OBJECTIVE

- To observe and record reactions between three classes of macromolecules in the presence of simple chemical indicators.
- To be able to distinguish positive from negative test results.
- To study macromolecular composition of selected plant and animal products.
- To be able to indicate which of the tested substances are normally found in various biological extracts.

INTRODUCTION

Carbohydrates, lipids, proteins and nucleic acids are four classes of large organic molecules (macromolecules) found in living things. Each macromolecule is composed of many identical or similar subunits (monomers) bonded together. Each type of macromolecule has a characteristic shape and organization, which determines how they function. Knowing this, we can devise tests that relate to the differences of these macromolecules and identify these macromolecules accordingly.

Carbohydrates are the class of macromolecules called sugars. They can range from small simple sugars consisting of only a single monomer (monosaccharide) to larger and more complex polymers consisting of two monomers (disaccharide) or many monomers (polysaccharide). Glucose is a monosaccharide derived from sugars (fructose, sucrose, galactose, etc.). Glucose is the body’s main preferred source of fuel for cellular respiration. As glucose is absorbed into the bloodstream as a result of digestion, it can be used immediately for cellular respiration or stored as glycogen in the liver and skeletal muscles for use later. Glucose is the only nutrient (as opposed to fats or proteins) utilized as a source of energy by the tissues in the brain. In plants, glucose can be used to form various storage or structural polysaccharides. Excess sugars in plants are stored as starch in roots and other tissues. Cellulose is the most abundant structural polysaccharide in plants and a major component of the cell wall for protecting and supporting the whole organism. Although cellulose is in great abundance, it is generally an inaccessible source of carbohydrates to most animals.

Lipids are a class of organic macromolecules composed primarily of carbon and hydrogen atoms; they include fats, phospholipids, waxes and steroids. Fats are stored in the body in the form of triglycerides (body fat) and provide a second source of energy utilized by the body for metabolic processes. Lipids also serve an important role as structural components in cell membranes, synthesis of lipoproteins, hormones, cushioning body organs against injury and insulating the body.

Proteins are polymers of amino acids and form the third group of organic macromolecules. Proteins are the least preferred source of energy by the body. This is because proteins are “expensive” to construct and better used for other metabolic processes. However, proteins are a vital component of an organism’s anatomy and physiology. They are essential for biochemical reactions, necessary for metabolic processes to take place and are integral to the structure of DNA.
Nucleic acids are polymers of nucleotides and form the fourth group of organic macromolecules. Nucleotides are composed of three components: a nitrogenous base, a pentose sugar and a phosphate group. When linked, the sugars and phosphate components of the nucleotides form the backbone, repeating sugar-phosphate-sugar-phosphate-etc. Deoxyribonucleic acid (DNA), a double stranded helical structure, contains an organism’s genetic programming and the inherited structure of a cell’s proteins. It is the nitrogenous base sequence along a DNA polymer that is unique for each gene and results in differences between individuals. Ribonucleic acid (RNA) is a single stranded nucleic acid that is involved in the synthesis of proteins and the genome of some viruses.

MATERIALS

<table>
<thead>
<tr>
<th>Test tube rack</th>
<th>2% Glucose solution</th>
<th>Benedict’s solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beakers for water bath</td>
<td>2% Starch solution</td>
<td>Biuret solution</td>
</tr>
<tr>
<td>Homogenized plant &amp; animal tissue</td>
<td>2% Protein solution</td>
<td>Sudan III or IV</td>
</tr>
<tr>
<td>Hot plate</td>
<td>Fat (oil)</td>
<td>Lugol’s Iodine solution</td>
</tr>
</tbody>
</table>

A. INDICATOR TESTS FOR THE PRESENCE OF MACROMOLECULES

- **Benedict’s solution**: small sugars + Benedict’s + HEAT => green/yellow/orange/red
- **Iodine**: starch + iodine => dark blue / black color
- **Sudan**: oil + Sudan => colored oil
- **Biuret**: protein + Biuret => purple/lavender  
  amino acid + Biuret => pink

Procedure:

1. Fill a 600 ml beaker **1/3 full** with deionized water, place it on a hot plate and heat until it is boiling. Keep this water bath at a boil as you will need it later.
2. Keep an eye of the boiling water so that it does not completely evaporate.
3. The boiling water will be needed for several tests.
4. Label four test tubes and fill them as listed below:
   - **Test tube #1** = 1 ml of glucose solution
   - **Test tube #2** = 1 ml of starch solution
   - **Test tube #3** = 1 ml of lipid solution
   - **Test tube #4** = 1 ml of protein solution

Test for the presence of simple sugars:
1. Add **1 ml of Benedict’s solution** to each of the four test tubes.
2. Note the initial color of each mixture for each tube.
3. Place all four test tubes in the **heated** water bath and note the color change when it is heated sufficiently (5 minutes or so).
4. Use the test tube holder when removing the **HOT** test tubes.
5. In the presence of small sugars, Benedict’s will change to either: green, yellow, orange or red color.
6. Record your observations on the worksheet.
Test for the presence of starch:
1. Clean the four test tubes thoroughly and prepare them as listed above.
2. Add 3 drops of iodine.
3. In the presence of starch, iodine changes to a navy blue/black color.
4. Record your observations on the worksheet.

Test for the presence of fats:
1. Clean the four test tubes thoroughly and prepare them as listed above.
2. Add 3 drops of Sudan.
3. In the presence of fats, you will notice red colored oil throughout.
4. Record your observations on the worksheet.

Test for the presence of protein:
1. Clean the four test tubes thoroughly and prepare them as listed above.
2. Add 1 ml of Biuret.
3. In the presence of proteins, Biuret will change to a purple/lavender color.
   In the presence of amino acids, Biuret will change to a pink color.
4. Record your observations on the worksheet.
5. Thoroughly clean each test tube.

B. MACROMOLECULE ANALYSIS IN VARIOUS FOOD ITEMS

In the above tests you determined simple identifying tests for the presence of macromolecules. You will now use these tests on various food items to see if they contain all or some of the four classes of macromolecules.

Procedure:

1. Develop a hypothesis or hypotheses regarding the macromolecules that may be present in each food item to be tested.
2. Setup four test tubes for the first food item to be tested and label #1 – #4.
3. Add 1 ml of the first food item to each test tube. Use only the liquid portion.
4. To test tube #1, add 1 ml of Benedict’s solution, mix and heat for 5 minutes.
   NOTE: Allow the solution to cool and observe the precipitate for any color changes.
5. To test tube #2, add 4 – 5 drops of iodine solution and mix.
6. To test tube #3, add 3 – 4 drops of Sudan solution and mix.
7. To test tube #4, add 1 ml of Biuret and mix.
   NOTE: All at least 2 – 5 minutes for a reaction to occur.
8. Record your observations on the worksheet.
9. Thoroughly clean and dry each test tube.
10. Repeat steps 3 – 9 for the remainder of the food items to be tested.
A. INDICTOR TEST RESULTS

Table 3.0: Positive and negative tests for indicator solutions:
Fill in the table below by placing a mark in the appropriate box for a positive (+) and a negative (-) result for each test.

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Starch</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benedict’s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sudan</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Biuret</td>
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</tbody>
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1. What are the building blocks (monomers) for each of the following complex molecules?
   a) Carbohydrates:
   b) Proteins:
   c) Fats:
   d) Nucleic acids:

2. If Biuret was added to a test tube and resulted in a lavender color, what does that mean?

3. What is the indicator for starch?

4. A student added Benedict’s solution to a lemonade solution and waited 5 minutes. Did he get an accurate reading? Briefly explain.

5. Benedict’s, iodine, Sudan and Biuret are chemical indicators. What is a chemical indicator?

6. What is a dehydration synthesis?
B. MACROMOLECULE ANALYSIS IN VARIOUS FOOD ITEMS

7. Develop a hypothesis regarding the possible macromolecules in food items.

8. Did your experimentation confirm your hypothesis?

Table 3.1: The presence of macromolecules in food items:
Fill in the table below by placing a mark in the box that corresponds to the presence of a particular macromolecule in each substance indicating a positive test reaction.

<table>
<thead>
<tr>
<th></th>
<th>Benedict’s (small sugars)</th>
<th>Iodine (starch)</th>
<th>Sudan (fats)</th>
<th>Biuret (protein &amp; amino acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td></td>
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<td></td>
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<tr>
<td>Chicken broth</td>
<td></td>
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<tr>
<td>Mayonnaise</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Banana</td>
<td></td>
<td></td>
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<tr>
<td>Potato</td>
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</tbody>
</table>

9. Were there any results that did not agree with the composition of foods?

10. Did you have any results that were unexpected? If so, briefly list and explain them.

11. From your experience with the taste of the food items above, did any substance test positive with the Benedict’s solution that normally does not taste sweet?

12. Let’s say you tested an unknown sample with Biuret and iodine solutions that resulted in a purple/lavender and black color respectively. What can you conclude about this sample?