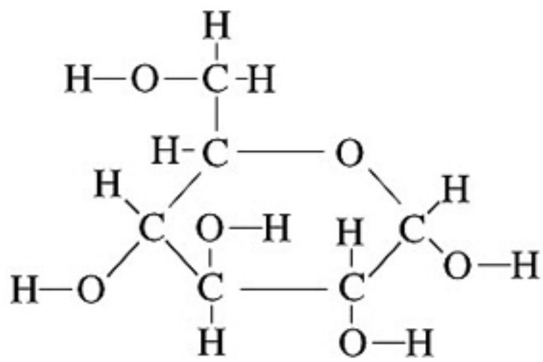
Carbohydrates

a) Introduction

Macromolecules are large molecules found in all living things. There are four major macromolecule types: Carbohydrates, Lipids, Proteins, and Nucleic acids. This lab will review the carbohydrates and demonstrate their presence in certain foods. It may be helpful to review your lecture notes on carbohydrates and the textbook section on carbohydrates before beginning this lab.

b) Monosaccharides

The smallest carbohydrates are called monosaccharides. The illustration below shows glucose, the most abundant of the monosaccharides.



Glucose ($C_6H_{12}O_6$)

The names of some other common monosaccharides are ribose, fructose, and galactose. All these monosaccharides share the following features with glucose: (1) They are ring-shaped molecules containing only carbon, hydrogen, and oxygen atoms. (2) Almost every carbon atom is attached to an OH group, and (3) The molecular formula fits the pattern $C_nH_{2n}O_n$. This means that number of carbons is equal to the number of oxygens, and the hydrogens is twice that number. For example, the monosaccharide ribose has a formula of $C_5H_{10}O_5$.

All monosaccharides are "simple sugars", which means that they taste sweet.

c) Disaccharides

Disaccharides are molecules made from two monosaccharides linked together. For example, the disaccharide sucrose (also called table sugar) is a disaccharide made from a glucose monosaccharide and a fructose monosaccharide linked together. The illustration below represents sucrose (G stands for a glucose molecule and F stands for a fructose molecule).

G–F

The sugar we sweeten our foods with is sucrose isolated from sugar cane or sugar beet. Other common disaccharides are maltose and lactose. Maltose is made from two glucoses linked together. Lactose (also called milk sugar) is made from a glucose and a galactose linked together.

All disaccharides are "simple sugars", meaning that they taste sweet.

d) Polysaccharides

Polysaccharides are molecules made of many glucose molecules linked together. The number of glucose molecules linked together in a polysaccharide can be hundreds or even thousands! The illustration below represents a polysaccharide (each G represents one glucose molecule)

G–G–G–G–G–G–G–G–G–G–G–G–G–G–G–G–G–G–G–G–G–G–G–G

A common polysaccharide in plants is starch. Plants make starch as a way to store the glucose molecules they obtain from photosynthesis. Many of our foods from plants contain starch. In animals, the most common polysaccharide is called glycogen. It is abundant in muscles and liver, but not in other tissues.

Plants also make a polysaccharide called cellulose. Cellulose (also called fiber) is a strong inedible material that is abundant in plant stems, wood, and cotton. Paper, cotton clothing, and lumber are some of the ways we use cellulose.

Although polysaccharides are made of glucose molecules, they are not sugars (meaning, they do not taste sweet).

e) Chemical tests for carbohydrates

There are two common chemical tests that detect carbohydrates. The exact procedure of how to perform these tests will be given in a later section of this handout, but here we will just introduce the basic concepts of each test.

<u>The iodine test for starch:</u> This chemical test detects starch and certain other polysaccharides. It uses a special solution of iodine that is yellow in color. To perform the test, the iodine solution is mixed with a substance that might contain starch. If no starch is present, the iodine solution remains its original color (yellow). If a large amount of starch is present, the iodine solution turns a black color. Colors between yellow and black indicate various amounts of starch:

Yellow/Orange	**gray-yellow**	**light gray**	**blue/purple**	**black**
No Starch	*Very little starch*	*Some starch*	*Much starch*	*Very much starch*

<u>Benedict's test for simple sugars:</u> This chemical test detects simple sugars (monosaccharides and disaccharides). It uses a copper-containing solution called Benedict's reagent that is sky blue in color. To perform the test, Benedict's reagent is mixed with a substance that might contain simple sugars, then boiled. If no sugars are present, the boiled solution stays blue in color. If a large amount of sugar is present, the boiled solution turns brick red. Colors between blue and red indicate various amounts of sugar:

Blue	**green**	**yellow**	**orange**	**red**
No sugar	*Very little sugar*	*Some sugar*	*Much sugar*	*Very much sugar*

<u>A note on experimental controls:</u>

All good experiments should have a negative control and a positive control. It is easiest to explain what these control are by using an example.

Imagine that you are trying to see if there is any lead metal in your drinking water. To do this, you add a dye that is supposed to detect lead by a color change: If the water that has lead, the dye is supposed to turn green. But if no lead is present, the dye turns blue.

To make sure that the dye is working properly, you should do two control tests: (a) You should add the dye to water that you know has no lead. If the dye is working correctly, you should see that water turn blue. This test is the negative control. (b) You should add the dye to water that you know has lead. If the dye is working correctly, you should see that water turn green. This test is the positive control.

So when you are trying to detect a substance by a color change...

The negative control verifies that you get the "negative" color when the substance is not present.

The positive control verifies that you get the "positive" color when the substance is present.

f) Testing foods for sugars and starch

1) Obtain a hot plate and a 500 ml beaker. Fill the beaker with 200 ml of deionized water (from the white-ended facet of the sink) then set the water to boil on the hotplate. While it is coming to a boil, proceed with steps 2 – 8 below.

2) Obtain a test tube rack and 11 clean test tubes. Use a marker to make the following labels on the tubes: 1A, 1B, 2B, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6B. Also obtain 6 disposable pipettes, and a mortar and pestle.

3) Next to the sink is a glass beaker of deionized water with a pipet in it. Using the water in the beaker, add 10 drops of water to tubes 1A and 1B. Using another pipette, add 10 drops of starch solution to tube 2B. The water test tube is the negative control of the experiment. The starch test tube is the positive control of the experiment.

4) Clean the mortar and pestle with a clean damp paper towel. Place a small piece of egg white (about the size and thickness of a dime) into the mortar. Using the water pipette from the small beaker near the sink, add 2 droppers full (**not** 2 drops!) of water to the egg white in the mortar. Use the pestle to homogenize the egg white with the water by grinding forcefully for a minute. Add another two droppers full of water and homogenize for another minute. Using a clean pipette, put 10 drops of the egg homogenate into tubes 3A and 3B. Try to avoid transferring any big chunks of egg since these will clog the dropper.

The mortar will contain some unused food pulp. Dispose of the unused food pulp by dumping it into a plastic tray with paper towels on the side counter.

5) Repeat step 4, but put 1/4 of a teaspoon of rice instead of egg into the mortar and pestle. Put 10 drops of the rice homogenate into tubes 4A and 4B.

6) Repeat step 4, but put a dime-sized piece of bread instead of egg into the mortar and pestle. Put 10 drops of the bread homogenate into tubes 5A and 5B.

7) Repeat step 4 with a new piece of bread, but instead of adding droppers full water to the mortar as you grind the bread, instead add droppers of amylase enzyme. Amylase enzyme is a digestive enzyme in saliva that digests starch into maltose sugar. By adding amylase enzyme to the bread in your mortar you are simulating chewing the bread in your mouth. After homogenizing the bread in your mortar put 10 drops of the bread homogenate into tubes 6A and 6B. We will call this the "chewed bread".

8) Using a clean disposable pipette, transfer 3 ml of Benedict's solution to each of the five "A" tubes. Place the five "A" tubes into the boiling water for 2 minutes, then remove them with a metal test tube tongs.

9) Using the water pipette, add 3 ml of water to each "B" tube.

10) Add 9 drops of iodine solution to each of the "B" tubes. Mix the tube contents of each tube by flicking the bottom of the test tube for 10 seconds.

11) Using section (e) of this handout as a guide for converting color to "amounts", fill out the table below.

Substance	**Amount of sugar (A)**	**Amount of starch (B)**
Water (1) (negative control tube)	________________	________________
Starch solution (2B) (positive control tube)		________________
Egg white (3)	________________	________________
Rice (4)	________________	________________
Bread (not chewed) (5)	________________	________________
Bread (chewed) (6)	________________	________________

12) **Have your instructor inspect your results** before cleaning up.

13) Clean up
• Clean the mortar and pestle thoroughly with soap and water.

• All test tubes containing the Benedicts reagent must be emptied into the Benedict's reagent special waste container. The test tubes with iodine solution must be emptied into the Iodine special waste container. Put the empty test tubes into the glass waste container.

• Dump out the boiling water into the sink but catch the boiling beads in a sieve to prevent them from going down the drain. Dry the beaker and put the boiling beads back into the dried beaker.

• The disposable pipettes can go into the garbage.

g) Review questions

1) What is the molecular formula for glucose? _____________

2) A researcher discovers a new monosaccharide with 4 carbons. Write the complete molecular formula of the new monosaccharide: _______________

3) After each statement below, write M if it applies to Maltose, write L if it applies to Lactose, write S if it applies to sucrose, write ST if it applies to starch, write G if it applies to Glycogen, and C if it applies to Cellulose. Some blanks will require more than one answer.

a) It is composed <u>only</u> of glucose: ____________________________
b) It is called table sugar: ____________________________
c) It is a polysaccharide: ____________________________
d) It is made in plants: ____________________________
e) It is called milk sugar: ____________________________
f) It contains the monosaccharide galactose: ____________________________
g) It contains glucose and another type of monosaccharide: _____________________
h) It is inedible to all humans: ____________________________
i) It is stored in the liver and muscles: ____________________________

4) What carbohydrate(s) does iodine test for? __________________________________
What color indicates it is present? ______ What color indicates it is absent? _______

5) What carbohydrate(s) does Benedict's reagent test for? ________________________
What color indicates it is present? ______ What color indicates it is absent? _______

6) What food item had the most starch? _______ The least starch? _______. What food item had the most sugar? _______ What food item had the least sugar? _______

7) How did the sugar content of chewed bread compare to the sugar content of non-chewed bread?

8) How did the starch content of chewed bread compare to the starch content of non-chewed bread?

9) Explain the change in the sugar content of the chewed bread compared to non-chewed. (Hint: Saliva contains digestive enzymes.) If you are not sure, ask your instructor.

g) Review question answers

1) $C_6H_{12}O_6$

2) $C_4H_8O_4$

3)
  a) M, ST, G, C
  b) S
  c) ST, G, C
  d) S, ST, C
  e) L
  f) L
  g) L, S
  h) C
  i) G

4) Starch (and other polysaccharides, such as glycogen)
  Dark blue   Yellow

5) Monosaccharides and disaccharides (the "simple sugars")
  Yellow, orange, or red   Sky blue

6) [Use your own data to answer this question]

7) Chewed bread had higher sugar than non-chewed bread

8) Chewed bread should have a lower starch content than non-chewed bread [although the difference in starch content might be too small to detect with the iodine stain]

9) Saliva contains amylase enzyme. Amylase breaks down starch into sugars. That is why chewing bread increases the sugar content of the bread: Amylase in the saliva breaks down the bread's starch into sugar.