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Research Article

STUDY ON PHYSICOCHEMICAL AND MICROBIAL QUALITY OF AVAILABLE RAW, PASTEURIZED AND UHT MILK DURING PRESERVATION

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ABSTRACT

Milk is a very popular drink in Bangladesh. This study was conducted to evaluate the microbial content of milk during preservation and to make a suitable decision on the effect of preservation on the microbial population in milk. Major tests considered in the research work were titratable acidity, COB test, total viable bacteria count (TVC) and Coliform count. The initial average TVC in raw milk was 5.49±0.69 log c.f.u. /ml. which increased to 6.25±0.10 log c.f.u. /ml. indicated deterioration in milk quality. In case of pasteurized milk samples initial average total viable count was 4.43±0.17 log. c.f.u. /ml. increased to 5.92±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 3.32±0.06 log c.f.u. /ml. and 3.59±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 3.55±0.12 log c.f.u. /ml. and 2.08±0.11 log c.f.u. /ml. for pasteurized and UHT milk samples which increase to 3.81±0.06 log. c.f.u. /ml. for pasteurized milk after six days of preservation and 2.43±0.10 log c.f.u. /ml. for UHT milk samples after four months of preservation. These result of the experiment suggests that both raw and pasteurized milk tends to increased in microbial population during refrigeration on the other hand, UHT milk which regards as a readily drinkable drink must not be purchased or consumed after three months from the production due to the microbial content especially coliform bacteria in milk sample increased by substantial amount.

Key word: Milk, Preservation, Bacterial count, Coliform Bacteria.

INTRODUCTION

Milk is considered as nature's single most complete food (O'Mahony, 1988) and is definitely one of the most valuable and regularly consumed foods. But at the same time, it is highly vulnerable to bacterial contamination and hence is easily perishable (Kim *et al.,* 1983; OECD, 2005). Though it is provided with high nutritional value, but is an excellent medium for microbial growth (Uddin, 1999). Chemically, milk is a complex mixture of fat, protein, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water, make it a complete diet (Haug *et al.,* 2007). Except high nutritional value, presence of pathogenic bacteria in milk can results with high health danger and eventually may cause death of consumers. In Bangladesh, milk is produced mostly in non-standardized way and is usually supplied to the consumers from the urban and rural areas by milkmen. Although there is little milk pockets specially milk vita, and some established dairy farms where surplus milk is readily available in Bangladesh, this perishable product has never received particular attention in hygienic distribution to the consumers (Khan *et al.,* 2008). The concept of pasteurized and UHT milk in Bangladesh is not very old idea and proved to be very popular among consumers. The microbial status of these heat treated milk gets attention nowadays. Although heat treated milk like pasteurized and UHT milk shouldn't contain pathogenic bacteria but if milk dose not processed properly, it may results with high microbial load in milk. Pasteurized milk is recommended to be consumed within seven days from the production and for UHT milk it is six months from the production date. But poor initial milk quality, faulty processing, problem in preservation at the consumer side may results into microbial contamination in milk and thus there are great chances of deterioration of milk much prior than the recommended preservation time. The Bangladesh Standards and Testing Institution (BSTI) oblige various chemical and sanitary requirements for the pasteurized milk (BSTI 2002). However, no standard is known to be established for the raw and UHT-treated milk.

So far, no work had been conducted on the quality evaluation of raw and processed milk during prolonged preservation in Bangladesh. The objectives of this study were to determine total bacterial count and coliform bacterial count during preservation of raw and processed milk samples in order to evaluate the soundness of milk processing plants available in Bangladesh as well as preservation condition in consumer's level. To make people aware of the milk quality they consumed every day is another objective of the present study.

MATERIALS AND METHODS

Collection of samples:

In Bangladesh, 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 a local daily market while brand milks were bought from different shops. The samples were chosen randomly. A total of nine samples were examined where three (designated as R-1, R-2, R-3) were of raw milk samples bought from different vendors. Of the remaining six, three (P-1, P-2, P-3) were pasteurized milks each of different brand and the other three (U-1, U-2, U-3) were UHT-processed also from different brands. All the samples were collected in the sellers' usual form (plastic packets), instantly transported to the laboratory maintaining cold state and examined immediately.

Chemical Analysis:

COB test: Approximately 2ml. of milk sample was taken in a test tube and was treated over sprit lamp. Then it was allowed to boil for 1 to 2 minutes and was noted whether the milk sample in test tube clotted or not.

Acidity test: Acidity was measured by titration with 0.1 N sodium hydroxide solutions and using 1% ethanol solution of phenolphthalein as indicator (Agrarwala and Sharma, 1961). Bacteriological analysis

Standard Plate Count (SPC) method recommended for dairy products (APHA, 1960) 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 having 30 to 300 colonies were considered for counting using colony counter (Gallenkamp, England) and total count was 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 in the enumeration of total viable bacteria. The medium used for coliform was MacConkey agar. Inoculated plates 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

Acidity Percentage:

Titratable acidity is a measure of freshness and bacterial activity in milk. Popescu and Angel (2009) reported that high quality milk essentially needs to have less than 0.14 percent acidity. Acidity percentage of milk samples are given in the Table 1. The acidity of the raw milk samples varied largely from one sample to another during the storage period. The average acidity percentage for raw milk samples was 0.211±0.008 for the first day of preservation and after six days of preservation the average acidity percentage was 0.241±0.010 which indicating high bacterial activity and risk of consuming milk with such high acidity

percentage. The average acidity of the pasteurized milk samples ranged from 0.169±0.010 to 0.200±0.013 during the six days examination period, where BSTI (2002) allows a maximum acidity of 0.15% for the pasteurized milks. Elmagli and El Zubeir (2006) observed a greater range of acidity (0.14 to 0.86%) in pasteurized milks. The most thrilling result was found with UHT milk samples during the preservation period. The average initial acidity percentage was recorded for UHT milk samples was 0.145±0.011 which emphasize two possibilities. The initial high acidity may suggest that the high acidity might have developed prior to the heat treatment and in the same time improper heat treatment may results into presence of bacterial population in treated milk which might also results into high initial acidity in UHT milk (Hossain *et al.,* 2011). After six months of preservation the average acidity percentage in UHT milk samples was 0.199±0.008, suggesting deterioration in milk quality.

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
1st	4.70	5.86	5.91	5.49±0.69	4.25	4.44	4.60	4.43±0.17	3.34	3.25	3.36	3.32±0.06
2nd	5.95	6.01	6.14	6.03±0.09	4.51	4.63	4.72	4.62±0.12	3.44	3.39	3.47	3.43±0.12
3rd	6.06	6.09	6.20	6.12±0.07	4.79	4.85	4.92	4.85±0.06	3.51	3.43	3.53	3.49±0.02
4th	6.14	6.27	6.34	6.25±0.10	5.87	5.93	5.98	5.92±0.05	3.63	3.55	3.61	3.59±0.04

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

[For raw and pasteurized milk samples difference between two stages is two days, in case of UHT milk, difference between two stages is one month, starting from one month of preservation to four months of preservation. R-1, R-2, and R-3 stands for Raw milk samples, P-1: Aarong pasteurized milk, P-2: Pran pasteurized milk, P-3: Milk Vita pasteurized milk, U-1: Pran UHT milk, U-2: Farm Fresh UHT milk and U-3: RD UHT milk]

Titratable acidity of milk has long been recognized and employed as an indicator of quality (Griffiths *et al.,* 1988). It is expressed in terms of percentage lactic acid since lactic acid is the principal acid produced by fermentation after milk is drawn from the udder. Fresh milk, however, 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 (O'Mahony 1988; Lampart 1947). Within a short time after milking, the acidity increases perceptibly due to bacterial activity. The degree of bacterial contamination and the temperature at which the milk is kept are the chief factors influencing acid formation. Therefore, the amount of acid depends on the cleanliness of production and the temperature at which milk is kept. For this reason, determination of acid in milk is an important factor in judging milk quality. Acidity affects taste as well. When it 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 (Heineman 1919; Torkar and Teger 2008).

Bacterial Distribution:

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
1st	0.203	0.210	0.220	0.211±0.008	0.160	0.169	0.180	0.169±0.010	0.147	0.153	0.155	0.145±0.011
2nd	0.217	0.221	0.227	0.226±0.009	0.172	0.175	0.198	0.183±0.012	0.154	0.161	0.160	0.158±0.005
3rd	0.224	0.234	0.236	0. 231±0.009	0.178	0.179	0.207	0.188±0.016	0.184	0.172	0.180	0.178±0.007
4th	0.232	0.241	0.250	0.241±0.010	0.195	0.190	0.216	0.200±0.013	0.191	0.200	0.208	0.199±0.008

Table 2: Total viable bacterial count in milk samples

[For raw and pasteurized milk samples difference between two stages is two days, in case of UHT milk, difference between two stages is one month, starting from one month of preservation to four months of preservation. R-1, R-2, and R-3 stands for Raw milk samples, P-1 : Aarong pasteurized milk, P-2: Pran pasteurized milk, P-3: Milk Vita pasteurized milk, U-1: Pran UHT milk, U-2: Farm Fresh UHT milk and U-3: RD UHT milk.

The results of bacterial distribution in the samples are presented in Table 2. All the raw milks had high bacterial load which ranged from 4.70 to 6.34log 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 ranged from 4.25 to 5.98 log c.f.u./ml, much higher than that recommended by BSTI and USPHS (not exceeding 20,000 c.f.u./ml) (BSTI 2002; Jay 2003). 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. During the preservation period of pasteurized milk in refrigeration temperature, it was also monitored that the bacterial population tends to increase by many fold. At the end of six days monitoring it was found that the average TVC in pasteurized milk sample was 5.92±0.05 log. c.f.u. / ml. which were 4.43±0.17 log. c.f.u./ml. in the initial stage of preservation. However, TVC of each of the UHT-processed milks were very little although according to the definition of UHT process, UHT milk should contain very little or no active bacteria (Hassan *et al.,* 2009). After four months of preservation, the average bacterial count in UHT milk samples was 3.59±0.04 log c.f.u./ml..Bacterial presence indicating that there might be problem in UHT process. 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). The high bacterial content in UHT process milk after four months of preservation also unearth the fact that though milk companies recommend high quality of UHT milk till six months from the manufacturing date, in reality UHT milk quality deteriorate

much prior than six months.

Coliform Count:

Coliforms are considered as 'indicator organisms' because their presence in food indicates some form of contamination. The results of coliform count in the samples are presented in Table 3. Average coliform count in the raw milks ranged from 3.97±0.13 log c.f.u../ml. to 4.40±0.26 log c.f.u./ml. during the six days preservation which was higher than that obtained by Saitanu *et al.* (1996), who found TCC (total coliform count) of <1000 c.f.u./ml. However, TCC obtained in the study of Sraïri *et al.* (2006) varied from less than 30 to 2.08 × 10^7 c.f.u./ml. in raw milk. Poor herd hygiene, contaminated water, unsanitary milking practices, and improperly washed and maintained equipment can all lead to higher coliform counts in raw milk (CDFA 2008). Pasteurized milk shouldn't contain any coliform bacteria as though coliform bacteria can't survive the pasteurization temperature but the presence of TCC of the pasteurized milk samples indicates either defect in pasteurization process or post pasteurization contamination which includes contamination in packaging materials, defects in pipe lines. The average TCC in pasteurized milk after four days of refrigeration was 3.72±0.17 log c.f.u./ml. which was very high than the standard threshold set by BSTI,2002 which recommend less than 10 colonies/ml. in milk samples. USPHS allows not over 10 colonies for 'Grade A' pasteurized milk (Jay, 2003). Coliforms do not survive pasteurization (CDFA 2008). So their presence in the pasteurized milks indicates recontamination after pasteurization. The experiment also demonstrated that UHT-milks under consideration were not free from coliform. The initial average coliform count for UHT milk samples was 2.08±0.11 log c.f.u. /ml. which became 2.43±0.10 log c.f.u./ml after four months of preservation at room temperature. These daunting results of coliform bacteria test indicates that processed milk available in Bangladesh are not properly processed and may cause high health risk to consumers.

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
1st	3.82	4.02	4.08	3.97±0.13	3.60	3.65	3.42	3.55±0.10	2.17	2.11	1.95	2.08±0.11
2nd	3.93	4.18	4.11	4.07±0.12	3.74	3.72	3.51	3.66±0.13	2.30	2.17	2.04	2.17±0.13
3rd	4.21	4.30	4.27	4.26±0.04	3.82	3.89	3.58	3.72±0.17	2.27	2.30	2.16	2.24±0.07
4th	4.35	4.48	4.39	4.40±0.26	3.85	3.97	3.61	3.81±0.09	2.39	2.36	2.55	2.43±0.10

Table 3: Total Coliform Bacteria Count in milk samples

[For raw and pasteurized milk samples difference between two stages is two days, in case of UHT milk, difference between two stages is one month, starting from one month of preservation to four months of preservation. R-1, R-2, and R-3 stands for Raw milk samples, P-1: Aarong pasteurized milk, P-2: Pran pasteurized milk, P-3: Milk Vita pasteurized milk, U-1: Pran UHT milk, U-2: Farm Fresh UHT milk and U-3: RD UHT milk].

CONCLUSION

All the milk samples under consideration failed to maintain the standard quality of milk both chemically and microbiologically. The presence of bacterial population in processed milk indicates defect in processing plants. The presence of the pathogenic organisms, the high counts of coliforms and the high levels of adulteration in milk are indicative of a potentially hazardous product which is likely to be posing a serious health risk to the consumers. The government therefore should conduct frequent inspection of the marketed milks to check whether they meet the minimum legal standards and should monitor the overall hygienic condition surrounding the production and handling of milk. Realistic standards for the raw milks need to be devised and appropriate training should be given to the raw milk producers in hygienic handling of milk.

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