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Study of Bacterial Diversity of Raw and Pasteurized Milk

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Abstract: Milk is considered as a wholesome food containing protein, fats, sugars, vitamins and minerals. Milk, due to its high nutritional value, can support a rich microbiota. Even though, raw milk is sterile during secretion and pasteurized milk is packed sterile, contamination of milk may take place during handling, pre-processing activities and during storage. The main aim of this study was to detect adulteration, chemical and physical qualities and to assess the microbial load in raw and pasteurized milk. The current study's preliminary goal was to determine the quality of milk samples using physical tests (such as pH, color, taste, and texture) and to detect adulteration using various parameters such as (presence of starch, sugarcane, benzoic acid, etc), chemical tests (acidity, alcohol, phosphatase, protein, etc), and microbiological tests. Raw milk is appearing more contaminated with coliforms and normal flora than pasteurized milk. Adulteration of starch, sugarcane, and benzoic acid was discovered in milk samples analyzed. All of the milk samples did not meet the nutritional requirements.
Keywords: Microbial Load in the Milk, Pasteurized Milk, Raw milk, Adulteration, Nutritional Quality of Milk

I. INTRODUCTION

Milk is regarded as nature's complete food, and it is being consumed by over 6 billion people worldwide. For consumption we get milk from different animal sources such as cow, buffalo, goat, sheep, as well as from humans [1]. Composition of milk includes proteins, vitamins, milk fat, phospholipids, minerals, and lactose (milk sugar). India is the world's leading milk producer, accounting for more than 23% of worldwide milk production. In 2020-21, India's average milk output is 209.06 million litres, and this number is expected to rise in the next years [2]. Milk provides an optimal habitat for microorganism growth due to its high nutritional value and high water activity. Raw milk is produced in specific cells of the mammary glands and is unpasteurized. Milk is easily contaminated by a wide range of germs. Milk which is drawn from animals is unprocessed. Milk includes microscopic dirt particles that are not visible to the human eye. Microbial contamination of milk originates primarily from three sources: inside the udder, outside the udder, and the surface of milk handling and storage equipment [3]. Escherichia coli, staphylococcus, streptococcus, enterococcus, lactobacillus, and pseudomonas, as well as different yeasts and moulds, make up the microbial composition of milk. Milk develops faults such as flavour deterioration, discoloration, souring, and gassiness as a result of the presence of unwanted bacteria. Lactic acid bacteria are the most common group of bacteria which convert lactose (milk sugar) into lactate [4]. To increase the quality of milk, it is generally adulterated by adding water, sugar, and flour. Consumers suffer major health consequences as a result of adulteration. Maintaining standards is one of the most critical needs in the manufacturing of milk. To improve the quality of milk, good milk hygiene practices such as healthy and clean cows, washing the udder, washing hands with soap, using clean water should be maintained [5)].

II. MATERIALS AND METHOD

A. *Sample collection*

A total 7 milk samples were gathered from Surat, Gujarat's various districts. 5 samples of raw milk were taken from dairy farms in the Tapi, Surat, and Navsari districts, and 2 samples of pasteurized milk were taken from Surat and Navsari district stores [6]. All the samples were collected in a sterile container and brought into lab within 2 hours and processed for physical, chemical, adulteration and for microbiological analysis [7].

B. *Physical Testing*

1) *pH:* The pH of milk is tested using pH paper with a universal indicator [8].
2) *Colour*: For the physical test, colour judgment was done by observation [9].
3) *Flavour*: Flavour can be detected by sniffing milk samples or by putting them in the mouth [10].
4) *Texture*: The texture of milk was determined by sight [11].

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C. Chemical Testing

1) Clot on boiling (COB) Test

The COB test is used to detect increasing acidity in milk. In a test tube, place 2 ml of milk and boil for 4 minutes. Formation of clots in test tube indicate positive COB test [12].

2) Alcohol Test

This test is done to know the quality of raw milk. Tale 2 ml of milk sample in test tube and add 68% of 2 ml of alcohol in test tube. When alcohol is added to milk, it causes flakes to form, and this sort of milk is rejected [13].

3) Phosphatase Test

Phosphatase test is used to know whether the milk is pasteurized or not. Label two sterile MBR tubes with the words "control" and "test." Fill both tubes with 1 ml of milk sample. Add 5 ml of phosphatase dye to a test tube and mix thoroughly. After 2 hours, incubate both tubes at 37°C in a water bath and check for colour change [14].

4) Protein and casein Test

The test is used to determine whether or not protein and casein are present in milk. Add 4-5 drops of phenolphalein indicator to 10 ml of milk samples in two flasks, followed by 0.4 ml of saturated potassium oxalate. Titrate it against 0.1 N Noah solutions after 5 minutes [15].

D. Adulteration Test

1) Detection of starch

The test is used to detect presence of starch in milk. Take 3 ml of milk sample in test tube. Boil and cool at room temperature and add 1 drop of iodine solution in it. Appearance of blue colour indicates presence of starch [16].

2) Detection of cane sugar

The test helps to detect sucrose in milk. Take 10 ml of milk in jumbo tube and add 0.1 gm of resorcinol. The appearance of red colour after 5 minutes implies that sucrose is present [17].

3) Detection of soap

This test is used to check adulteration of milk with soap and detergent. Take 10 ml of milk and mix it with 10 ml of boiling water. Using a phenolphthalein indicator, add 1-2 drops. Positive results are indicated by the presence of pink colour [18].

4) Detection of Formalin

Formalin (40% formaldehyde) is very poisonous chemical. It attaches to proteins and interferes with their function. Take 5 ml of milk and dilute it with the same amount of ferric chloride. For 3-5 minutes, place the tubes in a boiling water bath.Positive results are indicated by the presence of a brownish pink colour [19].

5) Detection of ammonium sulphate

To enhance the amount of SNF in milk, ammonium sulphate is added. Take 5 ml of milk sample in test tube and add 1 ml of 10% of sodium hydroxide solution in it. Observe for the colour change [20].

E. Microbiological analysis

1) Standard Plate Count (Quantitative Analysis)

Presence of aerobic bacteria in raw milk is estimated using a standard plate count. In this approach, the samples are plated on agar media for bacterial growth for 48 hours at 32 degrees Celsius, and the growth of bacteria is measured in colony forming units (CFU) units [21].

2) Detection of coliforms

The test is based on the principle that members of this group are capable of producing acid and gas. The milk samples is analysed with presumptive test, confirmed test and followed by complete test [22].

3) Detection of Acid Fast Bacteria

The Ziehl-Neelseen method is used to detect acid fast bacteria in milk. The smears were stained with carbol fuchsin, and the AFB counts were determined under immersion microscopy in the stained smears [23].

4) Presence Absence (PA) Test

Take 100 ml of milk sample and add into the PA bottle containing BGLB (Brilliant Green Lactose Bile Broth) medium. Incubate the sample for 24 hours at 35°C. After 24 hours, look for a colour change [24].

III.RESULTS AND DISCUSSION

In this study, Raw and Pasteurized milk sample collected from four districts of Gujarat i.e. from Valsad, Navsari, Surat and Tapi were analysed for physical parameters like pH, colour, texture, flavour. Chemical quality of milk tested by Clot on boiling test (COB), Acidity test, Alcohol test, Phosphatase test, Protein and Casein test and Methylene blue reduction time test (MBRT). The adulteration of both raw and pasteurized milk was done by starch, sugar cane, benzoic acid and salicylic acid, soap,formalin, and ammonium sulfate. Results obtained from these experiments are discussed below.

A. Physical parameter

1) pH: The average pH of raw milk is 6.9, while the pH of pasteurized milk is 7.0.
2) Colour: Pasteurized milk samples are creamy white in colour, while raw milk samples are yellowish in colour.
3) Flavour: All raw milk samples had a slightly sweet flavour, while pasteurized milk samples had a sweet flavour.
4) Texture: All of the milk samples had a normal texture.

The result of chemical- adulteration tests conducted in raw milk and pasteurized milk sample are given in tables:

B. Results of chemical parameter

Table I chemical analysis of raw and pasteurized milk.

Sr. No.	Name of Sample	COB Test	Alcohol Test	Phosphatase Test	Casein Test	MBRT	
						Hours	Quality
1	RC-1	Negative	Negative	<2 , Poor	Positive	<1	Poor
2	RB-1	Negative	Negative	<2 , Poor	Positive	<1	Poor
3	RC-2	Negative	Negative	<2 , Poor	Positive	1-2	Fair
4	RB-2	Negative	Negative	<2 , Poor	Positive	<1	Poor
5	RG	Negative	Negative	<2 , Poor	Positive	<1	Poor
6	P-1	Negative	Negative	4-5 , Good	Positive	3-4	Good
7	P-2	Negative	Negative	>5 , Good	Positive	3-4	Good

C. Methylene Blue Reduction Time test (MBRT)

The quality of raw milk was discovered to be poor. Pasteurized milk, on the other hand, was of good quality.
Raw milk changed colour within two hours; however pasteurized milk remained colourless for more than two hours, showing that raw milk contains a large number of microorganisms.

D. Results of Adulteration Test

Table II Adulteration test of raw and pasteurized milk

No. of sample	Name of isolate	Starch	Sugarcane	soap	formalin	Ammonium sulfate
1	RC-1	Positive	Positive	Negative	Negative	Negative
2	RB-1	Positive	Positive	Negative	Negative	Negative
3	RC-2	Positive	Positive	Negative	Negative	Negative
4	RB-2	Positive	Positive	Negative	Negative	Negative
5	RG	Positive	Positive	Negative	Negative	Negative
6	P-1	Positive	Positive	Negative	Negative	Negative
7	P-2	Positive	Positive	Negative	Negative	Negative

The milk sample showed positive results for starch and sugarcane test while it showed negative results in soap, formalin and ammonium sulphate. The results suggest the starch and sugarcane adulteration in milk samples to increase the shelf life of milk.

E. Results of Microbiological Analysis

1) Results of Standard Plate Count

Table III Quantitative analysis (Standard plate count) of raw and pasteurized milk

Sr. no	Name of sample	No. of colony	Average no. of colony	CFU/ml
1	RC-1	138	$5.04 * 10^6$	$1.38*10^7$
2	RB-1	132	$4.66 * 10^6$	$1.32*10^7$
3	RC-2	125	$4.23 * 10^6$	$1.25*10^7$
4	RB-2	101	$3.51 * 10^6$	$1.01*10^7$
5	RG	90	$2.95 * 10^6$	$9.0*10^6$
6	P-1	<30	-	-
7	P-2	<30	-	-

In the case of the coliform count, raw milk samples had counts > $1.38x10^7$CFU/ml which is higher than the given international standard set for the acceptable coliform count. Whereas, pasteurized milk samples had a counts of less than 30 colonies. The results depicts that the microbial contamination was high in raw milk as compared to pasteurized milk.

2) Detection of Coliforms

a) Presumptive Test



Figure 1 Positive presumptive test

From the above observation, all tubes show acid and gas production in all milk. Samples indicates positive presumptive test.

b) Confirmed Test

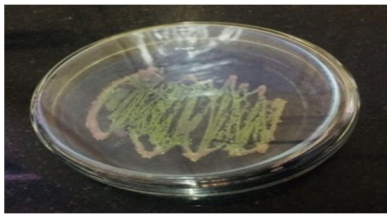


Figure 2 Positive complete test

All positive presumptive tubes tested On EMB agar plate showed small nucleated with or without greenish black metallic sheen observed.

c) Completed Test



Figure 3 Positive confirmed test

Presence of gram negative non-spore forming short rods coliforms, produce acid from lactose broth indicates positive results for completed test .

3) Acid Fast Staining

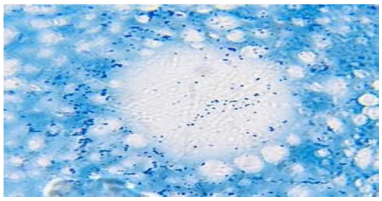


Figure 4 Non acid fast staining

The detection of acid fast organisms using the ZNCF method indicates that non acid fast organisms are detected in all milk samples.

4) Presence Absence Test

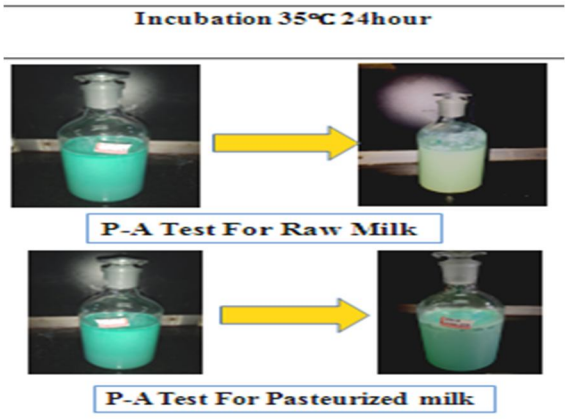


Figure 5 Presence Absence test of raw and pasteurized milk

The results of the P/A test show that dye in raw milk degrades after 24 hours of incubation, whereas dye in pasteurised milk degrades only partially after 24 hours of incubation. This indicates that raw milk had a higher microbial burden than pasteurised milk.

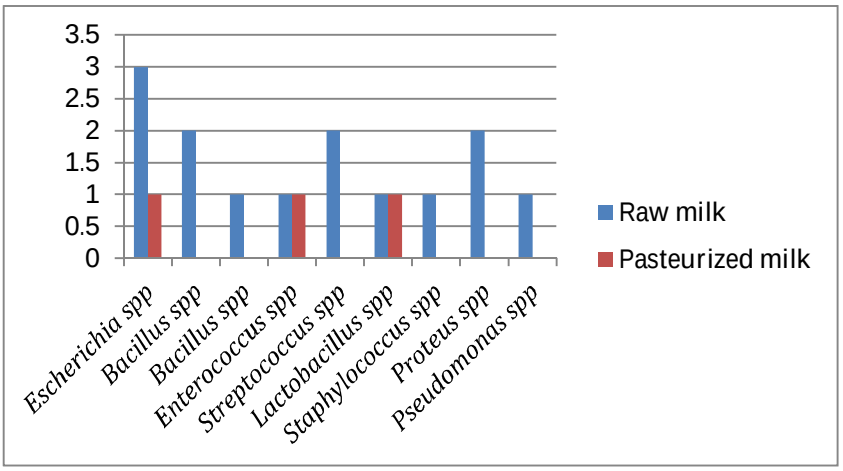


Figure 6 Bacterial isolates from raw & Pasteurized Milk Samples

IV. CONCLUSIONS

Milk is considered as wholesome food for daily consumption. In spite of remarkable developments in Indian milk industry there are still great difficulties with regard to quality as well as quantity of milk served to the consumers. The bacteriological analysis of raw milk in India is poor, resulting in an enormous loss through souring and spoilage and also greatly affecting the health of consumers with such milk. Bacterial count was high in raw milk as compared to pasteurized milk. 17 isolates were obtained from studying 7 milk samples, among 17 isolates 14 isolates were from raw milk while other 3 were from pasteurized milk. Adulteration and chemical testing of milk showed that quality of raw milk was poor as compared to pasteurized milk. From the results of hemolytic activity it is showed that isolate no. VRC1, TRC2, NRG2 and TRB2 were showing beta hemolytic.

V. ACKNOWLEDGMENT

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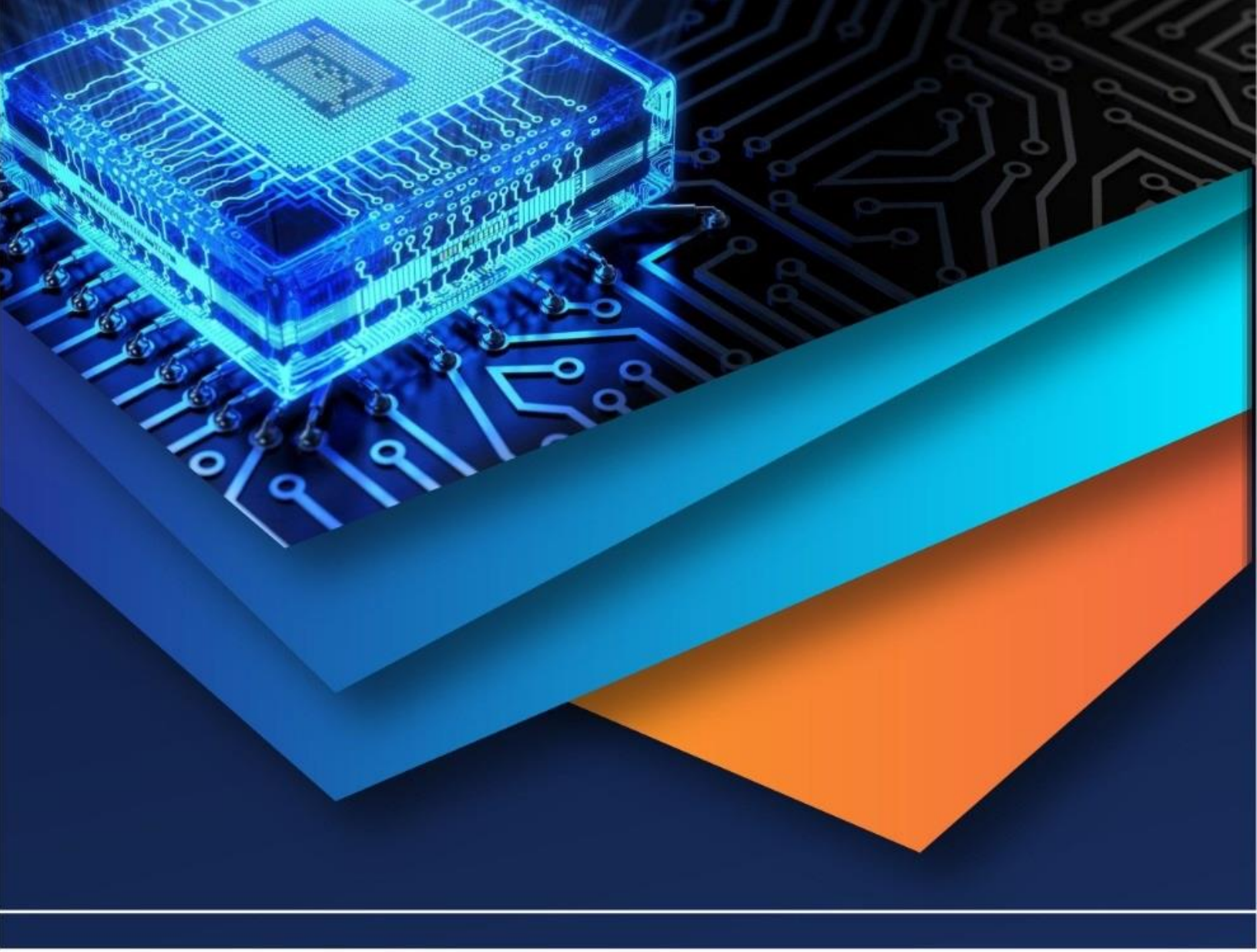
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