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Microbial load assessment of milk and milk products in and around Hyderabad

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Abstract

The present study was undertaken to assess the incidence of microbial load of pasteurized, Ultra High Temperature treated and badam milk sold in and around Greater Hyderabad Municipal Corporation in Telangana State. A total of 150 samples (50 each of pasteurized, Ultra High Temperature treated and badam milk) from 5 commonly available brands were collected and analyzed for total viable count, *Escherichia coli*, coliforms, *Staphylococcus aureus* and *Salmonella* spp. In pasteurized milk, the mean total viable counts were 3.5×10^4 , 1.81×10^4 , 2.5×10^3 , 2.85×10^3 and 4×10^4 in B1, B2, B3, B4 and B5 brands respectively. The mean coliforms and *Escherichia coli* counts were 3 and 0.2/gm in B5 brand, whereas all other four brands were negative. The *Salmonella* and *Staphylococcus aureus* are negative in all 5 brands. In Ultra High Temperature treated milk the total viable counts were nil upto 30th day at both 4 °C and 25 °C storage temperatures and on 45th day the counts were 2.5×10^1 and 3×10^2 at 4 °C and 25 °C of storage respectively. The coliforms count was nil at 4 °C and 25 °C upto 30th day and on 45th day the counts were 3 and 5 at 4 °C and 25 °C respectively. The *Escherichia coli* counts were nil at both temperatures upto 30th day and on 45th day the counts were 2 and 3 at 4 °C and 25 °C respectively. The *Salmonella* and *Staphylococcus aureus* were negative during entire storage period. In badam milk the total viable counts were nil upto 30th day at 4 °C and 25 °C and on 45th day the counts were 4×10^1 and 3×10^2 at 4 °C and 25 °C respectively. The coliforms counts were nil at 4 °C and 25 °C upto 30th day and on 45th day the counts were 4 and 6 at 4 °C and 25 °C respectively. The *Escherichia coli* counts were nil at 4 °C and 25 °C upto 30th day and on 45th day the counts were as 2 and 3 at 4 °C and 25 °C respectively. The *Salmonella* and *Staphylococcus aureus* were negative during entire storage period 45th day.

Keywords: pasteurized milk-UHT milk-badam milk-foodborne pathogens-microbial quality

Introduction

Milk is an ideal food for human being for all age groups due its nutrients, at the same time it is an excellent medium for microorganisms based on the temperature of the storage. The presence of microorganisms in milk poses serious health problems due to food borne illness or diseases of public health importance (Adak *et al.*, 2005). In India and other developing countries milk is produced under unhygienic conditions and handled carelessly which leads to spoilage and danger of milk borne diseases (Prajapathi, 1995) [19]. Microorganisms related to food borne illness are destroyed by proper heat treatment like pasteurization, boiling, sterilization etc. The outbreaks of food borne illness through consumption of heat-treated milks have been linked to post heat treatment contamination (Bryan, 1983) [7] in many countries. *Escherichia coli* 0157:H7, *Salmonella* and *listeria* were reported in pasteurized milks (Upton and coia, 1994) [23].

Post pasteurization contamination has been found to contribute most of the bacteria in milk that are capable of growth and subsequent spoilage (Meir, 1996) [14]. The Ultra High Temperature (UHT) treatment and aseptic package protect milk from bacteria and external contamination. The shelf life of milk is extended from one week in traditional pasteurization in over 4 months, with UHT and aseptic technology (Scott, 2008) [21]. Challenges are present in every type of processing and UHT is not an exception. Commercial sterilizing is defined as a condition in which all the microorganisms of public health significance are completely destroyed, but the sterilized milk available in the market still containing some quantity of micro flora may be due to inadequate processing, poor packing and are poor storage.

The present study was carried out to examine the microbial load of different brands of pasteurized milk, UHT milk and sterilized badam milk sold in and around Greater Hyderabad Municipal Corporation (GHMC), Telangana State.

Materials and Methods

Sample collection

Fifty samples each of pasteurized milk, UHT milk and sterilized badam milk from 5 different brands (B1, B2, B3, B4 and B5) were collected from the local markets in and around GHMC, Telangana State. The samples were packed in ice box and transported to the Department of Veterinary Public Health and Epidemiology laboratory, College of Veterinary Science, Rajendranagar, Hyderabad and stored under refrigeration till analysis.

Microbiological analysis

Total viable count, coliform count, counts of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. were conducted using standard procedures.

Total Viable Count was estimated by using total plate count agar, coliforms by using MacConkey agar, *Escherichia coli* by using Eosin methylene blue agar (EMB), *Staphylococcus aureus* by using Mannitol salt agar (MSA) and *Salmonella* by using Brilliant green agar (BGA) the media were prepared according to the guidelines given by the manufacturers.

Standard plate count (SPC): The total bacterial count was made by adding 1 ml of milk sample into sterile test tube having 9ml pep tone water. After thoroughly mixing, the sample was serially diluted up to $1:10^{-7}$ and duplicate samples (1ml) were pour plated using 15-20 ml standard plate count agar solution and mixed thoroughly. The plated sample was allowed to solidify and then incubated at 30°C for 48 hours. Colony counts were made using colony counter (Marth, 1978) [13].

Coliform count (CC): One ml of milk samples was added by into sterile test tube having 9 ml pep tone water. After mixing, the sample was serially diluted up to $1:10^{-5}$ and duplicate samples (1ml) were pour plated using 15-20 ml Violet Red Bile Agar solution (VRBA). After thoroughly mixing, the plated sample was allowed to solidify and then incubated at 30°C for 24 hours. Finally, colony counts were made using colony counter (Marth, 1978) [13]. Typical dark red colonies were considered as coliform colonies.

Escherichia coli count was determined by using EMB agar, serial dilutions of the milk samples were done up to $1:10^{-5}$ and duplicate samples (1ml) were pour plated using 15 to 20 ml of EMB agar the plates were incubated at 30°C and colony counts are made using colony counter for estimation of salmonella and staph aureus the diluted milk samples were pour plated in MSA and BGA respectively counts were made after incubating for 37°C 28 to 48 hours.

Result and Discussion

The microbiological parameters of total viable count (TVC),

coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp of 5 brands of pasteurized milk were present in Table 1. The mean TVC was high ($4 \times 10^4/\text{gm}$) for the B5 brand milk with a range of 2×10^3 to $5.62 \times 10^4/\text{gm}$ followed by $3.5 \times 10^4/\text{gm}$ with a range of 0 to 6×10^4 in the brand B1 samples, $1.81 \times 10^4/\text{gm}$ with a range of 1×10^3 to 3×10^4 in B2 brand sample, $2.85 \times 10^3/\text{gm}$ in the brand B4 sample with a range of 0 to 4×10^3 in B4 brand and least ($2.5 \times 10^3/\text{gm}$) with a range of 0 to 5×10^3 in B3 samples.

The mean TVC in all the 5 brands were below the accepted count of 30000/ml for satisfactory grade of pasteurized milk (BIS, 1998). Zhner *et al.* (1986) [26] and Fenlon *et al.* (1995) [8] reported low bacterial counts in pasteurized milk samples than in the present study and explained that these counts were due to post pasteurization contamination or inefficient pasteurization and poor packing. Hamida Abid *et al.* (2009) [12] reported nil TVC count in various brands of pasteurized milk sold in Peshwara city, Pakistan. A count of 2.8×10^2 CFU/ml in UHT milk samples sold in Bangladesh was reported by Muzahidul Islam *et al.* (2021), which was less than the counts observed in the present study.

Very high TVC counts of 5.5×10^6 , 10^6 to 10^7 and 8.8×10^7 CFU/ml were reported by Aaku *et al.* (2004) [2], Arenas *et al.* (2004) [3] and Hassan *et al.* (2015) [11] respectively, whereas very high counts of 4.4×10^8 , 1×10^9 and 5.6×10^9 CFU/ml were reported by AL-Taraziet ai (2003) [6], Moustafa *et al.* (1998) and Mohammed & EI-Zubeir (2007) [15] respectively.

The mean total coliform count in B1, B2, B3 and B4 brand milk samples was nil whereas the counts in B5 brand milk samples were 0.3 /gm with a range of 0 to 3 /gm. All the first 4 brands have given nil count which is as per BIS standards, where in it specified that no coliform count in satisfactory grade of pasteurized milk. Presence of coliforms in B5 brands samples might be due to post pasteurization contamination and/or leakages in the milk sachets (Meir, 1996) [14]. Higher counts ranging between 1.07×10^4 to 3.7×10^6 were reported by Fekadu (1994) [9], Alganesh (2002) [4] and Abebe *et al.* (2012) [5]. Low counts 5.1 to 8.4 CFU/ml in pasteurized market milk samples in Lahore city was reported by Muhammed *et al.* (2009) [17], whereas Muzahidul Islam *et al.* (2021) reported a count of 21.35 CFU/ml in pasteurized milk samples.

The *Escherichia coli* count in B1, B2, B3, and B4 were nil, which was coinciding the BIS standards, whereas the *Escherichia coli* count in B5 brand milk samples was 0.2/gm with a range of 0 to 2. The probable cause for the presence *E. coli* in pasteurized milk samples may be due to post pasteurization contamination and/or leakages.

All the 5 brand samples were negative for *Salmonella* and *Staphylococcus aureus* organisms. As per international and national standards the processed milk samples intended for human consumption shouldn't contain any pathogenic organisms.

Table 1: Microbiological analysis of pasteurized milk

Parameters	Counts	B1	B2	B3	B4	B5
TVC	Minimum	Nil	1×10^3	Nil	Nil	2×10^3
	Maximum	6×10^4	3×10^4	5×10^3	4×10^3	5.62×10^4
	Average	3.5×10^4	1.81×10^4	2.5×10^3	2.85×10^3	4×10^4
Coliform	Minimum	Nil	Nil	Nil	Nil	Nil
	Maximum	Nil	Nil	Nil	Nil	3
	Average	Nil	Nil	Nil	Nil	3
<i>Escherichia coli</i>	Minimum	Nil	Nil	Nil	Nil	0
	Maximum	Nil	Nil	Nil	Nil	0.2
	Average	Nil	Nil	Nil	Nil	0.2
<i>Salmonella</i>	Minimum	Nil	Nil	Nil	Nil	Nil

<i>Staphylococcus aureus</i>	Maximum	Nil	Nil	Nil	Nil	Nil
	Average	Nil	Nil	Nil	Nil	Nil
	Minimum	Nil	Nil	Nil	Nil	Nil
	Maximum	Nil	Nil	Nil	Nil	Nil
	Average	Nil	Nil	Nil	Nil	Nil

The total viable count, coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp of UHT milk were present in Table 2. The TVC, in UHT milk samples stored at 4 °C was nil up to 30th day of storage, whereas on 45th day of storage the count was 2.5x10¹/gm. The samples stored at 25 °C the TVC were nil at 30th day of storage, on 45th day the count was 3x10²/gm, which was higher than the count observed in the UHT milk samples stored at 4 °C. Hamida Abid *et al.* (2009) [12] also observed no TVC in UHT milk samples sold in Peshawar city, Pakistan up to 30 days of storage at both 4 °C and 25 °C and counts of 1.6x10¹/gm and 3x10⁴ /gm TVC were observed in UHT milk samples at 45 days of storage at 4 °C and 25 °C respectively. As the storage period advances, it was high on 90 days of storage in samples stored at 4 °C and 25 °C. The increase in the counts in the samples stored at 4 °C may be due to multiplication and also due to disruption of bacterial cell aggregates (Villanueva *et al.*, 1991 and Godden *et al.*, 2002) [24, 11]. Riadh AL -Tahiri (2005) [20] also observed, no TVC counts in the UHT milk, whereas Srikandakumar *et al.* (2004) [22] reported low counts of TVC. A count of 8.6x10¹ CFU/ml in UHT milk samples sold in Bangladesh was reported by Muzahidul Islam *et al.* (2021), which was slightly higher than the counts observed in

the present study.

The total coliform count in UHT milk samples stored at 4 °C was nil up to 30th day of storage whereas on 45th day of storage the count was 3 and samples stored at 25 °C coliform count was nil up to 30th day of storage on 45th day the count was 5 which was higher than the count observed in the UHT milk samples stored at 4 °C. This might be due to post treatment contamination and through leakages. A count of 6.53 CFU/ml in UHT milk samples sold in Bangladesh was reported by Muzahidul Islam *et al.* (2021).

The *Escherichia coli* count in UHT milk samples stored at 4 °C and 25 °C was nil up to 30th day of storage whereas on the 45th day storage the count was 2 for the samples stored at 4 °C and the samples stored at 25 °C, count was 3, which was higher than the count observed in the UHT milk samples stored at 4 °C, which might be due to post treatment contamination. A count of 3.11CFU/ml in UHT milk samples sold in Bangladesh was reported by Muzahidul Islam *et al.* (2021)

The UHT milk samples at 4 °C and 25 °C, were negative for *Salmonella* spp and *Staphylococcus aureus* organisms from 0 to 45th day of storage.

Table 2: Microbiological analysis of UHT milk

Parameters	Temperature	Day of storage			
		0	15	30	45
TVC	4 °C	Nil	Nil	Nil	2.5x10 ¹
	25 °C	Nil	Nil	Nil	3x10 ²
Coliform	4 °C	Nil	Nil	Nil	3
	25 °C	Nil	Nil	Nil	5
<i>Escherichia coli</i>	4 °C	Nil	Nil	Nil	2
	25 °C	Nil	Nil	Nil	3
Salmonella	4 °C	Nil	Nil	Nil	Nil
	25 °C	Nil	Nil	Nil	Nil
<i>Staphylococcus aureus</i>	4 °C	Nil	Nil	Nil	Nil
	25 °C	Nil	Nil	Nil	Nil

The commercial sterility is defined as a condition which will cause no viable microorganisms of both public health and non-health significance (Scott, 2008) [21]. The badam milk samples studied under this investigation were sterilized badam milk. The microbiological of total viable count, coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. of badam milk were presented in Table 3.

The TVC in badam milk samples stored at 4 °C and 25 °C was nil up to 30th day of storage, whereas on 45th day of storage the count was 4x10¹/gm and 3x10²/gm in the samples stored at 4 °C and 25 °C.

The coliform count in badam milk samples stored at 4 °C and 25 °C was nil up to 30th day of storage, whereas on the 45th day storage the counts were 4 and 6 /ml respectively. The count observed in the badam milk samples stored at 25 °C were slightly above the counts observed in the samples stored at 4 °C. These counts might be due to post pasteurization

contamination most probably through leakages. Krishnasree *et al.* (2018) [25] reported the average coliform count in badam milk collected in streets of Chennai as 11x10³ CFU/ml which was higher than the counts in the present study.

The *Escherichia coli* count in badam milk samples stored at 4 °C and 25 °C was nil up to 30th day of storage whereas on the 45th day storage the count was 2 and 3 in the samples stored at 4 °C and 25 °C respectively. This count might be due to post treatment contamination. Krishnasree *et al.* (2018) [25] observed average *Escherichia coli* in badam milk collected in streets of Chennai as 10 which was higher than the present study.

The badam milk samples stored at 4 °C and 25 °C were negative for *Salmonella* spp and *Staphylococcus aureus* organisms during entire storage period. No sample of milk which has undergone heat treatment should contain pathogenic microorganism BIS (1998).

Table 3: Microbiological analysis of badam milk

Parameters	Temperature	Day of storage			
		0	15	30	45
TVC	4 °C	Nil	Nil	Nil	4x10 ¹
	25 °C	Nil	Nil	Nil	3x10 ²
Coliform	4 °C	Nil	Nil	Nil	4
	25 °C	Nil	Nil	Nil	6
<i>Escherichia coli</i>	4 °C	Nil	Nil	Nil	2
	25 °C	Nil	Nil	Nil	3
Salmonella	4 °C	Nil	Nil	Nil	Nil
	25 °C	Nil	Nil	Nil	Nil
<i>Staphylococcus aureus</i>	4 °C	Nil	Nil	Nil	Nil
	25 °C	Nil	Nil	Nil	Nil

Conclusion

Based on the results of present study, it can be concluded that the consumers are advised to take UHT and badam milk on or before 30 days of manufacture and it is also recommended to boil the pasteurized milk before consumption to counter the post pasteurization contamination. Therefore, it is highly recommended to follow the good hygienic practices both during production and processing to get hygienic milk for human consumption.

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