NAME_____PER_____ DATE_____

Lab: Carbohydrate Digestion

Saliva contains salivary amylase (ptyalin) that digests starch into sugars. By testing for the transformation of substrate (starch) into product (sugar), the effects of extreme pH and heat denaturation on enzyme activity can then be determined.

QUESTION: What is the most optimal environmental condition for effective enzyme activity?

OBJECTIVES

1. Describe the action of salivary amylase, and explain how its enzymatic activity can be demonstrated.
2. Explain how the activity of salivary amylase is influenced by changes in pH and how high temperature affects enzyme activity.

MATERIALS

- (8) labelled test tubes. (1, 1B, 2, 2B, 3, 3B, 4, 4B)
- bunsen burner
- test tube clamps
- 10ml graduated cylinder.
- Benedict's Reagent Solution
- Hydrochloric Acid (HCl)
- Warm Water bath (37^o C)
- starch solution
- Iodine Indicator (IKI)
- distilled water
- hot plate

The digestion of starch begins in the mouth where it is mixed with saliva containing the enzyme salivary amylase, or ptyalin. Starch, which is a long chain of repeating glucose subunits, is hydrolyzed first into shorter polysaccharide chains and eventually into the disaccharide maltose, which consists of two glucose subunits. Fructose, glucose and galactose are known as ***reducing sugars*** or ***monosaccharides.***

In this exercise, the effects of pH and temperature on the activity of ptyalin will be tested by checking for the disappearance of substrate (starch) and the appearance of product (maltose) at the end of the incubation period. The appearance of maltose or monosaccharides in the incubation medium will be determined by the **Benedict's test**, where heat and the presence of simple sugars causes the solution to change colors. A color change indicates a positive test.

PROCEDURE

1. Begin with the 4 test tubes you already labeled (1, 2, 3, 4)
2. Collect 10ml of saliva into the small, graduated cylinder.
3. Add 3.0ml of distilled water to Tube 1.
4. Add 3.0ml of saliva to Tube 2, Tube 3, and Tube 4.
5. Add 3 drops of HCl to Tube 3.
6. Using a test tube clamp, place **only** Tube 4 containing saliva over the flame until it boils.
7. Add 5.0ml of starch solution to each of the four test tubes.
8. Place the tubes in the 37^o C incubator bath for 1 hour.
9. Split half of the contents of each test tube into tubes labeled 1B, 2B, 3B, and 4B.

10. Test the first group of tubes (1,2,3,4) for starch by adding a few drops of Iodine (IKI). A purplish-black color indicates a positive test for starch.
11. Test the other tubes (1B, 2B, 3B, and 4B) for reducing sugars in the following way: a) Add 5.0ml of Benedict's reagent to each of the tubes and immerse in rapidly boiling water for 3 minutes. b) Remove the tubes from the boiling water with a test tube clamp, and rate the amount of reducing sugar present according to the following scale.

Blue	-
Green	+
Yellow	++
Orange	+++
Red	++++

Data Table:

Contents before Incubation	**Starch Presence with Iodine indicator.**	**Maltose Presence with Benedicts indicator.**
1. Starch + Distilled water		
2. Starch + Saliva		
3. Starch + Saliva + HCl		
4. Starch + Boiled Saliva		

QUESTION ANALYSIS

1. Which tube(s) contained the most starch following incubation? Which tubes(s) contained the most sugar? What conclusions can you draw from these results?

2. What conclusion can you draw if both the test for starch and the test for sugar are positive for a particular tube? What might be the results if you let the tubes incubate for a longer period of time?

3. Reviewing your data, predict what would happen to salivary amylase activity once saliva is swallowed? Explain.

4. What effect does cooking have on enzyme activity? Explain why this effect occurs.