

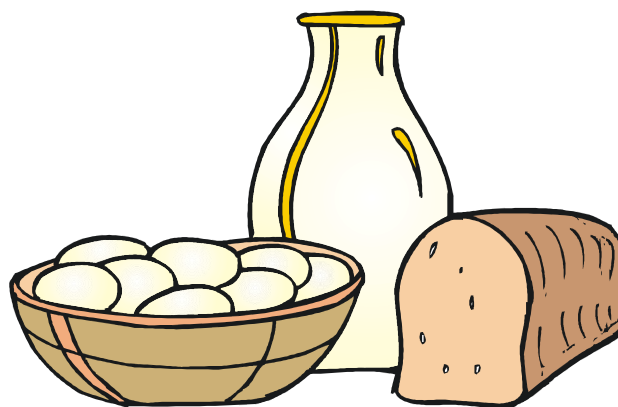
LAB 4: PROTEIN CHEMISTRY**aka FUN WITH MILK & EGGS!****Purpose:**

The purpose of this experiment is to demonstrate the effect of pH and temperature on the structure of proteins. Also to introduce the concept of denaturing proteins.

Background

Most people think of milk as a liquid. Yes, it is a liquid, but milk is really a mixture of fat and protein molecules in a watery solution.

As we discussed in class, proteins are large organic molecules that are built as a chain (or polymer) of amino acids. The behavior and function of the protein is caused by the specific amino acids that are linked together in the chain. These amino acids react with each other and cause the protein chain to twist and fold up into a large 3-D shape, forming a globular protein.



The “R” groups (or side groups) of each amino acid can be either hydrophobic (water-fearing) or hydrophilic (water-loving). When a section of the protein is made of hydrophobic amino acids, it will cause that part of the protein to try to stay away from water. When a section of the protein is made of hydrophilic amino acids, it will cause that part of the protein to try to stay in water.

The protein molecules in milk, called caseins, are very hydrophobic. They try to get away from the watery liquid of the milk, so they fold in on themselves a lot to hide from the water. This folding makes the milk protein molecules into globules in the milk. You can’t see them because even though they are large molecules, molecules are still too small to see with the human eye.

Because pH (the acidity of a liquid) and high temperature both disrupt chemical bonds, they can affect how a molecule forms or how it behaves. This is especially true for proteins, since how they are shaped directly controls how well they function. When a protein loses its 3-D shape and unravels back into a long chain, it is called “denaturing.”

We will investigate these concepts and effects in this lab.

SAY "CHEESE!"



In this experiment we are investigating the effect of pH on protein structure.

Vinegar is a mild acid. It has a lower pH than water. An acid affects how the milk protein molecule holds together. When you add a mild acid to milk, the hydrogen atoms (H^+) floating in the acid try to bond with parts of the amino acids in the milk protein globules. They break some of the internal bonds that hold the milk protein in a 3-D shape and force the protein to unravel back into a long chain. This "denatures" the proteins in the milk.



All these long protein chains in the milk start to twist around each other trying to get away from the watery part of the milk, like lots of loose string getting all tangled up. All the proteins clump up and coagulate. You see this as milk curds, or cheese curds like in cottage cheese. We will now investigate this by making our own cheese. You see, cooking is all about chemistry too!

Materials:

- 600 mL beaker
- 500 mL of milk (4%)
- hot plate
- 10 mL of vinegar
- stirring rod

Procedure:

1. Place milk in the beaker and warm on hot plate to a little above 37°C .
 37°C is roughly body temperature, so the milk should be just hot to the touch.
2. Add vinegar and remove from hot plate. Stir **very gently** only once and then let sit. Coagulation, or curdling, occurs quickly.
3. Drain excess liquid through a filter. This is the whey.
You may let the curds (the solids) sit in the filter (or cheese cloth) until well-drained, for a drier cheese.
4. Make observations.
5. Taste your cheese! You may want to add some salt for extra flavor.
6. Make observation and drawings in your lab notebook

Hints for success:

If the mixture does not coagulate quickly, add more vinegar or lemon juice. Heating the mixture further may help as well.

EGGS-ACTLY!

In this experiment we are investigating the effect of temperature on protein structure.

Some proteins can be denatured by heat alone. Egg proteins are a good example. The behavior of eggs in the kitchen is also all about protein chemistry.

The protein in egg whites is called albumen. The albumen proteins are also long chains of amino acids that are folded up onto themselves in a compact, 3-D, globular shape. Each protein molecule is held in this shape by various kinds of bonds between different parts of its chain.

The bonding that shapes the egg protein molecules is very easily disturbed by changes in temperature (or pH or salt). These changes can cause the protein molecules to bond together into a solid mass — to coagulate.



Take as an example the changes that occur during cooking. When eggs are heated, the increased energy of all the molecules in the egg breaks some of the bonds that keep the albumen proteins in their 3-D shape, and the individual protein molecules begin to unfold. This unfolding exposes more of each molecule's length to others, and so bonding between different protein molecules can occur. As the temperature of the egg rises, then, the proteins unfold more, bump into parts of each other more, and bond to each other more. Eventually, the initially separate, globular protein molecules floating around in the egg white form a mass of extended, intricately interconnected proteins. The liquid has become a solid, because the proteins have clustered together. In other words, the egg coagulates into a boiled egg!

Materials:

- 400 mL beaker
- hot plate
- 1 uncooked egg
- 300 mL water

Procedure:

1. Heat water to almost boiling. Swirl water in a fast vortex.
2. Crack raw egg and drop into vortex of hot water and leave undisturbed.
3. Make observations of changes.
4. Drain and taste your egg! You may want to add some salt for extra flavor.
5. Make observation and drawings in your lab notebook

Questions

1. What are proteins made of?
2. How are amino acids different from one another?
3. What is meant by a “hydrophobic” amino acid? How does it behave around water?
4. What is meant by a “hydrophilic” amino acid? How does it behave around water?
5. Proteins are long chains of amino acids, but they don’t stay as just long winding chains. What happens to their shape and why? Explain.
6. What bonds hold together the primary structure of a protein? Are they strong or weak bonds?
7. What bonds hold together the secondary & tertiary structures of a protein? Are they strong or weak bonds?
8. Describe what happens to protein molecules when you change pH. Also specifically discuss how low pH affects the secondary and tertiary structures of a protein.
9. Describe what happens to protein molecules when you heat them. Also specifically discuss how high temperature affects the secondary and tertiary structures of a protein.
10. What is meant by denaturing a protein?
11. Would cold temperature denature proteins? Explain why or why not.
12. Why when you cool the egg down after cooking does it not become liquid again and why when you neutralize the pH of the cheese, does it not become liquid milk again?

EXTRA CREDIT

- a. What organic and inorganic molecules are in the curds of the cheese?
- b. What organic and inorganic molecules are in the whey of the cheese?
- c. Give another example of how cooking involves chemistry.
- d. Could all the King’s horses and all the King’s men put Humpty Dumpty back together again?
- e. So what is a tuffet, Miss Muffet?