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Hygienic Quality of Indian Sweet Milk Products from Different Sources

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Seventy samples of milk sweets including 30 samples of khoa, 10 samples of burfi, 10 samples of gulabjamun, 10 samples of kalakand and 10 samples of peda from local vendors, private manufacturers and organized dairies in and around Chennai, India were collected. The milk products were examined for microbial quality that includes enumeration of total viable bacteria, coliforms, phychrotrops, aerobic spore farmers, yeast and mould and isolation, identification of mould species were carried out. The results revealed that the bacterial count, yeast and mould count of local vendor's samples were significantly (P<0.01) higher when compared to private manufacturers and organized dairies except psychrotrophic count in local and private vendor's samples were identical. The total percentage of occurrence of *Aspergillus* sp. isolated from khoa samples was found to be the highest (63.08 per cent) followed by *Penicillium* sp. (17.38 per cent), *Rhizopus* sp. (10.86 per cent), *Fusarium* sp. (4.34 per cent) and *Mucor* sp (4.34 per cent). However, the total percentage of occurrence of *Aspergillus* sp isolated from khoa based milk sweet samples was also found to be the highest (70.90 per cent) followed by *Penicillium* sp. (15.11 per cent), *Rhizopus* sp. (9.30 per cent), *Mucor* sp. (3.48 per cent) and *Fusarium* sp. (1.16 per cent). The present study concluded that the khoa, khoa based sweets produced by the local vendors were inferior in quality as compared private and organized dairies.

Key words: **Khoa, milk sweets, bacteriological count, yeast and mould, different sources**

Introduction

Milk and milk products constitute important nutritional components for all age groups; it is the only source of first class proteins especially for vegetarians and also essential food material for the young ones. It supplies most essential elements like calcium and phosphorus along with numerous other essential major and minor substances. Due to its complex biochemical composition and high water content, milk and milk products act as an excellent culture medium for growth and multiplication of varieties of microorganisms particularly under unhygienic production and storage at ambient temperature.

Among the Indian milk sweets, khoa and khoa based milk sweets are important products, which provide a good means of conserving and preserving surplus milk solids. Khoa is of greater importance to the confectionaries. India's annual milk production is over 78 million tons; nearly 50 per cent of total milk produced in India is utilized for the manufacture of variety of traditional milk products[1]. Approximately 50 percent of milk produced is consumed as fresh or boiled; remaining is utilized for manufacturing of indigenous varieties of milk products such as butter, ice cream, khoa, paneer, rabri, kheer, burfi, and gulabjamun[2]. The manufacture of these products is based on traditional method without any regard to the quality of raw material used and/ or the hygienic quality of the products. Under such conditions, many microorganisms can find access to the milk products[3]. The unhygienic conditions at the production units lead to contamination of products with different types of microorganisms leading to a low shelf life of the base products. Most of these products are sold in the market without proper packaging and unduly exposing them to atmospheric contamination[1].

Even though the khoa and khoa based milk sweets are produced under strict hygienic conditions in organized sector, they are prone for microbial contamination. It may gain entry into food at any stage of processing right from the farm to till the food is reached to the final consumer like at the time of packing, transport and storage etc, so it becomes imperative not only to take all kinds of preventive measures and also to evaluate at every stage which will subsequently influence the microbiological quality[4]. Spoilage of dairy products by moulds is of frequent occurrence in India due to the prevailing tropical climate and high humidity especially in coastal area like Chennai. Since the mould spores are transmitted through air, they are ubiquitous in nature. Gran[5] concluded that the hygienic aspects of dairy products are linked with transportation, preservation and handling.

Considering the above facts, the detection and enumeration of microorganisms in khoa and khoa based milk sweets is an integral part of any good quality assurance program and reflect the

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effectiveness of sanitation practices, processing and distribution schemes of local, private manufactures and organized dairies. Hence, the present study was taken to assess the microbial quality of milk sweet products in and around Chennai, India.

Materials and Methods

Source and Analysis of samples

A total 70 samples of milk sweets including 30 samples of khoa, 10 samples of burfi, 10 samples of gulabjamun, 10 samples of kalakand and 10 samples of peda from the following sources viz., local vendors, private manufacturers, organized dairies were collected aseptically and subjected to further analysis of total viable count, coliform count, psychrotrophic count, aerobic spore forming bacterial count, yeast and mould count and isolation, identification of mould species. Total viable, coliform and psychrotrophic count, were done as per standard methods for examination of dairy products[6,7]. For aerobic spore forming bacterial count, the vegetative cells were destroyed by heat treatment at 80°C for 10 min then the sample were plated in standard plate count agar in duplicate using lower dilutions according to the standard procedure[8].

The procedure adopted for the enumeration of yeast and mould count was as per IS: 3507[9]. Moulds colonies were isolated and identified by representative mould colonies from plates were picked, isolated and sub cultured on potato dextrose agar slants at a pH of 3.5. The cultures were maintained as slant cultures in the refrigerator and renewed at every 14 days of intervals and different species of *Aspergilli, Penicillium, Rhizopus, Fusarium* and *Mucor* were identified as per the procedure described by Smith[10] and Raper and Fennel[11]. The Morphological characters of rate of growth, colour of colony, colour changes of colony, colour on reverse side of the colony and texture of the colony on agar surface were studied after 10 days of incubation at 25°C on Czapek Dox agar.

Colony characters of the isolated moulds were examined under the stereomicroscope and the observations were recorded. Mounted preparations of the moulds on slides stained using lactophenol or cotton blue were examined using stage micrometer for finer details and reproductive structures under the low and high power of the microscope.

Statistical and Data Analysis

A total number of colonies of each category of the samples were enumerated and the collected data's were subjected to statistical analysis as per Snedecor and Cochran[12]. The rate of isolates of each mould in the khoa samples and khoa based milk sweets were calculated as a percentage of the total number of the isolates.

Results and Discussion

Bacterial enumeration

The results of bacterial enumeration of khoa and khoa based milk sweets which collected from local vendors, private manufacturers and organized dairies are shown in Table 1 and Table 2.

The result of this work showed that the total viable count of khoa samples obtained from the above three sources were ranged from $1.6x10^5$-$2.71x10^5$ cfugm^{-1}, $1.7x10^3$-$2.9x10^4$ cfugm^{-1} and $7x10^2$-$4x10^3$ cfugm^{-1} respectively; and of the khoa based milk sweets were $1.2x10^5$-$8x10^5$ cfugm^{-1},$1.9x10^3$-$2.3x10^5$ cfugm^{-1} and $8x10^2$-$3.1x10^4$ cfugm^{-1} respectively. This may be due to poor quality of raw milk as pointed out by Boor *et al.*[13] who reported that the bacterial quality of raw milk is increasingly important to final product quality and also due to storage of khoa for long period for making sweets[14]. The presence of a very high total viable count in local vendors' samples indicates serious faults in raw material selection, production hygiene, unsatisfactory sanitation and unsuitable storage temperature etc. These findings were in accordance with Bandekar *et al.*[15], who reported that the total bacterial count was higher in khoa samples collected from B grade shops than the A grade shops.

The high coliform count of khoa and khoa based milk sweet samples of local vendors shows the range of $3x10^2$-$2.9x10^3$ cfugm^{-1} and $4x10^2$-$3.1x10^3$ cfugm^{-1} with the mean values of 1.7 $x10^3$±4.2 cfugm^{-1}, $1.9x10^3$±3.85 cfugm^{-1} respectively. This findings were in agreement with Vijayakumar and Sinha[16] who recorded that the higher coliform count of khoa samples obtained from local vendors than compare with organized sectors, and Dwarakanath and Srikanta[17] also reported that coliform count of peda, burfi and kalakand were 460,1.61×10^4 and 4×10 cfugm^{-1} respectively. Similarly, other studies reported that the traditional products with a high count of coliforms is indicative of unsanitary conditions[18] and also *E. coli* and coliforms may indicate evidence of contamination or pollution especially of fecal nature[19].

The aerobic spore forming bacterial count of khoa samples of the above mentioned three sources were ranged from $3x10^3$-$1.7x10^4$ cfugm^{-1}, $2x10^2$-$5x10^2$ cfugm^{-1} and $1x10^1$-$2x10^2$ cfugm^{-1} respectively. These findings were in accordance with Kamat *et al*.[20], who recorded a range of 2×10^2"5×10^4 cfu.gm^{-1} of sample. The results of khoa based milk sweets are ranged from $3x10^2$-$1.7x10^4$ cfugm^{-1}, $3x10^2$-$1.1x10^3$ cfugm^{-1}and $4x10^1$-$5x10^2$ cfugm^{-1} respectively. These results were in close agreement with the findings of Magadam *et al.*[21] who recorded a range of 1×10^2 to 5.8×10^3 cfugm^{-1}. This may be due to better surviving ability of spore farming bacteria even in heat treatment during processing. In dairy industry the spore-farming bacteria like *Bacillus* and *Clostridium* species determine the shelf-life of a variety of heat-treated milk products, mainly if the level of post-process contamination is low[22]. In order to minimize problems caused by bacterial spores in foods and food production process a chain management approach, from raw material, ingredients and environmental sources to final product storage conditions, is more effective.

The mean psychotropic count of khoa samples collected from the three sources were 21 $x10^2$±3.26 cfugm^{-1}, 8.91 $x10^2$±3.05 cfugm^{-1} and 0.8 $x10^2$±0.38 cfugm^{-1} and khoa based milk sweets were $18.55x10^3$±7.71 cfugm^{-1}, $1.53x10^3$±0.42 cfugm^{-1} and $0.81x10^3$±0.27 cfugm^{-1} respectively. These values were higher than the psychrotrophic count recorded by Sharma and Joshi[23] (1991). The higher values in this study may be due to individual's unhygienic practices, through packaging material and environmental factors as reported previously[24].

Table 1: *Microbiological quality of Khoa obtained from three different sources*

Sl.No.	Counts	Local Vendors•			Private manufacturers•			Organized dairies•			F Value
		% of occurrence	Range cfugm^{-1}	Mean cfugm^{-1}	% of occurrence	Range cfugm^{-1}	Mean cfugm^{-1}	% of occurrence	Range cfugm^{-1}	Mean cfugm^{-1}	
1.	Total viable count	81.81	$16x10^4$-$2.71x10^5$	$218x10^3$±23.62^a	66.66	$1.7x10^3$-$2.9x10^4$	$21.83x10^3$±2.04^b	66.66	$7x10^2$-$4x10^3$	$4.8x10^3$±2.06^c	185.62^{**}
2.	Coliform count	63.63	$3x10^2$-$2.9x10^3$	$17x10^2$±4.2^a	55.55	$1x10^2$-$6.0x10^2$	$3.33x10^2$±0.80^b	54.54	1x10-$3x10^2$	$0.61x10^2$±0.31^c	36.74^{**}
3.	Aerobic spore formers count	72.72	$3x10^3$-$1.7x10^4$	$6.6x10^3$±2.11^a	54.54	$2x10^2$-$5x10^2$	$0.35x10^3$±0.05^b	54.54	$1x10^1$-$2x10^2$	$0.07x10^3$±0.02^c	56.10^{**}
4.	Psychrotrophic count	72.72	$10x10^2$-$3.1x10^3$	$21x10^2$±3.26^a	55.55	$1x10^2$-$1.7x10^3$	$8.91x10^2$±3.05^a	55.55	$1x10^1$-$2.5x10^2$	$0.8x10^2$±0.38^b	22.10^{**}
5.	Yeast & mould count	81.81	$1.1x10^3$-$1.9x10^3$	$14.83x10^2$±1.22^a	55.55	$1x10^2$-$9x10^2$	$3.75x10^2$±1.2^b	54.54	$2x10^1$-$2x10^2$	$0.76x10^2$±0.29^c	27.41^{**}

Number of Samples : ** Highly significant (p<0.01), means bearing same superscripts in the same row do not differ significantly.
Local vendors : 12
Private manufacturers : 9
Organized dairies : 9

Table 2: *Microbiological quality of Khoa based milk sweets obtained from three different sources*

Sl.No.	Counts	Local Vendors•			Private manufacturers•			Organized dairies•			F Value
		% of occurrence	Range cfugm^{-1}	Mean cfugm^{-1}	% of occurrence	Range cfugm^{-1}	Mean cfugm^{-1}	% of occurrence	Range cfugm^{-1}	Mean cfugm^{-1}	
1.	Total viable count	100	$1.2x10^5$-$8x10^5$	$345x10^3$±112.8^a	100	$1.9x10^3$-$2.3x10^5$	$14.33x10^3$±3.69^b	100	$8x10^2$-$3.1x10^4$	$6.76x10^3$±4.87^b	30.32^{**}
2.	Coliform count	100	$4x10^2$-$3.1x10^3$	$19x10^2$±	100	$2x10^2$-$1.1x10^3$	$4.65x10^2$±1.41^b	54.54	2x10-$1x10^2$	$1.16x10^2$±0.58^c	21.38^{**}
3.	Aerobic spore formers count	76.92	$3x10^2$-$1.7x10^4$	$83.83x10^2$±27.08^a	63.63	$3x10^2$-$1.1x10^3$	$6x10^2$±1.37^b	54.54	$4x10^1$-$5x10^2$	$1.91x10^2$±0.74^b	11.58^{**}
4.	Psychrotrophic count	69.23	$1.2x10^3$-$5.1x10^4$	$18.55x10^3$±7.71^a	54.54	$1.9x10^2$-$3.2x10^3$	$1.53x10^3$±0.42^b	54.54	$3x10^1$-$1.9x10^3$	$0.81x10^3$±0.27^b	12.73^{**}
5.	Yeast and mould count	84.61	$32x10^2$-$8x10^3$	$54x10^2$±6.62^a	72.72	$2x10^2$-$1.7x10^3$	$7x10^2$±2.38^b	63.63	$5x10^1$-$7x10^2$	$3.28x10^2$±1.14^b	25.41^{**}

• Number of Samples : ** Highly significant (p<0.01), means bearing same superscripts in the same row do not differ significantly.
Local vendors : 16
Private manufacturers : 12
Organized dairies : 12

Yeast and mould count

The yeast and mould count of khoa samples of three sources were ranged from $1.1x10^3$-$1.9x10^3$ cfu.gm^{-1}, $1x10^2$-$9x10^2$ cfu.gm^{-1} and $2x10^1$-$2x10^2$ cfu.gm^{-1}. This result agrees with the findings of Vijayalakshmi *et al*. [25], who reported that the total yeast and mould count of sweet khoa at 3, 4, 5 and 6 days of storage were 3.0×10^1, 1.0×10^2, 1.5×10^2 and 2.7×10^2 cfu.gm^{-1} respectively and also the mean yeast and mould count of khoa based milk sweets were $5.4x10^3$±6.62 cfu.gm^{-1}, $7x10^2$±2.38 cfu.gm^{-1}and $3.28x10^2$±1.14 cfu.gm^{-1} respectively. Yadav *et al.* [14] has pointed out the yeast and mould usually enter the product from air along with spores of some of the bacilli and also khoa is sometimes carried in bamboo basket lined with tree leaves, polythene bags/ pouches and tins etc., in such cases contamination with yeast and mould is inevitable. The mould growth is favored by the presence of high moisture content in khoa and khoa based milk products, air leakage in package, high humidity and sufficient aeration in storage room.

The mean total viable, coliform, aerobic spore formers, yeast and mould count of khoa samples and khoa based milk sweets from local vendors were highly significant (P<0.01) when compared to private manufacturers and organized dairies. The mean psychrotrophic count of khoa samples and khoa based milk sweets from local vendors and private manufacturers were highly significant (P<0.01) when compared to samples collected from organized dairies.

The total viable, coliform, yeast and mould count of khoa samples collected from organized dairies were found to be within the prescribed

limit, whereas in the samples collected from private manufacturers and local vendors exceeded the prescribed limits. Khoa based milk sweets collected from the organized dairies, private manufacturers the total viable count was found with in the prescribed limit, where as local vendor's samples exceeds the limits. However the samples collected from organized dairies, private manufacturers and local vendors the yeast and mould count was found to exceed the prescribed limits[7]. This might be due to usage of khoa stored for long period for making sweets[14] and also the higher microbial load may be due to contamination during post-preparation handling, transportation and storage of the finished product. The method of production, handling, transportation and marketing of these local vendors products are entirely depend upon traditional system. Such system could pose favorable environment for bacterial contamination. The unclean hands of workers, poor quality of milk, unhygienic conditions of manufacturing unit, inferior quality of material used and water supplied for washing the utensils could be the source of accelerating the bacterial contamination of milk products and post manufacturing contamination[26-30].

Table 3: *Distribution pattern of moulds in khoa from three different sources*

Sl.No.	Mould Isolates	Total No. of Isolates	Sources			Percentage	Total Percentage of
			*Local Vendors	*Private Manufacturers	*Organized Dairies		
1.	*Aspergillus niger*	12	7	2	3	26.08	*Aspergillus* sp 63.07
2.	*Aspergillus flavus*	10	7	2	1	21.78	
3.	*Aspergillus versicolor*	7	6	1	-	15.21	
4.	*Penicillium citrinum*	6	1	3	2	13.04	*Penicillium* sp 17.38
5.	*Penicillium frequentans*	2	1	1	-	4.34	
6.	*Rhizopus stolonifer*	5	-	3	2	10.86	*Rhizopus* sp 10.86
7.	*Fusarium sp*	2	-	-	2	4.34	*Fusarium* sp 4.34
8.	*Mucor sp*	2	-	1	1	4.34	*Mucor* sp 4.34
	Total	46	22	13	11		

*No. of Samples: Local Vendors: 12, Private Manufacturers: 9, Organized dairies: 9

Table 4: *Distribution pattern of moulds in khoa based milk sweets from three different sources*

Sl.No.	Mould Isolates	Total No. of Isolates	Sources			Percentage	Total Percentage of
			*Local Vendors	*Private Manufacturers	*Organized Dairies		
							Aspergillus sp 70.90
1.	*Aspergillus niger*	32	22	7	3	37.20	
2.	*Aspergillus flavus*	19	13	4	2	22.09	
3.	*Aspergillus versicolor*	6	4	2	-	6.97	
4.	*Aspergillus fumigatus*	2	2	-	-	2.32	
5.	*Aspergillus parasiticus*	2	-	1	1	2.32	
							Penicillium sp 15.11
6.	*Penicillium citrinum*	9	4	2	3	10.46	
7.	*Penicillium frequentans*	4	2	1	1	4.65	
8.	*Rhizopus stolonifer*	8	5	-	3	9.30	*Rhizopus* sp 9.30
9.	*Mucor sp*	3	-	1	2	3.48	*Mucor* sp 3.48
10.	*Fusarium sp*	1	-	1	-	1.16	*Fusarium* sp 1.16
	Total	86	52	19	15		

*No. of Samples: Local Vendors: 16, Private Manufacturers: 12, Organized dairies:12

Mould isolates

The results of moulds isolated from khoa and khoa based milk sweets which collected from local vendors, private manufacturers and organized dairies are shown in Table-3 and Table-4.

Aspergillus sp. were isolated more frequently (63.07 per cent in khoa and 70.90 per cent in khoa based milk sweets) than the *Penicillium* sp. (17.38 per cent and 15.11 per cent), *Rhizopus* sp. (10.86 per cent and 9.30 per cent), *Fusarium* sp. (4.34 per cent and 1.16 per cent) and *Mucor* sp. (4.34 per cent and 3.48 per cent). This result is agreeable to other studies[31, 32].

This could be due to the ability of *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. to grow well in wider range of temperature of 20°C to 50°C, with high relative humidity of 80 per cent to 95 per cent which usually persist in Chennai, India and with water content at 16 per cent to 17 per cent in the sample[33]. Burge[34] stated that keeping the indoor air dry is essential to prevent fungal growth and Nugari *et al*.[35] also reported that painting the walls is essential to prevent the fungal growth. Hence their presence in the indigenous milk products is an indication of the unhygienic conditions prevailing during manufacture and subsequent storage.

Conclusion

This investigation was undertaken with a view to assess the microbiological quality of traditional Indian milk sweets (khoa and khoa based milk sweets) with a special reference to isolation and identification of mould species. A total of 70 samples were collected from local vendors, private manufacturers and organized dairies in and around Chennai, India, examined for the microbiological quality. The results revealed that the bacterial count, yeast and mould count of local vendor's samples were significantly ($P<0.01$) higher when compared to private manufacturers and organized dairies except psychrotrophic count in local and private vendor's samples were identical. The total percentage of occurrence of *Aspergillus* sp. isolated from above samples was found to be the highest followed by *Penicillium* sp., *Rhizopus* sp., then *Fusarium* sp. and *Mucor* sp. In conclusion, the present study is recommended to local vendors that the strict hygienic measures should be practiced during pre and post-preparation handling, storage and marketing of the finished products to reduce the microbial load in the finished products.

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