Microbiological Evaluation of Ultra-High-Temperature (UHT)-Treated Milk Close to Expiry Date and Routine Home Practices for Preservation of Milk

Udayalaxmi Jeppu, Namitha Jayaram, Sneha Satya, Amrutha Kandathil Purayil
Department of Microbiology, Kasturba Medical College, Manipal University, Mangalore, Karnataka, India

ABSTRACT

Background: As milk is an excellent medium for growth of microorganisms, milk gets contaminated very easily leading to its early spoilage and to milk-borne diseases. Objectives: To compare the quality of pasteurized milk with milk cooker-treated milk and to compare the quality of fresh ultra-high-temperature (UHT) milk with that of UHT milk close to expiry date. Materials and Methods: Samples of pasteurized milk, milk heated in milk cooker, fresh UHT milk, and UHT milk nearing expiry date were tested by methylene blue reduction test, coliform test, and colony count test. The results were analyzed statistically using Statistical Package for Social Sciences (SPSS) version 11.5. Results: All milk samples passed the methylene blue test and UHT-treated milk passed the coliform test also. Out of the 30 fresh pasteurized milk samples, seven (23.3%) passed the coliform test; but the same after milk cooker treatment, 16 (53.3%) passed the coliform test ($P < 0.05$). Fourteen (46.6%) out of 30 fresh pasteurized milk samples failed the colony count test, but the same after milk cooker treatment only eight (26.6%) failed the test ($P = 0.583$). Only seven out of the 60 UHT milk samples failed the colony count test and out of these six (20%) were close to expiry date ($P < 0.05$). Conclusions: Milk cooker-treated milk appears to be safer than the fresh pasteurized milk. Fresh UHT-treated milk is safer then UHT milk close to expiry date.

Keywords: Coliform count test, colony count test, methylene blue test, pasteurized milk, UHT milk

Introduction

Milk is an important diet of children and some adults due to its high nutritional value. Milk is an excellent medium for growth of microorganisms.$^{(1,2)}$ So, the introduction of a few pathogens into milk becomes a serious problem as it leads to tremendous increase in bacterial numbers.$^{(1,2)}$ If milk is handled carelessly and if proper hygiene is not maintained, it gets contaminated very easily leading to its early spoilage and also leads to milk-borne diseases.$^{(1,2)}$ Many milk-borne epidemics of human disease have been spread by contamination of milk by soiled hands of dairy workers, unsanitary utensils, flies, and polluted water supplies.$^{(5)}$ Due to post process contamination and ineffective pasteurization, the quality and shelf-life of pasteurized milk deteriorated in two dairy plants in Gaborone, Botswana, South Africa.$^{(4)}$ Pasteurization cannot guarantee the absence of microorganisms, when they are present in large numbers in raw milk or due to post-pasteurization contamination.$^{(5)}$

There are a few studies on microbiological examination of milk of which none of them focus on reliability of
routine practices for milk preservation followed at home like milk cookers, the usual practice of keeping milk at room temperature for almost a day, and consuming UHT-sterilized milk close to expiry date. Presence of coliform bacteria in processed milk is suggestive of contamination due to unsanitary conditions or practices during the processing of milk. Hence we use coliform, colony count, and methylene blue test to evaluate the quality of milk in the present study. The objectives of the present study are to compare the quality of fresh pasteurized milk with milk heated in milk cooker and kept at room temperature for at least 4 h during the daytime and to compare the quality of fresh UHT-treated milk with UHT milk close to expiry date.

Materials and Methods

It is a cross-sectional comparative in vitro study. With 95% confidence level and 80% power with reference to previous studies, the sample size comes out to be 30 in each group. Thirty samples of pasteurized commercial milk and the same sample of milk kept for 4 h at room temperature after heating in milk cooker and 60 samples of UHT-sterilized milk of which 30 were nearing expiry date (expiry date ±3 days) were tested by methylene blue reduction test, coliform test, and colony count test.

Methylene blue reduction test
Milk sample was mixed well and 10 ml of the sample was taken in a sterile test tube. To this 1 ml methylene blue solution was added. The tube was closed with a rubber stopper and incubated at 37°C for 30 min in a water bath. Ten milliliter of milk that has been held at 100°C for 4 min + 1 ml methylene blue and 10 ml milk + 1 ml tap water served as controls. Those milk samples which fail to decolorize the dye in 30 min were considered to have passed the test.

Coliform test
Milk was mixed repeatedly and three 10-fold dilution in quarter strength Ringer’s solution was prepared. One milliliter of each dilution was dispensed into each of the three tubes containing 10 ml single strength Mac Conkey’s broth with Durham’s tube. The tubes were incubated at 37°C for 48 h. The absence of acid and gas from at least two of the three tubes indicates that the milk has passed the test.

Colony count test
Milk sample was mixed well and 1 in 10, 1 in 100, and 1 in 1,000 dilutions of the milk were made in quarter strength Ringer’s solution. One milliliter of each dilution was dispensed into three sterile Petri dishes to which 10 ml of yeast extract milk agar (melted and cooled to 45-50°C) was added and incubated at 30°C for 72 h. Colonies in each plate were counted and the mean number of colonies per plate was determined. By multiplying this number by the dilution factor, we got the number of viable bacteria per ml of milk. Those milk samples which yield <1,000 cfu/ml are considered to have passed the colony count test.

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 11.5. Chi-square test and Student’s t-test was used wherever appropriate.

Results and Discussion

All the milk samples passed the methylene blue test. Out of the 30 fresh pasteurized milk samples, seven (23.3%) passed the coliform test. These samples when tested 4 hours after milk cooker treatment, 16 (53.33%) passed the coliform test [Figure 1]. This difference when analyzed by the chi-square test was found to be statistically significant ($P < 0.05$). All the UHT-treated milk samples (fresh and nearing expiry date) passed the coliform test.

Out of the 30 fresh pasteurized milk samples, 10 (33.3%) yielded an average of $<10$ cfu/ml of milk and 14 (46.6%) grew an average of $>1000$ cfu/ml of milk. These samples when tested 4 h after milk cooker treatment, 15 (50%) yielded an average of $<10$ cfu/ml of milk and eight (26.6%) grew an average of $>1000$ cfu/ml of milk. Even though we find a better result after milk cooker treatment, the difference was not statistically significant by the Student’ t-test ($P = 0.583$) [Figure 2].

Out of the 30 fresh UHT-treated milk samples, 15 samples (50%) showed sterile plates; only one sample (0.03%) grew an average of $>1000$ cfu/ml of milk. Out of 30 UHT-treated milk samples close to expiry date, six (20%) yielded an average of $<10$ cfu/ml of milk and six (20%) grew an average of $>1000$ cfu/ml of milk. This difference...
was found to be statistically significant by the Student’s t-test ($P < 0.05$) [Figure 2].

All the milk samples passed the methylene blue test and UHT-treated milk passed the coliform test. But there are differences in the results when the milk samples were tested by the colony count test. So the colony count test appears to be the most sensitive among the 3 tests. Milk cooker-treated milk appears to be safer than the fresh pasteurized milk. But the either milk does not seem to be perfectly safe when tested by colony count test. As the milk borne diseases are not so very common, the other two tests appear to be fairly reliable.

UHT-treated milk appears to be safer than the pasteurized milk as it has successfully passed the coliform test and the methylene blue test. In the present study, only seven out of the 60 samples (both fresh and close to expiry date) grew $>1,000$ cfu/ml. Of these six are close to expiry date. So it is safe to avoid buying UHT-treated milk close to expiry date.

**Conclusions**

Milk cooker-treated milk appears to be safer than the fresh pasteurized milk so it is advisable to subject fresh pasteurized milk to heat treatment before consumption. Fresh UHT-treated milk is safer than UHT milk close to expiry date so it is better to avoid consumption of UHT milk close to expiry date.

**References**

1. Ananthanarayan R, Paniker CK. Bacteriology of water, air, milk and food. In: Arti Kapil, editor. Text Book of Microbiology. 9th ed. India: University press; 2013. p. 630-2.
2. Collee JG, Fraser AG, Simmons A, BP Marmion. Examination of water, milk, food and air. In: Collee JG, Fraser AG, Simmons A, Marmion BP, editors. Mackie & McCartney’s Practical Medical Microbiology. 14th ed. London: Churchill Livingstone; 2007. p. 892-8.
3. Ruegg PL. Practical food safety interventions for dairy production. J Dairy Sci 2003;86:E1-9.
4. Aaku EN, Collison EK, Gashe BA, Mpuchane S. Bacterial quality of milk from two processing plants in Gaborone Botswana. Food Control 2004;15:181-6.
5. Salmeron J, DeVega C, Perez-Elortondo FJ, Albisu M, Barron LJ. Effect of pasteurization and seasonal variations in the microflora of ewe’s milk for cheese making. Food Microbiol 2002;19:167-4.
6. Bernadette DG. Quality of pasteurized milk influences the performance of ready-touse systems for enumeration of aerobic microorganisms. Int Dairy J 2002;12:413-8.
7. Chatterjee SN, Bhattacharjee I, Chatterjee SK, Chandra. Microbiological examination of milk in Tarakeswar, India with special reference to coliforms. Afr J Biotechnol 2006;5:1383-5.