

Lab Exercise: Digestion of Starch by Salivary Amylase

The digestion of a carbohydrate such as starch begins in the mouth, where it is mixed with saliva containing the enzyme salivary amylase. Starch, a long chain of repeating glucose molecules, is hydrolyzed (cut) by amylase into shorter polysaccharide chains and eventually into the disaccharide maltose (known as a reducing sugar), which consists of two glucose subunits:

In this experiment, you will examine the effects of pH and temperature on the activity of salivary amylase. You will measure the activity of salivary amylase by measuring the amount of product formed using Benedict's reagent, which consists of an alkaline solution of cupric ions (Cu^{++}). Cupric ions will be reduced to cuprous ions (Cu^+) in the presence of maltose, forming a visible yellow-colored precipitate of cuprous oxide (Cu_2O).

Procedure: Use **plastic tubes** except for the one you will boil

1. Label 5 clean test tubes 1-5.
2. Obtain 10 ml of saliva in a small, graduated cylinder. Think about chocolate chip cookies if necessary. If this doesn't work, chew a piece of paraffin. If you can get only 5 ml of saliva, dilute it with distilled water to get the 10 ml you need.
3. Add 3.0 ml of distilled water to tube 1.
4. Add 3.0 ml of saliva to tubes 2 and 3.
5. Add 3 drops of concentrated HCl to tube 3.
6. Boil the remaining saliva in a **glass test tube** by passing the tube through the flame of a Bunsen burner. Use a test-tube clamp and keep the tube at an angle, pointed away from your face and from your neighbors. When it is cool, add 3.0 ml of the boiled saliva to tube 4.
7. Add 3.0 ml of maltose to tube 5.
8. Add 5.0 ml of starch to all 5 tubes.
9. Incubate all tubes in a 37°C water bath for 1-1.5 hours.
10. Label 5 new test tubes 1-5.
11. Divide the contents from the first set of 5 tubes in half by pouring half of the contents from each into the newly labeled tubes.
12. Test one set of 5 tubes for starch by adding a few drops of Lugol's reagent (containing iodine) to each tube. A positive result is indicated by the development of a purplish black color. Record your results.

13. Test the other set of 5 tubes for the presence of maltose by adding 5.0 ml of Benedict's reagent to each of the tubes and then immersing them in a rapidly boiling water bath for 2 minutes.

14. Remove the tubes from the water bath and rate the result using the following scale:

Blue (no maltose) -

Green +

Yellow ++

Orange +++

Red (most maltose) ++++