EXPERIMENT 3- ANALYSIS OF MILK FOR THE LIPIDS, CARBOHYDRATES AND PROTEINS

Milk is an emulsion or colloid of butterfat globules within an aqueous fluid. Each fat globule is surrounded by a membrane consisting of phospholipids and proteins which emulsifiers that keep the individual globules from joining together into noticeable grains of butterfat.

The largest structures in the fluid portion of the milk are casein protein micelles (aggregates of several thousand protein molecules) bonded with the help of nanometer-scale particles of calcium phosphate. The casein proteins make up around 80% of the protein in milk, by weight.

The type of carbohydrate found in the milk structure is lactose (the combination of glucose and galactose) which gives milk its sweet taste and contributes about 40% of whole calories of milk.

- Human milk contains, on average, 1.1% protein, 4.2% fat, 7.0% lactose, and supplies 72 kcal of energy per 100 grams.
- Cow milk contains, on average, 3.4% protein, 3.6% fat, 4.6% lactose, 0.7% minerals, and supplies 66 kcal of energy per 100 grams.

Procedure:

A) Determining the Percent Fat in Whole Milk:

- Weigh a dry, clean, empty 100 mL beaker and record the mass.
- Using a 10 mL graduated cylinder, measure out 5 mL of milk and pour into the beaker and record the mass of the beaker and milk.
- Determine and record the mass of just milk. Then, add 20 mL of water into the beaker.
- Pour all of the milk into a large test tube. In the fume hood add 25 mL 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.
- Shake the test tube for 30 seconds trying not to get the cork wet. Let the contents of the test tube separate into layers.
- Using a pasteur pipet remove the milk layer leaving behind the methylene chloride/fat layer. Put the milk back into the 100 mL beaker. Try to get as much of the milk as possible but do not take the nonpolar organic layer out of the tube.
- Weigh the beaker again with the milk and record your data.

Questions:

✔ Determine the mass of the fat you removed from the milk and record. Find the percent of the fat in the milk and compare with the known values.
B) **Determining if the Milk Fat Contains Unsaturated Fat:**

- Do this part of the experiment in the hood!!
- To the test tube with the methylene chloride and removed fat, add 3 drops of bromine solution.
- Record the color of the solution in the test tube after the bromine has been added.
  - Keep the milk for the next part, pour the organic layer with the fat, methylene chloride and bromine solution into the liquid waste container.

**Questions:**

- Is the fat content saturated or unsaturated? Explain by giving your reasons.
- Is there any other chemical test to determine the unsaturation of the structures? If any, explain the test briefly.

C) **Determining the % Protein in Whole Milk:**

- Do this part of the experiment in the hood!!
- To the milk layer from part one, add 1 drop of concentrated acetic acid. **DO NOT smell the fumes of the acid.**
- Swirl the beaker for 30 seconds and then let sit for a few minutes.,
- Record your observations.
- Using a buchner funnel and filter paper, filter out the liquid from the precipitate. **DO NOT discard the liquid. You will use it later.**
- Once the liquid has been separated from the precipitate, 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 a hot plate). The purpose is to dry the solid so that you can weigh it.
- Weigh your solid and record the mass.

**Questions:**

- Calculate the percent of protein in the milk and compare with the known values.
- What happens when you add the concentrated acetic acid into the milk solution. Explain briefly by giving your reasons.

D) **Test for the Presence of Protein:**

- 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 that you collected in part C and about 1 mL of water. Place test tube #1 in the water bath. In test #2 put some of the filtered liquid from part C.
- Still, DO NOT discard the liquid.
- Add about 1 mL of Biuret Reagent (0.01M CuSO4) to both test tubes, #1 and #2. 
- Add 3 drops of 6M NaOH to each test tube and mix. 
- Record your observations.

**Questions:**

- Why Biuret test is used to determine the absence or presence of proteins? Explain by giving your reasons and reactions if necessary.
- Are your observations consistent with your expectations? Explain briefly.

**E) Determining the Percent Water and Percent Carbohydrate:**

- Label 4 test tubes as #1, #2, #3 and #4. Add 2 mL of Benedict's (Fehling's) Solution to test tubes #1 and #2. Place them in a water bath for about 4 minutes.
- Put about 10 drops of the liquid you filtered in a test tube #1 and #3 and a few flakes of the solid in the other test tubes #2 and #4. Leave tubes #1 and #2 in the water bath but remove from the heat. Record your observations.
- To test tubes #3 and #4, add 10 drops of iodine solution and record your observations.

**Questions:**

- What is the aim of performing Benedict's Test?
- What is the aim of performing Iodine Test?

- Weigh a clean dry evaporating dish and record the mass.
- Add 5 mL of fresh milk to the evaporating dish and weigh again and record the mass.
- Set the evaporating dish with milk on top of the water bath. Stir the milk continuously to prevent burning.
- 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). Also, take off the dried milk from the stirring rod on the edge of the evaporating dish.
- Remove the evaporating dish and dry its bottom before you weigh it.
- Weigh the dish with the dried milk and record.

**Questions:**

- Calculate the percent water in the milk.