International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

Microbial and qualitative analysis of milk

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Submitted: 13-03-2024 Accepted: 23-03-2024

ABSTRACT

Milk is an important diet of vast population on earth due to its nutritional value. Milk adulteration is a common social problem today. Apart from the ethical and economical issues, it also creates health hazards. It is a common practice by the milk supplier to add water. Addition of water changes specific gravity of the milk, its color, texture flavor etc. To compensate the specific gravity, different types of salt and sugars are added. Chemical adulterants are used for various purposes. The common adulterants are urea, starch, sugars etc. So to prevent this adulteration, detection and control of milk are very important. Adulterants in the milk are detected by various chemical tests. Qualitative detection of adulterant in milk are simple color based chemical reaction. Due to high nutritive value of milk, it forms ideal medium for rapid multiplication of bacteria. Microbial load in milk was determined by microbiological tests such as Standard plate count (SPC) followed by quality check including Methylene blue reductase test (MBRT).Here four milk samples were collected which includes two domestic milk (cow and goat) samples and two other commercial milk samples. Density, fat, solid non-fat and total solids of these milk samples were calculated and acidity is also determined. Then various adulteration tests are carried out to detect hazardous chemicals present in milk samples. Then the quality of milk sample is determined by microbial analysis using Standard plate count and MBRT. Finally using these data, comparative study of milk samples is carried out.

Keywords: Microbiological test;Microbial load; Standard plate count; Methylene blue reductase test; Solid non-fat.

I. INTRODUCTION

Milk may be termed as the whole, fresh, lacteal secretion obtained by the complete milking of healthy animals. Milk is a whitish liquid containing proteins (2.5%), lactose (5%), fats (3.6%), water (87.5%) and various minerals and vitamins (0.7%) produced by the mammary glands of all mature female mammals. The milk produced by cows, goats and other animals is used for human consumption.It is one of the most popular and in-demand-product in India because of its nutritional value. India is the world's largest producer and consumer of milk around the globe. Besides being used in original form, it is utilized in many other ways. Milk is a valuable nutritious product that has a concise life and requires careful handling. Milk is a highly perishable item because it is an excellent medium for the growth of microorganisms that can cause hazardous health issues for consumers.

Milk is an excellent growth medium for microorganisms when suitable temperature exists. If it is produced unhygienically and handled carelessly, it gets contaminated very easily leading to its early spoilage. The quality of milk is determined by the aspects of its composition and hygiene. Due to its complex biochemical composition and high-water activity, milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms. These microbes can cause various diseases, so its proper processing is very important.

But every time, the milk and its products are not pure, as it may have some unwanted substances such as urea, water, detergents, starch, coal, tar, dyes, formalin, etc. which can cause health hazards to the consumers. These substances are called adulterants.

One of the most common adulterants in milk is water, which increase the bulk of the milk but decrease its specific gravity. Normal cow's milk has a specific gravity of (1.027-1.035), while the specific gravity of the skim milk produced by removal of fat is 1.042. Since the milk fat globules have a specific gravity of less than 1, their removal in the manufacture of skim milk causes an increase in specific gravity.

Adulteration of milk and milk products is a global concern. Milk adulteration is reported from many countries of world such as Pakistan, China, India and Brazil etc. The author worked for over seven years on UNDP assignment in Ethiopia, and observed that milk was frequently adulterated with water. According to Food Supply and

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

Standards Authority of India (FSSAJ), 2011, over 68 % of milk in India is found adulterated. The most common adulterants found in milk are detergents, caustic soda, white paint, refined oil and glucose. In rural areas of India, 8-13% of milk is adulterated mainly with water.

Figure 1. Lactometer

Figure 2. Butryometer

1.1Objectives

The primary objective of the project is to determine the qualitative and microbial analysis of milk sample and also the detection of adulterants if any present.

The other objective of milk is to determine best quality milk among the milk samples by comparative study.

1.2 Scope of the project

The world population is increasing day by day which creates an alarming situation for the adequate supply of the milk to each individual along with the optimum quality of the product. It is a highly perishable commodity hence; it should be consumed within a definite span of the time or otherwise should be preserved with a suitable preservative.

Since adulteration of food is becoming a common practice due to exploding population, it is essential that consumer be aware of the methods for detecting these adulterants and most importantly about the ill effects on human health by short term and long-term consumption.

The microbial quality of milk is an important parameter in determining its safety. Processes such as pasteurization are meant to ensure milk is safe for consumption; however, post-pasteurization activities could lead to milk contamination, hence threatening the health of consumers.

II. REVIEW OF LITERATURE

Milk is a wholesome nutritious dairy product and is consumed by a majority of the population worldwide for drinking as such, as well as via dairy products. However, the practice of adulteration of milk invariably reduces its quality and may introduce hazardous substances into the dairy supply chain jeopardizing consumers' health. Various instances of adulteration of milk have been reported globally, wherein substances such as extraneous water, foreign proteins, whey proteins, melamine and urea, vegetable or animal fats, plus many minor constituents of milk fat have been added as potential adulterants in milk and milk products. This review focusses on the different methods of detection of these adulterants in milk using techniques such as DSC, RP-HPLC, LC-GC, HPTLC, immunoassays: CE, ELISA, FAMPST, FTIR, NIR spectroscopy, PAGE, IEF, DNA-based methods and MALDI-MS that have been developed and employed for the last 25 years. The combination of advanced IR spectroscopy and chemometrics provides a powerful tool for quality and authenticity analysis of milk. An electronic tongue is an easy and economic tool for the detection of caprine milk adulterations with bovine milk. Biosensors having the ability to furnish

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real-time signals have been developed for the detection of urea in milk. An attempt has been made to give a clear understanding of the most suitable methods for the determination of various sources of adulteration.

Food Safety and Standards Authority of India (FSSAI) carried out a survey on quality of liquid milk from May 2018 to October 2018 covering all States and UTs. In this survey, a total of 6,432 samples of raw and processed milk were collected from 1,103 towns/cities with population above 50,000. The survey has shown that 12 out of 6,432 samples of milk were adulterated that render such milk unsafe for human consumption. A major finding in the survey was the presence of aflatoxin M1 residues beyond permissible limits in 368 (out of 6,432) samples, that is 5.7 % of the samples. This is the first time that the presence of Aflatoxin M1 in milk has been assessed. Aflatoxin M1 comes in the milk through feed and fodder, which are currently not regulated in the country. The survey further showed that 77 (out of 6,432) samples, that 1.2 % of the samples had residues of antibiotics above the permissible limits. Only one raw milk sample was found to contain pesticide residue above the permissible level. Overall, above 93% of the samples that is 5976 out of 6,432 samples were found to be absolutely safe for human consumption. The survey has shown that about 41% samples, though safe, fall short of one or another quality parameter or standard.

Both raw and processed milk samples have failed on account of low fat or low SNF (solids not fat). Further processed milk was found to have maltodextrin and sugar. These are not unsafe but are added to raise the level of fat and SNF of milk. The survey did not find any non-compliance on account of other parameters viz. cellulose, glucose, starch and vegetable oil.

Milk adulteration is a current fraudulent practice to mask the quality parameters (e.g. protein and fat content) and increase the product shelf life. Milk adulteration includes addition of toxic substances, such as formaldehyde, hydrogen peroxide, hypochlorite, dichromate, salicylic acid, melamine, and urea. In order to assure the food safety and avoid health risks to consumers, novel analytical procedures have been proposed for detection of these adulterants. The innovations encompass sample pretreatment and improved detection and data processing, including chemometric tools. This review focuses on critical evaluation of analytical approaches for assay of milk adulteration, with emphasis on applications published after 2010. Alternatives for fast, environmentally friendly and in-situ detection of milk adulterants are highlighted.

A Study on Milk Adulteration Of SavarUpazilain Bangladesh: The study involved a laboratory-based investigation aimed to assess the quality of milk marketed in Savar town. Total ten samples were collected from local market purposively. Ten milk samples were collected (five liquids and five powders). Five powder milk samples and five pasteurized milk samples were collected from different sites in SavarUpazilla purposively. Subsequently, samples were labelled and immediately kept in an ice box. Then immediately the samples were transported to laboratory to analyze. These samples examined for the presence of formalin and melamine. Two adulteration tests were conducted to detect formalin and melamine in milk samples collected from SavarUpazilla. Ten brands of milk from eight companies were collected from different markets of Savar and tested at Bangladesh Council of Scientific and Industrial Research (BCSIR) as per Bangladesh Standards. To know the presence of formalin in milk a qualitative test was done. Total ten samples were tested (five liquid and five powders). Method is non-destructive, cheap, little amount of sample preparation and having sensitivity level less than 2% level of formalin adulteration. 5 ml of milk sample in a test tube was taken and two drop of formaldehyde reagent-1 was added and mixed. Then 1 ml of formaldehyde reagent-2 was added very slowly and carefully along the side of the test tube forming a violet color ring at the junction of the milk and the reagent indicates the presence of formaldehyde in milk. Normal milk gives a light brown color ring at the junction. To know the presence of melamine in milk, a quantitative test was done. Total two samples were tested (a liquid and a powder). The Method was HPLC.

A total of ten samples were tested for presence of formalin and two samples were tested for melamine. The study found the concentration of formaldehyde in all ten analyzed products was the level of detection, i.e.,0.4 ppm. As well as, melamine level also below the level of detection in 2 samples.

III. METHODOLOGY

3.1 Collection of milk samples

Milk sample i.e., both domestic (cow and goat milk) and commercial milk were collected from its source. Then the physical properties of

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

milk were noted which includes color, pH, acidity, temperature, odour, density, fat and total solids.

3.2Determination of acidity

3.2.1 Reagent

Sodium hydroxide 0.1 N

Phenolphthalein indicator solutions

3.2.2 Procedure

- Measure out 10ml of milk in a proclaim milk.
- Add equal amount of distilled water.
- Add about 2-3 drops of phenolphthalein indicator solution.
- Titrate against 0.1 N NaOH solution from a burette stirring vigorously

The time taken for complete titration shall not exceed 20 seconds.

3.2.3 Calculation

$$\text{Titratable acidity} = \frac{\text{volume of titrant} \times N \times 90}{\text{weight of sample} \times 1000} \times 100$$

3.2.4Determination of density

Lactometer: Determine the density of milk.

3.2.4.1Procedure

- Take sample milk in a lactometer jar.
- Place lactometer in it.
- Note the temperature and lactometer reading of the milk.
- Find out the corrected lactometer reading

3.2.4.2Calculation

$$\text{Density} = 1 + \frac{\text{CLR}}{1000}$$

3.2.5Determination of fat

3.2.5.1Reagent

90% Sulfuric Acid

Amyl Alcohol

3.2.5.2Procedure

- Measure out 100ml of 90% sulfuric acid into a dry butyrometer.
- Pipette out 10.75ml of milk into the butyrometer.
- Pipette out 1 ml of amyl alcohol into the butyrometer.
- Close the neck of the butyrometer firmly with the stopper.
- Shake the butyrometer carefully to miss the content.
- Centrifuge the butyrometer at the maximum speed for 4-5 minutes.
- Take out the butyrometer and note the scale reading corresponding to the fat column.

3.2.2 Determination of solid non fat (SNF) and total solids (TS)

3.2.2.1 Procedure

- Take temperature and lactometer reading and find CLR

3.2.2.2 Calculation

$$\text{SNF\%} = {}^{\text{CLR}}/_{4 + 0.2F + 0.5}$$

$$\text{TS} = \text{SNF\%} + \text{Fat\%}$$

3.2.3 Adulteration test

Table 1. Adulteration test

#	ADULTERANT	PROCEDURE	OBSERVATION
1	Alcohol	Alcohol precipitation test: Take 2ml milk sample and 68% of ethyl alcohol in test tube with cork. Tubes were inverted many times in order to mix it thoroughly. Milk samples were examined for precipitation of casein present in milk.	If precipitation occurs, milk samples were near souring point and were of not good quality.

International Journal of Pharmaceutical Research and Applications
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#	ADULTERANT	PROCEDURE	OBSERVATION
2	Formalin	Hehner's test: Take 10 ml milk sample in test tube. Add 5 ml conc. Sulfuric acid with a little amount of ferric chloride without shaking.	Appearance of violet color orblue color at junction of two liquid layer indicates presence of formalin.
3	Urea	A) Take 5ml milk sample in test tube. Add 5ml p-dimethyl amino benzaldehyde reagent (Ehrlichr's reagent).	Appearance of distinct yellow color indicates added urea in milk.
		B) Take 5 ml milk in test tube Add 0.2 ml urease (20mg/ml) Shake well at room temperature. Add 0.1 ml Bromothymol blue (BTB) solution (0.5%)	Appearance of blue color after 10-15 minutes indicates added urea in milk. Normal milk shows faint blue color
4	Starch	5 ml of milk sample was taken and boiled. Allowed to cool at room temperature. Add 1-2 drops of iodine solution.	Formation of blue color indicates presence of starch.
5	Benzoic acid and Salicylic acid	Take 5 ml sample in test tube. Upon acidification with sulfuric acid, 0.5% ferric chloride solution is added to it drop by drop. Mix it well.	Development of buff color indicates benzoic acid. Development of violet color indicates salicylic acid.

3.1 Microbial analysis

The presence of microbes in milk sample were detected by MBRT test. Methylene Blue Reduction Test also known as MBRT test. It is a qualitative test for milk used to check the quality of raw and pasteurized milk.

The Methylene Blue Reduction Test is based on the fact that in the presence of oxygen the methylene blue solution forms blue color, and it will lose the color as the oxygen is depleted.

The bacteria present in the milk will ferment lactose (milk sugar) to form lactic acid. During this fermentation process, the oxygen is used up which causes the depletion of oxygen in milk and electrons are released. These electrons react with the methylene blue solution. As a result, it decolorizes the methylene blue.

Mainly bacteria are responsible for the oxygen in milk. It is assumed that greater the number of bacteria in milk, quicker will be the oxygen consumption.

3.1.1 Aim of methylene blue reduction test

This test is performed to check the bacteria contamination in milk. It will visually indicate the presence of bacteria in a given milk sample and will also indicate the level of milk quality.

3.1.2 Methylene blue reduction test principle

Milk has sufficiently low redox potential which reduces the methylene blue immediately. During the milking, cooling and dumping, the oxidation-reduction potential of milk is increased

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

to +0.3V. At this point, the methylene blue remains in oxidized state.

When the bacterial cells start to increase their number in milk it consumes more dissolved oxygen from the milk and as a result oxygen gets depleted. Then the Methylene Blue starts acting as an electron acceptor instead of oxygen. The methylene blue gets reduced due to the decrease of oxidation-reduction potential from + 0.06V to 0.01 V.

The double-bonded nitrogen atom of Methylene Blue dye accepts 1 atom of hydrogen. As a result the dye is converted into a colorless state. The greater is the number of microorganisms in milk, the greater is the metabolic activity and faster is the reduction of methylene blue.

3.1.3 Materials

1. Methylene blue solution (1% aqueous).
2. Milk sample.
3. Test tube.
4. Test tube stopper.
5. Pipette.
6. Water bath.

3.1.4 Methylene blue reduction test procedure

1. Mix the milk sample thoroughly to distribute the fat uniformly.
2. Add 10ml of milk sample in a test tube.
3. Then add 1ml of standard methylene blue solution in this test tube and invert the testtube to mix it properly.
4. After that, place the test tube in a water bath at 37°C (99°F) for 30 minutes and cover the bath with a lid.
5. After 30 minutes of incubation, observe the sample and check for discoloration and make subsequent readings at hourly intervals thereafter.
6. After each reading, remove the decolorized tubes and then slowly make one complete inversion of remaining tub.
7. Record reduction time in whole hours between last inversion and decolorization. For example, if the sample were still blue after 5 hours but was decolorized (white) at the 2.5-hour reading, the methylene blue reduction time would recorded as 2 hours. Decolorization is considered complete when four-fifths of the color has disappeared.

3.1.5 Estimation of bacterial population in milk sample

It is done through standard plate count method.

3.1.5.1 Plate count method

Referred to as total plate count (TPC), standard plate count (SPC) or aerobic plate count (APC).
Most widely used conventional method for determining viable cells or colony forming units (CFU) in foods.

3.1.5.2Standard Plate Count (SPC)

This method consists of growing the bacteria in a nutrient culture petridish or (petrifilm) and counting colonies which develops.
Procedure:
1. Wash the hands and disinfect the stage.
2. Mixing of milk sample.
3. Make a series dilutions of milk sample.
4. Transfer a suitable dilution to a petridish by sterile pipette.

3.1.6 Detection of E. coli in milk

3.1.6.1 By EMB agar technique

Eosin Methylene Blue (EMB) agar is a differential microbiological medium, which slightly inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose (e.g., E.coli) and those that do not (e.g., Salmonella, Shigella. Eosin Methylene Blue (EMB) agar is a differential microbiological medium, which slightly inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose (e.g., E.coli) and those that do not (e.g., Salmonella, Shigella).
In EMB agar, most of the strains of E. coli colonies have a characteristic green sheen.
Procedure:

- Suspend 35.96 grams in 1000 ml distilled water.
- Mix until the suspension is uniform. Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING.
- Cool to 45-50°C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue color) and to suspend the flocculent precipitate.
- Pour into sterile Petri plates.
- Allow plates to warm to room temperature.
- The agar surface should be dry before inoculating.
- Inoculate and streak the specimen as soon as possible after collection.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

- If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface and streak for isolation with a sterile loop.
- Incubate plates aerobically at 35-37°C for 18-24 hours and protect from light.
- Examine plates for colonial morphology.

IV. RESULT AND DISCUSSION

4.1 Sample A (Cow milk)

Appearance -Yellowish color
pH - 6.5
Density –1.0295

Calculation:
Density=1+CLR/1000
CLR (Lactometer reading) = 29.5
Density = 1 + CLR /1000
= 1 +29.5/1000
=1.0295
Acidity- 0.135%
Titratable acidity = (volume of titrant x N x 90 x 100) / (weight of sample x 1000)
Weight of sample = volume of milk x specific gravity of milk

Table 2. Table of sample A.

Trial no.	Volume of milk	Initial burette reading(ml)	Final burette reading (ml)	Volume of titrant
1.	9	0	1.4	1.4
2.	9	0	1.6	1.6
3.	9	0	1.4	1.4

Volume of titrant = 1.4
Specific gravity of milk =1.0295
Normality (N) of milk = 0.1
Titratable acidity = $\frac{1.4 \times 0.1 \times 90 \times 100}{9 \times 1.0295 \times 1000}$
= 0.135%

4.2Sample B (Goat milk)

Appearance- Whitish color
pH-6.34

Density- 1.033
Calculation:
Density=1+CLR/1000
CLR (Lactometer reading) =33
Density = 1 + CLR /1000
= 1 + 33/1000
=1.033
Acidity = 0.25%
Titratable acidity = (volume of titrant x N x 90 x 100) / (weight of sample x 1000)

Table 3. Table of sample B.

Trial no.	Volume of milk	Initial burette reading(ml)	Final burette reading (ml)	Volume of titrant
1.	9	0	2.5	2.5
2.	9	0	2.6	2.6
3.	9	0	2.6	2.6

Volume of titrant = 2.6
Specific gravity of milk =1.028
Normality (N) of milk = 0.1
Titratable acidity = $\frac{2.6 \times 0.1 \times 90 \times 100}{9 \times 1.033 \times 1000}$
= 0.25%

4.3Sample C (commercial milk)

Appearance- Whitish color
pH-6.3

Density- 1.028
Calculation:
Density = 1 + CLR /1000
CLR(Lactometer reading) = 28
Density = 1 + CLR /1000
=1 + 28/1000
= 1.028
Acidity - 0.21%
Titratable acidity = (volume of titrant x N x 90 x 100) / (weight of sample x 1000)

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

Table 4. Table of sample C.

Trial no.	Volume of milk	Initial burette reading(ml)	Final burette reading (ml)	Volume of titrant
1.	9	0	2.2	2.2
2.	9	0	2.3	2.3
3.	9	0	2.2	2.2

Volume of titrant = 2.2
Specific gravity of milk =1.028
Normality (N) of milk = 0.1
Titratable acidity = $\frac{2.2 \times 0.1 \times 90 \times 100}{9 \times 1.028 \times 1000}$
= 0.21%

4.4Sample D (commercial milk)
Appearance – Whitish color
pH- 6.5
Density –1.029
Calculation:
Density = 1 + CLR /1
CLR(Lactometer reading) =29
Density = 1 + CLR /1000
= 1 + 29/1000
= 1.029
Acidity- 0.174%
Titratable acidity = (volume of titrant x N x 90 x 100) / (weight of sample x 1000)

Table 5. Table of sample D.

Trial no.	Volume of milk	Initial burette reading(ml)	Final burette reading (ml)	Volume of titrant
1.	9	0	1.6	1.6
2.	9	0	1.8	1.8
3.	9	0	1.8	1.8

Volume of titrant = 1.8
Specific gravity of milk =1.029
Normality (N) of milk = 0.1

Titratable acidity = $\frac{1.8 \times 0.1 \times 90 \times 100}{9 \times 1.029 \times 1000}$
= 0.174%

4.5Fat
The value of fat is determined from butyrometer reading. Gerber butyrometer is graduated on 0-10 scale and calibrated in such a way that each 1% division represents 0.125 ml of fat.
Sample A (cow milk):
Fat =3.4
Sample B (goat milk):
Fat=1.8
Sample C (commercial milk):
Fat=3.5
Sample D (commercial milk):
Fat=2.7

4.5.1 Solid non fat (SNF) and total solid (TS) results

$$SNF\% = {}^{CLR}/_{4} + 0.2F + 0.5$$

$$Total\,Solids\% = SNF\% + Fat\%$$

Sample A (cow milk):
CLR = 29.5
F(fat)=3.4
SNF% (Solids Not Fat) =CLR/4+0.2×F+0.5, where F is fat% in milk sample.
SNF%=29.5/4+0.2×3.4+0.5
SNF% =8.5
Total Solids (TS) =SNF%+Fat%
=8.5+3.4
TS = 11.9
Sample B (goat milk)
SNF%=CLR/4+0.2×F+0.5
=33/4+0.2×1.8+0.5
SNF%=9.11
TS=SNF%+Fat%
=9.11+1.8
TS=10.91
Sample C
SNF%=CLR/4+0.2×F+0.5
=28/4+0.2×3.5+0.5
SNF%=8.29
TS=SNF%+Fat%
=8.29+3.5
TS=11.7
Sample D
SNF%=CLR/4+0.2×F+0.5
=29/4+0.2×2.7+0.5
SNF%=8.29
TS=SNF%+Fat%
=8.29+2.7
TS=10.99

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

4.6Adulteration test

Table 6. Adulteration test observation

Test	Observation	Inference
1.Alcohol precipitation test		
Sample A	No precipitation	Since no precipitation occurs it is a good quality milk.
Sample B	No Precipitation	Good quality milk because no precipitation occurs.
Sample C	Precipitation occurs (high precipitation)	Bad quality milk due to the formation of precipitation.
Sample D	Precipitation occurs (low precipitation)	Bad quality
2. Hehner's test		
Sample A	No observation	Absence of formalin
Sample B	No observation	Absence of formalin
Sample C	No observation	Absence Of formalin
Sample D	No observation	Absence Of formalin
3.Urea		
SampleA	SlightYellow	Formation of slight yellow indicates the presence of natural urea in milk.
SampleB	SlightYellow	Formation of slight yellow indicates the presence of natural urea in milk.
Sample C	Distinct Yellow formed	Appearance Of distinct yellow color indicates the presence of added urea in milk.
Sample D	Distinct Yellow formed	Formation of distinct yellow color indicates presence of added urea in milk
4.Benzoic and Salicylic acid		
Sample A	No observation	Absence of benzoic acid and Salicylic acid.
		Absence of benzoic acid and Salicylic acid.
		Absence of benzoic acid and Salicylic acid.
		Absence of benzoic acid and Salicylic acid.
Sample B	No observation	
Sample C	No observation	
Sample D	No observation	

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

5.Starch		
Sample A	No observation	Absence of Starch
Sample B	No observation	Absence of starch
Sample C	No observation	Absence of starch
Sample D	No observation	Absence of starch

4.7MBRT test result

Methylene Blue dye Reductase Test for assessing the raw milk quality:

Sample A (cow milk):

The reduction of methylene blue occurs within 3-4 hours, hence it is a good quality milk.

Sample B (goat milk):

The reduction of methylene blue occurs within 5 hours and above, hence the milk is of very good quality.

Sample C (commercial milk):

The reduction of methylene blue occurs within 1-2 hours, therefore the milk quality is fair.

Sample D (commercial milk):

The reduction of methylene blue occurs within 1-2 hours, therefore the milk quality is fair.

The test relies on the fact that methylene blue solution is blue in the presence of oxygen, but will lose color as oxygen is depleted. Bacteria in milk ferment the lactose (milk sugar) to form lactic acid. During this process oxygen is used up and electrons are released, which react with methylene blue. Because methylene blue is a redox indicator, which loses its color when it comes under the effect of lack of oxygen.

4.8Estimation of bacterial population in milk sample

Standard Plate Count (SPC) results:

Sample A

Colonies per plate = 290

Dilution factor =10^2

Volume of dilution added to the plate =0.1ml

$$\text{Number of cells per ml} = \frac{\text{Number of colonies}}{\text{Volume of sample taken}} \times \text{dilution factor}$$

= 290/0.1 x 10^2

= 290000

Sample B

Colonies per plate = 67

Dilution factor =10^2

Volume of dilution added to the plate =0.1ml

$$\text{Number of cells} = \frac{\text{Number of colonies}}{\text{Volume of sample taken}} \times \text{dilution factor}$$

=67/0.1 x 10^2

=67,000

Sample C

Colonies per plate = 40

Dilution factor =10^2

Volume of dilution added to the plate =0.1 ml

$$\text{Number of cells per ml} = \frac{\text{Number of colonies}}{\text{Volume of sample taken}} \times \text{dilution factor}$$

= 40/0.1 x 10^2

=40,000

Sample D

Colonies per plate =42

Dilution factor =10^2

Volume of dilution added to the plate =0.1ml

$$\text{Number of cells per ml} = \frac{\text{Number of colonies}}{\text{Volume of sample taken}} \times \text{dilution factor}$$

= 42/0.1 X 10^2

=42,000

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

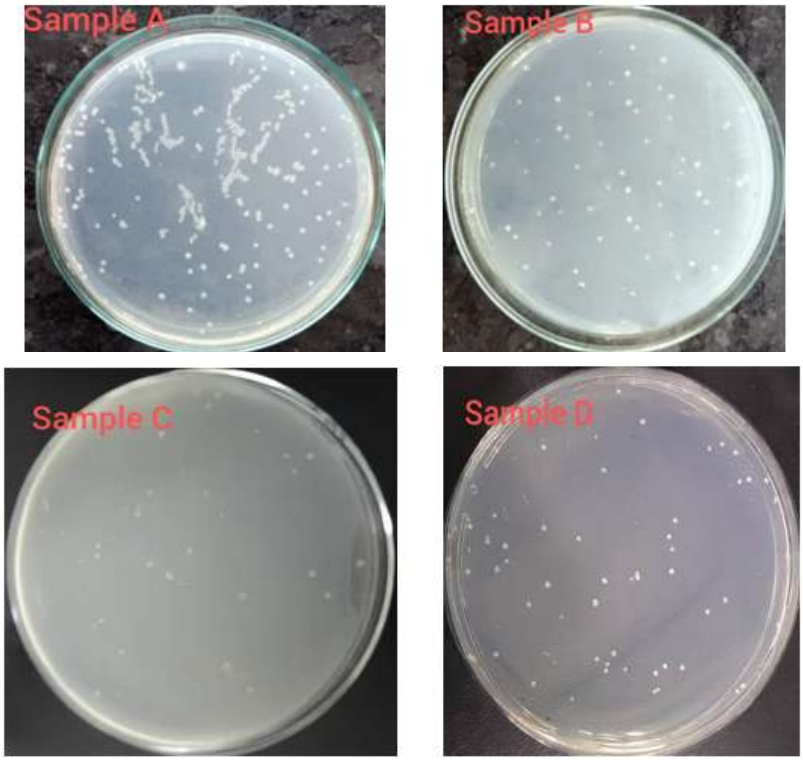

Figure 3. Sample A, B, C and D.

4.9EMB agar detection method

Green metallic sheen of colonies were obtained this indicates the presence of Escherichia coli. In EMB agar, most of the strains of Escherichia coli colonies have a characteristic green sheen. Rapid fermentation of lactose & production of strong acids, thus a rapid reduction in the pH of the EMB agar the critical factor in the formation of the green metallic sheen observed with Escherichia coli, rapid fermentation of lactose and formation of strong acids.

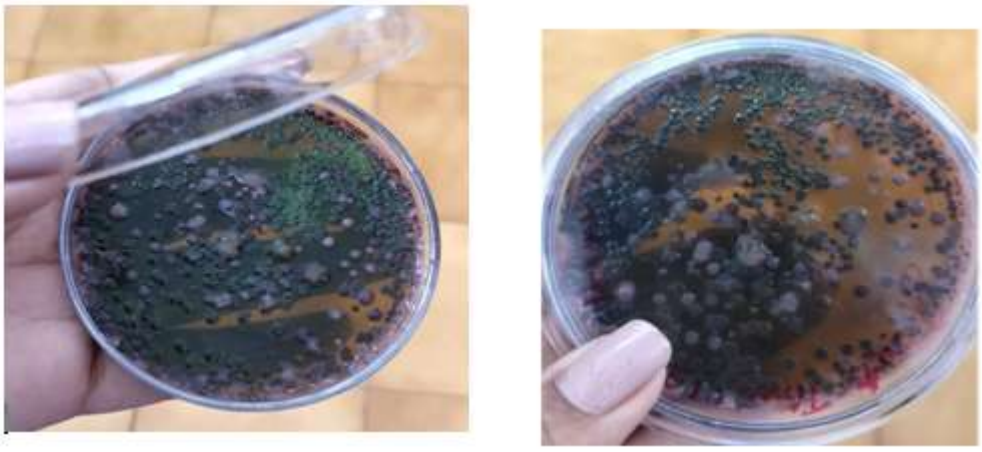

Figure 4. Sample A and B.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

Table 7. Comparative study of milk samples

PHYSICAL PROPERTIES	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLED
Colour	Yellowish Colour	Whitish Colour	Whitish Colour	Whitish Colour
Density	1.0295	1.033	1.028	1.029
Fat	3.4	1.8	3.5	2.7
Solid non-fat (SNF)%	8.5	9.11	8.2	8.29
Total solid	11.9	10.91	11.7	10.99

Table 8. Comparative study of milk samples

CHEMICAL PROPERTIES	SAMPLEA	SAMPLEB	SAMPLEC	SAMPLED
PH	6.5	6.28	6.3	6.4
Acidity	0.135%	0.25%	0.21%	0.174%
Urea Test	Slight Yellow colour	Slight Yellow colour	Intense yellow colour	Intense yellow colour
Alcohol precipitation test	No precipitation	No precipitation	Precipitation occurs	Precipitation occurs.

Table 9. Standard plate count and MBRT test

Sl. No.	NUMBER OF COLONIES PER PLATE	CFU/ML	REDUCTION TIME	QUALITY OF MILK
SampleA	290	290000	3-4hrs	Fair quality
SampleB	67	67,000	5hrsand above	Good quality
SampleC	40	40,000	5hrs and above	Good quality
SampleD	42	42,000	5hrs and above	Good quality

4.1 EMB agar detection method

The study on composition, adulterant and microbial analysis of milk samples shows fat concentration of3.4%(Sample A),1.8%(Sample B),3.5%(Sample C),and 2.7%(Sample D).The minimum fat content was recorded as 1.8% and maximum as 3.5% while milk has an average of 3.5% .SNF content was found to be 8.5%(Sample A), 9.11%(Sample B),8.2%(Sample C),8.29%(Sample D) .Fat is more sensitive to change in diet compared to protein .Composition of milk is also affected by breed and species of animals, by season, age of animal, environment, and stage of lactation, level of milk production, disease and genetics. The study reported that milk adulteration in commercial milk samples in which the formalin, boric acid and starch are 0%. Adulteration results in raw milk samples collected from different milk points in which the adulteration of water was reported but no trace of starch, formalin and benzoic acid. Two types of preservatives that are commonly used is formalin and starch. Added urea is detected in processed milk.

The MBRT result of two samples of raw milk, in which one sample was of good quality (Sample B) with microbial load of 67 CFU/ml and other was of fair quality (Sample A) with microbial concentration of 290 CFU/ml. MBRT result of commercial samples show good quality with 40 CFU/ml and 42 CFU/ml in Sample C and Sample D respectively. In raw milk (Sample B,) the bacteria isolated includes mostly of Escherichia coli. The coliforms colonies were less in number in pasteurized milk sample as compared to raw milk samples. Contamination of milk may be due to unhygienic milking practices and contaminated water. In our experiment the reduced number of

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

CFU/ml may be due to reason that the milk collected were processed. Presence of bacteria can minimize the keeping quality and their toxins and enzymes can survive in pasteurized milk. From above studies, it is clear that the best quality milk is Sample B (goat milk) and poor quality milk is Sample D.

V. SUMMARY AND CONCLUSION

Milk is a global food source as it is rich in nutrients. At present, India is the largest producer of milk in the world with over 150 million tonnes of production and per capita availability of over 300 grams per day. The dairy industry has seen tremendous growth and as demand is increasing, it is expected to grow more in future too.

The basic ingredient for all the dairy products is milk. Raw milk has a very short shelf life. To overcome this, various adulterants are added to the milk. Adulterant food is dangerous for health as it may contain toxic components.

Milk adulteration with dangerous preservatives is a huge challenge for the dairy industry. Driven by greed and lack of responsibility towards society few dealers add chemicals such as formaldehyde, hydrogen peroxide, boric acid, and antibiotics into the milk. The dairy industry has strict quality control parameters to overcome this issue. The Chemical and molecular biology-based testing is done to make sure that the product reaching the consumer is safe and is of high quality.

Commercial milk is composed of the components such as urea, detergents and neutralizers which are very harmful or toxic in nature. Other components used in commercial milk such as water, sugar and starch do not have severe health problems but their poor quality (food or microbial) may cause health problems. Regular intake of commercial milk in place of natural cow/goat milk not only causes serious health problems but can also makes consumers deficient in the nutrients which are obtained from natural milk.

It is obvious from the study that a large number of samples procured did not conform to the legal standards prescribed by the Food Safety and Standards Authority of India (FSSAI). Most of the milk samples were found adulterated. The extent of adulteration varied significantly. This portrays that most of the milk samples were prepared with added adulterants during their production and processing or added intentionally according to one's own choice to generate money. In India, where milk and milk products play an important role of daily human lives through different processed food products, the findings of this study may bring more awareness to the general public about the malpractices or negligence in milk production.

The need of the hour is to address the growing fraudulent acts of adulteration of 'milk' which is regarded as the common man's balanced diet. The regulatory bodies, public administration, market intelligence and scientific communities should work in tandem to bring an end to all such unethical malpractices in large. Injecting right information to the consumers for easy detections of and exercising awareness campaigns can drastically reduce and check such expanding malpractice.

From our project it is found that milk from natural source is of good quality and nutritionally rich but one of its disadvantage is that there is high risk of microbial contamination. A biosensor that incorporates qualitative test for adulterants based on pH change, color change resulting due to chemical reaction can be devised and made available in household. This helps consumer to make a primary assumption about the milk quality. Furthermore, researchers can be done to increase the sensor's sensitivity and repeatability by considering the extraneous factors like temperature etc. An embedded portable biosensor system for bacterial concentration detection that is consistent with Standard Plate Count (SPC) technique while exhibiting significant advantages in terms of response time (3-12 hours vs. 24-72 hours) and of the possibility of in-situ measurements without shipping samples to microbiology laboratories has been developed and is under study. Such biosensors are based on different transduction techniques, such as optics, bioluminescence, piezoelectricity, flow cytometry etc. The biosensor has been tested measuring bacterial concentrations in cow's raw milk samples. The results show a good linear relation between biosensor response and bacterial concentrations measured by SPC, with non-negligible dispersions mainly due to difference in growth rate between the different bacteria present in the samples. This offers a great promise in future.

VI. ACKNOWLEDGEMENTS

The authors are grateful for the cooperation of the management of Mar AugusthinoseCollege for necessary support. Technical support from Binoy A Mulanthra is also acknowledged.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 385-398 www.ijprajournal.com ISSN: 2249-7781

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