Macromolecules: Carbohydrates, Proteins, Lipids

All of the foods you eat contain biologically important macromolecules such as carbohydrates, proteins, and lipids. Many foods contain more than one type of macromolecule and may also contain ones that you didn't realize before. Scientists have tests to determine the presence of each of these macromolecules in food and today that is what you will be experimenting with.

Controls:
With all of these tests we will need two controls, a **negative** and a **positive** control. All of the tests we will be using involve a visual change (usually color) when the molecule being tested for is present. The **negative control** is used to show you what the test result looks like when the molecule of interest is not present. In order for this to work you must choose an item that you are 100% sure does not contain the molecule you will be testing for. For all of our tests we will use water as the negative control as it does not contain carbohydrates, proteins or lipids. The **positive control** is the opposite of this; it is used to show us what the test will look like when the molecule of interest is present. In order for this to work you must choose an item that you are 100% sure contains the molecule of interest. Then when you test food items that may or may not contain the molecule of interest you will compare the result to your negative and positive controls to determine the result of your unknown items.

Carbohydrates:
Carbohydrates consist of carbon, oxygen and hydrogen. There are many different types of carbohydrates including **monosaccharides** or simple sugars. Pairing two monosaccharides results in the formation of a **disaccharide** and linking three or more monosaccharides results in the formation of a **polysaccharide**. Starch, cellulose and glycogen are all polysaccharides. The different types of carbohydrates each require different tests. We will be performing two tests for carbohydrates, one for monosaccharides and one for the polysaccharide starch.

Benedict's test for simple sugars
Benedict's solution is a light blue solution that will change to an orange-red color in the presence of simple sugars (monosaccharides). The redder the solution is, the more monosaccharides are present. However, the reaction will not occur unless heat is provided.

Procedure for Benedict's test

1. Fill in the prediction section of table 1 indicating whether or not you think simple sugars will be present in each food item.
2. Obtain 6 test tubes and number them (your instructor may ask you to use more).
3. Add 3 ml of water to tube 1.
4. Add 3 ml of glucose solution to tube 2.
5. In tubes 3-6 we will be adding food items your instructor has for you to test for simple sugars. If your instructor asks you to test more or less foods or differs

from the foods listed in table 1 adjust accordingly. The liquids and solids must be dealt with differently.

- For liquids, add 3 ml to the tube.
- For the solids, obtain a small amount of the food item and crush it using a mortar and pestle, adding several drops of water until you have a somewhat liquid solution. Transfer this to your tube and **rinse off your mortar and pestle before obtaining the next food item.**

6. Add 2 ml of Benedict's solution to each tube.
7. Place the tubes in boiling water for 5 minutes
8. Record your results in table 1.

Table 1.

Tube	Predictions	Food	Benedict's Color	Simple sugar present?
1		Water		
2		Glucose		
3		Sucrose solution		
4		Onion		
5		Potato		
6		Apple		
7				
8				
9				

Iodine test for starch

Iodine-potassium iodide, I_2KI specifically stains the polysaccharide starch. I_2KI is a brownish solution and will remain that way if starch is not present. In the presence of starch, I_2KI turns a dark blue to blackish color.

Procedure for I_2KI test for starch

1. Fill in the prediction section of table 2 indicating whether or not you think starch will be present in each food item.
2. Obtain 6 test tubes and number them (your instructor may ask you to use more).
3. Add 3 ml of water to tube 1.
4. Add 3 ml of starch solution to tube 2.
5. In tubes 3-6 we will be adding food items your instructor has for you to test for starch. If your instructor asks you to test more or less foods or differs from the foods listed in table 2 adjust accordingly. The liquids and solids must be dealt with differently.
    - For liquids, add 3 ml to the tube.
    - For the solids, obtain a small amount of the food item and crush it using a mortar and pestle, adding several drops of water until you have a somewhat liquid solution. Transfer this to your tube and **rinse off your mortar and pestle before obtaining the next food item.**
6. Add 7-10 drops of I_2KI to each tube
7. Mix thoroughly and record results in table 2.

Table 2.

Tube	Predictions	Food	I_2KI Color	Starch present?
1		Water		
2		Starch solution		
3		Glucose solution		
4		Onion		
5		Potato		
6		Apple		
7				
8				
9				

Proteins:
Proteins are polymers of amino acids. They are found in many parts of your body including muscle, hair and finger nails. Additionally, they are found in many foods that you eat on a regular basis (some more than others). Biuret's reagent is used to test for the presence of proteins. Biuret's reagent is a pale blue color and will turn light to dark violet in the presence of protein; the amount of protein present is proportional to the intensity of the violet color.

Procedure for Biuret's reagent test for protein

1. Fill in the prediction section of table 3 indicating whether or not you protein will be present in each food item.
2. Obtain 6 test tubes and number them (your instructor may ask you to use more).
3. Add 3 ml of water to tube 1.
4. Add 3 ml of albumin (pure protein) to tube 2.
5. In tubes 3-6 we will be adding food items your instructor has for you to test for starch. If your instructor asks you to test more or less foods or differs from the foods already listed in table 3 adjust accordingly. The liquids and solids must be dealt with differently.
    - For liquids, add 3 ml to the tube.
    - For the solids, obtain a small amount of the food item and crush it using a mortar and pestle, adding several drops of water until you have a somewhat liquid solution. Transfer this to your tube and **rinse off your mortar and pestle before obtaining the next food item.**
8. Add 7-10 drops of Biuret's reagent to each tube
9. Mix thoroughly and record results in table 3

Table 3.

Tube	Predictions	Food	Biuret's Color	Protein present?
1		Water		
2		Albumin solution		
3		Egg White		
4		Deli meat		
5		Potato		
6		Apple		
7				
8				
9				

Lipids:
The category of lipids contains many molecules including dietary fats (triglycerides). A triglyceride consists of three fatty acid chains attached to a glycerol molecule. There are two tests we will use for lipids. One (Sudan IV) is similar to the tests above; the other is a test that you have likely unknowingly performed at your home or a restaurant more than once. Sudan IV is a red substance, when added to lipids Sudan IV will dissolve in the lipid and color the entire solution red. If exposed to a non-lipid Sudan IV will separate.

Procedure for Sudan IV lipid test

1. Fill in the prediction section of table 4 indicating whether or not you think lipids will be present in each food item.
2. Obtain 6 test tubes
2. Add 3 ml of water to tube 1.
3. Add 3 ml of vegetable oil to tube 2.
4. In tubes 3-6 we will be adding food items your instructor has for you to test for starch. If your instructor asks you to test more or less foods or differs from the foods already listed in table 3 adjust accordingly. We will only be using liquids this time. Add 3 ml of each liquid.
5. Add 7-10 drops of Sudan IV to each tube
6. Mix thoroughly and let stand for five minutes.
7. Record results in table 3.

Table 4.

Tube	Predictions	Food	Sudan IV Appearance	Lipids present?
1		Water		
2		Vegetable Oil		
3		Egg White		
4		Whole Milk		
5		Skim Milk		
6		Juice		
7				
8				
9				

Procedure for paper towel test for lipids

This test is different from the others above, mainly because it does not involve the addition of a solution to the food items. If you have ever set a piece of pizza on a napkin before or wiped grease off of a surface with a paper towel you may have noticed that lipids will turn brown napkins or paper towels translucent, while non-lipids will not.

1. Fill in the prediction section of table 5 indicating whether or not you think lipids will be present in each food item.
2. Obtain a brown paper towel
3. Draw six circles on it and label them as indicated on table 5. Make adjustments as indicated by your instructor if different items will be used.
4. Place a drop of water in the circle labeled "water."
5. Place a drop of oil in the circle labeled "oil."
6. Allow the liquids to evaporate and compare.
7. Now add the remaining food items to their respective circles. For the solids, place the item on the circle and use your pestle to crush it into the paper. Remove the solid components and observe.
8. Record your results in Table 5.

Table 5.

Circle	Predictions	Food	Appearance	Lipids present?
1		Water		
2		Vegetable Oil		
3		Nuts		
4		Coconut		
5		Oats		
6				
7				
8				
9				

Questions:

1. What is the purpose of a positive and negative control?

2. What was the positive and negative control for each test?

3. Which food item had the most simple sugars?

4. Why didn't sucrose react with the Benedict's solution?

5. Was there a difference in the carbohydrates found within the onion and potato? Why might this be?

6. Which food had the most protein?

7. Were there any foods you thought would contain protein that did not?

8. What are the advantages and disadvantages of the two tests for lipids?

9. Which test for lipids did you feel was more accurate?

10. Discuss any of your predictions from all the tests that were not correct. Did any of the results surprise you?