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Microbial quality of Khoa and Khoa based milk sweets from different sources

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Abstract

A total 66 samples of Indian indigenous milk sweets such as khoa (27), burfi (10), gulabjamun (10), kalakand (9) and peda (10) were collected from local vendors, private manufacturers and organized dairies for enumeration of total viable bacterial count, coliform count, psychotropic count, aerobic spore farmers, yeast and mould count, isolation and identification of mould species were also carried out. The total viable bacterial count, yeast and mould count of local vendor's samples were higher than private manufacturers and organized dairies. The occurrence of *Aspergillus* sp. isolated from Khoa samples was found to be the highest (63.08%) followed by *Penicillium* sp.(17.38%), *Rhizopus* sp.(10.86%), *Fusarium* sp (4.34%) and *Mucor* sp. (4.34%). However the occurrence of *Aspergillus* sp. isolated from Khoa based milk sweet samples was also found to be highest (70.90%) followed by *Penicillium* sp.(15.11%), *Rhizopus* sp. (9.30%), *Mucor* sp.(3.48%) and *Fusarium* sp. (1.16%) respectively. The higher microbial load of these local vendor's products may be due to contamination at various sections of production and until marketing of the finished products. It can be concluded that regular quality assessment programme together with good manufacturing practices, would pave the way to maintain safety and quality of various indigenous milk products.

Introduction

Milk and milk products constitute important nutritional components serve as the source of first class proteins especially for children and vegetarians. 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 the growth and multiplication of varieties of microorganisms particularly stored at ambient temperature.

Among the Indian indigenous dairy products, khoa and khoa based milk sweets are 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 106 million ton; nearly 50 per cent of total milk produced in India is utilized for the manufacture of variety of traditional milk products (Khan, 2006). Approximately 50 percent of milk produced is consumed as fresh or boiled, one sixth as yoghurt or curd and remaining is utilized for manufacturing of indigenous varieties of milk products such as butter, ice cream, khoa, paneer, rabri, kheer, burfi and gulabjamun. 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 (Soomro *et al*., 2002). 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 finished products, most of the products are sold in the market without proper packaging and unduly exposing them to atmospheric contamination (Khan, 2006).

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 (Agarwal and Rachappa, 2006) which will subsequently influence the microbiological quality. Spoilage of dairy products by moulds is of frequent occurrence in India due to the prevailing tropical climate and high humidity. Since the mould spores are transmitted

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through air, they are ubiquitous in nature.

Considering the above facts, the detection and enumeration of microorganism in khoa and khoa based milk sweets is an integral part of any good quality assurance program and reflect the effectiveness of sanitation practices, processing and distribution schemes of local, private manufactures and organized dairies.

Materials and Methods

A total 66 samples of Indian indigenous milk sweets such as khoa (27), burfi (10), gulabjamun (10), kalakand (9) and peda (10) from the following sources viz., local vendors, private manufacturers, organized dairies were collected aseptically and subjected to further analysis of total viable bacterial count, coliform count, psychrotrophic count, aerobic spore forming bacterial count, yeast and mould count and isolation, identification of mould species. Total viable bacterial count, coliform count and psychrotrophic count were done as per standard methods for examination of Dairy products (Bureau of Indian Standards 1479, 1977). For aerobic spore forming bacterial count, the vegetative cells were destroyed by heat treatment at 80^{o}C for 10 min then the sample were plated in standard plate count agar in duplicate using lower dilutions according to the standard procedure (WHO, 1962).

As per the Bureau of Indian Standards 1479 (1977), the enumeration of yeast and mould count was made. Mould colonies from the representative agar plates were picked, isolated and sub cultured on potato dextrose agar slants at a pH of 3.5. These 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 (Smith, 1981). The growth rate and the morphological colony characters such as colony colour, colour changes, colour on reverse side of the colony and texture of the colony on agar surface were studied after 10 days of incubation at 25^{o}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 by using Lactophenol cotton blue were examined using stage micrometer for finer details and reproductive structures under the low and high power of the microscope. 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 (1980). 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

The total viable count of khoa samples obtained from Local vendors, Private manufacturers and Organized dairies ranged (Table 1) from $16x10^4$ to $2.71x10^5$, $1.7x10^3$ to $2.9x10^4$ and $7x10^2$ to $4x10^3$ respectively. Similarly the coliform count of khoa samples obtained from the above three sources ranged from $3x10^2$ to $2.9x10^3$, $1x10^2$ to $6.0x10^2$ and $1x10^{-3}x10^2$ respectively.

The aerobic spore farming bacterial count of khoa samples obtained from Local vendors, Private manufacturers and Organized dairies ranged from $3x10^3$ to $1.7x10^4$, $2x10^2$ to $5x10^2$ and $1x10^1$ to $2x10^2$ respectively. And also psychotropic count of khoa samples collected from the three sources ranged from $10x10^2$ to $3.1x10^3$, $1x10^2$ to $1.7x10^3$ and $1x10^1$ to $2.5x10^2$ respectively. The yeast and mould count of khoa samples three sources were ranged from $1.1x10^3$ to $1.9x10^3$, $1x10^2$ to $9x10^2$ and $2x10^1$ to $2x10^2$ respectively.

The total viable count of khoa based milk sweets obtained from local vendors, private manufacturers and organized dairies ranged (Table 2) from $1.2x10^5$ to $8x10^5$,$1.9x10^3$ to $2.3x10^5$ and $8x10^2$ to $3.1x10^4$ respectively. Similarly the coliform count was ranged from $4x10^2$ to $3.1x10^3$, $2x10^2$ to $1.1x10^3$ and 2x10 to $1x10^2$ respectively.

The percentage distribution of mould species isolated from khoa samples (Table 3) were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus versicolor*, *Penicillium citrinum*, *Rhizopus stolonifer*, *Penicillium frequentans*, *Fusarium* sp. and *Mucor* sp. were 26.08, 21.78, 15.21, 13.04, 10.86, 4.34, 4.34 and 4.34 respectively.

The aerobic spore farming bacterial count of khoa based milk sweets obtained from the above three sources were ranged from $3x10^2$ to $1.7x10^4$, $3x10^2$ to $1.1x10^3$ and $4x10^1$ to $5x10^2$ respectively. Similarly the psychrotrophic count of was ranged from $1.82x10^3$ to $5.1x10^4$, $1.9x10^2$ to $3.2x10^3$ and $3x10^1$ to $1.9x10^3$ respectively. The yeast and mould count of khoa based milk sweets obtained from the above mentioned three sources were ranged from $32x10^2$ to $8x10^3$, $2x10^2$ to $1.7x10^3$ and $5.4x10^1$ to $7x10^2$ respectively.

The percentage distribution of mould species isolated from khoa based milk sweets (Table 4) were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus versicolor*, *Aspergillus fumigatus*, *Aspergillus parasiticus*, *Penicillium citrinum*, *Penicillium*

frequentans, *Rhizopus stolonifer*, *Mucor* sp. and *Fusarium* sp. were 38.20, 22.09, 6.97, 2.32, 2.32, 10.46, 4.65, 9.30, 3.48 and 1.16 respectively.

The results of mean values of the samples collected from local vendors, private manufacturers and organized dairies were summarized in the respective tables. 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.

Discussion

The total viable and coliform count of Khoa samples (Table 1) obtained from local vendors were high as compare to private manufacturers and organised dairies. The total bacterial count was higher in Khoa samples collected from B grade shops than the A grade shops (Bandekar *et al*., 1998). Similarly, Kumar and Sinha (1989) also recorded higher coliform count of khoa samples obtained from local vendors was high as compare with organized sectors. And also the total viable count and coliform count of Khoa based milk sweets (Table 2) collected from local vendors were high as compared to the private manufacturers and organized dairies: Garge and Usha, (1984), recorded a range of 1.1×10^3 to 5.6×10^5cfu/gm. The coliform count of peda,burfi and kalakand were 460,1.61×10^4 and 4×10 cfu/gm respectively (Dwarakanath and Srikanta, 1977). The unhygienic condition ofpreparation of these Khoa and Khoa based milk products and water used for washing of utensils has enhanced the bacterial contamination of these products.

The aerobic spore formers count of Khoa samples (Table 1) collected from local vendors were high as compare to private manufacturers and organised dairies (Kamat *et al*., 1989), recorded a range of $2\times10^2-5\times10^4$ cfu /gm of sample. The aerobic spore formers count of Khoa based milk sweets (Table 2) were in close agreement with the findings of Magadam *et al.* (1988) who recorded a range of 1×10^2 to 5.8×10^3 cfu/gm. The higher incidence of spore formers may be due to marketing in dusty, dirty and open environment .

The psychrotrophic counts of Khoa samples (Table 1) and khoa based milk sweets (Table 2) from all three sources were higher than the psychrotrophic count recorded by Sharma and Joshi (1991). The higher values in this study may be due to individual's unhygienic practices, through packaging material and environmental factors.

The yeast and mould count of Khoa samples obtained from local vendors were high as compare with private manufacturers and organized dairies. 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 respectively (Vijayalakshmi and Tamilarasi, 2001). This is mainly due to unhygienic conditions prevailing during manufacture and subsequent storage.

The yeast and mould count of Khoa based milk sweets (Table 2) obtained from local vendors were high as compare with private manufacturers and organized dairies and are in agreement with the findings of Ghodeker *et al*. (1980). Who reported that the yeast and mould count of burfi and pera were 20 to 3,700/gm and 30 to 4,000/gm respectively. 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.

The total viable, coliform, yeast and mould count of khoa samples collected from organised dairies were found to be within the prescribed limit, whereas in the samples collected from private manufacturers and local vendors the total viable, coliform, yeast and mould count exceeded the prescribed limits of Bureau of Indian Standards 1479 (1977).

Khoa based milk sweets collected from the organized dairies, private manufacturers the total viable count was found within the prescribed limit, where as local vendor's samples exceeds the limits. But the samples collected from organized dairies, private manufacturers and local vendors the yeast and mould count was found to exceed the prescribed limits of Bureau of Indian Standards 1479 (1977). This might be due to usage of stored khoa for long period for making sweets (Yadav *et al.*, 1993). In general, the total viable, coliform, aerobic spore formers, psychrotrophic and yeast and mould count in local vendors samples were higher than that of private manufacturers and organized dairies and also highly significant ($P < 0.01$). 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

Table 1. Microbiological quality of Khoa obtained from different sources

Sl. No.	Counts	Local Vendors*			Private manufacturers*			Organised dairies*			F Value
		% of occurrence	Range cfu/gm	Mean cfu/gm	%of occurrence	Range cfu/gm	Mean cfu/gm	% of occurrence	Range cfu/gm	Mean cfu/gm	
1.	Total viable count	81.80	$16x10^4$ to $2.71x10^5$	$218x10^3 \pm 23.62^a$	66.66	$1.7x10^3$ to $2.9x10^4$	$21.83x10^3 \pm 2.04^b$	66.66	$7x10^2$ to $4x10^3$	$4.8x10^3 \pm 2.06^c$	185.62**
2.	Coliform count	63.63	$3x10^2$ to $2.9x10^3$	$17x10^2 \pm 4.2^a$	55.55	$1x10^2$ to $6.0x10^2$	$3.33x10^2 \pm 0.80^b$	54.54	1x10 to $3x10^2$	$0.61x10^2 \pm 0.31^c$	36.74**
3.	Aerobic spore formers count	72.73	$3x10^3$ to $1.7x10^4$	$6.6x10^3 \pm 2.11^a$	54.54	$2x10^2$ to $5x10^2$	$0.35x10^3 \pm 0.05^b$	54.54	$1x10^1$ to $2x10^2$	$0.07x10^3 \pm 0.02^c$	56.10**
4.	Psychrotrophic count	72.72	$10x10^2$ to $3.1x10^3$	$21x10^2 \pm 3.26^a$	55.55	$1x10^2$ to $1.7x10^3$	$8.91x10^2 \pm 3.05^a$	55.55	$1x10^1$ to $2.5x10^2$	$0.8x10^2 \pm 0.38^b$	22.10**
5.	Yeast & mould count	81.81	$1.1x10^3$ to $1.9x10^3$	$14.83x10^2 \pm 1.22^a$	55.55	$1x10^2$ to $9x10^2$	$3.75x10^2 \pm 1.2^b$	54.54	$2x10^1$ to $2x10^2$	$0.76x10^2 \pm 0.29^c$	27.41**

** Means bearing different superscripts between treatments differ significantly (P < 0.01)

Table 2. Microbiological quality of Khoa based milk sweets obtained from different sources

Sl. No.	Counts	Local Vendors*			Private manufacturers*			Organized dairies*			F Value
		% of occurrence	Range cfu/gm	Mean cfu/gm	% of occurrence	Range cfu/gm	Mean cfu/gm	% of occurrence	Range cfu/gm	Mean cfu/gm	
1.	Total viable count	100	$1.2x10^5$ to $8x10^5$	$344x10^3 \pm 112.8^a$	100	$1.9x10^3$ to $2.3x10^5$	$14.38x10^3 \pm 3.69^b$	100	$8x10^2$ to $3.1x10^4$	$6.96x10^3 \pm 4.87^b$	30.32**
2.	Coliform count	100	$4x10^2$ to $3.1x10^3$	$19.2x10^2 \pm 3.85^a$	100	$2x10^2$ to $1.1x10^3$	$4.55x10^2 \pm 1.41^b$	54.54	2x10 to $1x10^2$	$1.18x10^2 \pm 0.58^c$	21.38**
3.	Aerobic spore formers count	76.92	$3x10^2$ to $1.7x10^4$	$83.83x10^2 \pm 27.08^a$	63.63	$3x10^2$ to $1.1x10^3$	$6x10^2 \pm 1.37^b$	54.54	$4x10^1$ to $5x10^2$	$1.91x10^2 \pm 0.74^b$	11.58**
4.	Psychrotrophic count	69.23	$1.82x10^3$ to $5.1x10^4$	$18.55x10^3 \pm 7.71^a$	54.54	$1.9x10^2$ to $3.2x10^3$	$1.53x10^3 \pm 0.42^b$	54.54	$3x10^1$ to $1.9x10^3$	$0.88x10^3 \pm 0.27^b$	12.73**
5.	Yeast & mould count	84.61	$32x10^2$ to $8x10^3$	$54x10^2 \pm 6.62^a$	72.72	$2.0x10^2$ to $1.7x10^3$	$7x10^2 \pm 2.38^b$	63.63	$5.4x10^1$ to $7x10^2$	$3.33x10^2 \pm 1.14^b$	25.41**

** Means bearing different superscripts between treatments differ significantly (P < 0.01)

Table 3. Distribution pattern of moulds in khoa from different sources

Sl. No.	Mould Isolates	Total number of Isolates	Sources			Percentage	Total Percentage
			Local Vendors*	Private Manufacturers*	Organized Dairies*		
1.	*Aspergillus niger*	13	7	3	3	26.08	*Aspergillus* sp. 63.07
2.	*Aspergillus flavus*	09	6	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		

** Means bearing different superscripts between treatments differ significantly (P < 0.01)

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. Our findings correlate with the reports of Kumar and Sinha (1989), Grewal and Tiwari (1990) and Kulshrestha (1990).

As shown in Table 3, the percentage distribution of mould species isolated from khoa samples were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus versicolor*, *Penicillium citrinum*, *Rhizopus stolonifer*, *Penicillium frequentans*, *Fusarium* sp and *Mucor* sp were 26.08, 21.78, 15.21, 13.04, 10.86, 4.34, 4.34 and 4.34 respectively. These findings were in close agreement with the findings of Ghodeker *et al.*(1980). It was seen that there was a higher incidence of *Aspergillus* sp 63.07% followed by *Penicillium* sp 17.38%, *Rhizopus* sp 10.86%, *Fusarium* sp 4.34% and *Mucor* sp 4.34%.

The percentage distribution of mould species isolated from khoa based milk sweets (Table 4) were *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrinum*, *Rhizopus stolonifer*, *Aspergillus versicolor*, *Penicillium frequentans*, *Mucor* sp, *Aspergillus fumigatus*, *Aspergillus parasiticus*, and *Fusarium* sp were 38.20, 22.09, 10.46, 9.30, 6.97, 4.65, 3.48, 2.32, 2.32 and 1.16 respectively. These findings were in accordance with findings of Dhand *et al*. (2001). It was found that the higher incidence of *Aspergillus* sp 70.90% followed by *Penicillium* sp. 17.38%, *Rhizopus* sp 10.86%, *Mucor* sp 4.34% and *Fusarium* sp 4.34 % in both khoa and khoa based milk sweets. 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, at a relative humidity of 80% to 95% and with water content at

Table 4. Distribution pattern of moulds in khoa based milk sweets from different sources

Sl. No.	Mould Isolates	Total number of Isolates	Sources			Percentage	Total Percentage
			Local Vendors*	Private Manufacturers*	Organized Dairies*		
1.	*Aspergillus niger*	34	22	7	3	38.20	*Aspergillus* sp. 70.90
2.	*Aspergillus flavus*	18	12	4	2	22.09	
3.	*Aspergillus versicolor*	5	4	1	-	6.97	
4.	*Aspergillus fumigatus*	2	2	-	-	2.32	
5.	*Aspergillus parasiticus*	2	-	1	1	2.32	
4.	*Penicillium citrinum*	10	4	2	2	10.46	*Penicillium* sp. 15.11
5.	*Penicillium frequentans*	4	2	1	1	4.65	
6.	*Rhizopus stolonifer*	8	5	-	3	9.30	*Rhizopus* sp. 9.30
7.	*Mucor sp*	3	-	1	2	3.48	*Mucor* sp. 3.48
8.	*Fusarium sp*	1	-	1	-	1.16	*Fusarium* sp. 1.16
	Total	86	52	19	15		

** Means bearing different superscripts between treatments differ significantly (P < 0.01)

16% to 17% in the sample (Claude and Maurice, 1979). Hence their presence in the indigenous milk products is an indication of the unhygienic conditions prevailing during manufacture and subsequent storage.

In conclusion the present study is recommended to Local vendors should keep in view the public health importance of consumer, the strict hygienic preventive 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, so as to increase quality of the product.

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