Introduction

Milk supplies complete nutrition to young mammals. The basic elements of all foods are **proteins, carbohydrates**, and **fats**. These compounds are present in milk in amounts balanced for the needs of each species. The proportions vary widely, as seen below.

Human milk is rich in carbohydrate but relatively low in protein. The quality of milk is typically judged by the fat content.

ANIMAL	FAT	PROTEIN	CARBO-HYDRATES
Northern Elephant Seal	54.5	9.0	<0.3
Northern Fur Seal	49.4	9.8	0.1
CA Sea Lion	43.7	8.9	0.6
Blue Whale	42.3	10.9	1.3
Polar Bear	33.1	10.9	0.5
Humpback Whale	33.0	12.2	1.1
Otter	23.9	11.0	0.1
Human	4.1	0.9	6.8
Cow	3.7	3.2	4.6
African Elephant	5.0	3.6	5.4
Hippopotamus	3.5	5.3	4.3
Domestic Cat	4.6	7.7	4.8
Horse	1.3	1.9	6.9
Black Rhinoceros	1.8	1.1	5.6

Table 1–Weight % of Milk Components

The separation of milk into its components for the production of whey, cheese, and butter has been carried out for many centuries. Most of the fat can be obtained simply by skimming the layer that rises after standing, or by centrifuging. To prevent cream separation, most whole milk is homogenized by pumping it through a small orifice, which breaks up fat globules to such a small size that a stable colloidal suspension results. To make cheese, the major protein, casein, is precipitated, together with the fat if it has not been removed. The carbohydrate, lactose, remains in the whey, or filtrate, together with a mixture of salts, which includes about 12 metallic cations plus chloride, phosphate and sulfate. A substantial amount of the phosphate in milk is precipitated with the casein in the form of phosphate esters of hydroxyl groups in the protein.

The main goal of the experiment is the isolation of the main nutrient components of milk. This will be carried out in the laboratory using the different solubility behaviors of the carbohydrates, proteins, and lipids. This lab will introduce the concepts of stepwise reaction logic in a laboratory setting.

The polypeptide chain in casein (i.e. protein) contains more acidic than basic groups, and casein is soluble as a polymeric anion at the pH of whole milk (about 6.0). The

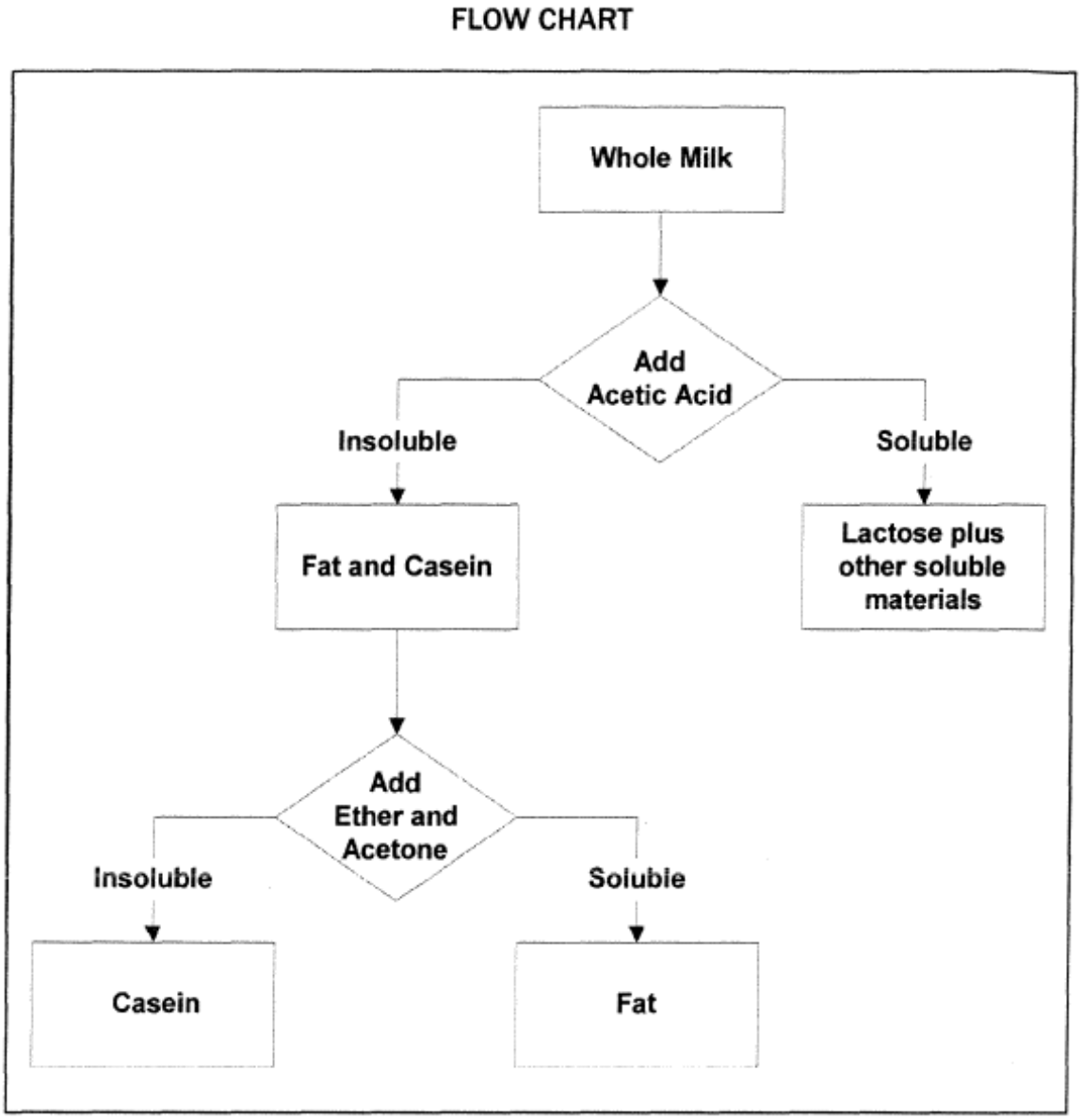
protein is very much less soluble at the isoelectric pH, 4.7, where the anionic and cationic charges are balanced, an addition of a small amount of an acid causes the casein to precipitate. The colloidal particles of butterfat, which are kept in suspension by the presence of protein, agglomerate and separate from the casein. The steps in the separation process are outlined in the flowchart below.

After this initial precipitation, the mixture is strained and the insoluble curd will be extracted using acetone and ether. In this step, the acetone acts as a co-solvent for both water and ether so that the fat globules dispersed in the curd can be dissolved in the ether. The extract is then evaporated completely to remove the acetone, and the fat is isolated.

The butterfat obtained by this procedure is sometimes referred to as "clarified butter", containing about 98% triglycerides plus small amounts of other lipids, including the fat-soluble vitamins. In contrast, creamery butter contains about 14% of water emulsified in the fat plus 1 to 2% of protein. The recovery of butterfat by this solvent extraction procedure is not complete, since some fat remains trapped in the casein. Quantitative analysis for butterfat is an important determination, because milk is graded and purchased on the basis of fat content. This analysis is carried out routinely by the Babcock method. A sample of milk is treated with a large amount of concentrated sulfuric acid to dissolve all of the protein and other constituents except fat, and the volume of the fat layer is measured in a special flask with a calibrated neck.

The carbohydrate in milk is almost exclusively the disaccharide called lactose. The aqueous whey contains a small amount of soluble proteins, lactalbumin and globulins, and these must be removed before the lactose is isolated. In commercial practice, the proteins are separated by neutralizing the solution with lime and heating until a compact mass of protein coagulates. The lactose is then isolated by evaporation of most of the water. In the laboratory procedure, the protein is precipitated by diluting the whey with a large volume of ethanol. After removal of the protein, the lactose crystallizes directly in nearly pure form from the alcohol solution. The procedure is very tedious and does not give good results, therefore we will only test for the presence of lactose in the whey, not isolate it.

Goals for Today's Lab:



Procedure

Separation of Curds and Whey:

1. Using a graduated cylinder, measure out 50 mL of homogenized fresh milk.

2. After measuring with a graduated cylinder, measure the mass of the milk (mass using the measuring cylinder or transfer to 100 ml beaker). This measurement will act as a double check. **How**?

3. Transfer the milk to a 125 ml erlenmeyer flask.

4. Place this Erlenmeyer flask with the milk on a hot plate and warm to around 40°C. Place a thermometer in the milk to measure the temperature. NOTE: You should never rely on the measurement of a hot plate, as these have not been calibrated and may be off by many degrees.

5. Add about ~1 mL of 50% acetic acid to this now-warming milk, swirling after every few drops, until a curdy precipitate forms. Use a glass pipette to transfer.

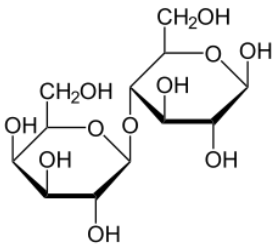
6. Allow the mixture to cool while the curd continues to coagulates.

7. With a large beaker, place a coffee filter over the top and have your lab partner pour the now coagulated liquid from the flask into the filter. This will separate the curds from the liquid. You may need to squeeze the filter in order to dry the solid pieces. Place this filter paper with the curds off to the side, we will use these later.

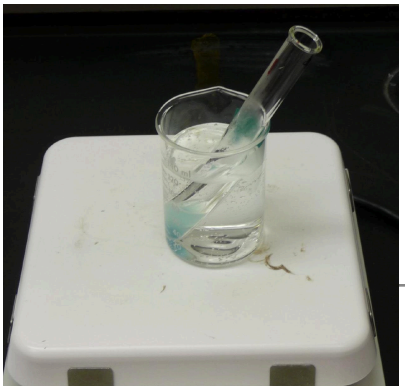
8. The liquid (referred to as the whey) will now be used to test for lactose. Measure the amount you were able to procure using a grad cylinder.

Lactose (Sugar) Testing:

1. Lactose is a **disaccharide**, so we will be testing for its presence using the Benedict's test.

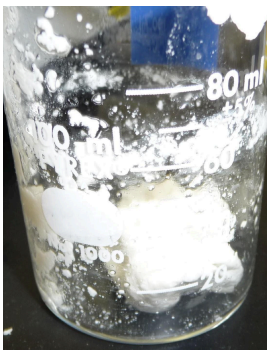


2. Using approximately 20 drops (~ 1 mL) of the whey that we saved earlier, add ~3 mL of Benedict's solution in a medium sized test tube.



3. Prepare a hot water bath using an oversized beaker and place the test tube in, allow the test tube to heat in a boiling hot beaker of water for around ten minutes. Observe the color before and after heating, was there a change? What color

indicates the presence of a reducing sugar?



Isolation of Butterfat (Lipids):

1. Grab the filter paper with the solid curds and transfer these curds into a clean beaker, we will now separate out the fats (lipids).

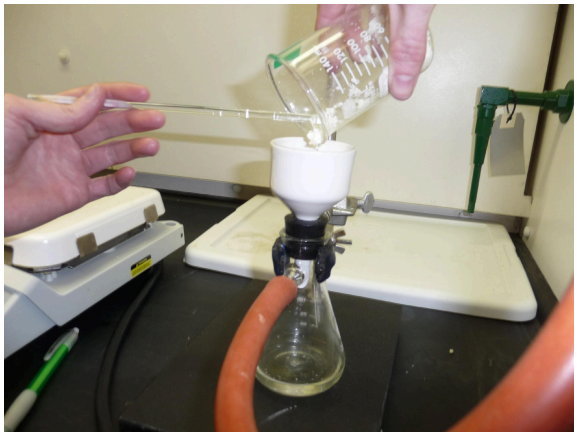
2. Setup your vacuum Erlenmeyer filtration equipment. Before you finalize the setup, weigh your vacuum Erlenmeyer flask. Use a **Buchner** funnel to avoid overwhelming the filter system.

3. After setting up your vacuum Erlenmeyer flask system, add ~10 mL of acetone and ~10 mL of ether into a beaker containing the solid curds.

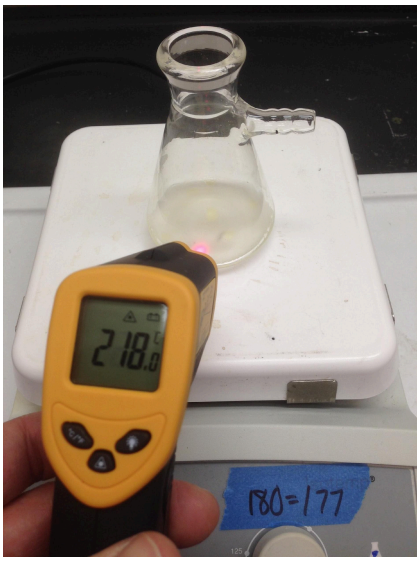
NOTE: The instructor may pour the ether. These must be keep within the hood at all times. Ether is a very volatile compound and the fumes are not pleasant.

4. Using a glass-stirring rod, smooth out all the lumps you can in the solid material. Work the solvent into the material.

5. Turn on the vacuum and pour out the beaker into the filter. Use another ~10 mL of acetone to wash any remaining solids from the beaker into the filter.



6. The vacuum Erlenmeyer flask now contains our lipids and the acetone:ether solvent.



7. Remove the Buchner funnel and place the Erlenmeyer flask containing the liquid on the hotplate and slowly boil away the acetone:ether mixture. You may find that maintaining a vacuum greatly speeds this process. Why?

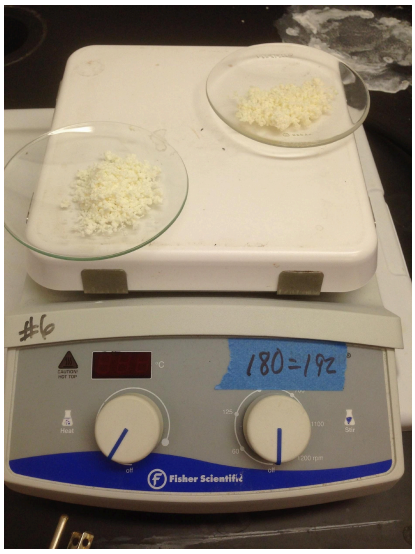
8. You should be left with a yellow residue. This is the butter fat. Weigh and compute the mass of the butterfat.

9. The solid pieces left on the filter paper are the proteins that were unable to dissolve. We will dry these next.

Isolation of Casein (Proteins):

1. Transfer the solid pieces from the last step to a pre-weighed watch glass.

2. Place the watch glass on top of a now warm hot plate (NOT HOT – Unplug the unit beforehand). The heat from the hot plate will help evaporate residual solvents.



3. After allowing ten to twenty minutes for any remaining solvent to evaporate, weigh.

Observations:

Carbohydrates:

Mass of Milk Used:		g
Volume of Milk Used:		mL
Volume of Whey:		mL

Observations of Benedict's Test:

Lipid:

Mass of vacuum Erlenmeyer flask:		g
Mass of vacuum Erlenmeyer flask and Lipids (After evaporation of acetone and the ether):		g
Mass of the Lipids:		g

%Butterfat: (assuming that you had a 50 mL or grams of milk): Use your actual values (i.e. how much milk did you actually use.)

$$\left(\frac{\text{Weight of butterfat collected}}{\text{50 g of milk}}\right) \times 100 = \text{\% butterfat}$$

Protein:

Mass of Watch Glass:		g
Mass of Watch Glass and Proteins		g
Mass of the Proteins:		g

%Casein (or Protein): (assuming that you had a 50 mL or grams of milk):

$$\left(\frac{\text{Weight of casein collected}}{50\text{ g of milk}}\right)\times 100 = \%\text{ casein}$$

Postlab:

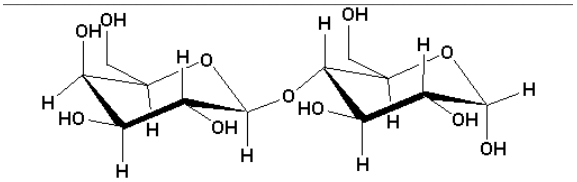
1. **Name** and **draw** the monosaccharide units that makes up lactose? (Haworth Format and use the **COMMON** Name)

(2 Points)

Monosaccharide 1	Monosaccharide 2
Name:	Name:

2. Under aqueous acid conditions and heat the lactose disaccharide can be hydrolyzed into its monosaccharide units. Draw a reaction mechanism showing the hydrolysis of the lactose molecule

(2 Points)



Lactose

3. In the lab we can induce curdling and separation of the protein by adding acetic acid.

Describe using a chemical reaction scheme what is happening chemically when milk sours and curdles naturally. (**What causes** the curdling and what is responsible for the sour taste?)

(2 Points)

4. Lactose can exist in two diasteriomeric forms, either the α form or the β form. Explain the structural difference between these two forms.

(2 Points)

α	β

Explain the Difference:

5. Lactose can be classified as a reducing sugar. What is meant by the term, "**Reducing Sugar**"?

(2 Points)

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- Post Lab