Experiment 14: Biochemistry

Analysis of milk for the lipids, carbohydrates, and proteins.

A. Determining the % Fat in Whole Milk

1. Weigh a clean, dry, empty 50 ml beaker and record the mass in the table on page 2.
2. Using a 10 ml graduated cylinder, measure out 15 ml of whole milk and pour into the 50 ml beaker. Record the mass of the beaker and milk.
3. Determine the mass of **just** the milk and record in the table on page 2.

Calculation:

4. Pour all of the milk into a large test tube. **In the fume hood** add 10 ml of methylene chloride to the milk and cork the tube. Methylene chloride is a nonpolar solvent which will not mix with the water but will take the fat out of the water since fat is also nonpolar.
5. Shake the tube for 30 seconds trying not to get the cork wet. Let the contents of the tube separate into layers.
6. Using a Pasteur pipet remove the milk layer leaving behind the methylene chloride/fat layer. Put the milk back into the 50 ml beaker. Try to get as much of the milk as possible but do not take the nonpolar organic layer out of the tube.
7. Weigh the beaker again with the milk and record.
8. Determine the mass of the milk without the fat and record in the table.

Calculation:

9. Determine the mass of the fat you removed from the milk and record in the table.

Calculation:

10. Find the percent of the fat in the milk by dividing the mass of the fat (# 6 in table) by the mass of the milk (# 3 in table) and multiply by 100.

Calculation:

Table: Components of Milk

1. Mass of empty beaker	
2. Mass of milk and beaker	
3. Mass of milk	
4. Mass of milk w/o fat and beaker	
5. Mass of milk w/o fat	
6. Mass of fat	
7. % Fat in the milk	
8. Mass of protein in milk	
9. % Protein in milk	
10. Mass of evaporating dish	
11. Mass of evaporating dish and 5 ml of milk	
12. Mass of evaporating dish and dried milk	
13. Mass of dried milk	
14. % Water in milk	
15. % Carbohydrates in milk	
16. Mass milk to be dried	
17. Mass of water in milk	
18. % Water in milk	
19. % Carbohydrates in milk	

B. Determining if the Milk Fat contains Unsaturated Fat

1. Do this in the hood. To the test tube with the (don't take the methylene chloride tube out of the hood uncorked!) methylene chloride and removed fat add 3 drops of the solution of **bromine**. Record the Color of the tube after the bromine has been added.

Observation:

Is the fat saturated or unsaturated?_____ How do you know?

_____

_____

_____

_____

Waste Disposal: Keep the milk for the next part, pour the organic layer with the fat, methylene chloride and bromine solution into the waste container labeled "Halogenated Organic Waste".

C. Determining the % protein in Whole Milk

1. Do this in the fume hood. To the milk layer from part one add 30 drops of concentrated acetic acid. **Do not smell the fumes of the acid. If the acid gets on you wash your hands immediately.** Swirl the beaker for 30 seconds and then let sit for a few minutes. Record your observations

Observation:

2. Using a buchner funnel and filter paper with suction filter out the liquid from the precipitate (curds). You may need to press the liquid out of the mixture. Do not discard the liquid. You will use it later.
3. Once the liquid has been separated from the curds, spread the filter paper with the solid onto a watch glass and put that on top of a water bath (250 ml beaker 2/3 full of water on hot plate in the hood). The purpose is to dry the solid so you can weigh it.
4. Weigh the solid by putting a piece of paper towel onto the balance and pushing the tare button. Put the dried solid onto the balance and record the mass.
5. Calculate the percent of protein in the milk the same way you calculated the percent fat in the milk by dividing the mass of the protein (# 8 in table) by the mass of the milk (# 3 in table) and multiply by 100. Record in the table.

Calculation:

D. Test for the Presence of Protein

6. Test for protein in the liquid and in the solid. Label 2 test tubes #1 and #2. In test tube #1 put a few flakes of the solid and about 1 ml of water. Place tube #1 in the water bath. In test tube #2 put some of the filtered liquid. Do not discard the liquid.
7. Go to the reagent bench and get no more than 5 ml of **Biuret reagent (0.01M $CuSO_4$)**. Add about 1 ml of the $CuSO_4$ to test tubes #1 and #2.
8. At your bench pour about 1-2 ml of **6 M NaOH** (sodium hydroxide) into a clean beaker. Add 3 drops of the NaOH to each test tube and mix. Record your observations. Be careful with the NaOH. NaOH can be found on your lab bench in bottle #7.

Test tube #1	
Test tube #2	

A positive Biuret test is a violet color. This means that there is the presence of peptide bonds which are the links between to amino acids. This confirms the presence of protein

Protein in test tube #1? Yes or No	
Protein in test tube #2? Yes or No	

E. Determining the percent water and percent carbohydrate

1. Label 4 test tubes 3, 4,5 and 6. Go to the reagent bench and add about 2 ml of **Benedict's solution** to tubes 3 and 4. Place them in a water bath for about 4 minutes. Remove the flame.
2. Put about 10 drops of the liquid you filtered in a test tube #3 and # 5 and a few flakes of the solid in the other test tube #4 and #6. Leave tubes 3 and 4 in the water bath but remove from the heat. Record your observations.
3. To test tubes 5 and 6 add 10 drops of iodine solution and record observations

Observations: Test tube #3: Test tube #4: Test tube # 5 Test tube # 6

A positive Benedict's test is orange. This demonstrates the presence of lactose.

Lactose in test tube #3? Yes or No	
Lactose in test tube #4? Yes or No	

A positive Idodine test is dark blue. This demonstrates the presence of starch.

Starch in test tube #5? Yes or No	
Starch in test tube #6? Yes or No	

4. Weigh a clean dry evaporating dish and record.
5. Add 5 ml of fresh milk to the evaporating dish and weigh again and record.
6. Set the dish and milk on top of the water bath. (250 ml beaker) Stir the milk to prevent burning. Scrape dried milk from the stirring rod on edge of evaporating dish.
7. Stop heating when the water is gone from the dish. You will no longer see steam coming from the top of the dish. The dried milk will stop changing at this point and keep its consistency. It may look like a paste.
8. Remove the evaporating dish and dry the bottom.
9. Weigh the dish with the dried milk and record.
10. Determine the mass of the water that was evaporated and record.

Calculation:

11. Calculate the percent of water in the milk by dividing the mass of the water (# 17 in the table) by the mass of the milk (# 16 in table) and multiply by 100. Record in the table.

Calculation:

12. Calculate the percent of carbohydrates in milk by adding up the percents of protein, fat, and water and subtracting the total from 100. Record.

Calculation: 100- % fat-% protein-% water = % carbohydrates

Of Course you know that there are minerals in milk such as calcium. Although these are very important nutrients, they make up only about 1% of the mass of milk. So we will disregard that mass here.

Serving size of milk: 244 grams
8 grams of fat
13 grams of carbohydrates
8 grams of protein
146 Calories
From the above label data from a milk container determine the % of fat, protein, and carbohydrates in one serving of milk (244 g). This will serve as theoretical data that you can compare with your experimental results.

Calculations
Theoretical % fat in one serving:
Theoretical % protein in one serving:
Theoretical % carbohydrate in one serving::

Components of Milk		
	Theoretical %	Experimental % (from your table)
% Fat		
% Protein		
% Carbohydrate		

Using your **experimental** percent calculate the mass of each component in a 244g serving. Follow the example for fat using your numbers. Then do the same for protein and carbohydrates

Calculations:
_____% fat (experimental) x 244 g/serving = _____g fat/serving _____g protein/serving _____g carbohydrates/serving

There are 146 Calories in a serving of milk. Fat generally provides 9 Cal/g, proteins provide 4 Cal/g and carbohydrates provide 4 Cal/g.

Using your calculated grams of fat, protein and carbohydrates above, calculate the number of calories from each component and total the Calories in a serving.

Calculations:
_____g fat/ serving x 9 Cal/g = _____Cal/serving from fat _____Cal/serving from protein _____Cal/serving from carbohydrates Total Calories in a serving of milk determined by your experiment: _____Cal/serving

Calories in Milk		
	Theoretical %	Experimental C/g (from your table)
Cal/serving Fat		
Cal/serving Protein		
Cal/serving Carbohydrate		
Total Calories per serving		

Why do you think milk is such an important part of our diet?
