International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.6, pp 2804-2809, Oct-Dec 2013

Microbiological And Chemical Analysis Of Raw, Pasteurized And UHT Milk During Preservation In India.

Monika Saxena[1]*, Poonam Rai[2]

Department of Applied Chemistry, Bansal Institute of Research, Technology and Science, Bhopal, India.

***Corres.author: msaxena72@gmail.com**

Abstract: Milk is very popular drink in India and is used for various purposes like for making tea coffee, making sweets, making dairy products etc. Milk contains up to 87% of water which can be easily adulterated by unscrupulous middlemen and uneducated farm workers. The aim of this study was to diagnose chemical, microbial content of milk during preservation. Major tests considered in the research work were titratable acidity, COB test; total viable bacteria count (TVC) and Coliform count. The result of the experiment suggest that both raw and pasteurized milk tends to increasing microbial population during refrigeration. On the other hand, UHT milk which regards as a readily drinkable drink must not be purchased after three months from production due to microbial content in milk sample increased by substantial amount.
Key Words: Milk, Preservation, Bacterial count, Coliform Bacteria.

Introduction

Milk is considered as nature's single most complete food Moreover; its high nutritive value makes it an ideal medium for the rapid multiplication of bacteria, particularly under unhygienic production and storage at ambient temperatures[1,2]. We know that, In order for any processor to make good dairy products, good quality raw materials are essential. A milk processor or handler will only be assured of the quality of raw milk if certain basic quality tests are carried out at various stages of transportation of milk from the producer to the processor. Milk is complex mixture of fat, protein, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water, make it a complete diet[3]. Except high nutritional value, presence of pathogenic bacteria in the milk can results with high health danger and may cause death of consumers. In India, milk is produced mostly in non-standardized way and is usually supplied to the consumers of the urban and rural areas by milkmen and by some established dairy farms where surplus milk is readily available. Contaminated raw milk can be a source of harmful bacteria. Different heat and other treatments are given to raw milk in order to remove pathogenic microorganism and to increase the shelf life. Pasteurization process is largely applied to certain food products in order to decrease the microbiological risk and to increase their preserving ability. Pasteurization is not intended to kill all pathogenic microorganism in food or liquid. UHT (ultra heat treatment) is also used for milk treatment. UHT processing holds the milk at a temperature of 138 degree celcius for a fraction of second. The microbial status of these heat treated milk is a concern and garbing attention of the authorities nowadays. Heat treated milk like pasteurized & UHT milk should not contain pathogenic bacteria but if the milk does not processed properly, it may results with high microbial load in milk. The problems of post treatment contamination in containers which are not sterilized properly can cause the contamination in the milk. The contamination can either be through poor seal or through pin hole in the containers. Organism will probably

have entered from ineffectively sterilized plant down stream from heat treatment stage of the process which includes spores of some organism. The types of spores which have been investigated as of particular relevance in UHT are those of Bacillus subtilis and Clostridium botulinum has been studied. In India till now no standard is known to be established for raw and UHT treated milk.

So far, less work had been conducted on the quality evaluation of raw and processed milk during prolonged preservation in India. The present investigation will throw light on quality of raw, pasteurized & UHT milk in India by chemical and bacteriological tests.

Materials And Methods

Collection Of Samples:

In India, milk is generally sold in two ways. In most cases, the farmers bring milk in open pots and sell it directly in the market without any processing and packaging. In other cases, milk companies collect milk from the farmers or dairy farms, process it via pasteurization or UHT treatment and package the processed milk which is then sold in shops under specific brand name. In this study, raw milks were purchased from different vendors. While brand milks were bought from different shops. A total of nine samples were examined where three (designated as R-1, R-2, and R-3) were of raw milk samples bought from different vendors. Three (P-1, P-2, and P-3) were of pasteurized milks samples each of different brand and the other three (U-1, U-2, and U-3) were of UHT-processed also from different brands. During the whole sampling process, its transportation to the laboratory and storage and all precautionary measures were observed.

Chemical Analysis:

Milk Samples For Chemical Tests.

Milk samples for butterfat testing may be preserved with chemicals like Potassium dichromate (1 Tablet or ½ ml 14% solution in a ¼ litre sample bottle is adequate.) Milk samples that have been kept cooling a refrigerator or ice-box must first be warmed in water bath at 40 ºC, cooled to 20ºC, mixed and a sample then taken for butterfat determination. Other preservative chemicals include Sodium azide at the rate of 0.08% and Bronopol (2-bromo-2-nitro-1, 3-propanediol) used at the rate of 0.02%.

If the laboratory cannot start work on a sample immediately after sampling, the sample must be cooled to near freezing point quickly and be kept cool till the work can start. If samples are to be taken in the field e.g. at a milk cooling centre, ice boxes with ice pecks are useful.

Clot On Boiling (C.O.B) Test

The test is quick and simple. It is one of the old tests for too acid milk (pH<5.8) or abnormal milk (e.g. cholesterol or mastitis milk). If a milk sample fails in the test, the milk must contain many acid or rennet producing microorganisms or the milk has an abnormal high percentage of proteins like cholesterol. Such milk cannot stand the heat treatment in milk processing and must therefore be rejected.

Procedure:

Boil a small amount of milk in a spoon, test tube or other suitable container. If there is clotting, coagulation or precipitation, the milk has failed the test. Heavy contamination in freshly drawn milk cannot be detected, when the acidity is below 0.20-0.26% Lactic acid.

Acidity Test

Bacteria that normally develop in raw milk produce more or less of lactic acid. In the acidity test the acid is neutralized with 0.1 N Sodium Hydroxide and the amount of alkaline is measured[4]. From this, the percentage of lactic acid can be calculated. Fresh milk contains in this test also "natural acidity" which is due to the natural ability to resist pH changes. The natural acidity of milk is 0.16-0.18%, Figures higher than this signifies developed acidity due to the action of bacteria on milk sugar.

Apparatus:

- A porcelain dish or small conical flask
- 10 ml pipette, graduated
- 1 ml pipette
- A Burette, 0.1 ml graduations
- A glass rod for stirring the milk in the dish
- A Phenolphthalein indicator solution, 0.5%in 50% Alcohol
- N Sodium hydroxide solution

Procedure:

- 9 ml of the milk measured into the porcelain dish/conical flask, 1 ml Phenolphthalein is added and then slowly from the burette, 0.1 N Sodium hydroxide under continuous mixing, until a faint pink colour appears. The number of mls of Sodium hydroxide solution divided by 10 expresses the percentage of lactic acid.

Sampling Milk For Bacteriological Testing

Sampling milk for bacteriological tests require a lot of care. Dippers used must have been sterilised in an autoclave or pressure cooker for at least 15 minutes at 120° C before hand in order not to contaminate the sample. On the spot sterilisation may be employed using 70% Alcohol swab and flaming or scaling in hot steam or boiling water for 1 minute.

Bacteriological Analysis:

Standard Plate Count (SPC) method recommended for dairy products[5] was followed for quantitative analysis of bacteria.

Enumeration of total viable bacteria: Nutrient agar medium (Difco) was used for enumeration of total viable bacteria. pH of the medium was adjusted at 6.8 prior to sterilization. Inoculated plates were incubated at 37°C for 24 to 72 hours to facilitate viable bacterial growth. After incubation, the inoculated plates having30 to 300 colonies were considered for counting using colony counter (Gallenkamp, England) and total countwas expressed as colony forming units per milliliter (c.f.u. /ml).

Enumeration of total coliform bacteria: Total coliform was determined by the same method used inthe enumeration of total viable bacteria. The medium used for coliform was MacConkey agar. Inoculatedplates were incubated at 37°C for 24 hours. After incubation, typical pinkish and centrally red colonies were counted by using colony counter and total coliform was calculated.

Results And Discussion

1. Acidity Percentage:

Titratable acidity is a measure of freshness and bacterial activity in milk.

High quality milk essentially needs to have less than 0.14 percent acidity.

Acidity percentage of milk samples were given in Table 1.

Table 1:- Acidity (%) test of different milk samples

Stage	Raw Milk				Pasteurized Milk				UHT Milk			
(Time)	R-1	R-2	R-3	Average	P-1	P-2	P-3	Average	U-1	U-2	U-3	Average
1	0.203	0.210	0.220	0.221 ±0.008	0.160	0.169	0.180	0.179 ±0.010	0.147	0.150	0.152	0.143 ±0.010
2	0.217	0.221	0.227	0.226 ±0.009	0.172	0.175	0.198	0.183 ±0.012	0.154	0.161	0.158	0.158 ±0.010
3	0.224	0.234	0.236	0.231 ±0.009	0.178	0.179	0.207	0.188 ±0.016	0.184	0.172	0. 170	0.178 ±0.006
4	0.232	0.241	0.250	0.251 ±0.010	0.195	0.190	0.216	0.210 ±0.013	0.191	0.200	0.182	0.189 ±0.007

The average acidity % for raw milk samples was 0.221±0.008 for the first day of preservation and after six days of preservation the average acidity percentage was0.251±0.010 which indicating high bacterial quality. The average acidity of the pasteurized milk samples ranged from 0.179±0.010 to 0.210±0.013during the six days examination period[6], where Indian standards allow a maximum acidity of 0.15% for the pasteurized milks.

The most unexpected result was found with UHT milk samples during the preservation period. The average initial acidity percentage for UHT milk samples was 0.143±0.010. After six months of preservation the average acidity percentage in UHT milk samples was 0.189±0.007, suggesting deterioration in milk quality.

Titrable acidity of milk is expressed in terms of percentage lactic acid[7]. Fresh milk does not contain any appreciable amount of lactic acid and therefore an increase in acidity is a rough measure of its age and bacterial activity. Within a short time after milking, the acidity increases due to Bacterial activity. The amount of acid depends on the cleanliness of Production and the temperature at which milk is kept. Determination of acid in milk is an important factor in judging milk quality. Acidity affects taste when acidity reaches about 0.3%, the sour taste of milk becomes sensible. At 0.4% acidity, milk is clearly sour, and at 0.6% it precipitates at normal temperature. At acidity over 0.9%, it moulds.

2. Bacterial Count:

The results of bacterial distribution in the samples are presented in Table 2. All the raw milks had high bacterial load which average ranged from 4.19±0.69 to 6.35±0.11 log c.f.u./ml during the preservation period. The most frequent cause of high bacterial load is poor cleaning of the milking system. Bacterial count was high due to milking dirty udders, maintaining an unclean milking and housing environment and failing to rapidly cool milk to less than 40°F. The TVC (total viable bacterial count) of the pasteurized milk samples average ranged from 3.43± 0.17 to 4.82±0.05 log c.f.u./ml. c.f.u./ml). The reason for high bacterial count in the pasteurized milks may include defective pasteurization machinery, surviving pasteurization, and post-pasteurized contamination due to poor processing and handling conditions and/or poor hygienic practices by workers. According to the definition of UHT process, UHT milk should contain very little or no active bacteria[8]. After four months of preservation, the average bacterial count in UHT milk samples was 3.49±0.04 logc.f.u./ml. The presence of bacteria in UHT milk might be due to many factors including the milk quality, sanitation of process plant, status of packaging material and also the handling process (Tekinsen *et al*., 2007). Milk companies recommend high quality of UHT milk till six months from the manufacturing date, But in reality UHT milk quality deteriorate much prior than 6 months.

Table 2: Total viable Bacteria Count in milk samples

Stage	Raw Milk				Pasteurized Milk				UHT Milk			
(Time)	R-1	R-2	R-3	Average	P-1	P-2	P-3	Average	U-1	U-2	U-3	Average
1	3.75	4.86	4.91	4.19±0.69	3.25	3.24	3.30	3.43±0.17	2.14	2.15	2.26	2.22±0.06
2	4.90	5.01	5.24	5.13±0.09	3.51	3.33	3.52	3.60±0.12	2.14	2.19	2.27	2.23±0.12
3	5.00	5.20	5.10	5.22±0.17	3.59	3.45	3.82	3.87±0.06	2.31	2.40	2.53	2.39±0.02
4	5.16	6.17	6.21	6.35±0.11	4.67	4.73	4.78	4.82±0.05	3.53	3.55	3.65	3.49±0.04

3. Coliform Count:

The results of coliform count in the samples are presented in Table 3. Colifor are considered as indicator organisms because their presence in food indicates some form of contamination. Average coliform count in the raw milks average ranged from 2.97±0.13 log c.f.u../ml. to 3.40±0.26 log c.f.u./ml. during the six days preservation(Saitanu et al,1996) . Poor hygiene, contaminated water, unsanitary milking practices, and improperly washed and maintained equipment can lead to higher coliform counts in raw milk .Pasteurized milk shouldn't contain any coliform bacteria as though coliform bacteria can't survive the pasteurization temperature but the presence of TCC (Total coliform count)of the pasteurized milk samples indicates either defect in pasteurization process or post pasteurization contamination which includes contamination in packaging materials(Srairi et al,2006), defects in pipe lines. The average TCC in pasteurized milk after four days of refrigeration was2.81±0.09 log c.f.u./ml. which was very high. The experiment also demonstrated that UHT-milks under consideration were not free from coliform. The initial average coliform count for UHT milk samples was 2.00±0.11 log c.f.u. /ml. which became 2.23±0.10 log c.f.u./ml after four months of preservation at room temperature. These results of coliform bacteria test indicates that processed milk available in India are not properly processed and may cause high health risk to consumers.

Table 3: Total Coliform Bacteria Count in milk samples

Stage	Raw Milk				Pasteurized Milk				UHT Milk			
(Time)	R-1	R-2	R-3	Average	P-1	P-2	P-3	Average	U-1	U-2	U-3	Average
1	2.85	3.02	3.08	2.97±0.13	2.60	2.65	2.42	2.55±0.10	2.27	2.12	1.55	2.00±0.11
2	2.90	3.18	3.11	3.07±0.12	2.74	2.72	2.51	2.66±0.13	2.40	2.19	2.00	2.11±0.13
3	3.25	3.30	3.27	3.26±0.04	2.82	2.89	2.58	2.72±0.17	2.37	2.35	2.12	2.21±0.07
4	3.37	3.48	3.39	3.40±0.26	2.85	2.97	2.61	2.81±0.09	2.49	2.38	2.50	2.23±0.10

Conclusion

It is concluded from the whole study that the initial average TVC (total viable count) in raw milk was 4.19±0.69 log c.f.u. /ml. which increased to 6.35±0.11 log c.f.u. /ml. a clear indication of deterioration in milk quality. In case of pasteurized milk samples initial average total viable count was 3.43±0.17 log. c.f.u. /ml. increased to 4.82±0.05 log c.f.u. /ml. after six days of preservation. UHT milk samples which should not contain microbial contamination also provided with initial average total viable count of 2.22±0.06 log c.f.u. /ml. and 3.49±0.04 log c.f.u. /ml. during preservation at room temperature for four months. Coliform bacteria usually cannot survive at the pasteurization temperature .The initial average coliform bacteria were estimated for raw milk 2.97±0.13 log c.f.u. /ml. and increased to 3.40±.20 log c.f.u. /ml. The initial average coliform bacteria were estimated for pasteurized milk 2.55±0.10 log c.f.u. /ml. and increased to 2.81±.09 log c.f.u. /ml. The initial average coliform bacteria were estimated for UHT milk 2.00±0.11log c.f.u. /ml. and increased to 2.23±.10 log c.f.u. /ml. These findings may be helpful for authorities, branded milk companies, researchers and consumers to monitor the quality of milk products in the Indian market. The presence of bacterial population in processed milk indicates

defects in processing plants. The presence of pathogenic organism, the high counts of coliforms and the high levels of adulteration in milk are indicative of a hazardous product. The concerned authorities therefore should monitor the overall hygienic condition surrounding the production and handling of milk.

References

1. Kim, H., Hardy, J., Novak, G., Ramet, J. P. and Weber, F. 1983. Off-tastes in raw and reconstituted milk. FAO Animal Production and Health Paper, 35: 2.
2. OECD 2005. Dairy policy reform and trade liberalization. Organisation for economic co-operation and development, p. 98, OECD Publishing.
3. Haug, A., Hostmark, A.T. and Harstad, O.M. 2007. Bovine milk in human nutrition – a review. Lipids in Health and Disease 6:25.
4. Aggawala, A.C. and Sharma, R.M. 1961. A Laboratory Manual of Milk Inspection. Bombay, Calcutta, New Delhi, India.
5. APHA 1960. Standard Methods for the Examination of Water and Waste Water (A. E. Eaton, L. S. Clesceri and A.E. Greenberg, eds.). American public health association, Maryland, United Book Press Inc.
6. Elmagli, A. A. O. and El Zubeir, E.L. 2006. Study on the compositional quality of pasteurized milk in Khartoum State (Sudan). International Journal of Dairy Sciences 1 (1): 12-20.
7. Griffiths, M.W., Phillips, J.D., West, I.G. and Muir, D.D. 1988. The effect of extended low-temperature storage of raw milk on the quality of pasteurized and UHT milk. Food Microbiology. 5(2): 75-87.
8. Hassan, A., Amjad, I. and Mahmood, S. 2009. Microbiological and physicochemical analysis of different UHT milk available in local market. As. J. Food Ag-Ind. 2009, 2(03), 434-447.
9. Sraïri, M. T.; Moudnib, J.; Rahho, L. and Hamama, A. 2006. How do milking conditions affect the hygienic quality of raw milk? Case study from Moroccan dairy farms. Livestock Research for Rural Development. Hassan II Agronomy and Veterinary Medicine Institute, 18: 97.
10. Jay, J. M. 2003. Modern Food Microbiology. 4th Edition, First Indian Edition: 1996, Reprint: 2003, p. 447, CBS Publishers & Distributors, New Delhi.
11. BSTI 2002 BDS 1702: 2002. Bangladesh Standard: Specification for Pasteurized Milk.pp.2-3, Bangladesh Standards and Testing Institution, Tejgaon Industrial Area, Dhaka.
12. Saitanu, I. A., Chuanchuen, K. R., Nuanuarsuwan, S., Koowatananukul, C. and Rugkhaw, V. 1996. Microbiological quality of raw cow milk. Thai Journal of Veterinary Medicine. 26(3): 193-214. Internet Journal of Food Safety, 7: 45-48.

*****