ASSESSMENT OF KEEPING QUALITY AND EFFICIENCY OF PASTEURIZATION OF PACKAGED MILK SAMPLES FROM VENDORS OF THANE, MAHARASHTRA, INDIA

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ABSTRACT

High nutritive value of milk makes it an ideal medium for the rapid multiplication of bacteria, particularly under unhygienic production and storage at ambient temperatures. Contamination & easy spoilage of milk due to poor keeping quality is a serious and widely reported issue. In view of this, the keeping quality and efficiency of pasteurization of 20 packaged milk samples of different brands, procured from different vendors in Thane was tested. Seventeen samples out of twenty were of 'fair' keeping quality, while three were of 'good' keeping quality as per results of Methylene Blue Reduction Test; while Resazurin Reduction Test revealed sixteen samples to be of 'excellent' keeping quality, while four of 'good' keeping quality. Phosphatase tests results indicated that all the samples were appropriately pasteurized. Thus, samples procured from these vendors from Thane, Maharashtra, India, seem to be of acceptable keeping quality. Further tests need to be performed to exactly determine the microbial count.

Keywords: Milk, keeping quality, pasterization, phosphatase test, MBRT, RRT

INTRODUCTION

Milk, with its high nutritional value, around 87.80% water, 3.20% protein, 3.50% fat, 4.80% lactose, and 0.70% mineral content is considered to be 'complete meal'. It also serves as rich source of energy, 100 gm milk providing around 66 kcal of energy (Pehrsson, 2000).

During last few decades, India has transformed from a country of acute milk shortage to the world's leading milk producer. Several measures initiated by the government to increase the productivity of livestock has resulted in increasing the milk production significantly. According to a survey by Economic Times, the milk production in India has increased from 146.3 million tonnes in 2014-15 to 198.4 million tonnes in 2019-20.

High nutritive value of milk also makes it an ideal medium for the rapid multiplication of bacteria, particularly under unhygienic production and storage at ambient temperatures. Contamination & easy spoilage of milk due to poor keeping quality is a serious and widely reported issue. Sometimes, dairy animals serve as major reservoirs for many milk-borne

pathogens such as *Staphylococcus aureus* and *Escherichia coli* (*E. coli* O157:H7) (Mohammad *et al.*, 2014). A number of milk-borne epidemics and outbreaks, such as tuberculosis, typhoid, diphtheria, dysentery, etc., have been occurred through consumption of milk and their product in humans (Khan *et al.,* 2018). These microbes may gain entry into raw milk in numerous way such as directly from dairy buffaloes experiencing sub clinical or clinical mastitis, contaminated water source used for washing and utensils used for the storage of milk on farm, or during transportation. Animals affected with mastitis might shed large numbers of microorganisms into the milk. A number of pathogenic bacteria including *S. aureus, Escherichia coli* and *Salmonella spp.* have been recovered from raw milk Many milk-borne epidemics of human diseases, that occur due to contamination of milk by inappropriate handling by dairy workers, unclean utensils; non-potable water used as adulterant, improper or delayed chilling or long-term storage have been described (Chatterjee *et al.*, 2006). Thus, monitoring the quality of milk before consumption or processing is an essential prerequisite.

Keeping quality is an expression used to indicate the length of time for which milk remains sweet and otherwise palatable and suitable for direct consumption. Several quality tests have been described in the literature to test the keeping quality of milk. Tests like Direct Microscopic Count (DMC), Standard Plate Count (SPC), Methylene Blue Reduction Test (MBRT) and Resazurin Reduction Test (RRT) are routinely being used to determine the quality of milk. Determination of DMC and SPC is lengthy and requires expertise. MBRT and RRT serve as standard tests to determine keeping quality of the milk sample in lesser time, and are also simple to perform.

The principle of MBRT is based on the fact that the colour imparted to the milk by addition of appropriate concentartion of the dye methylene blue will disappear more or less quickly. Because methylene blue is a redox indicator which loses its colour under lack of oxygen. Oxygen is utilized during bacterial metabolic processes. Thus, more the bacterial load in milk sample, lesser is the oxygen content. In RRT, resazurin, another redox dye is used for this purpose. The test is conducted similar to MBRT. In RRT, however, determination of keeping quality is based on colour produced after a stated period of incubation (Khan *et.al.*, 2017).

Since its introduction in 1929, RRT has been widely used to monitor the sanitary quality of milk. Conventional RRT involves incubation of considerable amount of milk sample (10 ml) with appropriate concentration of resazurin (7-hydroxy-10-oxidophenoxazin-10-ium-

3-onesodium), a blue redox indicator dye. During growth, bacteria in the milk sample utilize oxygen, the rate of oxygen removal being proportional to milk keeping quality. Reduction of the dye is indicated by gradual colour change through various shades of purple and mauve to full pink, at which point the resazurin is changed to resorufin. This stage of reduction is irreversible. The second stage, consisting of further reduction of resorufin to dihydroresorufin and characterized by a fading of the pink colour, is reversible. The keeping quality of milk sample is determined by noting either (a) degree of colour change after a stated period of incubation, or (b) time required for reducing the dye to a predetermined end-point (APHA, 1953). Conventional RRT requires the use of significant amount of milk sample, reagents and glassware. Also, sterile conditions are needed to be maintained throughout the protocol.

Pasteurization, a preservation technique for milk, is mainly performed to destroy or inactive all the harmful or pathogenic microorganisms by using heat treatment. Pasteurized milk is obtained by heating milk for a minimum time of 15 sec (at a temperature of 72°C) or 30 min (at a temperature of 63°C). Ultra-High Temperature (UHT) pasteurization is a process of heating milk at a temperature of 135°C–150°C for a fraction of a second holding time to prolong the shelf life of milk. Pasteurization kills any pathogens that might be present in the milk sample (especially *Mycobacterium tuberculosis*, *Salmonella* spp., enteropathogenic *E. coli*, *Campylobacter jejuni*, and *Listeria monocytogenes*). Most of the spoilage microorganisms in raw milk, such as coliforms, mesophilic lactic acid bacteria, and psychrotrophs, are also killed by pasteurization. Pasteurisation is an essential process in the production of milk safe and free from pathogens.

Alkaline Phosphatase is an enzyme which is naturally present in milk but is destroyed at a temperature just near to the pasteurization temperature. Alkaline phosphatase test is based on the principle that the alkaline phosphatase enzyme in milk, if not inactivated by appropriate pasteurization, liberates phenol from substrate disodium para-nitro phenyl phosphate, to form yellow coloured complex at alkaline pH. The intensity of yellow colour produced is directly proportional to the activity of the enzyme. The test is not applicable to sour milk and milk preserved with chemical preservatives.

In view of the importance of maintenance of keeping quality and efficient pasteurization in prevention of milk-borne diseases, current study aimed at analysis of these criteria for randomly collected milk samples from different areas of Thane, Maharashtra, India.

MATERIALS AND METHODS

Sample collection: Packaged milk sample of different brands were procured aseptically in sterile containers and immediately processed for MBRT, RRT and phosphatase tests.

Preparation of methylene blue dye (1:30000): 0.1 g of methylene blue dye was completely dissolved in 9 ml of sterile distilled water after which volume of the solution was made to 10 ml. This solution was 10 fold serially diluted, twice, and 3 fold, once, using sterile distilled water to obtain 1: 30,000 concentration of the dye. The dye was then stored in sterile amber coloured bottle in dark to prevent photo oxidation.

Methylene Blue Reduction Test: 10 ml milk sample was added aseptically in sterile test tube and one ml of methylene blue dye (1:30000) was added to it. The contents were thoroughly mixed and tubes incubated at 37°C for up to 8 hours. Appropriate positive and negative control tubes were also set as per the procedure detailed in IS: 1479 (Part – III) 1962. The time required for complete decolorization of dye in test tubes was noted in comparison with the control tubes.

Preparation of resazurin dye (1:30000): 0.1 g of resazurin blue dye was completely dissolved in 9 ml of sterile distilled water after which volume of the solution was made to 10 ml. This solution was 10 fold serially diluted, twice, and 3 fold, once, using sterile distilled water to obtain 1: 30,000 concentration of the dye. The dye was then stored in sterile amber coloured bottle in dark to prevent photo oxidation.

Resazurin Reduction Test: 10 ml milk sample was added aseptically in sterile test tube and one ml of resazurin dye (1:30000) was added to it. The contents were thoroughly mixed and tubes incubated at 37°C for 30 mins. Appropriate positive and negative control tubes were also set as per the procedure detailed in IS: 1479 (Part – III) 1962. The test tubes were checked for colour change, if any, as compared to the control tubes.

Preparation of substrate reagent for phosphatase test: 0.35g of anhydrous sodium carbonate and 0.15g of para-nitrophenyl phosphate (PNPP) were dissolved in 90 ml of sterile distilled water and volume made up to 100ml in a volumetric flask. The reagent was stored at 4^0C in amber coloured bottle (IS:8479 (Part-1)).

Phosphatase test:

1ml of milk sample was transferred to three sterile test tubes under aseptic conditions and labelled as positive control, negative control, and test respectively. Negative control tube was kept in boiling water bath for 10 minutes, cooled down to room temperature and 5ml

of PNP (42 µg/ml in distilled water) was added aseptically. 5ml of phosphatase reagent was added aseptically in negative colour control tube and to the 'test' tube. All tubes were incubated at 37°C for 30 minutes and observed for colour change (IS:8479 (Part-1)).

RESULTS AND DISCUSSION

On one hand, India has become a leading milk producing country, milk-borne diseases have become a cause for concern on the other. Singh et al. (2018) have reported presence of *Salmonella* in 210 milk and milk product samples analysed from Maharashtra. In view of the risk associated with milk-borne infections, the keeping quality of twenty different packaged milk samples was determined using MBRT and RRT tests. Also, efficiency of pasteurization of these samples was tested by performing the phosphatase test. The following results were obtained.

Table 1: MBRT, RRT and phosphatase test results of milk samples

Sample Number	MBRT (time for de-colourization)	RRT (Colour after incubation for 30 mins at 37^0C)	Phosphatase Test (Colour after incubation for 30 mins at 37^0C)
S1	2 to 6 hours	Lilac	white
S2	6 to 8 hours	Blue	white
S3	2 to 6 hours	Blue	white
S4	2 to 6 hours	Blue	white
S5	2 to 6 hours	Blue	white
S6	2 to 6 hours	Blue	white
S7	2 to 6 hours	Blue	white
S8	2 to 6 hours	Blue	white
S9	2 to 6 hours	Blue	white
S10	6 to 8 hours	Blue	white
S11	2 to 6 hours	Lilac	white
S12	2 to 6 hours	Lilac	white
S13	2 to 6 hours	Blue	white
S14	2 to 6 hours	Blue	white
S15	2 to 6 hours	Blue	white
S16	2 to 6 hours	Lilac	white
S17	6 to 8 hours	Blue	white
S18	2 to 6 hours	Blue	white
S19	2 to 6 hours	Blue	white
S20	2 to 6 hours	Blue	white

The dye reduction time in MBRT indirectly reflects the microbial load in the milk and the total metabolic activity of the microorganisms in the milk sample. The interpretation criteria for MBRT as per BIS (Bureau of Indian standards) 1977 are as follows.

Table 2: interpretation criteria for MBRT

Decolourization time	Quality of milk
More than 8 hours	Very good
6 to 8 hours	Good
2 to 6 hours	Fair
Less than 2 hours	Poor

Thus, in current study, seventeen samples out of twenty were of 'fair' keeping quality, while three were of 'good' keeping quality as per results of MBRT and referring the BIS standards.

In RRT, resazurin imparts blue colour to the milk which when reduced to resorufin changes to pink and finally to white when reduced to di-hydroresorufin. The time required for complete decolourization i.e. reduction of two resazurin and the degree of colour changed is directly related to the number of bacteria in the milk. As per ISI Standards after 30 minutes of incubation at 37°C, the milk samples are graded as follows: -

Table 3: interpretation criteria for RRT

Colour	Quality of milk
Blue	Excellent
Lilac or mauve	Good
Mauve or Pink	Poor
White	Grossly abnormal

Thus, in present study, sixteen samples out of twenty were of 'excellent' keeping quality, while four were of 'good' keeping quality as per results of RRT and referring the ISI standards.

Alkaline Phosphatase test is routinely used to indicate whether milk has been adequately pasteurised or whether it as been contaminated with raw milk after pasteurisation. After incubation of tubes at 37°C for 30 minutes, if yellow colouration is seen in the 'test' tube then it indicates the presence of active alkaline phosphatase enzyme and that the milk is not efficiently pasteurized; whereas if no yellow colouration is seen then the milk is said to be efficiently pasteurized (Sonawane, 2018). None of the twenty samples in current study showed yellow colouration after incubation, indicating that they were properly pasteurized and not contaminated with raw milk after pasteurization.

Fig .1: MBRT results for samples 1 to 5

Fig.2 : RRT results for samples 1 to 5

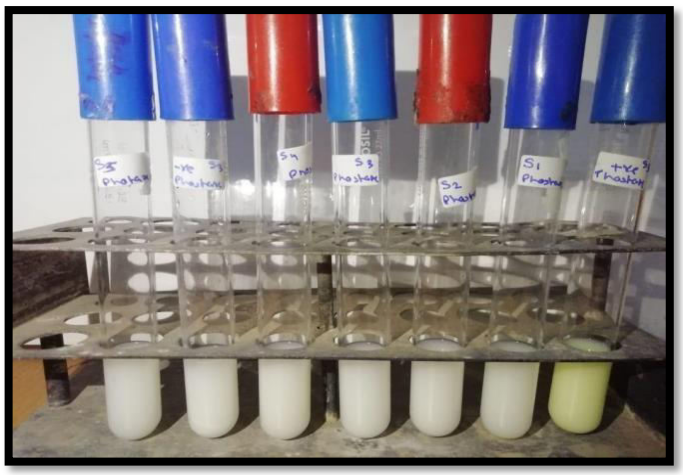

Fig.3: Phosphatase test results for samples 1 to 5

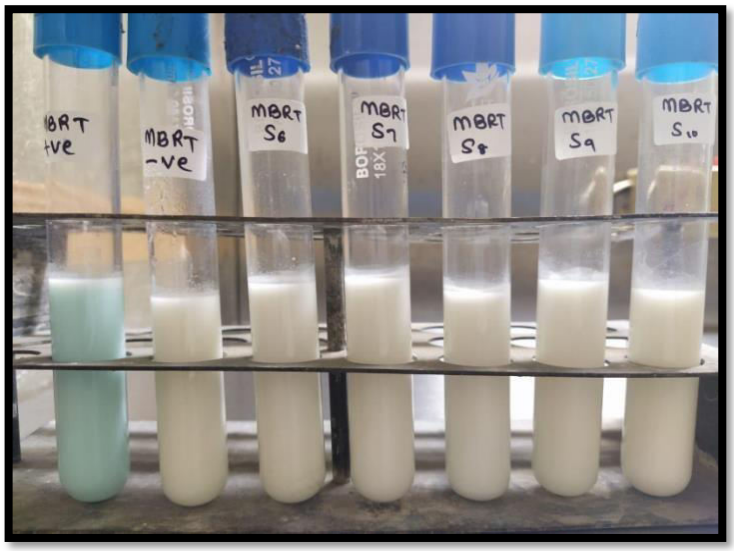

Fig.4: MBRT results for sample 6 to 10

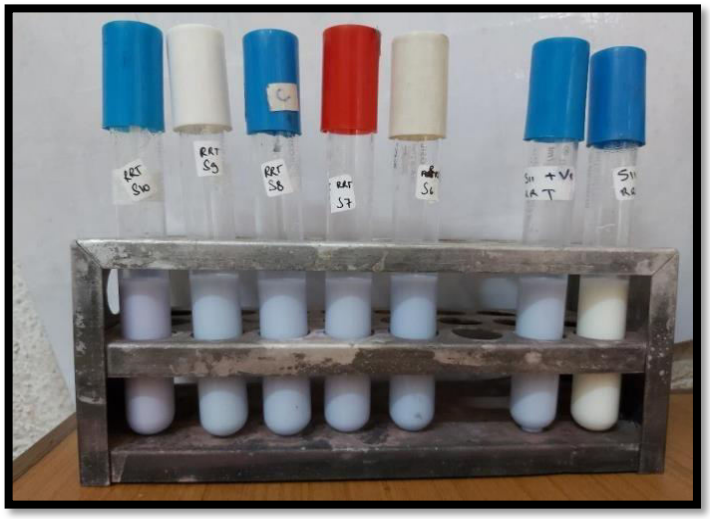

Fig.5: RRT results for samples 6 to 10

Fig.6: Phosphatase test results for samples 6 to 10

Fig.7: MBRT results for samples 11 to 15

Fig. 8: RRT results for samples 11 to 15.

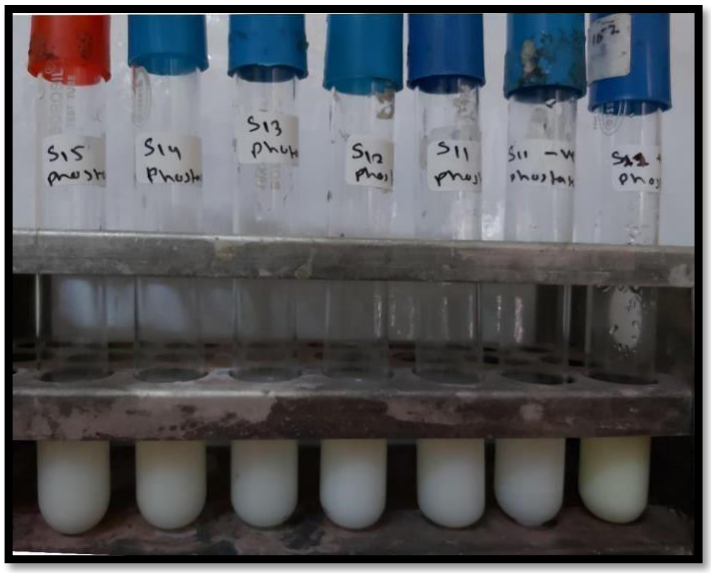

Fig.9: Phosphatase test results for samples 11 to 15

Fig.10: MBRT results for samples 16 to 20

Fig.11: RRT results for samples 16 to 20

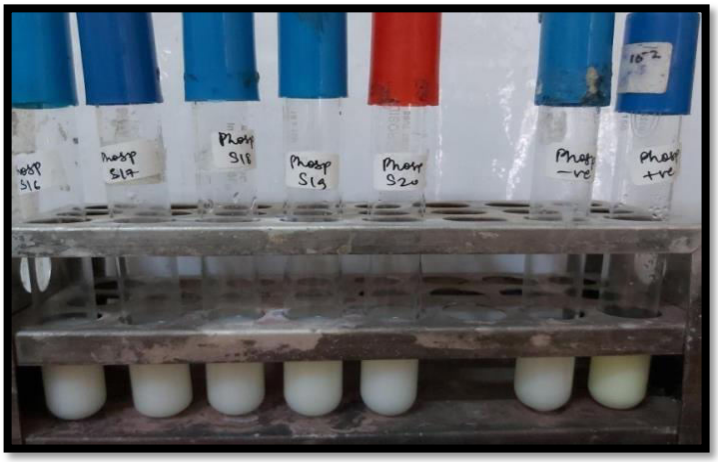

Fig.12: Phosphatase test results for samples 16 to 20

CONCLUSION

The keeping quality and efficiency of pasrerization of 20 packaged milk samples of different brands, procured from different vendors in Thane was tested. Seventeen samples out of twenty were of 'fair' keeping quality, while three were of 'good' keeping quality as per results of MBRT. RRT revealed sixteen samples to be of 'excellent' keeping quality, while four of 'good' keeping quality. All the samples were appropriately pasteurized and not contaminated with raw milk after pasteurization.. Thus, samples procured from these vendors from Thane, Maharashtra, India, seem to be of acceptable keeping quality. Further tests need to be performed to exactly determine the microbial count.

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