Analysis of the Chemical Components of Milk

Objectives:
- to identify the main chemical components of milk
- to gain experience in natural products separation
- to complete and interpret a lactase analysis

Materials/Equipment:
1. sugar (sucrose) 1. baby food jars or test tubes 1. thermometer
2. water 2. small saucepan 2. aluminum foil
3. 100 mL whole milk 3. funnel 3. 5 bathroom cups
4. paper coffee filters (Mr. Coffee® style) 4. balance 4. 20 mL of vinegar (white preferably)
5. measuring cups 5. small plate, butter knife and spoon
6. lactase enzyme tablets found at any drugstore, grocery or discount store (usually in the antacid area)
7. 2-3 large coffee mugs that can safely handle hot liquid
8. 15 mL mineral spirits (optional: ½ gallon at Walmart is $2.47, but you’ll only need about ~15 mL: your call)
9. reagent strips for urinalysis specific to glucose test strips found at any pharmacy (drugstore, grocery—may have to ask pharmacist) Two common brands are Diastix® and Ketostix®, both test for both glucose and ketones, but Ketostix has a variety that only tests for ketones, so avoid that variety

Introduction

Got milk? Most of us know that milk and other dairy products provide a useful dietary source of calcium, which is important for bone and tooth growth and maintenance, as well as numerous metabolic processes, including cell division, ATP synthesis, muscle contraction, nerve impulse transmission, and blood clotting. Milk is our first source of nourishment and has always played an important role in human nutrition. Indeed, the preparation of milk products such as cheese or yogurt predates recorded history. From a mammal’s perspective, milk has to be an almost perfect food. For the first few weeks or months of life, milk must fill all of an infant mammal’s nutritional needs. Each mammal’s milk is uniquely suited to the metabolic and nutritional needs of that particular mammal.

In this experiment you will isolate and identify some of the major components of milk. The major components of milk are water, protein, fat and the carbohydrate, lactose (milk sugar). It also contains a variety of vitamins and minerals. The exact proportions of these components vary among types of milk (human, cow, goat, dolphin, etc.), among types of commercial varieties of cow’s milk (skim, 2%, whole, etc.) and among brands. You will isolate some of the protein, some of the fat, and test for the presence of sugar.

Proteins. Protein molecules are composed primarily of amino acids linked together through amide bonds (a.k.a. peptide bonds.) Each amino acid has at least one amine function and one carboxylic acid function. An amide bond results from a condensation reaction between these two functional groups, catalyzed in biochemical systems by enzymes.
Enzymes themselves are proteins.

\[
\begin{align*}
R_1 & \quad O \\ H-N-CH-C-O-H & + H-N-CH-C-O-H & \rightarrow & H-N-CH-C-N-CH-C-O-H & + H_2O \\
| & | & | & | & | \\
H & | & H & | & H & | & H & | & H
\end{align*}
\]

Two amino acids condensing to form a dipeptide. This repeats over and over until long chains of peptide residues are strung together to form a protein molecule.

The surfaces of protein molecules display a large number of functional groups, represented in the diagram above as R1 and R2. Many of these are amine or carboxylic acid functions. Amines act as bases. At normal physiological pH's the amine groups are protonated and carry a positive charge:

\[
R-NH_2 + H^+ \rightarrow R-NH_3^+ \quad \text{high pH} \\
\text{low pH}
\]

Amines accept protons to become cations as the solution pH falls.

Conversely, the carboxylic acid functional groups become charged at high pH. They donate protons at physiological pH to become negatively charged:

\[
R-CO_2H \rightarrow R-CO_2^- + H^+ \quad \text{low pH} \\
\text{high pH}
\]

Carboxylic acids donate protons and become anions as the pH rises.

Many water soluble proteins, such as those found in milk, have an excess of either positive or negative surface charges due to their amino or carboxylate groups. As long as one charge or the other predominates, the protein will remain soluble. However, if the pH of the solution is changed dramatically (higher or lower, depending upon the functional groups), so that the number of positive and negative charges on the protein becomes equal, the protein will become less soluble because it will tend to aggregate (clump together) with other protein molecules. The pH at which this charge equality occurs is called the isoelectric point.

A major protein constituent of milk, casein, can be precipitated from milk by acidifying the milk. By adding acetic acid until the final acid concentration is about 1%, the pH of milk (normally about pH 7) is lowered almost to the isoelectric point of casein, pH 4.6. Butterfat will precipitate with the protein and can be separated from it with a bit of effort. Curdled solids and a sour taste are the hallmarks of spoiled milk. Both are caused by lactic acid, a by-product of bacterial growth in milk. Casein and fat are also the main constituents of the curd used in making cheese.

The other major proteins in milk are lactalbumin and lactoglobulin. These two proteins are soluble at both neutral and acidic pHs. They do not precipitate in the curdling process and are often referred to as the "whey proteins" (Remember Little Miss Muffet and her curds and whey? The curds were the casein, discussed above; the whey is the liquid remaining after the curds have precipitated. The whey contains these whey proteins, as well as the minerals and carbohydrate.) Whey proteins can be precipitated by heating. Heat tends to unfold the compact structure of a soluble protein. The extended protein chains become entangled and form insoluble
aggregates. The unfolding process is called denaturation, and is usually irreversible. Heat denaturation of proteins is responsible for the “skin” on boiled milk and for the solidification of egg whites when they cook.

Fats. The fat found in milk is commonly called butterfat. It is suspended as globules in raw milk, but because the density of butterfat is less than that of water, it will rise and separate as cream. Homogenization is a process, which reduces the average size of the fat globules so they will remain homogeneously mixed throughout the milk without separating out for long periods of time. Churning, on the other hand, is a method of causing the butterfat in cream to coagulate to a semi-solid mass called butter, which is about 80% fat.

When the casein is precipitated by acidification of milk, most of the butterfat aggregates with it. The two can be separated based on their chemical differences. Fats are esters formed between long-chain carboxylic acids (fatty acids) and glycerol, a molecule having three carbon atoms, each possessing an -OH group.

\[
\begin{align*}
\text{Glycerol} & \quad + \quad \text{Fatty acids} \\
\text{Fat} & \quad \rightarrow \\
H-C-O-H + H-O-C-C_{x}H_{y} & \quad + \quad H-C-O-C-C_{m}H_{n} \\
H-C-O-H + H-O-C-C_{m}H_{n} & \quad \rightarrow \quad H-C-O-C-C_{n}H_{n} + \quad 3H_{2}O \\
H-C-O-H + H-O-C-C_{p}H_{q} & \quad \rightarrow \quad H-C-O-C-C_{p}H_{q}
\end{align*}
\]

The structures of a typical fat molecule and of glycerol are shown above. The C_{x}H_{y}, C_{m}H_{n}, etc. groups on the fatty acid may be the same or different. They are typically long carbon chains (C6-20) with single or double bonds. As milk contains animal fats, the chains would be saturated, meaning they would have no double bonds. Unlike proteins, fats do not have charged surface groups. Consequently they are much more soluble in organic solvents and much less soluble in water. You will be able to separate the butterfat from the casein precipitate by extracting it with a non-polar solvent.

Carbohydrates: Carbohydrates are hydrated carbon chains, that is, carbon-containing molecules with -OH groups attached to the carbons. Sugars, starches and cellulose are well known hydrated carbon compounds. The carbohydrates found in milk are sugars, the major one being lactose. Lactose is a disaccharide, meaning it is composed of two sugars, glucose and galactose, bonded together. Glucose and galactose are each monosaccharides. Sucrose (table sugar) is another disaccharide, consisting of glucose covalently bonded to fructose. In order to digest lactose, the bond between the two simple sugars must be broken. The enzyme lactase accomplishes this cleavage.

\[
\text{Lactose (glucose–galactose)} \quad \xrightarrow{\text{lactase}} \quad \text{glucose} + \text{galactose}
\]

Lactose is produced by all mammalian infants. Adults of mammalian species, except for some humans, cease the ability to produced lactase and consequently are lactose intolerant. The common symptoms of lactose intolerance in adults are gas, cramping, bloating, or diarrhea after consuming dairy products. This is a different condition than a milk allergy, in which the proteins in milk trigger an allergic response. Lactose remains soluble in the whey even after acidification and heating. Its presence can be detected with common test for sugars, the Fehling's test, which
is not easily done at home. However, lactase can be purchased and when it acts upon lactose, glucose is formed. Glucose tests (used by diabetics to monitor the urinary presence of glucose) are available commercially for home use.

**Other Milk Constituents.** Many vitamins, including A, much of the B complex and C, are present in milk. Vitamin A is yellow in color and fat soluble. It is primarily responsible for the color of butter and cheese. Most of the vitamin C is destroyed by heating during pasteurization. Vitamin D is not found naturally in milk, but it is commonly added. Among the more important mineral constituents found in milk are calcium and phosphate ions (Ca\(^{2+}\) and PO\(_4^{3-}\)). Combined with hydroxide ions, these two form hydroxyapatite, the major constituent of tooth enamel and the solid component of bone.

\[
5\text{Ca}^{2+} + 3\text{PO}_4^{3-} + \text{OH}^- \rightarrow \text{Ca}_5(\text{PO}_4)_3\text{OH}
\]

The formation of hydroxyapatite from its ionic constituents is an equilibrium process, which normally favors the forward, or mineralization, reaction. The reverse reaction, demineralization, is favored by high acid content. Why?

Nursing mothers who lack sufficient amounts of these minerals in their diets are prone to loss of bone or tooth mass through demineralization. As calcium and phosphate ions are consumed in milk production, the blood level of these ions drops. Bone and tooth hydroxyapatite are normally stable relative to its constituent ions, but it will dissociate in response to low levels of these minerals in the blood (simple application of Le Châtelier’s Principle). Bacteria living on your teeth produce lactic acid, especially in response to high sugar levels. As acid levels rise, the rate of tooth demineralization increases. In whole milk the calcium ions are mostly bound to protein.

**Procedural Overview**

Part I: You will separate the casein and butterfat from the rest of the constituents by changing the pH of the milk (effectively curdling it).

Part II (optional): You will separate the casein from the butterfat by their different solubilities in mineral spirits.

Part III: You will separate the lactalbumins and lactoglobulins from the rest of the constituents by heating.

Part IV: You will test for the presence of glucose after submitting the solution to the enzyme lactase.

**Procedure**

I. Isolation of Casein and Butterfat

Measure 100 mL of milk into the coffee mug.

Place 1 – 1½ inches of water in your saucepan. (Check to make sure the mug can stand up in the saucepan with water without spilling). Heat the water on your stove. Do not boil; a temperature of 60°C is more than sufficient. Remove your warm water bath from the heat.
Place the mug in the saucepan of warm water and heat until the temperature reaches 40°C. Monitor with the thermometer.

Remove the mug from the water bath and add ~30 mL of white vinegar to the warm milk. Gently stir the milk and vinegar by swirling jar in circular motion. You should see a separation of the milk.

Filter the coagulated suspension, which contains the casein and the butterfat, through two coffee filters (stacked one inside the other). Collect and save the filtrate (the liquid) in a jar or another coffee mug.

Pour two ~10 mL portions of water through the coffee filter to rinse the gooey solid. Combine the liquid from these washings with the initial liquid and set it aside for now. Continue with the isolation of casein.

Take a piece of aluminum foil big enough to cover the saucepan on the stove. You will be putting your casein/butterfat mixture on top of this aluminum foil so make sure you have enough aluminum foil so that you can bend some extra over the sides of the sauce pan for stability.

Gently scrape the precipitate onto the aluminum foil and spread it out a bit. Do not puncture the foil. Carefully place the aluminum foil over the pot of water and bend the edges down around the pot. DO NOT make an airtight seal. You need room for the steam to escape.

Start heating the pot on the stove. As the heating progresses, water will separate from the precipitate and the precipitate will dry out. Use a wadded up paper towel to absorb the water. Carefully stir up the casein/butterfat precipitate with a spoon or rubber spatula to help it dry out.

When the solid looks fairly dry, remove it from the heat. When it has cooled sufficiently to touch, remove the aluminum foil from the top of the saucepan and set it on the counter.

II. Separating Butterfat from Casein (optional)

If you choose not to perform this part of the experiment, you should determine the weight of the solid you isolated above. Record it on your data sheet. Note its odor and appearance.

**CAUTION: Mineral spirits are toxic and flammable. This part of the experiment should be done outside and away from any flames, heat sources, or sources of electrical spark.**

Place the casein/butterfat precipitate into a small jar or other container. Add ~10 mL of mineral spirits to the casein/butterfat precipitate and use a spoon or end of a drinking straw to mix and mash the mixture trying to break up casein/butterfat. Doing so will allow butterfat to dissolve in mineral spirits separating the two.

Filter this mixture through a new coffee filter. Let the filtrate (liquid) stand so mineral spirits evaporate to reveal butterfat. As best you can, determine the weight of the butterfat and record it on your data sheet. Remove a small portion of the solid; note its odor and appearance.
Weigh the casein after the filter paper has dried. Record it on your data sheet. Note its odor and appearance.

III. Whey Proteins

Take aqueous filtrate (the liquid part you set aside before) from the procedure (Part I) above and place it in a coffee mug and heat as you did in Part I. Turn the stove heat up, as you want the liquid to boil (10 min or more). Add water to the saucepan if it looks in danger of boiling dry.

After ~10 minutes of heating at boiling you should see some solids appear in the liquid. This is the whey protein being denatured by heat.

Continue to boil for 2-3 minutes after you see the whey proteins appear. Remove from heat and carefully filter the whey proteins through a coffee filter.

Let the filter paper dry and note the substance on the filter paper; it is whey protein. If you have enough, weigh it. Record your observations on your data sheet.

You will use the remaining liquid filtrate in the next step (IV).

IV. Test for Carbohydrates

Take the liquid filtrate from Part III above and distribute more or less equally into three (3) small cups or test tubes. Label these test tubes 1–3.

Obtain two (2) additional test tubes or small cups. Label them 4 & 5.

1. Test tube #1 is your control; add nothing else to it.
2. Grind up a lactase tablet with the bowl of a spoon. Place ½ of the resulting powder into test tube #2.
3. To test tube #3, add ½ teaspoon of sugar.
4. In test tube #4, place 10 mL of water and ½ a teaspoon of sugar.
5. In test tube #5, place 10 mL of water, ½ a teaspoon of sugar, and the other half of the powder from the ground up lactase tablet.

Follow the directions on the test strips for urinalysis (only test the solutions in test tubes 1-5 NOT your own urine!) Typically you dip the test strip in the solution and wait 15 seconds to compare its color to the colors the color chart provided. Use one test strip per solution and test each of the 5 solutions. If you have both kinds of test strips, test each solution with each kind of test strip. Record your observations on the data sheet provided.
Experiment 9 Data Sheet

Name___________________________________________

1. Isolation of casein
   mass ___________________
   Describe its odor and appearance.

2. Isolation of butterfat (optional)
   mass ___________________
   Describe its odor and appearance.

3. Isolation of whey proteins
   mass ___________________
   Describe odor and appearance.

4. Analysis of lactose

<table>
<thead>
<tr>
<th>Sample</th>
<th>Contents</th>
<th>Glucose test results (positive or negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>milk filtrate</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>milk filtrate</td>
<td>lactase</td>
</tr>
<tr>
<td>3</td>
<td>milk filtrate</td>
<td>½ tspn sugar (sucrose)</td>
</tr>
<tr>
<td>4</td>
<td>10 mL water</td>
<td>½ tspn sugar</td>
</tr>
<tr>
<td>5</td>
<td>10 mL water</td>
<td>½ tspn sugar lactase</td>
</tr>
</tbody>
</table>

What conclusions can you draw from the analysis of lactose? What did the lactase accomplish?