# Pre-Lab Information

Purpose Conduct an investigation to explore the presence of macromolecules in various foods. Add digestive enzymes to determine how they participate in breaking down larger subunits.

Time Approximately 60–75 minutes spread over two class periods

Question How can macromolecules be detected and how can digestive enzymes assist in breaking them down into smaller subunits?

Summary Digestion of food consists of mechanical and chemical processes. In mechanical digestion, large pieces of food are broken down into smaller pieces. In chemical digestion, macromolecules undergo chemical reactions to produce smaller molecules that can be more easily absorbed into the bloodstream.

Our digestive system consists of organs (i.e., esophagus, stomach, and small intestine) that food directly passes through, and accessory organs (i.e., pancreas and liver) that produce chemicals and enzymes designed to assist in chemical digestion. Enzymes, such as amylase and lipase, are protein molecules that facilitate certain types of chemical reactions and food digestion.

Both mechanical and chemical digestion begins in our mouths. Our teeth begin the mechanical digestion of food by shredding and grinding. Our saliva, which contains salivary amylase produced by the salivary glands, begins the chemical digestion of carbohydrates. Carbohydrates are a large group of molecules that includes sugars, starch, and cellulose. Starch is a polysaccharide, which means it is composed of many simple sugars joined together. Glucose and fructose are examples of simple sugars that are called monosaccharides.

In this investigation, you will use indicators to determine the presence or absence of starch, carbohydrates, and protein in a variety of solutions. You will examine the role of amylase in carbohydrate digestion and protease in protein digestion.

# Safety

* Always wear a lab coat, safety goggles, and gloves when performing an experiment.
* Although food-based substances are used in this lab activity, do not eat or drink anything in the lab.
* Make sure all behavior in the lab is purposeful.
* Use caution with glassware such as beakers, test tubes, and stirring rods. Check glassware for cracks and chips prior to use.
* Be careful not to spill when transferring chemicals. Notify your teacher immediately of any spills.
* Use caution with chemical solutions, as several will stain clothing and can be harmful if ingested or spilled on your skin. Do not inhale the solutions. Follow safety precautions and do not mix solutions.
* Use test tube holders to lift the test tubes from the hot water bath. Monitor the hot water bath for bubbling, boiling over, or boiling dry.
* Point open test tubes away from you so that solutions do not bubble toward your face.
* Report all accidents—no matter how big or small—to your teacher.

# Procedure

1. **Gather materials.**

|  |  |  |
| --- | --- | --- |
| * 9 test tubes * Hot plate or hot water bath * Iodine * Alpha amylase (2% solution or saliva) * Test tube rack * Crayon, pencil, or labeling tape | * Stirring rod for each solution * Benedict’s solution * 4 mL glucose solution * Test tube clamp * 2% gelatin solution * Rice water solution * Biuret solution | * 500 mL beaker * Pipettes for each solution * Distilled water * 2% syrup solution * 2% pepsin solution * 2% hydrochloric acid * Whole milk |

## Part I: Starch Detection

1. **Label and fill the test tubes.** 
   1. Label test tubes 1–5 and place them in the test tube rack.
   2. Fill the test tubes with the following solutions. Be sure you designate one pipette and one stirring rod for each solution you are testing as well as each indicator solution so they do not cross contaminate.

Tube 1 = 3 mL distilled water

Tube 2 = 3 mL rice water solution

Tube 3 = 3 mL 2% syrup solution

Tube 4 = 3 mL whole milk

Tube 5 = 3 mL 2% gelatin solution

1. **Add iodine to the test tubes.**
2. Add three drops of iodine to each test tube**. Caution: Iodine can stain clothing and skin. It is poisonous if ingested.**
3. If the test tube turned dark blue/purple or black, the solution had a positive reaction for starch. If test tube remained yellow/red or brown, the solution had a negative reaction for starch. Record the results in Table A under “Starch.” Use a +sign for a positive reaction and a **–** sign for a negative reaction.
4. **Empty your test tubes in the manner indicated by your teacher.**
5. **Clean your test tubes.**

## Part II: Glucose Detection

1. **Label and fill the test tubes.** 
   1. Refill test tubes 1–5 as above.
   2. Label test tubes 6–10. Fill the test tubes with the following solutions.

Tube 6 = 3 mL distilled water

Tube 7 = 3 mL rice water solution

Tube 8 = 3 mL 2% syrup solution

Tube 9 = 3 mL whole milk

Tube 10 = 3 mL 2% gelatin solution

1. **Add amylase or water.**
   1. Add 3 mL amylase solution to test tubes 1–5. You may use saliva that you collect in a test tube or the 2% amylase solution provided by your teacher.
   2. Add 3 mL water to test tubes 6–10.
2. **Add Benedict’s solution.** 
   1. Add 3mL Benedict’s solution to each test tube. **Caution: Benedict’s solution is hazardous to the skin, or if it is ingested or inhaled.**
   2. Use the stirring rods to mix the solutions.
   3. Record the starting color of each test tube in Table A under “Glucose Start Color.”
3. **Boil the test tubes.**
4. Place all 10 test tubes in a boiling water bath for 3–5 minutes. If you are creating your own boiling water bath at your lab station with a 500 mL beaker and a hot plate, do not fill your beaker above midway with water before putting in the test tubes. It is necessary to immerse the test tubes in boiling water so that the reaction between the indicator, Benedict’s solution, and glucose can occur.
5. **Remove the test tubes.** 
   1. Use a test tube clamp to remove the test tubes from the boiling water bath and place them in your test tube rack.
   2. Record the end color of each test tube in Table A under “Glucose End Color.”
   3. If the test tube turned yellow/orange or red/brown and formed a precipitate, the solution had a positive reaction for simple sugars such as glucose. If the test tube turned yellow/orange, it indicates a weaker reaction. If the solution remained a clear blue, the solution had a negative reaction for simple sugars. Record the results in Table A under “Glucose (+/–).” Use a *+* sign for a positive reaction and a **–** sign for a negative reaction.
6. **Empty your test tubes in the manner indicated by your teacher.**
7. **Clean your test tubes.**

## Part III: Protein Detection

1. **Fill the test tubes.** 
   1. Refill test tubes 1–5 as above.
2. **Add Biuret solution.** 
   1. Add 10–15 drops of Biuret solution to each test tube. **Caution: The sodium hydroxide in Biuret solution is caustic. Do not allow it on your skin or ingest any.**
   2. Use the stirring rods to mix the solutions.
   3. Record the color of the solution in Table A under “Protein Color.”
   4. If the test tube turned lavender/purple, the solution had a positive reaction for protein.

If the test tube remained blue or clear, the solution had a negative reaction for protein.

Record the results in Table A under “Protein (+/–).” Use a + sign for a positive reaction and a **–** sign for a negative reaction.

1. **Empty your test tubes in the manner indicated by your teacher.**
2. **Clean your test tubes.**

## Part IV: Protein Digestion

1. **Fill the test tubes.** 
   1. Place three pieces of tofu in test tubes 1–4.
2. **Add solutions.** 
   1. Add 10 mL of water to test tube 1.
   2. Add 10 mL of 2% pepsin solution to test tube 2.
   3. Add 10 mL of HCl to test tube 3.
   4. Add 5 mL of HCl and 5 mL of pepsin to test tube 4.
3. **Record your observations.** 
   1. Look for signs of the solutions reacting with the tofu. Record your observations in the Table B under “Initial Observations.”
4. **Store the test tubes.** 
   1. Follow your teacher’s directions about where to store the test tubes overnight.
5. **Record your observations.** 
   1. Look for signs of the solutions reacting with the tofu. Record your observations in Table B under “Day 2 Observations.”
6. **Empty your test tubes in the manner indicated by your teacher.**
7. **Clean your test tubes.**

# Data

Record your data either in your lab notebook or in the space below.

**Table A**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **Starch** | **Glucose** | | | **Protein** | |
| **Test Tube** | | (+/–) | Start Color | End Color | (+/–) | Color | (+/–) |
| 1 | Water |  |  |  |  |  |  |
| 2 | Rice water |  |  |  |  |  |  |
| 3 | Syrup water |  |  |  |  |  |  |
| 4 | Whole milk |  |  |  |  |  |  |
| 5 | Gelatin |  |  |  |  |  |  |
| 6 | Water |  |  |  |  |  |  |
| 7 | Rice water |  |  |  |  |  |  |
| 8 | Syrup water |  |  |  |  |  |  |
| 9 | Whole milk |  |  |  |  |  |  |
| 10 | Gelatin |  |  |  |  |  |  |

**Table B.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Test Tube** | | Initial Observations | Day 2 Observations |
| 1 | Water |  |  |
| 2 | Pepsin |  |  |
| 3 | HCl |  |  |
| 4 | Pepsin + HCl |  |  |

# Follow-Up Questions

Answer the following questions.

1. Which solutions tested positive for starch? How did you know that starch was present?
2. Which test tubes tested positive for simpler sugars? Was the enzyme, amylase, required for this reaction?
3. Which solutions tested positive for protein?
4. Which test tube showed the digestion of protein? What substances are necessary for the digestion of protein?
5. What is the role of enzymes in digestion?