

Microbiological study of khoa sold in Chambal region (Madhya Pradesh): A case study

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Abstract Present study was conducted to analyze bacterial contaminants /pathogens in Khoa samples sold in Chambal region of Madhya Pradesh. Total Fifty samples of Khoa were brought from different localities of Chambal region at random and processed. Bacterial colony counts were also performed. *Staphylococcus* species and *Streptococcus* species were the predominant isolates. The viable counts obtained ranged from 1.3×10^4 to 2.1×10^6 CFU/g. Contamination of Khoa by pathogenic bacteria could be an important factor of gastrointestinal infections including food poisoning and food borne illness. Adequate consumer protection can be achieved by measuring the microbiological data of product.

Keywords Khoa · Bacteriological study · Viable count · *Staphylococci*

Introduction

Indian system is using various types of milk products including, Khoa a partially desiccated milk which is traditionally been used in preparation of variety of sweets, vegetable curry etc. In India, Khoa is prepared by condensing milk by regular heating to remove water. It is perishable food product and has short shelf life. During preparation of khoa temperature of milk is raised enough to destroy most of the vegetative cells of bacteria. Since, the product is manufactured by traditional method without any regard to quality of raw material used and hygienic storage, so the shelf life of the product is adversely affected by the thermophilic organisms and organisms acquired during storage. High nutritional value and high water activity (0.96) of khoa is conducive for the growth of bacteria¹. Microbial content of heat dried dairy product is temperature dependent and time of preheating evaporation process is also a predisposing factor contamination and growth during storage also affect microbial population of heat dried dairy products. Psychrotrophic bacteria may also affect quality and flavor of heat-treated product, some heat tolerant enzymes produced by some psychrotrophic species cause spoilage both before and after heating. Microbes produce undesirable effects like change in odor, color, taste and texture of food. Besides this contamination of products with pathogenic bacteria can result into outbreaks of gastrointestinal infection and thus threat to consumer. Plethora of studies carried out in different part of India evidenced that pathogenic organism as *Staphylococcus aureus*, *Bacillus cereus*, often contaminate Khoa.^{2, 3} Probably the microbes access to Khoa is mainly by improper handling of workers and contaminated utensils used during processing. The present study is designed to find out bacterial load of Khoa sold in Chambal region.

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Table 1 Bacteriological study of Khoa

Sr. No.	Sample code	Viable count Log cfu	Gram staining reaction				Bacterial isolates
			Gram positive		Gram negative		
			Bacilli	Cocci	Bacilli	Cocci	
1	G1	4.54	–	+	–	–	F, T
2	S1	4.89	–	+	–	–	F
3	G2	5.02	–	+	–	–	F, T
4	S2	4.91	–	+	+	–	R, S, P
5	R1	4.83	–	+	+	–	F, T, R, E, S
6	C1	4.77	–	+	–	–	F, T
7	R2	4.67	–	+	–	–	F, T
8	R3	5.04	–	+	–	–	F, T
9	C2	4.65	–	+	–	–	F, T
10	G3	4.85	–	+	+	–	F, T, R, S, P
11	G4	4.95	–	+	+	–	R, P
12	K1	4.78	–	+	–	–	F, T
13	K2	4.85	–	+	–	–	T, L
14	M1	4.71	–	+	–	–	F, T, S
15	M2	4.76	–	+	–	–	F, T
16	N1	4.90	+	+	–	–	F, T, L
17	N2	4.84	+	+	–	–	F, T, L
18	C3	4.94	+	+	–	–	F, T, L
19	C4	4.95	–	+	–	–	F, T
20	C5	4.81	+	+	–	–	F, T, L
21	K3	4.90	–	+	–	–	F
22	M3	4.27	–	+	–	–	F
23	J1	No growth	–	–	–	–	–
24	J2	4.91	–	+	–	–	F, T
25	A1	4.84	–	+	–	–	F, T
26	L1	5.13	–	+	–	–	F, T
27	L2	4.47	–	+	–	–	F, T
28	J3	4.30	–	+	–	–	F, T
29	R4	4.60	–	+	–	–	F, T
30	K3	No growth	–	–	–	–	–
31	R5	4.90	–	+	–	–	F
32	S3	4.81	–	+	–	–	F
33	J3	5.32	–	+	–	–	F
34	G5	4.57	–	+	–	–	F, T
35	L3	4.49	–	+	–	–	F, R
36	A2	4.36	–	+	–	–	F, T
37	C6	4.11	–	+	–	–	F, T
38	S4	4.95	–	+	–	–	F, T
39	L3	4.14	–	+	–	–	F, T
40	B1	4.47	–	+	–	–	F, T
41	A3	5.08	–	+	–	–	F

42	B2	4.77	–	+	–	–	F, T
43	K1	4.85	–	+	–	–	F, T
44	B3	4.60	–	+	–	–	F
45	G6	4.25	–	+	–	–	F
46	S5	4.64	–	+	–	–	F
47	G7	4.61	–	+	–	–	F, T
48	G8	4.85	–	+	–	–	F
49	B4	4.79	–	+	–	–	F
50	G9	4.81	–	+	–	–	F

Data mean of three separate determinations

+ = Present, - = Absent, F= *Staphylococcus* sp. , T= *Streptococcus* sp ,

L= *Lactobacillus* sp, R= *Proteus* sp., E= *Enterobacter* sp., S= *Serratia* sp. P= *Pseudomonas* sp.

Table 2 Bacterial isolates in Khoa samples

S. No.	Name of Bacteria	Number of sample with bacteria
1	<i>Staphylococcus</i> sp.	45
2	<i>Streptococcus</i> sp.	32
3	<i>Lactobacillus</i> sp.	05
4	<i>Proteus</i> sp.	05
5	<i>Pseudomonas</i> sp.	03
6	<i>Serratia</i> sp.	04
7.	<i>Enterobacter</i> sp.	01

Materials and Methods

Total 50 samples of khoa were collected at random in pre-sterilized containers from vendors of various parts of Chambal region and then these samples were transported in ice bucket to Department of Microbiology at College of Life Sciences, Cancer Hospital & Research Institute, Gwalior. Each sample of khoa was processed under sterile condition by taking 1 g of khoa with 10ml of physiological saline and homogenized by mortar pestle.

Inoculation of processed sample was carried out immediately on nutrient agar, MacConkey agar (HiMedia), Mannitol salt agar (HiMedia), DNase agar (HiMedia), Blood agar (HiMedia), Esculin azide broth (HiMedia). After incubation at 37°C ± 10°C for 24 hrs the identification of the colonies grown was made. Obtained isolates were identified by various biochemical and pathogenicity tests.^{4,5}

Samples were also subjected to methylene blue reduction test and SPC. To assess the bacterial colony count, dilutions of homogenized samples were made in normal saline and then inoculation was made on plate count agar, plates were incubated at 37°C ± 10°C for 24 hrs. The colony forming units per gram (CFU/g) of the original samples were obtained by multiplying the counts obtained with dilution factor.^{4,5,6}

Results and Discussion

Fifty samples of khoa were examined to determine bacterial load. All the 50 samples studied showed bacteriological count ranging from 1.3×10^4 to 2.1×10^6 CFU/g (Table 1). Results of Methylene blue reduction test showed that 30 samples were of poor quality and 20 samples were of fair quality.

Out of 50 samples (Fig. 3), 45 showed presence of *Staphylococcus* species. 32 were found to be *Streptococci*, 05 were found to be *Proteus*, 05 showed presence of *Lactobacilli*, 04 showed presence of *Serratia*, *Pseudomonas* sp. was present in 03 while *Enterobacter* was present in one sample (Table 2).

Food products are source of nutrition and substrates for the growth of microorganisms. Excess of microorganisms in foodstuff causes its spoilage, which in turn causes food borne illness. Moreover in tropical countries raw milk and its various products are responsible for many outbreaks of gastrointestinal tract. Our results indicate high degree of bacterial contamination in studied samples. The number of viable bacteria was assessed by colony count in samples, which was correlated with the results obtained by positive MBRT. Misra and Kulia, reported coliforms count from 10 CFU/g to 1.0×10^2 CFU /g in sandesh sweet prepared from khoa⁷. Reddy *et al*, reported SPC of 5×10^3 CFU / g to 2.1×10^5 CFU / g in khoa from Hissar market⁸. Sen and Rajorhia, reported that product sandesh showed count between 3×10^5 CFU/g to 7.5×10^7 CFU/g⁹.

Staphylococcus species was the preponderate organism isolated. The literature documents that *Staphylococcus* species is frequently occurring organism in sweet-based milk products such as Khoa, rabri, gulabjamun, etc^{10, 11}. It is reported in case of cheese, contamination can come from hands of sellers. Strains of *Staphylococcus* can cause gastroenteritis via production of heat stable enterotoxin¹². Millions of people are victims of food borne illness resulting from ingestion of toxin produced by food associated *Staphylococcus*.

Streptococcus species was also present in 34 sample, it is a thermophilic organism and its presence indicates that it may survive after heating process. Our study indicates the occurrence of *Pseudomonas*, *Proteus*, *Serratia*, *Enterobacter* sp. in khoa samples, all these organisms are potential pathogens with very low ID₅₀¹³. *Streptococci* and *Lactobacilli* have fermentative metabolism they can cause souring of khoa. *Pseudomonas* is proteolytic and lipolytic, it can also change the color of khoa.

Haq *et al* reported the presence of fecal and non-fecal contaminants in raw milk sample of Khoa. *E. coli* is frequently occurring organism in milk products like Mawa / Khoa, dahi, cheese etc¹⁴. Soomoro *et al* reported high percentage of Khoa contamination by *E. coli*¹⁵. It is ubiquitous organism; its pathogenic strains could be hazardous to consumers¹⁶. However, *E. coli* species was not isolated in studied samples.

Bacteria are not homogeneously distributed in Khoa; number of different factors makes it difficult to enumerate population present in a given lot. In addition, physical attributes of food matrix may make difficult detection of bacteria and processing steps like heating, dehydration also affect the degree of bacterial growth and development. Even with statistical significant sampling technique, it is difficult to analyze whether a whole lot is pathogen free or contaminated.

Results of present study revealed that the problem of Khoa contaminations in Chambal region. Heavy bacterial contamination is found in all samples. The unhygienic condition of preparation of these foodstuffs and water used for washing of utensils enhances the bacterial contamination of milk and milk products.¹⁷

The sample contamination can be attributed to the practice of preparing large bulk of product in advance prior to the requirement and storage of the product at room temperature for long duration.¹⁸ Keeping in view the public health importance of consumer more hygienic preventive measures are needed to address this problem of bacterial contamination in khoa samples and to increase quality of product.

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