**Macromolecule Identification Laboratory**

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We often hear that milk is a complete food. In this assignment, you will identify the components of different types of milk and 2 products that are made from milk.

There are several dairy by-products on the market. Since these products are derived from whole milk harvested on the farm, the first step is to determine the components of the milk.

Milk is a complex substance and its composition varies depending on the animal species, breed, diet and even the season. Complete the table below for each of the constituents of fresh cow’s milk.

|  |  |  |
| --- | --- | --- |
| **Constituents** | **Semi-developed formula description**  | **Quantity****(g/100 g)** |
| Water |  |  |
| Carbohydrate |  |  |
| Lipid |  |  |
| * Saturated fatty acids
 |  |  |
| * Monounsaturated fatty acids
 |  |  |
| * Polyunsaturated fatty acids
 |  |  |
| Protein (3 main) |  |  |
|  |  |
|  |  |
| Non-protein nitrogenous matter |  |  |
| Minerals |  |  |
| Vitamins |  |  |
| Enzyme |  |  |
| Dissolved gas |  |  |

The components of milk do not have the same behaviour towards water.

Cow’s milk consists of a true solution, a colloidal solution, a colloidal suspension and an emulsion.

|  |  |  |
| --- | --- | --- |
| **Physicochemical condition** | **Constituents** | **Dimensions (mm)** |
| True solution | Carbohydrate | 10-9 to 10-10 |
| Minerals | 10-3 to 10-4 |
| Colloidal solution | Serum protein  | 10-2 to 10-3 |
| Emulsion | Lipid | 0.1 to 20 |
| Colloidal suspension  | Casein micelle | 0.03 to 0.3 |

**Definition:**

* **True solution**: mixture of substances solute — solvent;
* **Colloidal solution**: mixture formed by a dispersed insoluble phase, present in the form of fine solid particles in a liquid phase whose particles have a strong affinity for the aqueous phase;
* **Colloidal suspension**: a mixture formed by a dispersed phase not solubilized, present in the form of fine solid particles in a liquid phase;
* **Emulsion**: mixture of a dispersed phase in a non-solubilized liquid, in the form of fine droplets in a liquid dispersing phase.

**Nutrient analysis**

**Objective:** Highlight nutrients in whole milk, skim milk and whey

**Material**

|  |  |
| --- | --- |
| * Whole milk (about 120 mL)
* Skim milk (about 20 mL)
* Whey (about 20 mL)
* Curd (about 20 mL)
* Glucose solution
* Vegetable oil
* Egg white
* A sheet of brown paper
* 6 3 mL plastic pipettes
* 2 500 mL beakers
* 1 glass rod
* A 10 mL and 100 mL graduated cylinder
* A test tube holder
* Test tubes
* Pliers
 | * Filter paper (or nylon filter)
* Benedict’s solution (Lugol or Fehling’s liquor)
* Sudan III dye
* Ammonium oxalate
* Silver nitrate
* Ammonium molybdate
* Concentrated nitric acid (Protein indicator tape or NaOH (1 mol/L) and CuSO4 (0.5%)
* Acetic acid 1 mol/L
* Distilled water
* Water bath
* Hot plate
* 1 funnel
 |

**Protocol**

**Step 1: Prepare the whey**

Whey is the liquid resulting from the coagulation of milk. It is the liquid residue obtained during the cheese-making process.

1. Pour 100 mL of 3.25% milk into a beaker
2. Bring milk to near boiling point (80 °C).
3. As soon as it starts to boil, add 5 mL of acetic acid
4. Turn off the heat.
5. Let stand for 10 minutes.
6. Place a funnel on a 100 mL graduated cylinder or a beaker. Place a filter paper in the filter.
7. Let drain for 15 minutes to remove whey.
8. Save the whey and curd for part 2 of the lab.
9. If the curd is too dense, add distilled water to the curd and mix well.

**Step 2: Identification of components**

To validate the presence of nutrients, known control samples will be used. Water that does not contain nutrients, glucose (sugar), vegetable oil (lipid), egg white (protein).

**Part A: Carbohydrates**

* 1. Prepare a hot water bath by filling a beaker halfway.
	2. Label the test tubes
		1. Water
		2. Glucose
		3. Whole milk
		4. Skim milk
		5. Whey
		6. Curd
	3. Pour 3 mL of each substance into the corresponding test tube.
	4. Add 2 mL of Benedict’s solution to each test tube.
	5. Place the test tubes in the water bath and heat gently for 5 to 10 minutes.
	6. Identify colour changes and record observations.

***Note :*** *another technique for identifying carbohydrates*

1. *Lugol test: add 2 drops of Lugol to each test tube*
2. *Fehling’s liquor : add 3 drops of Fehling’s liquor to each test tube. Place the test tubes in the water bath and heat gently for 5 to 10 minutes.*

**Part B: Lipids**

1. Paper test
	* 1. On a sheet of brown paper, draw 6 circles about 4 cm in diameter
		2. Identify the circles from 1 to 6 (**Note**: *glucose is replaced by vegetable oil*)
		3. Water
		4. Vegetable oil
		5. Whole milk
		6. Skim milk
		7. Whey
		8. Curd
		9. Using a glass rod, place and rub each sample in the corresponding circle. Use the same order as for the carbohydrate test.
		10. Let the sheet dry and observe the presence of translucid traces on the paper
		11. Record your observations.
	1. Microscope observation
		1. Identify slides from 1 to 6
		2. Place one drop of each substance on a slide and cover with a coverslip
		3. Add one drop of Sudan Red III dye
		4. Observe under the microscope and record your observations

**Part C: Proteins**

 a. Number and identify the test tubes from 1 to 6.

1. Water
2. Egg white
3. Whole milk
4. Skim milk
5. Whey
6. Curd
7. Pour 3 mL of each substance into the corresponding test tube
8. Carefully pour a few drops of nitric acid into each tube
9. Observe the colour changes and record your observations.

 ***Note:*** *other protein identification techniques*

1. *Albustix ribbon and colour chart. Pour 10 drops of each sample into marked watch glasses. Dip the ribbon in the solution and read the colour chart.*
2. *NaOH (1 mol/L) and CuSO4 (0,5 %) Pour in 5 mL of sample, add 20 drops of NaOH and 5 drops of CuSO4*

**Part D: Minerals**

* 1. **Calcium salts**
		1. Number and identify the test tubes from 1 to 5
		2. Water
		3. Whole milk
		4. Skim milk
		5. Whey
		6. Curd
1. Pour 3 mL of each substance into the corresponding test tube
2. Carefully pour a few drops of ammonium oxalate into each tube
3. Observe the formation of a white precipitate. Record your observations.
	1. **Chlorure**
		1. Number and identify the test tubes from 1 to 2
4. Water
5. Whole milk
6. Skim milk
7. Whey
8. Curd
	* 1. Pour 3 mL of each substance into the corresponding test tube
		2. Carefully pour a few drops of silver nitrate (AgNO3)
		3. Observe the formation of a white precipitate. Record your observations.
	1. **Phosphate**
		1. Number and identify the test tubes from 1 to 2
9. Water
10. Whole milk
11. Skim milk
12. Whey
13. Curd
14. Pour 3 mL of each substance into the corresponding test tube
15. Carefully pour a few drops of ammonium molybdate
16. Observe the formation of a yellow precipitate. Record your observations.

**Observation**

The report must contain the results of each test in a tabular format.

**Analysis**

* 1. Which of the products tested contained carbohydrates?
	2. Identify the reducing sugar in milk. Give its semi-developed formula.
	3. Which of the products tested contained protein?
	4. Which of the products tested contained lipids?
	5. There are several categories of fatty acids in milk: saturated, unsaturated, monounsaturated, polyunsaturated, cis, ω3, ω6, ω 9... Research and give an example of each category. Indicate which are the most abundant in milk.
	6. Make a comparison between the composition of whey and whole milk.
	7. Which of the products tested contained minerals?
	8. Cow’s milk contains vitamins A, D, E, K, B2, B3, B12. Research whether they are water-soluble or fat-soluble. In what other foods are they found? Gather the results of this research in the table below
	9. What vitamins are not found in skim milk?
	10. Make a comparison between the composition of whey and whole milk.
	11. Make a comparison between the composition of curd and whole milk.
	12. Knowing that cheese is made by the same process as curd production, what type of milk would be the best to obtain the maximum yield. Explain your answer.