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MICROBIAL QUALITY EVALUATION OF MILK PRODUCTS

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ABSTRACT

Milk products are considered as a complete diet and it is rich source for proteins, fat, sugar, minerals and vitamins which is required for the growth as well as for maintenance. However, it has been observed that it also acts as excellent medium/carrier for the pathogenic/spoilage microorganism. These milk products may be contaminated intentionally/unintentionally at different level at the time of production, processing and marketing. Therefore, the present study was conducted with an objective to assess the quality of market milk products available in and around Ludhiana. Milk products sample were collected hygienically and subjected to microbiological analysis. Among all milk products sample Burfi showed the average viable count of 3.1 x 10^4cfu/mL/g, rasgulla 2.8 x 10^4cfu/mL/g, paneer 6.7 x 10^4cfu/mL/g and ice-cream 8.3 x 10^4cfu/mL/g. Results for lactose fermenter and non-lactose fermenter bacteria showed that among the milk products, ice-cream has the highest average count (22600/mL/g), and lowest in burfi (10400 /mL/g). For the bacteriological quality milk products sample ice-cream had 12.5%, rasgulla and paneer had 14.3% Salmonella. In rasgulla Proteus was absent but was present in burfi and ice-cream (12.5%), paneer (14.2%). Klebsiella was isolated from burfi (25%), rasgulla (42.8%), paneer (14.3%) and ice-cream (37.5%).

INTRODUCTION

Sweetmeat prepared from milk is an integral part of the culinary habits of people all over India. But these products are extremely vulnerable to contamination with spoilage and pathogenic organisms as well as toxic metabolites of microbial origin if subjected to advertent and inadvertent abuse during their production and processing. The manufacture of various milk products is based on traditional methods without any regard to the quality of raw material used or hygienic quality of the product. Under such conditions many microorganisms can find access to the milk products (Grewal and Tiwari, 1990; Kulshrestha, 1978). More than 200 foods borne illnesses are now recognized and most of them require specific laboratory diagnosis (Prasad, 1998). To improve the microbiological quality of milk products, precaution against contamination must be taken at different Critical Control Points (Roy *et al*., 1998). Approximately 50% of the milk produced is consumed as fresh or boiled, one sixth as yoghurt or curd and remaining is utilized for manufacturing indigenous variety of milk products such as ice cream, butter, khoa, paneer, rabri, kheer etc. (Anjum *et al*., 1989). Approximately 90% of dairy related diseases in human being arise from unhygienic milk products (Ryser, 1998). To protect public health against milk-borne infections, there are regulations that require proper hygienic handling of milk and its pasteurization.

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MATERIALS AND METHODS

Indigenous milk products (burfi, rasgulla, paneer and ice-cream) were collected from different sources and analyzed for microbial and biochemical quality. The samples were collected in the sterile bottles, sealed properly, brought to the laboratory, kept at below 4^0C and tested within three to four hours after collection. All the samples were first of all evaluated for the microbial quality by standard plate count (SPC) then presumptive test were performed to observe the presence of lactose and non-lactose fermenting bacteria by using MacConkey agar. The samples were inoculated on MacConkey Agar and incubated aerobically at 37 ^{o}C for 24 hours. Then non- lactose fermenting colonies were sub cultured again on MacConkey agar for purification of the isolate. Gram staining was performed to ensure the purity of

the organism. Salmonella was identified by various biochemical tests e.g. Catalase test, Simmon Citrate Agar, Indole Production, Nitrate reduction, Urease production, Voges Proskaur, Methyl red.

Processing of samples

Raw and Pasteurized Milk Samples

1 ml of milk samples (raw and pasteurized) were mixed with 9 ml of sterile distilled water and mixed well. Serial dilutions were made up to 10^{-5} to 10^{-6}.

Processing of sample

11 g of the sample was weighed and added to 89 ml of sterile distilled water and kept on rotary shaker for 30 minute, to ensure even distribution of micro-organisms in the solution. The initial dilution of 10^{-1} was further diluted to 10^{-4} and 10^{-5}.

Standard Plate Count

1 ml of dilutions (milk/milk product) was added to petri plates and nutrient agar medium was poured in it. Triplicate plates were prepared and incubated at 37^0 C for 48 hours. Plates showing colonies between 30 - 300 were selected for total count. The average count of two plates was taken for the standard plate count/gram

Presumptive coliform test

1 ml of milk samples (raw and pasteurized) were mixed with 9 ml of sterile distilled water and serial dilution were made upto 10^{-5} to 10^{-6}. 1 ml of dilutions were added to petri plates with McConkey agar medium in triplicates and incubated at 37^0 C for 48 hours.1 ml of each dilution was transferred to McConkey broth for the detection of acid production and formation of gas.

Biochemical Characteristics

Production of Catalase

This illustrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide. Few drops of three percent hydrogen peroxide were added to 1 drop of bacterial culture. Formation of bubbles is a positive test for catalase.

Production of Oxidase

This test depends on presence of certain oxidases in bacteria that will catalyse the transport of electron donors in bacteria. Few drops of oxidase reagent were added on the bacterial culture grown on nutrient agar medium. Change of colony colour to pink and finally to black is the positive indicator of the presence of oxidase.

Nitrate Reduction Test

This test is used to differentiate the members of family enterobacteriaceae from other gram negative bacteria that do not produce the nitrate reeducates enzyme. This enzyme reduces nitrates into nitrite. The test was done by inoculating heavy growth of test organism into 2.0 ml nitrate broth. After 4-6 hour incubation at 37^0C, one drop of sulphanilic acid and one drop of alpha- naphthylamine was added. Development of red color after mixing was taken as positive.

IMVIC test

Test for Indole Production

This test demonstrates the ability of certain bacteria to decompose amino acid tryptophan to indole which accumulates in the medium .The test was performed by inoculating the culture into tryptone broth and incubating for 24±2 h at 35°C. 0.2-0.3 mL of Kovac's reagent was added into the tubes. Appearance of distinct red color in upper layer is the positive test.

Voges-Proskauer (VP)-Reactive Compounds

This test indicates the production of acetyl-methyl carbinol in peptone water containing glucose by some bacteria. The test was performed by adding few drops of VP solution (3 ml 5% alpha –naphthol + 1 ml 40% KOH) into 24- 48 hr cultures grown in MR-VP medium. Test is positive if pink color develops while no color change was observed in negative cases

Methyl Red-Reactive Compounds

After VP test, MR-VP tubes were incubated for additional 48±2 h at 35°C. After that, 5 drops of methyl red solution was added to each tube. Distinct red color is positive test. Yellow is negative reaction.

Citrate Utilization Test

This test demonstrates the capacity of organisms to use citrate as sole source of carbon and ammonium salt as sole source of nitrogen. Slants of Simmon's citrate agar medium were prepared and bacterial culture was streaked on it. Slants were incubated at 37^0C for 48 hours. Development of blue color due to utilization of citrate is a positive test.

RESULTS AND DISCUSSION

The present investigation was taken up to study the quality of milk products both chemically as well as microbiologically.

Standard plate count

Sweet prepared from milk is an integral part of the culinary habits of people all over India. But these products are extremely vulnerable to contamination with spoilage and pathogenic organisms as well as toxic metabolites of microbial origin if subjected to advertent and inadvertent abuse during their production and processing. More than 200 food borne illness are now recognized and most of them require specific laboratory diagnosis (Prasad, 1998). Precaution must be taken against contamination at different Critical Control Points to improve the microbiological quality of sweetmeat (Roy*et al*., 1998). In the present study SPC of various milk products showed a high degree of contamination (Table 1).

Table 1. Enumeration of micro-organisms in milk product samples by standard plate count (SPC)

SAMPLE TYPE	SAMPLE NUMBER	Cfu/ml(10^4)
Burfi	1	6.7×10^4
	2	1.9×10^4
	3	3.1×10^4
	4	2×10^4
	5	3.6×10^4
Rasgulla	1	1.7×10^3
	2	2.3×10^4
	3	1.9×10^4
	4	7.8×10^4
	5	3.1×10^3
Paneer	1	1.7×10^4
	2	5×10^4
	3	2.8×10^5
	4	2.3×10^4
	5	1.8×10^4
Ice-cream	1	2.3×10^5
	2	7.8×10^4
	3	1.9×10^6
	4	1.8×10^4
	5	2.7×10^4

Burfi samples showed the average viable count of 3.1 x 104 cfu/mL/g, rasgulla2.8 x 104 cfu/mL/g, paneer 6.7 x 104 cfu/mL/g and ice-cream 8.3 x 104 cfu/mL/g. it gives an indication of unhygienic practices followed by manufacturers. Table 2 shows that among the milk products, ice-cream has the highest average count (22600/mL/g), followed by rasgulla (17800/mL/g), paneer (16000 /mL/g) and burfi (10400 /mL/g). Two samples of burfi, one sample each of rasgulla and paneer did not show the presence of coliforms (Table 3). All the five samples of ice cream were positive for coliforms. In most of the samples percentage of lactose fermenters is more than non-lactose fermenters. Milk products have high nutritive value but they are less perennial because different types of microorganisms are often present in it due to unhygienic condition. The unsanitary conditions followed by the halwais in preparation, processing, packaging and storage of sweetmeats are often so poor that the products on reaching the consumers have an unbelievable microbial load, which may include the different strains of pathogens that causes the serious health hazards (Dwarkanath and Srikanta, 1978). Collins *et al*. (1995) reported that Escherichia coli and coliform bacilli which they are belong the family of Enterobacteriaceae may indicate evidence of contamination or pollution especially of fecal nature. Enterobacteriaceae include other organisms, like important pathogens such as salmonella and various non-lactose fermenters that may be present in human and animal faeces. Table 4 and 5 showed that no Salmonella was isolated from burfi whereas ice-cream had 12.5%, rasgulla and paneer had 14.3% Salmonella. E. coli could be isolated from all the milk products with burfi and ice-cream (25%), rasgulla (14.3%) and paneer (28.5%). In rasgulla Proteus was absent but was present in burfi and ice-cream (12.5%), paneer (14.2%). Klebsiella was isolated from burfi (25%), rasgulla (42.8%), paneer (14.3%) and ice-cream (37.5%).

Table 2. Number of Lactose and Non-Lactose Fermenting Bacteria (Presumptive test)

Particular of sample	No. of sample	Total No. of Coliforms (per ml per g)			Lactose Fermenting			Non-lactose Fermenting		
		Avg	Max	Min	Avg.	Max	Min	Avg.	Max	Min
Burfi	5	10400	22000	13000	6400	15000	13000	2000	8000	2000
Rasgulla	5	17800	33000	17000	15600	29000	13000	2200	7000	4000
Paneer	5	16000	22000	11000	11600	16000	2000	4400	13000	9000
Ice-cream	5	22600	36000	6000	16600	29000	6000	6000	20000	10000

Table 3. Result of presumptive test (Milk Products)

S NO.	Cfu /mL (1/1000)	Lactose Fermenting	Percentage (%)	Non-Lactose Fermenting	Percentage (%)
			BURFI		
1	13	13	100	0	0
2	0	0	0	0	0
3	17	15	88.2	2	11.8
4	0	0	0	0	0
5	22	14	63.6	8	36.4
			RASGULLA		
1	17	17	100	0	0
2	20	13	65	7	35
3	0	0	0	0	0
4	33	29	87.9	4	12.1
5	19	19	100	0	0
			PANEER		
1	11	2	18.2	9	81.8
2	31	31	100	0	0
3	16	16	100	0	0
4	22	9	40.9	13	59.1
5	0	0	0	0	0
			ICE-CREAM		
1	36	16	44.4	20	55.6
2	23	13	56.5	10	43.5
3	29	29	100	0	0
4	19	19	100	0	0
5	6	6	100	0	0

Table 4. Bacteriological quality of various milk products

S.NO.	Type of Microrganism	Burfi	Rasgulla	Paneer	Ice-Cream	Total samples
1	*Salmonella*	0	1(14.3%)	1(14.3%)	1(12.5%)	3(10%)
2	*Proteus*	1(12.5%)	0	1(14.3%)	1(12.5%)	3(10%)
3	*E.coli*	2(25%)	1(14.3%)	2(28.5%)	2(25%)	7(23.3%)
4	*Klebsiella*	2(25%)	3(42.8%)	1(14.3%)	3(37.5%)	9(30%)
5	Unidentified	1(12.5%)	1(14.3%)	1(14.3%)	1(12.5%)	4(13.3%)
6	No Coliform	2(25%)	1(14.3%)	1(14.3%)	0	4(13.3%)
	Total samples	8	7	7	8	30

Table 5. Biochemical attributes of different isolates from milk products

Sample No.	Isolate	Gram staining	Bacilli	Catalase production	Oxidase production	Nitrate activity	Indole production	Methyl red	VogesPros kauer	Citrate Utilization	Reaction on TSI			Lactose fermentation
											Alk. Red slant	Acid yellow but	H_2Sproduction	
								BURFI						
1	E	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
2	E	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
3	U													
4	K	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
5	P	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve
6	K	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
								RASGULLA						
1	E	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
2	K	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
3	S	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
4	K	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
5	U													
6	K	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
								PANEER						
1	S	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
2	U													
3	E	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
4	E	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
5	K	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
6	P	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve
								Ice-cream						
1	S	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
2	E	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
3	P	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve
4	K	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
5	K	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
6	U													
7	E	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
8	K	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve

Burfi and ice-cream had 12.5% whereas rasgulla and paneer had 14.3% unidentified coliforms. In the present study no Salmonella was detected in burfi. These findings are in accordance with Jayant *et al.* (2007). However Kulshrestha (1978) isolated S. enteridis from 6.25% samples of burfi. Patel *et al*. (1962) isolated S.enteridis from Basudi (khoya product). Tambekar and Bhutda (2004) could isolate 16.3% Salmonella from pedha. Ice-cream had 12.5% Salmonella. Ashfaq (1983) reported two percent Salmonella contamination in ice-cream, Patel and Vyas (1971) 8.75%. In contrast all the 36 samples examined by Tamminga *et al.* (1980) and 24 by Pasetto Falcao *et al*., (1983) were negative for salmonellae. 14.3% Paneer and rasgulla samples were detected with Salmonella in the present study. These findings are in consistence with the findings of other workers who had isolated Salmonella from 2.5 to 11.11 percent samples of cheese (Vaishnavi, *et al*., 2001).

Guven Uraz *et al*., 2008) isolated Salmonella from 27.2% of fresh urfa cheese. The absence of Salmonella in burfi might be due to sufficient heat treatment during its preparation that might have killed salmonellae. The growth of Salmonella might also have been inhibited by other fast growing bacteria isolated in the present study. The presence of other enteric organisms might be due to the soiled hands of the persons handling the product. The presence of Salmonella in rasgulla, paneer and ice-cream could be due to the use of contaminated milk for their preparation as reported by Mathur (1959) and Das and Nag (1986). Another reason could be the unhygienic handling and use of contaminated ingredients for the preparation of these products (Barret, 1986). *E. coli* could be isolated from all the milk products with burfi and ice-cream (25%), rasgulla (14.3%) and paneer (28.5%). In rasgulla *Proteus* was absent but was present in burfi and ice-cream (12.5%), paneer (14.2%). *Klebsiella* was isolated from burfi (25%), rasgulla (42.8%), paneer (14.3%) and ice-cream (37.5%). Burfi and ice-cream had 12.5% whereas rasgulla and paneer had 14.2% unidentified coliforms.

Burfi and ice-cream samples had 25% E.coli. Singh *et al.* (1994) isolated coliforms from 84.0 percent samples of burfi. Soomro *et al.* (2002) isolated *E.coli* from 51.66% samples. Tambekar and Bhutda (2004) reported 14.13% E.coli contamination of pedha samples. Haq *et al.* (1995) reported that E.coli is a frequently occurring organism in milk products like khoa, dahi, cheese etc. However, Bhatnagar *et al.* (2007) did not isolate any *E.coli*. Presence of *E.coli* in all the milk product indicate faecal contamination which may arise due to unhygienic preparation conditions in most of the sweetmeat shops or it may be from the hands of handlers. Garg and Mandokhot (1984) described *E.coli* as a potential pathogen isolated from milk product. *Klebsiella* was isolated from burfi (25%), rasgulla (42.8%), paneer (14.3%) and ice-cream (37.5%). Vaishnavi, *et al.* (2001) isolated *Klebsiella* from 27.5% of paneer samples studied. 90.2% *K. pneumoniae* were observed in Mozzarella cheese (Salvtore Massa *et al*., 1992) whereas Guven Uraz *et al*. (2008) observed 72.7% in urfa cheese. The occurrence of *Klebsiella* was reported to be 100% in ice-cream (Ojokoh, 2006).

Conclusions

In present study by the above results and finding, it can be clinched that due to higher demand and lower supply is one of the main reason for the milk contaminated with unhygienic addition of the adulterated water. Unhygienic processing, handling of milk products, failure in cold chain maintenance during transport, post preparation contamination, personal and utensil hygiene and contaminated adulterated ingredients are the main factors responsible for the microbial deterioration and contamination of the milk products.

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