How-To-Do-It

McMush Lab

Judy Brown

For the Student

Purpose

The purpose of this lab is to analyze the organic compounds found in an ordinary fast-food lunch. You will be working in laboratory teams. Each team will be responsible for testing for the presence of a specific organic compound. The results will be pooled for use by the entire class.

Background

Everything you eat is composed of three major components: carbohydrates, proteins and fats. In order to convert food into energy, the body must be able to break the food into these basic compounds and then further reduce them to the molecular level. The body can absorb food only when it is at the molecular level because it must be small enough to pass through cell membranes. Food is broken down by two processes: **hydrolytic**—breaking down in the presence of water and **enzymatic**—breaking down by enzymes. This process of food breakdown is called digestion.

Laboratory Groups

Simple Sugars (Monosaccharides) Testing Group

The building blocks of carbohydrates are sugars. The simplest sugar is a monosaccharide, glucose. Carbohydrates provide the body with the glucose necessary for cellular respiration and the synthesis of the ATP molecule. Amylase is a digestive enzyme found in the saliva of most humans. It breaks down carbohydrates into sugar. In humans, the digestion of carbohydrates begins in the mouth. Your laboratory group will be adding saliva to your McMush mixture and testing for the presence of carbohydrates by using Benedict's solution. When Benedict's solution is added to a mixture containing carbohydrates, the color changes from blue to orange in a boiling water bath.

Safety Warning: Benedict's solution is caustic and especially dangerous to the eyes. You **must** *wear goggles while working with it and be sure to wash your hands afterwards.* **Do not** *touch or rub your eyes before washing your hands.*

Materials

- 4 test tubes
- 1 test-tube rack
- 1 bottle brush
- 1 bottle of sugar solution
- 1 bottle of distilled water
- 1 bottle of Benedict's solution
- 1 cup of filtered McMush
- 1 empty paper cup
- Goggles
- 6 plastic transfer pipets
- Hot plate with 500-ml beaker of boiling water
- 1 marking pen

Use a separate transfer pipet for each sample or solution.

Procedure

1. Put 1 ml of distilled water into a test tube and label "water."
2. Put 1 ml of filtered McMush into a test tube and label "treated."
3. Put 1 ml of filtered McMush into a test tube and label "untreated."
4. Put 1 ml of sugar solution into a test tube and label "sugar."
5. Add 1 ml of amylase solution to test tubes with sugar, water and the "treated" McMush. **Do not add amylase to the untreated filtered McMush.**
6. Into *all* of the test tubes, add 1 ml of Benedict's solution and swirl tube to mix.
7. Put all test tubes in the boiling water bath for 10 minutes and watch for a color change.
8. Record your results on the data chart and use the bottle brush to clean the test tubes. Return all materials exactly as you received them.

Protein Testing Group

The building blocks of proteins are amino acids. In order for your body to manufacture the specific proteins it needs, the protein eaten in the diet must be broken down into amino acids ready for reassembly. In humans the digestion of proteins begins in the stomach where hydrochloric acid and the enzyme pepsin are present. Your laboratory group will be testing for the presence of amino acids in the McMush sample by using Biuret's reagent. If proteins have been broken down into amino acids, the mixture will turn purple.

Safety Warning: Biuret's solution is caustic and dangerous if it gets into your eyes. You **must** *wear goggles and wash your hands after using it.* **Do not** *touch or rub your eyes before washing your hands.*

Materials

- 4 test tubes
- 1 test-tube rack
- 1 bottle of pepsin
- Biuret's reagent
- 1 bottle of HCl
- 1 bottle of protein albumin
- 1 cup of filtered McMush
- 1 bottle brush
- Goggles
- 7 plastic transfer pipets
- 1 marking pen

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Use a separate transfer pipet for each sample or solution.

Procedure

1. Put 1 ml of water into a test tube and label tube "water."
2. Put 1 ml of albumin into a test tube and label tube "protein."
3. Put 1 ml of filtered McMush into a test tube and label tube "treated."
4. Put 1 ml of filtered McMush into a test tube and label tube "untreated."
5. Add 1 ml of Pepsin to the "water," "protein" and "treated" tubes.
6. Add 1 ml of HCl to the "water," "protein" and "treated" tubes. Swirl tubes to mix.
7. Add 10 drops of Biuret's reagent to all four tubes and swirl to mix.
8. Watch for a color change. Purple indicates the presence of amino acids.
9. Write down results on your data chart.
10. Use the bottle brush to clean the tubes. Return the supplies exactly the way you received them.

Fats Testing Group

Fats are important to your body because they are used to make up part of the cell membrane. The amount of fat permitted in ground beef is regulated by law: 30% maximum for regular ground beef and 15% maximum for lean ground beef. The amount of fat in french fries is not regulated. You will be testing for the presence of fats in the McMush sample by mixing your McMush with water and boiling to separate the fats from the rest of the mixture. You will pour the boiled mixture into a graduated cylinder and cool. The fats will form a layer near the top and solidify as they cool. You can then calculate the percentage of fat in your McMush sample by dividing the ml of fat by the total ml of your sample.

Materials

- 1 cup *unfiltered* McMush
- 1 200-ml graduated cylinder
- Hot plate
- 1 500-ml beaker
- 100 ml water
- Oven mitt
- Spoon or stirring rod

Procedure

1. Pour the entire cup of McMush into the 500 ml beaker.
2. Add 100 ml water to the 500 ml beaker. Stir to mix.
3. Boil the mixture in the beaker for 15 minutes.
4. Cool mixture for 5 minutes.
5. Using oven mitt to protect your hands, pour the mixture into your graduated cylinder and cool by placing it in a refrigerator.
6. Measure the fats accumulated near the top of the graduated cylinder and calculate the fat percentage of the McMush mixture.
7. Write down results on your data chart.
8. Clean your glassware and return the supplies exactly the way you received them.

Starch Testing Group

Starches are carbohydrates in the form of polysaccharides. Polysaccharides are stored by plants for energy reserve and are formed when many single sugars are joined together. A single starch molecule consists of hundreds of glucose molecules. The french fries and bun are both made up of starch. Iodine is often used to test for the presence of starch because it changes from brown to a blue-black when starch is present. You will be performing the iodine test to determine if any of the carbohydrates in your filtrate are in the form of starch.

Materials

- 4 test tubes
- 1 test-tube rack
- 1 bottle of cornstarch mixture
- 1 bottle of iodine
- 1 bottle brush
- 5 plastic transfer pipets
- 1 marking pen.

Use a separate transfer pipet for each sample or solution.

Procedure

1. Stir the starch mixture and add 1 ml to a test tube and label it "starch."
2. Add 1 ml of water to a test tube and label it "water."
3. Add 1 ml of filtered McMush into a test tube and label it "treated."
4. Add 1 ml of filtered McMush to tube marked "untreated."
5. Add 1 ml of iodine to all tubes ***except*** the sample labeled "untreated."
6. Observe any color changes and record in your data chart.
7. Use the bottle brush to clean all test tubes and return the supplies in exactly the same condition as you received them.

Summary

As a lab group, write a summary of this laboratory. You should include an explanation of the controls that were used for ***each test*** and the methods used for testing for the presence of each component. Your summary should include a concluding statement about the various components present or absent in an average fast-food lunch. Turn in one data sheet and one summary for each lab group. Be sure all names of the lab group are on the report.

McMush Lab Data Chart

+ positive control	−negative control
Carbohydrates	**Carbohydrates**
_____ water	_____ water
_____ McMush treated	_____ McMush treated
_____ McMush untreated −	_____ McMush untreated −
_____ sugar solution +	_____ sugar solution +
Proteins	**Proteins**
_____ water	_____ water
_____ McMush treated	_____ McMush treated
_____ McMush untreated −	_____ McMush untreated −
_____ Albumin protein +	_____ Albumin protein +
Fats	**Fats**
_____ % of fats in mixture	_____ % of fats in mixture
Starch	**Starch**
_____ starch +	_____ starch +
_____ water −	_____ water −
_____ McMush treated	_____ McMush treated
_____ McMush untreated −	_____ McMush untreated −

For the Teacher

A significant portion of the diet of young Americans is derived from fast foods. I have developed this variation of a rather standard lab analysis of proteins, sugars, starches and fats. This variation is designed to bring real world experiences into the laboratory as students analyze four classes of biomolecules found in a typical fast-food lunch. The body is able to digest these classes to meet its caloric needs:

1. Carbohydrates are converted into glucose that is circulated in the bloodstream to provide cells with an energy source. Excess carbohydrates are stored in the liver and muscle cell glycogens. If there is a continued excess, carbohydrates are stored as fats in fat cells.
2. Proteins are broken down into individual amino acids that are reassembled as proteins which act as hormones and enzymes to replace cell organelles.
3. Fats can be burned as a source of energy to fuel voluntary muscles.
4. Starches are complex carbohydrates and require more energy for the body to break down into glucose. Starches are initially broken down into maltose by salivary amylase and then to glucose by pancreatic amylase.

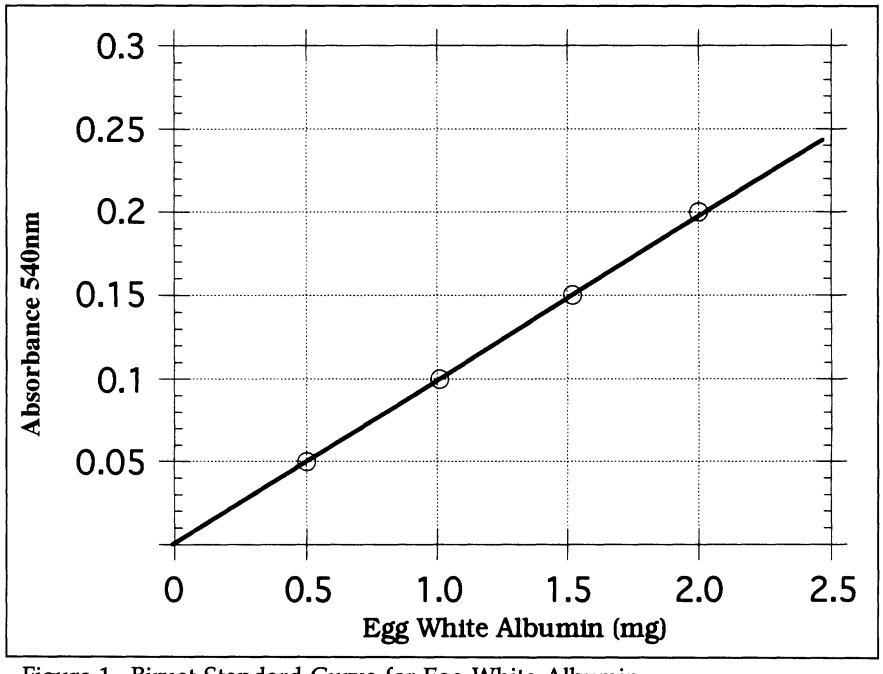

Figure 1. Biruet Standard Curve for Egg White Albumin.

Many fast-food establishments throw away food after it has been on the line for a certain period of time. Teachers can request this food for this lab experience. Use a quarter-pound hamburger sandwich, medium order of french fries and a medium-sized cola drink (12 oz) for each class. Add an 8 oz glass of water to this for a complete McMush luncheon.

On the day of the lab, I tell the students they are going to be given a fast-food lunch to "enjoy." The food is in the original containers in front of the classroom as I explain about the mechanical breakdown necessary for digestion. The lunch is broken up into bite-sized pieces and put into a blender. A flip of the switch produces "McMush." I pass a small cup of mush around for students to see and explain that this is approximately what the food looks like when it enters the stomach. The rest of the mush is filtered through two layers of cheesecloth or a large cone-shaped coffee filter lining a large funnel.

I divide an average-sized biology classes into eight lab groups. Two lab groups do the carbohydrate test, two groups do the fats test, two groups do the starch test, and two groups do the protein test. This cuts down on preparation time since each test only needs two setups.

50 ml (2 oz) of the filtrate is given to each of the protein, carbohydrate and starch lab groups for analysis. The fats lab groups need to use the *unfiltered* McMush. Since the filtering process is somewhat slow, I prepare the filtrate for the first class ahead of time and filter the filtrate of each succeeding class as part of the demonstration.

Students will probably need help understanding the concept of the positive and negative controls used in this lab experience. The lab has been designed so that most groups have controls. The amount of protein present in an average fast-food lunch is so low that the color change on the protein test will only be a slight pink rather than the purple color response of albumin. Students can use this information to analyze the nutritional value of a fast-food lunch (see Table 1).

A copy of the McMush Lab Data Chart is on the overhead projector or chalkboard for each group to record their results. The class uses this collected data for their lab summary. I collect only one data chart and lab summary from each lab group and assign a group lab grade. The solutions and lab setups can be saved in boxes to be ready for the following year.

Pre-Lab Preparation

Solutions: For five sections of this lab, mix two 100-ml bottles of each of the following solutions.

For Starch Testing Groups

Starch: 0.4% solution using cornstarch or soluble starch

Iodine solution: Use undiluted Lugol's iodine

Table 1. Expected values for demonstration lunch.

Quarter pound hamburger on a roll
Medium french fries
12-oz cola drink
Total grams 263
Protein—27 grams = 1% of meal
Carbohydrates—108 grams = 41% of meal
Fats—37 grams = 14% of meal
(Figures supplied by McDonald's Food: The Facts. 1991. McDonald's Corporation, OB#400-8499)

For Protein Testing Groups

HCl: 0.5% solution of 1 N hydrochloric acid

Pepsin: 0.5% solution in water

Albumin: Use powdered albumin or fresh egg whites and mix 1:4 with water.

Biuret's reagent (can be purchased ready-made): 300 ml 10% solution NaOH, 1.50 g $CuSO_4 \cdot 5\ H_2O$

Add distilled water for total volume of 1 liter.

The Biuret reaction can be used to qualify the presence of protein or to quantify amount of protein in the reaction. A Biuret reaction occurs with all compounds containing two or more peptide bonds. The reaction produces a purple color when a complex is formed between Cu^{++} and two nitrogen atoms from each adjacent peptide chain. This complex is called biuret.

In order to quantify the amount of protein in the McMush sample, the following assay can be used:

Prepare a protein standard using powdered albumin 2.0 mg/ml. Prepare a series of dilutions of this standard ranging from 0, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0. To 1.5 ml of each of the dilutions, add 1.5 ml Biuret reagent and mix. Incubate for 15 minutes at 37° C. Using a blank of 1.5 ml distilled water plus 1.5 ml Biuret reagent, calibrate the Spectrophotometer 20 with the frequency set at 540 nm. Plot a standard curve using absorbance readings for each of the dilutions. See Figure 1.

Dilute the McMush sample 1:10, 1:100 and 1:1000. Add 1.5 ml of each dilution to 1.5 ml of Biuret reagent; mix and incubate for 15 minutes at 37° C. Take spectrophotometer readings using absorbance scale. Plot these readings against the standard to quantify the amount of protein in the sample.

For Carbohydrate Testing Groups

Sugar: 15% solution in water

Benedict's (can be purchased premixed): 17.3 g copper sulfate, 17.3 g sodium citrate, 100 g sodium carbonate, distilled water

Dissolve sodium citrate and sodium carbonate in 800 ml *warm* water. Filter and pour into glass graduate. Add water to make a total volume of 850 ml. Dissolve copper sulfate in 100 ml water. Pour sodium carbonate/citrate mixture into large beaker. Slowly add copper sulfate stirring constantly.

Amylase: 0.2% solution in water

For Fats Testing Groups

These groups should be given 100 ml unfiltered McMush to test.

Materials Needed for Teacher Demonstration

For Each Class

- 1 quarter pound hamburger
- 1 medium french fries
- 12-oz cup of cola
- 12-oz cup of water
- Blender
- Large funnel lined with several layers of cheesecloth or large coffee filter
- Large beaker or flask to collect filtrate
- 14 bathroom-sized paper cups

Materials Needed for Student Lab Groups

- Goggles for each student
- 4 hot plates
- 28 test tubes
- 6 test-tube racks
- 6 bottle brushes
- 6 marking pens
- 4–7 plastic transfer pipets
- 2 small bathroom-size paper cups
- 4 500-ml beakers for hot plate
- 2 200-ml graduated cylinders
- 2 oven mitts
- 2 stirring rods or spoons
- 2 100-ml bottles distilled water
- 2 100-ml bottles Benedict's solution*
- 2 100-ml bottles Biuret's solution*
- 2 100-ml bottles albumin solution
- 2 100-ml bottles pepsin
- 2 200-ml bottles HCl
- 2 100-ml bottles cornstarch solution
- 2 100-ml bottles iodine

***Safe Disposal Procedure**

Benedict's Qualitative solution—Flinn #26b

Biuret Solution—Flinn #10

References & Resources

Alexander, R., Griffiths, J. & Wilkinson, M. (1985). *Basic biochemical methods* (Chapter 2). New York: John Wiley.

Flinn Scientific, Inc., 131 Flinn Street, P.O. Box 219, Batavia, IL 60510-0219.

McDonald's Nutrition Information Center. McDonald's Corporation, Oak Brook, IL 60521.

Morholt, E. & Brandwein, P.F. (1986). *A sourcebook for the biological sciences*. Orlando, FL: Harcourt Brace Jovanovich.