, and travel down the esophagus and into the stomach by peristalsis.

Bolus is further mixed with the gastric acid in the stomach by muscular contractions and starts protein digestion. Gastric acid mainly contains hydrochloric acid and pepsin. HCl denatures the tertiary structures of proteins, making them easier to digest, kills bacteria and converts inactive pepsinogen into the active enzyme pepsin. Pepsin catalyzes the breakdown of proteins into smaller polypeptides. When the pyloric sphincter valve opens, chyme enters the duodenum and then passes through the small intestine where most digestions take place. Most of the digestive enzymes are secreted by the pancreas and enter the small intestine via the pancreatic duct. After the chyme is fully digested, it is absorbed into the blood. 95% of absorption of nutrients occurs in the villi of small intestine. Water and minerals are reabsorbed back into the blood in the colon (large intestine). Waste material is eliminated during defecation.

The three major classes of nutrients that undergo chemical digestion are proteins, carbohydrates and lipids (fats): Protein digestion begins in the stomach, most others takes place in the small intestine. Proteolytic enzymes, including Trypsin and chymotrypsin, are secreted by the pancreas and cleave proteins into smaller peptides; Carboxypeptidase, splits one amino acid at a time; Aminopeptidase and dipeptidase free the end amino acid products. Carbohydrates are degraded into oligosaccharides, simple sugars, or monosaccharides (e.g., glucose) by pancreatic amylase, dextrinase, glucoamylase, maltase, sucrase, lactase, etc. Approximately half of the adult produce only small amounts of lactase and are unable to eat milk-based foods. This is commonly known as lactose intolerance. Some carbohydrates, such as cellulose, are not digested at all. Humans lack the enzyme for splitting the beta-glucose-bonds.

Lipids (fats) are degraded into fatty acids and glycerol. But the fatty triglycerides are hydrophobic and tend to orient towards each other and away from the watery intestinal surroundings. For efficient digestion, pancreatic lipase and intestinal lipase works with the help of the bile salts secreted from the liver and the gall bladder. Bile salts attach to triglycerides to help emulsify them, which aids the access by enzymes.

Reagents:

1. 1% starch
2. Benedict’s reagent

3. 0.02N HCl
4. Pepsin
5. Millon's reagent
6. Pancreatin
7. litmus paper

Procedure:

A. Carbohydrate digestion：

Add 5ml of the 1% starch solution、1ml of saliva into a clean flask and mix well. Place the flask in a water bath at 37-40 ℃ for 20 minutes or longer. Perform Benedict's test for reducing sugars and the iodine test for starch. Record and explain the results.

B. Protein digestion：

Prepare a cellophane tubing. Pour into the tubing 4ml of 0.02N HCl solution and add some cheese、~0.15g of pepsin into the tubing. Tie off the open end of the cellophane tubing, and put it into the 400ml beaker containing ~50ml of 0.02N HCl solution. Next week, perform Millon's test for tyrosine(red ppt.). Record and explain the results.

C. Lipid digestion：

Add ~5ml of whole pasteurized milk (or cream)、litmus paper into three test tubes separately. Add 2ml of pancreatin solution、2ml of boiled pancreatin solution、2ml of water into different test tubes and label it "fat + lipase"、"fat＋boiled-lipase"、"blank" in order. Place these tubes in a water bath at 37-40℃ for about 3 minutes, and record any change in color and odor that occur during the period. Commercial pancreatin contains pancreatic lipase、a potent fat-splitting enzyme. Write the equation for the reaction that occurs when one of the ingredients of buffer fat, tributyrin, is splited by lipase. Explain the changes in color and odor that you observe in the tube containing the lipase. Why do these changes not occur in the "boiled-lipase" tube？

Results：

Record your observations.

Discussions：