

Qualitative analysis of raw and pasteurized milk in Hyderabad by using Methylene blue Reduction Test (MBRT)

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Abstract : The study was conducted in the year 2018 in Hyderabad. The milk samples of total 50 was collected and categorized as raw and pasteurized milk samples. Among 50 samples raw milk samples are 35 no. and pasteurized milk 15 no. The samples were tested for MBRT following the procedure of APHA, 1978. The samples were tested for the change of colour with 0.1 % methylene blue dye color in 20 minutes to 5 hours at $36^{\circ} \text{C} \pm 1^{\circ}\text{C}$ by using water bath. The discoloration was observed in 26 (52%) samples with within 5 hours which indicates high microbial contamination and low quality samples. Whereas 24 (48%) samples retained the color even after 5 hours which indicates good quality of samples. 90% of the pasteurized samples and 70% of raw samples are good in quality.

1. Introduction: The milk can be defined in several ways. Milk is a complex fluid in which more than 100 separate chemical compounds have been found. It includes a major proportion of water and others in the form of carbohydrates, proteins, fats, minerals and vitamins etc., varying with milk of animals with genetic, species and ecosystem diversity of species. Milk is one of the food which is unavoidable from the diet of every household as it is considered as a holistic food as it meets the nutritional demand of every growing species [1]. The quality of raw milk is largely effected with its adulteration, climatic conditions, geographic location of milking, time of milking, unhygienic practices of milking and unsterilized dairy utensils, mode of its transportation, and delay in time of milking to its consumption etc. Milk is a very good medium for the growth of microorganisms. These active microorganisms reduce oxidation reduction potential of the milk due to their oxygen demand [2]. Contaminated milk can transfer the diseases from animals to humans. MBRT is widely used indirect method for estimation of microbial load in the raw milk [3]. The formation of methylene blue reductase plays vital role in determining the quality of milk. The basic principle involved in MBRT (Methylene Blue Reduction Test) is the introduction colour to the milk by adding methylene blue which will disappear with the time. The quality of the milk depends on how fast the milk loses its imported colour and which depends on the contamination of milk. Methylene blue is a redox indicator that loses its colour under the absence of oxygen. MBRT estimates the bacterial load in milk indirectly and permits faster grouping of raw and pasteurized milk samples into different grades. The reduction time appears to be inversely related to initial bacterial content of the sample. The use of MBRT would, however, necessitate establishing geographical type of classification as well as separate classification for can and for farm bulk tank supplies.

2. Study area: Hyderabad is located in the southern part of India, its climatic conditions are like hot and dry in day and cools in the night. Hyderabad is having a population of 9.482 million (2018) and growing approximately at the rate of 2.92% per annum, milk supply for the Hyderabad is accomplished by various sources, supplied as a raw milk (40%) from dairy farms located in outer part of Hyderabad apart from branded pasteurized (60%) suppliers. Per capita availability of milk in Telangana state is 422 gm.



Figure 1: Milk sampling locations in Hyderabad

3. Materials and Methods: The objective of our study is to test the quality of raw and pasteurized milk for their quality by using MBRT and to ensure the milk is safe, healthy, and meet the standards, purity, and level of contamination of micro-organisms, and finally comparing the quality of raw and pasteurized milk for its consumption. Large pool of non-refrigerated raw milk samples and refrigerated pasteurized milk samples (50 No.) were procured from various locations of the Hyderabad and the milk samples were incubated for methylene blue reduction test. 0.1% Methylene blue dye was procured as per the standards. The dye solution and milk sample were used in 1:10 proportion on ml basis. The methylene blue reduction test was done according to the standard method [2] 1 ml of dye was transferred in to test tube after placing 10 ml of milk sample. The test tubes were then incubated at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ by using thermometer in hot water bath for 5 hours and the observations are recorded for change in colour for first 20 min and the subsequent readings were taken at hourly intervals.

4. Results and discussions: A total of 50 milk samples out of which 35 (70%) are raw and 15 (30%) are pasteurized were collected from different geographic locations of Hyderabad and then tested for the MBRT test which is given in the table-1. Out of 35 raw samples 10 (29%) are found to be good quality, 15 (43%) are fair, 3 (8%) are in bad quality and 7 (20%) are very bad in their quality, are shown in Table-1 and figure-6. Whereas 15 pasteurized samples 14 (93%) are found to be very good in their quality and only 1 (7%) is observed as fair in its quality, which is shown in Table-2 and Figure-3. The observation on methylene blue reduction is shown in Figure-4 for pasteurized samples and Figure-8 for raw samples. Based on the time of decolorization approximate time for keeping their quality are shown in figure-5 and figure-9. Bacterial load of pasteurized and raw samples are shown in figure-6 and Figure-10 respectively. To meet the rapid growing population's milk demand in Hyderabad the local raw milk suppliers are encashing the people's mindset of consumption of fresh and natural food, misconception on milk preservation and packaging. Lack of awareness on milk preservation techniques and poor quality check at the suppliers end are leading to provision of very bad quality milk to the people [10]. MBRT is based on the fact that colour induced the milk by addition of a methylene blue color dye, the disappearance of blue colour is due to depletion of oxygen in the milk by the contaminated bacteria in the milk that why time of colour reduction is taken as a measure of bacterial load. The contaminated milk makes the people sensitivity to the diseases It is also observed that the % of good quality samples with less bacterial load, and high shelf life are more in pasteurized samples as compared to raw samples, and it is also noticed that there are no bad and very bad quality pasteurized milk samples. It is also known that pasteurization removes vitamin C and lactoferrin aggregation with the heat treatment [7], reasonably the high *E-coli* content was observed in raw and farm milk [5], according to WHO *E-coli* should not be present in any consumables [8].

Conclusion: From the results it shows that 90% of pasteurized samples are with good quality and 70 % of raw samples are good. Lack of awareness on hygienic practices of milking, preservation, and storage leads to the bad (8%) and very bad (20%) quality of raw milk. Even though the majority of raw samples are with good quality their national demand is to be maintained with well-trained health care professionals and there should be continuous assessment to take care of people's health from consumption of unsafe milk. It is also found that the quality of samples are degrading with the increase in distance of transportation from its milking to the supply.

Table1: Details of the sample locations and quality by MBRT

S.No.	Milk Supplier name	Sample location	MBRT	Quality
1	Vijay Milk	Lalapet, Hyderabad	4	Fair
2	Mallesham milk	Sainagar colony, alkapuri	4	Fair
3	Srisai dairy	kothapet, Hyderabad	2	Fair
4	Eeshwar milk	ankapuri, Hyderabad	1	Bad
5	Kamadenu Milk	Snehapuri coloney,Hyderabad	0.3	Very Bad
6	Sri Krishna Milk	Rnallavelly, yacharam, RR)	0.3	Very Bad
7	Kiran Milk	Vanasthalipuram, Hyderabad	0.3	Very Bad
8	Balaji Milk	kompally, Hyderabad	0.3	Very Bad
9	rajashekar	Nagole, Hyderabad	.>5	Good
10	Natural Milk	Medchal, Hyderabad	.>5	Good
11	fresh Milk	Brindavan coloney, Hyderabad	2	Fair
12	Revathi home	Shankar palli, Rangareddy	3	Fair
13	Murali krishna Milk	Prashanthi nagar Vanasthalipuram	3	Fair
14	Nandi daiy	Lb, Nagar	2	Fair
15	Gangadhar dairy	Survey coloney, Uppal	2	Fair
16	Sri milk	Church, coloney Ramanthapur	2	Fair
17	kmf Nandini milk	Ajay Nagar, Bandlaguda	2	Fair
18	Mohan farm	Jaipuri colony, Bandlaguda	2	Fair
19	Sri gopal Milk farm	Muttuguda, nagole	2	Fair
20	Srinivas Milk	lalithanagar, nagole	.>5	Good
21	Yashoda Milk	geetanagar, nagole	.>5	Good
22	Srinath milk farm	RK puram	1	Bad
23	varala dairy farm	vidyanagar, Hyderabad	.>5	Good
24	Nazveen Milk centre	Tatti annaram,	.>5	Good
25	Manikanta dodla farm	Shivani nagar, nagole	1	Bad
26	Mahaveer Milk	Mahaveer coloney, dilsukhnagar	3	Fair
27	Teja milk farm	Near saibaba temple, Dilsukhnagar	.>5	Good
28	Krishna Milk farm	kothapet, Hyderabad	.>5	Good
29	pasha dairy farm	kothapet, Hyderabad	.>5	Good
30	Madhav Dairy farm	Adarshnagar, Nagole	.>5	Good
31	Shakti dairy	Sriganesh nagar, nagole	2	Fair
32	Raja farm milk	Tarnaka	2	Far
33	Krishna Gede palu	Raghavendra coloney, LB Nagar	0.3	Very Bad
34	Srirama dairy farm	Rocktown resident coloney, Hyderabad	0.3	Very Bad
35	Raithu palu	Thyagarayanagar, Nagole, Hyderabad	0.3	Very Bad

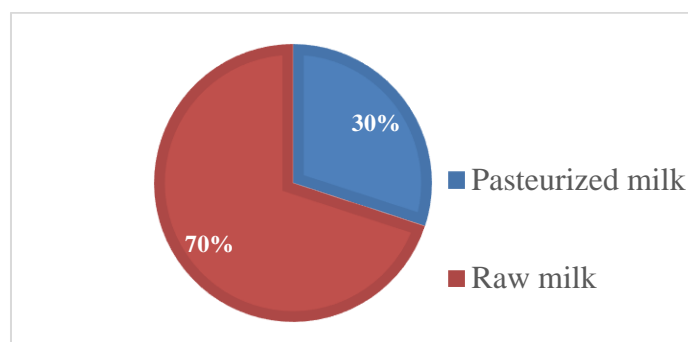


Fig.2 Distribution of samples tested, Raw and pasteurized

Table 2: MBRT of pasteurized milk samples

S.No.	Brand Name	Sample Location	MBRT (hrs)	Quality
1	SIDS private farm	Tallapally, Shabad, Rangareddy	.>5	Good
2	Vijaya dairy	Lalapet, Hyderabad	.>5	Good
3	Heritage	Cheruvugattuvillage, Narketpally.	.>5	Good
4	Amul	Nagole, Hyderabad	.>5	Good
5	Karimnagar dairy	Karimnagar, TS	.>5	Good
6	Model dairy	Nidamanuru, Vijayawada	.>5	Good
7	Tazza Milk	Kalas, Indapur, Pune, MH	.>5	Good
8	Mother dairy	Hayath nagar, Hyderabad	2	Fair
9	Country delight	Nagole, Hyderabad	.>5	Good
10	Jersey	ShadNagar Hyderabad	.>5	Good
11	Arokya	Medchal, Telangana	.>5	Good
12	Dodla dairy	Gundrampalli, Chityala, Nalgonda	.>5	Good
13	Masqati Dairy Milk	Turkayamjal, Rangareddy	.>5	Good
14	Thirumala Milk	Kodivedu, Chillakur, Nellore	.>5	Good
15	Vaishnavi milk	Gundrathimadugu, Konijerla, Kammam	.>5	Good

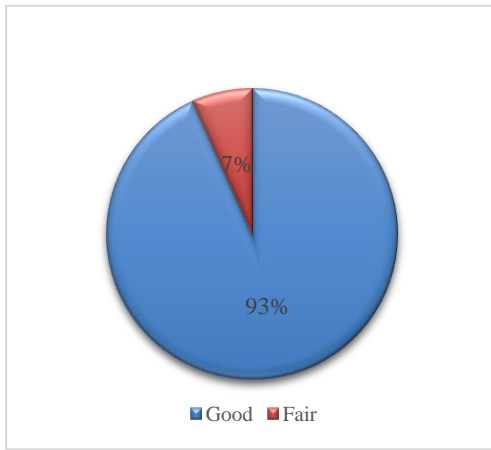


Fig.3 Quality of pasteurized milk samples

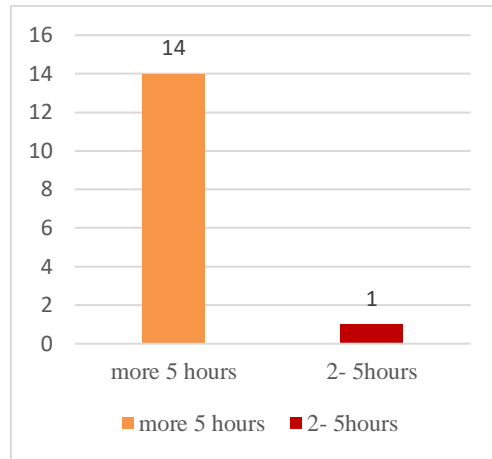


Fig. 4 MBR Time for pasteurized Milk samples

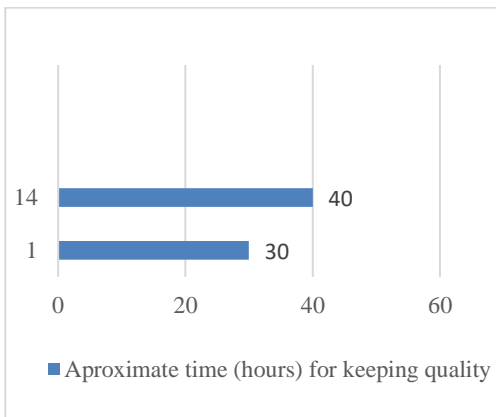


Fig. 5 Approximate time for keeping quality

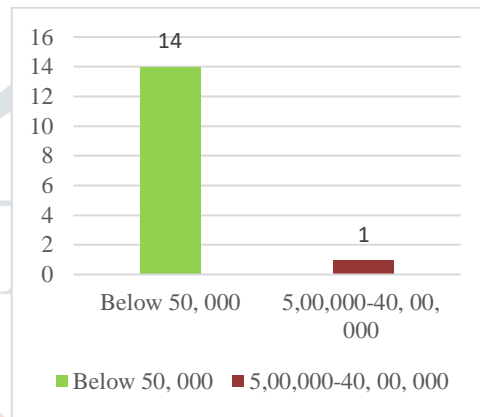


Fig. 6 Bacterial count of pasteurized milk samples

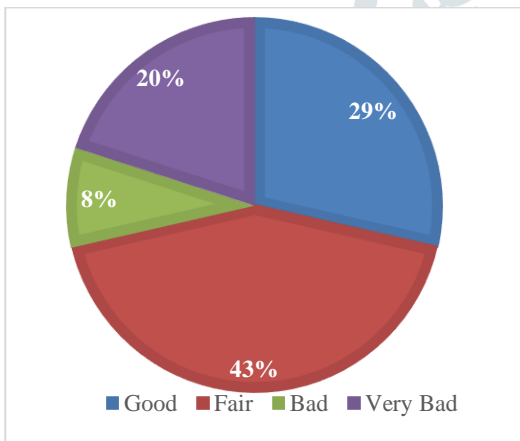


Fig.7 Quality of raw milk samples

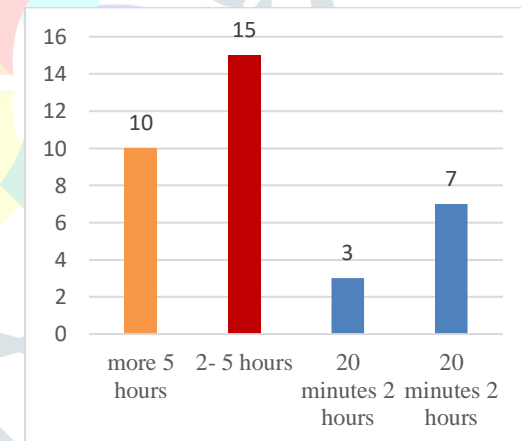


Fig. 8 MBR Time for raw Milk samples

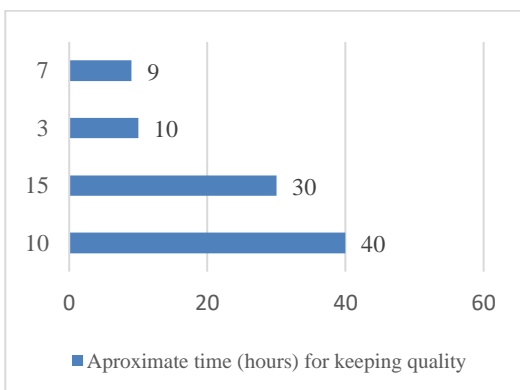


Fig. 9 Approximate time for keeping quality

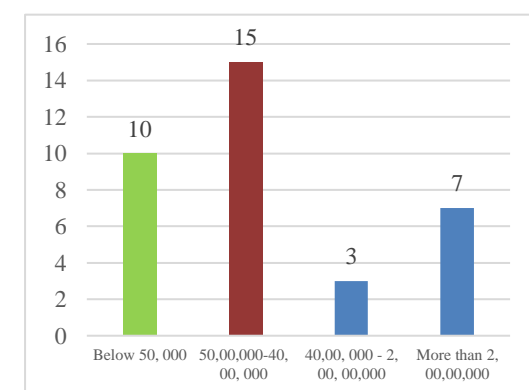


Fig. 10 Bacterial count of raw milk samples

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