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Determination of percentage of protein in the given sample of milk and its common adulterants

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Milk is an emulsion or colloid of butterfat globules within a water-based fluid. The largest structures in the fluid portion of the milk are casein protein micelles.The outermost layer consists of strands of one type of protein, kappa-casein, Caseins have an appropriate amino acid composition that is important for growth and development of the nursing young. Caseins are highly digestible in the intestine and are a high quality source of amino acids. Milk is a source of Conjugated linoleic acid. CLA has been shown to kill human skin cancer, colorectal cancer and breast cancer cells in vitro studies and may help to lower cholesterol level and prevent atherosclerosis. Milk consumption reduces the risk of arterial hypertension, coronary heart disease, colorectal cancer and obesity. The common adulterants of the milk are cane sugar, cereal flour and gelatin solution. The experiment was performed for the determination of percentage of protein in different samples of milk by formal titration – Pyne's metod (A. K. Gupta and M. L. Varshney, 1997). The experiments were also performed for the determination of cane sugar, cereal flour and gelatin solution in different milk samples. The percentage of protein in different milk samples ranges between 2.21 % to 3.91% Most of the milk samples contain of cane sugar but no milk sample was found to have cereal flours or gelatin solutions.

Keywords :- *Milk, Milk Protein, Adulterants, Cane sugar, Cereal flour, Gelatin Solution.*

Introduction : Milk is an emulsion or colloid of butterfat globules within a water-based fluid. Each fat globule is surrounded by a membrane consisting of phospholipids and proteins; these emulsifiers keep the individual globules from joining together into noticeable grains of butterfat and also protect the globules from the fat-digesting activity of enzymes found in the fluid portion of the milk[2]. In unhomogenized cow's milk, the fat globules average about four micrometers across. The fat-soluble vitamins A, D, E, and K are found within the milk fat portion of the milk.

The largest structures in the fluid portion of the milk are casein protein micelles[3]: aggregates of several thousand protein molecules, bonded with the help of nanometer-scale particles of calcium phosphate. Each micelle is roughly spherical and about a tenth of a micrometer across. There are four different types of casein proteins, and collectively they make up around 80 percent of the protein in milk, by weight.

Milk contains dozens of other types of proteins besides the caseins. They are more water-soluble than the caseins and do not form larger structures. Because these proteins remain suspended in the whey left behind when the caseins coagulate into curds, they are collectively known as *whey proteins*. Whey proteins make up around twenty percent of the protein in milk by weight. Lactoglobulin is the most common whey protein by a large margin (Harold McGee., 1984).

Caseins have an appropriate amino acid composition that is important for growth and development of the nursing young. This high quality protein in cow milk is one of the key reasons why milk is such an important human food. Caseins are highly digestible in the intestine and are a high quality source of amino acids. Most whey proteins are relatively less digestible in the intestine, although all of them are digested to some degree. When substantial whey protein is not digested fully in the intestine, some of the intact protein may stimulate a localized intestinal or a systemic immune response. This is sometimes referred to as milk protein allergy and is most often thought to be caused by ß-lactoglobulin. Milk protein allergy is only

one type of food protein allergy. Milk is a source of Conjugated linoleic acid. CLA has been shown to kill human skin cancer, colorectal cancer and breast cancer cells *in vitro* studies, and may help lower cholesterol and prevent atherosclerosis.

It appears to be effective at promoting muscle growth. Depending on the age, milk contains 8 grams of protein, and a number of other nutrients (either naturally or through fortification) including: Biotin, Pantothenic acid, Iodine, Potassium, Magnesium, Selenium, Thiamine , Vitamin A, Vitamin B12, Riboflavin, Vitamins D & Vitamin K (Composition and Structure of Milk, 2009).

Studies show possible links between low-fat milk consumption and reduced risk of arterial hypertension, coronary heart disease, colorectal cancer and obesity. Overweight individuals who drink milk may benefit from decreased risk of insulin resistance and type 2 diabetes (Diary's Role in Managing Blood Pressure) Other studies suggest that milk consumption may increase the risk of suffering from certain health problems. Cow's milk allergy (CMA) (Cow milk protein allergy presenting with Hirschsprung's disease imicking Symptoms) is as an immunologically mediated adverse reaction to one or more cow's milk proteins. Rarely is it severe enough to cause death. Milk contains casein, a substance that breaks down in the human stomach to produce casomorphin, an opioid peptide. In the early 1990s it was hypothesized that casomorphin can cause or aggravate autism, and casein-free diets are widely promoted.

The milk is commonly adulterated with the following adulterants:-

1. Cane sugar,
2. Cereal flours &
3. Gelatin solution.

Objective :

1. To recognise a sample of milk rich in protein i.e. determination of percentage of protein in the given sample of milk.
2. To find out the common adulterants in the given sample of the milk.

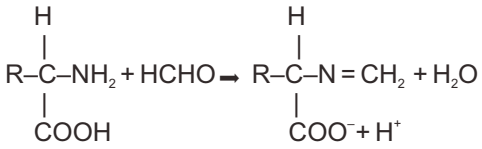
Area of study : PATNA

EXPERIMENTS :-

AIM I :- DETERMINATION OF PROTEIN IN DIFFERENT SAMPLES OF MILK BY FORMAL TITRATION BY PYNE'S METHOD.

Theory :-

When formaldehyde (HCHO) is added to the milk which was previously titrated against the standard alkali to the end point of an indicator phenolphthalein, it binds the amino groups of milk proteins and release an equivalent amount of hydrogen ($H^{+)}$ could be titrated against the alkali to the same end point. The amount of alkali used in the second titration is the measure of amino groups originally present in the protein.



Amino acid *Formaldehyde*

Procedure :-

1. 10 ml of the well mixed sample of milk was pipette into a flask. 5 drops of phenolphthalein indicator was added to it.
2. 0.4 ml of saturated potassium oxalate was added and was left undisturbed for few minutes.
3. The milk was titrated against the standard alkali to its end point of faint pink colour.
4. 2 ml of neutral formaldehyde solution was added and mixed.
5. The mixture was again titrated against the standard alkali to the same end point and the volume of alkali used in the second titration was recorded.

Calculations :

Volume of N/10 NaOH required by 10ml of milk treated with = V ml of HCHO

% of protein in the given sample per 10ml of milk = V x 1.7 sample of milk.

AIM II:- DETERMINATION OF CANE SUGAR IN MILK.

Theory :

Resorcinol when added to acidic solution of sucrose or cane sugar, it gives a red coloured complex.

Procedure :

1. 10 ml of milk in a test tube was taken.
2. 1 ml of Conc. HCl was added to it.
3. About 0.1 gm of Resorcinol powder was added and was mixed to the Solution in the test tube.
4. The test tube was kept in the boiling water bath for about 5 minutes.

Observation:

Red colouration indicates the presence of cane sugar in the milk.

AIM III:- DETERMINATION OF CEREAL FLOUR (STARCH) IN MILK

Theory :

Iodine solution gives intense blue colour with starch due to the formation of an unstable starch-iodo complex. The colour vanishes on heating which reappears again on cooling.

Procedure :

1. 3 ml of milk was taken in a test tube.
2. 10 ml of water was added to it and was boiled.
3. The boiling solution was cooled and few drops of Iodine solution were added to it.

Observations :

If the solution turned to blue colour, then the milk is adulterated with starch or cereal flours.

AIM IV:- DETERMINATION OF GELATIN SOLUTION IN MILK

Theory:

Gelatin solution produces yellow precipitate with Picric acid.

Procedure:

1. 5 ml of milk was taken in a test tube.
2. 5 ml of Mercuric nitrate solution was added to it and was shaken well.
3. 20 ml of water was added and the mixture was left undisturbed for few minutes.
4. The mixture was filtered and the filtrate was collected in a test tube.
5. Equal volume of saturated aqueous solution of picric acid was added to the filtrate in the test tube.

Observations:

If a yellow precipitate is obtained, then the milk sample is adulterated with gelatin solution.

OBSERVATIONS FOR ONE SAMPLE

SAMPLE I :- SUDHA SMART (YELLOW)

SIZE :- 500 ml

Observation:

Table 1:- WITHOUT FORMALDEHYDE

Observation No.	Volume of Milk Taken	Burette Reading (ml) (N/10 NaOH)			Concurrent Reading (ml)
		Initial	Final	Difference	
1.	10 ml	0.5	1.0	0.5	0.5
2.	10 ml	3.0	4.7	1.7	
3.	10 ml	5.7	6.2	0.5	
4.	10 ml	8.3	8.8	0.5	
5.	10 ml	10.6	11.1	0.5	

Table 2:- WITH FORMALDEHYDE

Observation No.	Volume of Milk Taken	Burette Reading (ml) (N/10 NaOH)			Concurrent Reading (V ml)
		Initial	Final	Difference	
1.	10 ml	1.0	3.0	2.0	2.0
2.	10 ml	4.7	5.7	1.0	
3.	10 ml	6.3	8.3	2.0	
4.	10 ml	8.8	10.6	1.8	
5.	10 ml	11.1	13.1	2.0	

Calculations:

Volume of N/10 NaOH required by 10 ml milk treated with formaldehyde = V ml

% of protein in the given sample per 10 ml of milk

= V x 1.7 sample of milk

= 2.0 x 1.7 = **3.40%**

ADULTERATION TESTS:

Cane Sugar – Not present
Cereal Flour – Not present
Gelatin Solution – Not present

OBSERVATIONS FOR ALL MILK SAMPLES:

Similarly all the observations for all the milk samples are tabulated in the following observation table 3.

Table:-3

S. No.	Sample	Volume of Milk (ml)	Vol. of N/10 NaOH (ml) (Concurrent Reading)		Percentage of Protein
			Without Formaldehyde	With Formidehyde	
1.	Sudha Smart	10	0.5	2.0	3.40
2.	Sudha Healthy	10	0.4	1.9	3.23
3.	Sudha Shakti	10	0.3	2.0	3.40
4.	Sudha Gold	10	0.5	2.0	3.40
5.	Sudha Cow Milk	10	0.8	2.2	3.74
6.	Raj Fresh	10	0.2	2.3	3.91
7.	Raj Shakti	10	0.2	2.0	3.40
8.	Raj Diamond	10	0.4	2.3	3.91
9.	Raj Double Tonned	10	0.2	2.1	3.57
10.	Raj Tonned	10	0.3	2.0	3.40
11.	Amulya Powder Milk	10	0.7	2.2	3.74
12.	Everyday Powder Milk	10	0.2	1.4	2.38
13.	Local Cow Milk	10	0.2	1.3	2.21

OBSERVATION FOR ADULTERANTS
Table:- 4

S. No.	Sample	Adulterants		
		Cane Sugar	Cereal Flour	Gelatin Sol^{n}
1.	Sudha Smart	A	A	A
2.	Sudha Healthy	A	A	A
3.	Sudha Shakti	P	A	A
4.	Sudha Gold	P	A	A
5.	Sudha Cow Milk	A	A	A
6.	Raj Fresh	A	A	A
7.	Raj Shakti	P	A	A
8.	Raj Diamond	P	A	A
9.	Raj Double Tonned	P	A	A
10.	Raj Tonned	P	A	A
11.	Amulya Powder Milk	A	A	A
12.	Everyday Powder Milk	A	A	A
13.	Local Cow Milk	P	A	A

Note :- P = Present: A= Absent

Conclusion :

1. Raj Fresh milk has the highest protein content of 3.91% per 10 ml of the milk and shows no adulteration.
2. Local cow milk has the least protein content of 2.21% per 10 ml of the milk and cane sugar is present as an adulterant.
3. In all the milk the protein content is approximately equal to 3% per 10 ml of milk.
4. Most of the milk sample contains cane sugar.
5. None of the milk sample contains cereal flour and gelatin solution

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4. *Diary's Role in Managing Blood Pressure, web page of the US National Dairy Council.*
5. *Cow milk protein allergy presenting with Hirschsprung's disease imicking Symptoms.*