

Topic 2: Biochemistry – 2b. Macromolecules in Butter

- Resources: Chu, M. Clarified Butter, *Cooking for Engineers*. [Internet]. 25 Aug. 2005. Available from:
<http://www.cookingforengineers.com/article/131/Clarified-Butter>
- Miller, K., Levine. J. (2004). *Biology*. Boston, MA: Pearson Prentice Hall.
- Margarine. *Chemistry Daily: The Chemistry Encyclopedia* [Internet]. 4 Jan. 2007. Available from:
<http://www.chemistrydaily.com/chemistry/Margarine>
- Building on: The study of the macromolecules that play such a large role in biochemistry requires an understanding of *chemical bonds* and *functional groups*. Macromolecules can act as the gateway to discussing *energy storage*, *photosynthesis*, *cellular respiration*, and *digestion*.
- While the importance of the structure of these molecules can seem rather abstract to high school students, the fact that they provide certain properties to food and that each type of macromolecule behaves differently in food is something they can better understand. This lab reinforces the differences in the properties of the various types of macromolecules and it provides the opportunity to do some science that is both colorful and deals with something they are already familiar with, butter.
- Links to Chemistry: Chemical bonds
Chemical reactions
Organic chemistry
Functional groups
- Links to Physics: Phase changes
Melting point
Molecular structure
Bond energy
- Stories: One of the questions that this lab will often raise is the difference between margarine and butter. Margarine was first produced in 1860 when Emperor Louis Napoleon III of France offered a prize to anyone that could make a substitute for butter that could be used to feed the army. A man named Hippolyte Mège-Mouries developed what he called oleomargarine. He did this by clarifying beef fat (the stuff that most people cut off of their steaks). He extracted the liquid fraction and solidified it with a chemical

called butyric. It tasted somewhat like butter and was an acceptable substitute.

The production of this cheap substitute for butter began to expand and the dairy industry got worried (this margarine didn't require any milk). They fought the expansion of margarine production. One strategy they used was outlawing the addition of color to the margarine. Under natural conditions the margarine is white in color and people wanted margarine to look like butter, so most producers added yellow color. After a court ruled that dairies could not prevent the use of color in the margarine, they attempted to pass a law requiring that red color be added to margarine, knowing that people would find that most disturbing. That law was later overruled and today we have yellow margarine.

You can get more information about margarine and trans fats at the following website:

www.chemistrydaily.com/chemistry/Margarine

Lab Instructions and Materials for the Teacher:

- Unsalted butter
- Test solutions: Benedict's, Biuret, Iodine (can be ordered through science supply houses)
- Brown paper bags cut into strips
- Hot plate with beakers of boiling water
- Test tube holders
- Well plates
- Glucose (dextrose)
- Unflavored gelatin
- Spray starch
- Vegetable oil

The day before the lab you need to prepare the butter. I would use about a pound of butter for one or two classes. You will get lots of the upper fraction (the lipid), but much less of the lower fraction (the protein and some sugar). By using well plates borrowed from chemistry, each lab group only needs a small amount of each fraction for testing, but I would suggest that you hand out their allotted lower fraction and impress on them that it is all they are going to get.

To clarify the butter, put it in a large glass beaker, place it in a microwave and melt it. I prefer to heat it a little at a time and check on it until it is completely melted. Then set it out for a couple of hours before you put it into the refrigerator. The protein and sugar will do two things: most will sink to the bottom and remain a white liquid even after refrigeration and some will rise and form a thin, frothy layer at the top. I skim off the frothy top layer with a spoon or spatula and throw it away. Then I insert a straw down the side of the beaker, all the way to the bottom. When I withdraw the straw I have created a pathway for the white liquid. I tilt the beaker and pour out the lower fraction. You may need to place the straw a couple of

places along the outer edge of the butter, as some of the lower white liquid may be trapped on the other side of the beaker.

When you are ready for the lab, heat up a part of the upper fraction to melt it to liquid form. Use distilled water for all of your negative controls. For your positive controls:

Glucose – Just put a couple of tablespoons of glucose (aka dextrose) into a 400 ml beaker of water and stir.

Protein – Fill a 400 ml beaker with warm (not hot) water and stir in about a teaspoon or two of gelatin; it should not be enough to solidify, but if it does, just heat it a little in a microwave.

Starch – Fill a 400 ml beaker with water and either spray in a generous amount of spray starch and stir or add some of the soluble packing pieces that come with some of your biology orders and stir.

Lipid – Use a little vegetable oil.

Cleanup is a big issue with this lab. I don't even try to get the kids to get that oil and upper fraction out of the well plates and test tubes. I have them place all their dirty tubes and plates in a bucket and I run them through a dishwasher. We have the luxury of having a dishwasher at school, but I used to take them home and run them through my own dishwasher.

Macromolecules in Food (The Butter Lab)

Introduction: We metabolize food in order to release energy to produce the ATP needed for cellular work. We also break down food molecules in order to use their subunits as raw materials for synthesizing our own macromolecules. In this exercise, you will investigate butter to learn which macromolecules it contains.

Objectives: After completing this exercise, you should be able to:

- Understand the tests and procedures that are used to identify the presence of reducing sugars, starch, lipids, and proteins.
- Recognize both the positive and the negative results from each of the macromolecule tests.
- Explain how the components of butter are distributed in two fractions by the process of clarification.
- Interpret the results of tests that indicate the presence of sugar, starch, lipid, and protein in the two fractions produced by the clarification of the butter.
- Understand the use of a positive and a negative control in an experiment.

Separation of Butter: Most foods are complex mixtures of substances. Butter, for example, may appear to be solid fat, but it is actually a mixture. It is an emulsion, which means that the lipids occur in very small droplets dispersed throughout the water-soluble portion of the butter.

The lipid can be separated from the water-soluble portion of the butter in a process called clarification. Clarification occurs when the butter is melted and allowed to slowly cool. Butter is often clarified for use in cooking. Once the water-soluble part has been removed, the lipid that remains can be used to cook at higher temperatures than would be possible using the whole butter because whole butter can easily scorch.

Procedure: In this lab you will be given a negative control (water) for each of the tests you will perform for the identification of the presences of specific macromolecules, reducing sugar (glucose), starch, protein, and lipid. You will also be given a known positive control for each of these types of macromolecules. You will be able to compare the results you get from the two fractions of butter with both the positive and the negative control to determine if a particular macromolecule is present or absent from each fraction of butter.

Macromolecule Tests:

Benedict's Test (reducing sugars – glucose):

1. Place one or two ml of your test substance (this could be your positive control, your negative control (water), your upper fraction of butter, or your lower fraction of butter) into a test tube.
2. Add about 10 drops of Benedict's solution.
3. Shake the test tube gently to mix the two substances.
4. Place the test tube into boiling water for 1 minute and look for a color change.

Biuret Test (proteins):

1. Place a small portion of your test substance in one of the wells of a well plate.
2. Add several drops of biuret solution and look for a color change.

Iodine Test (starch):

1. Place a small portion of your test substance in one of the wells of a well plate.
2. Add several drops of iodine solution and look for a color change.

Brown Paper Bag (lipids):

1. Place a couple of drops of your test substance on a small piece of brown paper bag.
2. Let it sit for at least 10 minutes and observe the spot to see if it has dried or remained translucent.

Butter Fraction Tests:

1. Your teacher will supply you with two fractions of butter that were separated by the process of clarification. One fraction will be called the upper and the other the lower.
2. Follow the procedures for the macromolecule test and for each procedure test the positive control, the negative control, the upper fraction and the lower fraction.
3. Record your results in the Evidence Table provided.

Evidence Table:

Tests	Positive Control		Upper Fraction		Lower Fraction		Negative Control	
	Color	Pos/Neg	Color	Pos/Neg	Color	Pos/Neg	Color	Pos/Neg
Brown Paper Bag								
Benedict's								
Biuret								
Iodine								

Analysis Questions:

1. The upper fraction of the clarified butter lacks the “butter” flavor. What does this tell you about the type of macromolecules that are responsible for the taste of butter?
2. Some students tried this procedure to clarify margarine. They warmed the margarine and left it on ice for 10 minutes as specified in the directions. They found that the margarine has re-solidified, but there is no liquid lower layer. What does this tell them about the margarine?
3. Why might a substance taste sweet, yet give a negative reaction with the Benedict's test?