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ANTIMICROBIAL ACTIVITY OF MILK

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ABSTRACT

Milk provides the new-born (neonate) with nutrients and an array of antimicrobial factors. These are believed to help protect neonates from infection until their own immune system has developed. Antimicrobial proteins naturally present in milk have the ability to kill and inhibit a broad spectrum of bacteria. The antibacterial properties of these proteins make them suitable for use in a variety of applications, including the prevention of mastitis in cattle and for improving the health of man. Milk contains several antimicrobial activities, including lactoferrin, lactoperoxidase, lysozyme, and possibly N-acetyl-ß-D-glucosaminidase, which may be involved in protecting against mastitis, protecting against bacterial growth post-harvest, and protecting the consumer of the milk product. Probiotics from milk were isolated and tested for their antimicrobial activity for *Bacillus subtilis*, *Bacillus cereus, salmonella typhi, Enterobacter aerogenes* using the agar overlay method.

INTRODUCTION:

Milk is a nutrient-rich, white liquid food produced by the mammary glands of mammals. It is the primary source of nutrition for infant mammals (including humans who are breastfed) before they are able to digest other types of food. Early-lactation milk contains colostrum, which carries the mother's antibodies to its young and can reduce the risk of many diseases. It contains many other nutrients including protein and lactose. Interspecies consumption of milk is not uncommon, particularly among humans, many of whom consume the milk of other mammals **[3]**.

As an agricultural product, milk, also called dairy milk, is extracted from farm animals

during or soon after pregnancy. Dairy farms produced about 730 million tones of milk in 2011, from 260 million dairy cows. India is the world's largest producer of milk, and is the leading exporter of skimmed milk powder, yet it exports few other milk products. The ever increasing rise in domestic demand for dairy products and a large demand-supply gap could lead to India being a net importer of dairy products in the future. The United States, India, China and Brazil are the world's largest exporters of milk and milk products. China and Russia were the world's largest importers of milk and milk products until 2016 when both countries became self-sufficient, contributing to a worldwide glut of milk **[2]**.

Throughout the world, more than six billion people consume milk and milk products. Over 750 million people live in dairy farming households.

HISTORY:

Humans are first learned to consume the milk of the other mammals regularly following the domestication of animals during the Neolithic Revolution or the development of agriculture. Initially the animals were kept for meat, and archaeologist Andrew Sherratt has suggested that dairying, along with the exploitation of the domestic animals for hair and labor, began much later in separate secondary products revolution in the fourth millennium BC. Sherratt's model is not supported by the recent findings, based on the analysis of lipid residue in prehistoric pottery, that shows that the dairying was practiced in the early phases of the agriculture in Southwest Asia and by at least the seventh millennium BC **[1]**.

From Southwest Asia residential dairy creatures spread to Europe hu(beginning around 7000 BC but did not reach Britain and Scandinavia until after 4000 BC), and South Asia (7000–5500 BC). The primary agriculturists in central Europe and Britain] drained their creatures. Peaceful and peaceful roaming economies, which depend transcendently or solely on residential animals and their items instead of trim cultivating, were created as European ranchers moved into the Pontic-Caspian steppe within the fourth thousand years BC, and hence spread over much of the Eurasian steppe. Sheep and goats were presented to Africa from Southwest Asia, but African cattle may have been autonomously tamed around 7000–6000 BC. Camels, tamed in central Arabia within the fourth thousand years BC, have moreover been utilized as dairy creatures in North Africa and the Middle eastern Promontory. The earliest Egyptian records of burn medicines portray burn dressings utilizing drain from moms of male babies **[1]**.

MATERIALS AND METHODS:

➢ **COLLECTION OF SAMPLE :-**

Eight different brands of commercially available packaged like Amul Gold, Amul Shakti, Amul Taja, Mahi Taja, Row Cow and Row Buffalo in plastic or paper box containers were purchased, market places and beverage stores in different locations in Vadodara, the milk were then refrigerated before the commencement of the analysis. Information like NAFDAC registration number, expiry date, batch number and other information on the labels of the yogurt were noted and recorded **[4]**.

➢ **Physicochemical properties of milk**

A) Physical

B) Chemical

C) Biological

A) PHYSICAL PROPERTIES OF MILK (FSSAI)

- Acid- base equilibrium
- Viscosity
- Density
- Foaming
- pH

1) ACIDITY AND ALKALINITY OF MILK:- (FSSAI)

- **METHOD**:-

Acidity

Total acidity- Took 50 ml sample & add 3 drops of phenolphthalein. Titrate over a white surface with 0.2N NaOH solution.

Alkalinity

Took 20 ml sample in flask. Add 2-3 drops phenolphthalein indicator. Then add 0.02N H_2SO_4 drop by drop to pH 8.3. Note down volume after solution become colourless. Add 2-3 drops of Methyl orange indicator. Titrate with 0.02N H_2SO_4 to yellow color **[7]**.

2) VISCOSITY:- (FSSAI)

Viscosity is a measure of a fluid's resistance to flow. It describes the internal friction of a moving fluid. A fluid with large viscosity resists motion because its molecular makeup gives it a lot of internal friction **[8-10]**.

➢ **Formula:-**

i. **Relative viscosity=** $\frac{dx \times tx}{dw \times tw}$

where,

dx = Absolute density

tx = Time of milk

dw = Density of water (0.9956)

tw = Time of water

ii. **Absolute Viscosity** $= \frac{dx \times tx \times nw}{dw \times tw}$

where,

nw = viscosity of water (0.00807 poise**)**

3) DENSITY :- (FSSAI)

Density is a measurement that compares the amount of matter an object has to its volume. An object with much matter in a certain volume has high density. [8-10]

- **Formula:-**

I. **Specific density=** $\frac{\text{Weight of liquid}}{\text{Weight of water}}$

II. **Absolute density** = Specific density × dw (0.9956)

4) FOAMING :- (FSSAI)

A collection of minute bubbles formed on the surface of a liquid by agitation, fermentation, etc.

- **Method**:-
- Shake the milk sample in a jar**.**
- In electric mixture heat milk sample.
- Once frothing wand then warm your milk.
- Pour your warm milk into a blender and blend on medium speed until frothy.
- Observe the foam**.**

B) CHEMICAL PROPERTIES OF MILK:-

i). Detection of Ammonium Compounds in Milk:

Method:-1. .

Procedure:-

1. Take 1.0 ml of milk add 0.5 ml of 2% sodium hydroxide, 0.5 ml of 2% sodium hypochlorite and 0.5 ml of 5% phenol solution **[6 & 28]**.

2. Heat for 20 seconds in boiling water bath, bluish colour turns deep blue in presence of ammonium sulphate.

3. The development of pink colour shows that the sample is free from Ammonium sulfate.

ii) .Tests for Presence of Sulphates in Milk:-

Presence of sulfate salts, which may be added to milk to raise its SNF level in milk, can be detected by using barium chloride **[11-13]**.

Procedure

1. Take 10 ml of milk in a 50 ml stoppered test tube. Add 10 ml of TCA solution. Filter the coagulated milk through Whatman filter paper Grade 42.

2. Take 5 ml of clear filtrate. Add few drops of barium chloride solution. Observe for any visible precipitates in the tube.

3. Formation of milky-white precipitates indicates the presence of added sulfates like ammonium sulfate, sodiumsulfate, zinc sulfate and magnesium sulfate etc. to milk. The limit of detection of method is 0.05%.

iii). Detection of Sodium Chloride in milk

The presence of extraneously added sodium chloride in milk can be detected by silver nitrate and potassium chromate reagent. **[11-13]**

Procedure

1. Take 2.0 ml of milk and add 1.0 ml of 5% potassium chromate, 2.0 ml of 0.1 N silver nitrate.

2. Appearance of red precipitate indicates the absence of dissolved chloride in milk

and appearance of yellow colour indicates presence of dissolved chloride.

3. The limit of detection of method is 0.02%.

Vii). Detection of casein in milk:-

Procedure:-

1. A clean dry beakers has been taken

2. Follow by putting 20 ml of cow's milk in to adding 20 ml saturated Ammonium sulphate solution slowing with stirring fat along with casein was precipitate out

3. The solutions were filtered and transfer the precipitates in another beaker

4. Add 30 ml water to the precipitate

5. Only casein dissolves in water forming milky solution leaving fat undissolved

6. The milky solution was heated about 40°C and add 1% acetic solution drop wise when casein got precipitated

7. Filtered the precipitate, washed with water precipitate was allowed to dry.

8. Weighted the dry solid mass in previously weighted watch glass The experiment was repeated with other sample of milk **[29]**.

C) BIOLOGICAL PROPERTIES

i). Coliform plate count (cpc)

Principle:-

The coliform group of bacteria comprises all aerobic and facultative anaerobic, gram negative, non-spore forming rods able to ferment lactose with production of acid and gas at 37oC within 48 h(department of the environment, 1994) . One source of these organisms is the intestinal tract of human and animals. Their presence in milk and milk products is indicative of possible faecal contamination although some species (e.g. *Enterobacteraerogens*) may be derived from feeding materials and soil.(Gleeson and Grey, 1991) As these organisms are heat labile, their presence in pasteurized milk is considered to indicate post-pasteurization contamination.

For testing presence of coliforms in milk and milk product, a small quantity of the product (1.0, 0.1 or 0.01 ml) is added to liquid or solid media containing lactose and bile salt with a suitable indicator. Production of acid and gas in liquid media and appearance of typical coliform colonies on the plates is taken as evidence of coliform contamination. A few other bacteria, such as those belonging to the genus Clostridium and Bacillus and certain. **[17 & 19]**

Procedure:-

- ✓ **Liquid media :-**

1. Prepare serial dilution of sample

2. Transfer 1ml of required dilution in to macconkey broth tube in triplicate

3. Incubate the tube for 24 hours at 37°C and observe for the production of acid & gas

4. The production of acid is exhibited by Chang of color of medium from purple to yellow in case of bromocresol purple and

orange to pink in the case of andrade's indicator

5. Production of gas is observed in the Durham's tube which may be particularly or completely filled with gas

6. In the case of no change further incubate for another 24 hours and record the observation

- ✓ **Solid media:-**

1. Prepare serial dilution of the sample

2. Incubate 1ml of the required dilution into sterile petriplatesin duplicates

3. Mix the contents by rotating plates

4. Allow to agar solidify

5. Pour additional layer (3-4ml) of the medium completely over the surface of solidify medium

6. Invert and incubate the plate at 37°C for 24 hours Once incubation is over examine the plates of presence of typical dark red colonies measuring at least 0.5 mm in diameter

7. Count such colonies and express the results as coliform count per ml of milk

ii) Methylene blue reduction time test :-

- ✓ **Introduction:-**

  Use this test to check the bacteria contamination in a sample of milk. It will visually indicate whether bacteria are present in the milk sample and give an approximate level of milk quality based on the level of viable bacteria in the milk.

- ✓ **Principle:-**

  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 lactose (milk sugar) to form lactic acid. During this process oxygen is used up and electrons are released, which react with methylene blue. One effect is to decolorize the methylene blue. More bacteria present in the milk leads to faster reduction (taking on electrons) of methylene blue and increased rate of color loss **[20-22]**.

- ✓ **Procedure:-**
  - Mix the milk sample well to distribute any bacteria it may contain. Pour 10ml into a clean, sterilized test tube.
  - Using a pipet, add 1ml of Methylene blue solution to the milk sample in the test tube.
  - Stopper the test tube and gently invert it to mix the contents.
  - Place the test tube in a water bath at 37°C (99°F) for 30 minutes making sure water level is high enough to cover most of the test tube. Cover bath with lid to so test tube sample is in the dark.

- After 30 min. examine the sample, checking for decoloration.

➢ **ENZYME ACTIVITY OF MILK SAMPLES** :-

- **Principle:-**

The sample/inoculum is diluted by streaking it across the surface of the agar plate. While streaking in successive areas of the plate, the inoculums is diluted to the point where there is only one bacterial cell deposited every few millimetres on the surface of the agar plate.

- **Procedure:-**

1. Sterilize the inoculating loop in the Bunsen burner by putting the loop into the flame until it is red hot. Allow it to cool.
2. Pick an isolated colony from the agar plate culture and stick on half plate of agar which already have substrate like lactose for lactase, tributyrin for lipase, starch for Amylase , Gelatine for gelatinises, casein for protease
3. Pick different milk samples and stick on half plate of agar.
4. Invert the tube and incubate all tube at 37°C for 24 to 48 hours.
5. Next day observed the zone **[27-30]**.

RESULTS AND DISCUSSIONS:

1. Acidity and alkalinity of milk sample:

Sample	Acidity (%)	Alkalinity
Raw Cow Milk	0.1395	2.4
Raw buffalo Milk	0.1513	6.5
Amul gold	0.1755	3.4
Amul Shakti	0.1620	3.2
Amul Taja	0.171	4.2
Mahi Taja	0.1782	5.5

2. Freezing Point of milk samples:

Sample	Freezing Point(°C)
Raw Cow Milk	-0.545
Raw buffalo Milk	-0.545
Amul gold	≤ -0.520
Amul Shakti	≤ -0.520
Amul Taja	≤ -0.520
Mahi Taja	≤ -0.520

3. Viscosity of the milk samples:

Sample	Relative viscosity(Pa.s)	Absolute viscosity(poise)
Raw Cow Milk	1.97	0.0158
Raw buffalo Milk	2.243	0.018
Amul gold	2.15	0.017
Amul Shakti	1.89	0.0152
Amul Taja	1.9265	0.015
Mahi Taja	1.77	0.0142

4. Density of the milk samples:

Sample	Specific density(kg/m³)	Absolute density(pounds/gallon)	Surface tension(dine/m²)
Raw Cow Milk	1.053	1.048	54.37
Raw buffalo Milk	1.049	1.044	48.50
Amul gold	1.000	0.995	53.00
Amul Shakti	0.990	0.9863	51.84
Amul Taja	1.050	1.045	54.22
Mahi Taja	1.055	1.051	49.98

5. Foaming of the milk sample:

Sample	Foaming
Raw Cow Milk	Low
Raw buffalo Milk	Medium
Amul gold	High
Amul Shakti	Medium
Amul Taja	Low
Mahi Taja	Medium

6. pH:-

Figure: pH of milk samples

All milk contain 7 pH (Nutral).

- ❖ **Chemical properties of milk sample :-**
- ✓ **Detection of Ammonium Compounds in Milk:** As there is no change in color of milk sample, so ammonium salt is absent in all milk.
- ✓ **Tests for Presence of Sulphates in Milk :-** Formation of milky-white precipitates indicates the presence of added sulfates like ammonium sulfate, sodium sulfate, zinc sulfate and magnesium sulfate etc. are in milk samples.

✓ **Detection of Sodium Chloride in milk samples :-**

Milk	Colour/Precipitate	Dissolved Cloride
Raw Cow Milk	Yellow	Present
Raw buffalo Milk	Red	Absent
Amul gold	Yellow	Present
Amul Shakti	Yellow	Present
Amul Taja	Yellow	Present
Mahi Taja	Red	Absent

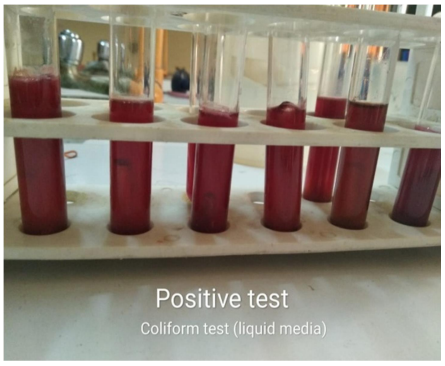
✓ **Detection of casein in milk samples:-**

MILK	CASEIN
Mahi taja	1.805
Amul shakti	2.702
Amul gold	2.347
Loose buffalo	1.114
Loose cow	2.467
Amul Taja	2.957

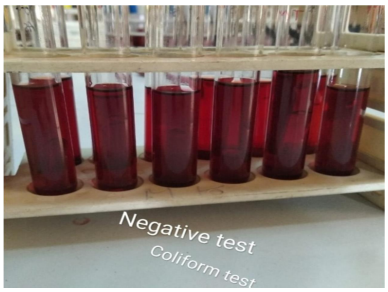
➢ **BIOLOGICAL PROPERTIES**

- **Coliform plate count (cpc)**

✓ **Liquid media :-**



Production of gas is observed in the Durham's tube which indicates positive coliform test



Absence of gas is observed in the Durham's tube which indicates negative coliform test.

Figure show the results of coliform count in liquid media

- ✓ **Solid media:-**

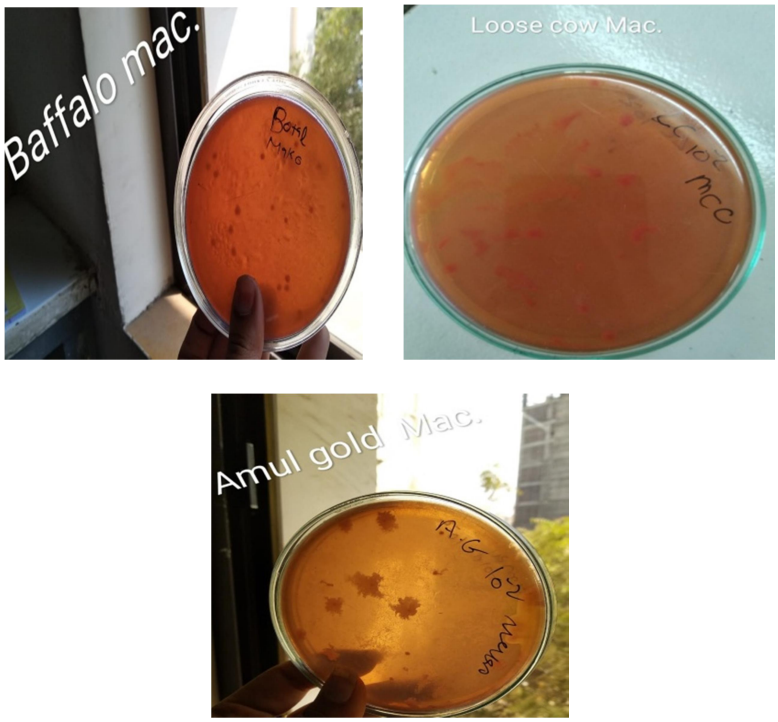


Figure: coliform count in solid media

Counting of Colonies of coliform plate:

Sample	Row cow	Row buffalo	Amul gold	Amul Shakti	Amul taja	Mahi super gold	Mahi taja
10^{-1}	-	5×10^{-3}	2.5×10^{-2}	2.5×10^{-2}	-	-	-
10^{-2}	-	-	5×10^{-4}	-	-	-	1×10^{-3}
10^{-3}	-	-	1×10^{-4}	5×10^{-5}	5×10^{-3}	-	1×10^{-4}
10^{-4}	-	-	2×10^{-5}	-	-	-	2.5×10^{-5}
10^{-5}	-	-	-	5×10^{-7}	-	-	5×10^{-7}
10^{-6}	-	-	-	5×10^{-8}	1.5×10^{-7}	-	1.5×10^{-7}
10^{-7}	5×10^{-9}	-	-	5×10^{-9}	-	-	5×10^{-9}
10^{-8}	-	-	-	5×10^{-10}	-	-	5×10^{-10}
10^{-9}	-	-	-	5×10^{-11}	-	3×10^{-10}	-
10^{-10}	-	2.5×10^{-11}	-	-	-	-	-

Methylene blue reduction time test:-

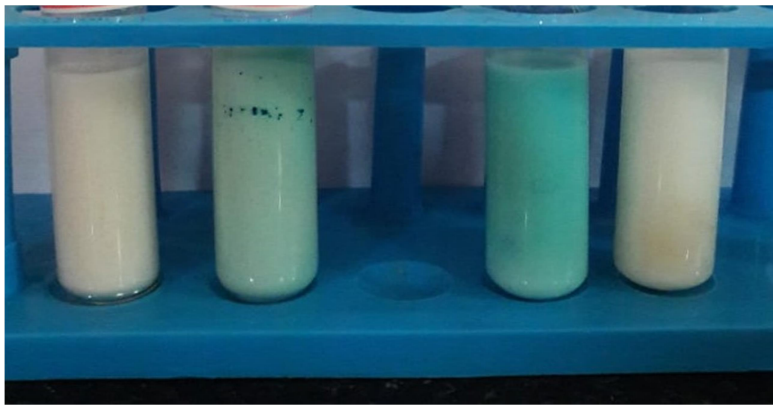


Figure: MBRT

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