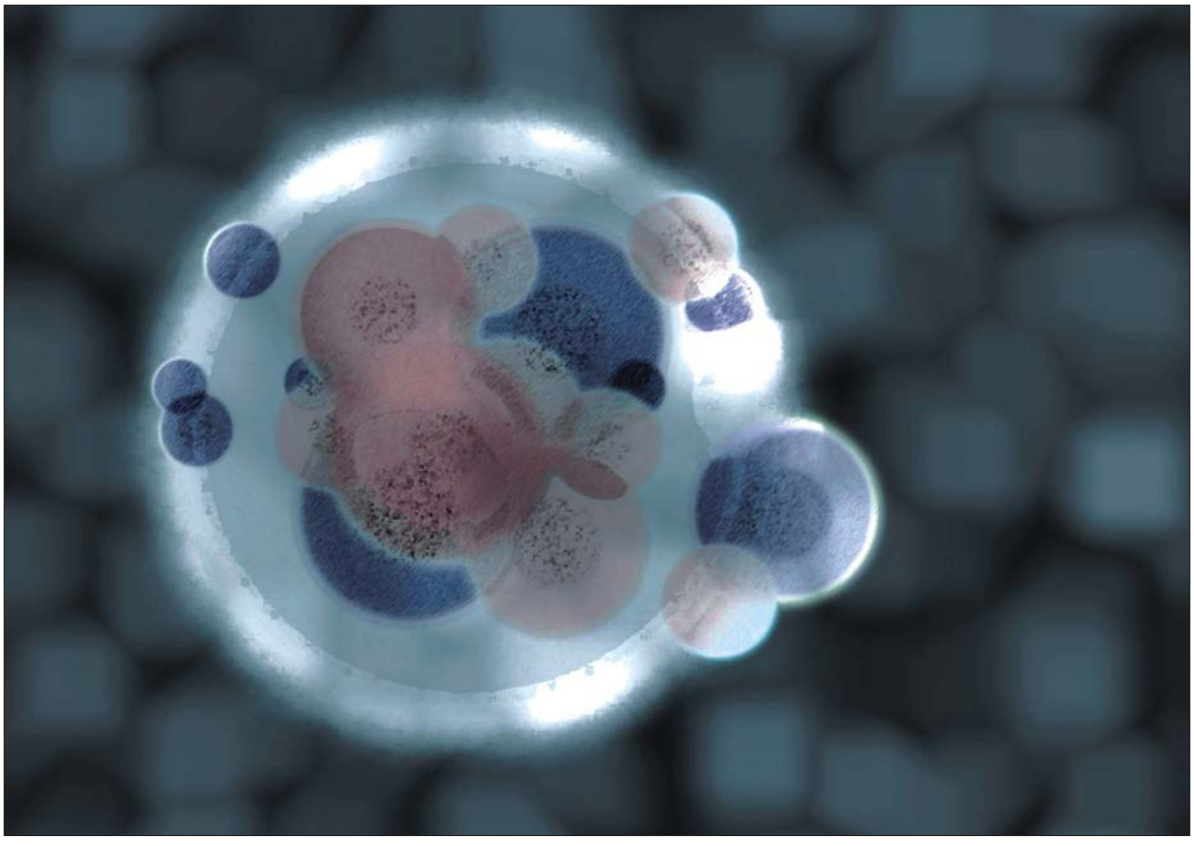

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Incidence of Coliform in Butter

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Abstract: Microbial examination of 50 samples of both packed and unpacked butter was carried out. The samples were examined for total viable count, mould and yeast count, spore formers and coliform. The microbial load in the unpacked sample of butter was highest i.e., 3.8 × 10^6/gm - 6.6 × 10^6/gm. The coliform count was found maximum in unpacked sample and one of the packed sample of butter (Kausar brand) i.e., 39/ml. The unpacked sample of butter contained highest number of aerobic spore formers i.e., 280/gm. The anaerobic spore-formers were found absent in 20 samples of butter and the rest contained in the range of 0-170/gm. The yeast cultures were found only in the sample of Lyallpur and Kausar butter. The mould count of these samples ranged from 0-280/gm.

Key words: Butter microscopy, coliforms, microbiology of butter

Introduction

Butter is a very delicate fermented milk product and it contains around 81% fat, 15% water, less than 0.5% carbohydrates, 1% protein and 3% ash (James and Coulter, 1998). Butter was used as medicine by the Greeks and the Romans (Eckles *et al.,* 1982). The main source of spoilage of this product by microorganisms in cream whether sour or sweet. The processing of raw and pasteurized creams of yield butter brings about a reduction in the total number of microorganisms with values for finished creams ranging from several hundred to over 100,000/gm having been reported for the finished salted butter (Macy *et al*., 1992).

Paula and Marth (1997) reported the occurrence of different species of mould and yeast in the samples of butter, available for public consumption. Moulds of the genera *Cladosporium, Alternaria, Aspergillus, Mucor* and *Rhizopus* cause surface discolouration. Yeasts and Moulds may be used as an index of cleanliness in butter. Various common moulds grow on the surface but often these grow only in water droplets or in pockets in the Wrapper (Murphy, 1981). Coliform organisms can be added in raw milk through contamination of teat canal by feed or manur. Califorms in milk products generally are considered to be of animal origin and tend to indicate the hygienic standard of the product and its keeping quality, rather than the presence of human pathogen. The more carefully the milk is produced and handled, the lower the coliform count (Trollar, 1994).

The object of microbial examination of any food or food product is to look their freedom from fecal pathogens, toxinogenic bacteria and high viable count. The main purpose of the present study was to examine the microbial contamination of the butter available in the market.

Materials and Methods

Forty-five samples from different brands of butter were collected from local market, whereas five samples of butter were prepared at home. The unpacked samples were collected in sterile polythene bags. Dilutions of samples were prepared using sterilized saline water as 10^{-2}-10^{-4} by Serial dilution method (Clark *et al.,* 1958).

Glassware was sterilized in an oven at 180°C for 2 hours while all the culture media were sterilized in autoclave at 121°C for 15 minutes under 15 lb/inch^2 pressure.

Nutrient agar medium consisting of g/l; peptone, 6.0 casein hydrolysate 4.0, yeast extract 3.0, glucose 2.0, beef extract, 1.5, agar, 15.0 was used for total viable count. The plates were incubated at 37°C for 24 to 48 hours and the colonies were counted with the help of colony counter. The nutrient agar medium was also used for both aerobic and anaerobic spore-formers i.e., samples suspended in saline water were given heat shock at 90°C in water bath for 10 minutes, before adding to the agar plates. The plates were incubated at 37°C for 24 hours. For anaerobic spore formers, however, sterile agar solution was poured on the incubated plates.

Agar malt extract medium consisting of g/l, malt extract, 20.0; agar, 20.0 was used for determination of mould and yeast count. The plates were incubated at 30°C for 24 to 48 hours.

The coliforms were determined by standard multiple tube fermentation technique (De Man, 1977) containing lactose broth consisting of meat extract, 3.0 gm; peptone, 10.0 gm; lactose, 5.0 gm; bromothymol blue indicator, 1.0 ml; distilled water, 1000 ml.

Gram's staining of colonies was done according to the method of Hucker and Conn (1923).

Results

Total viable count: Total viable count of coliforms in different butter samples is shown in Table 1. The number of microorganisms in general, varied from 1.5 × 10^5/gm -6.6 × 10^6/gm. The highest viable count 6.6 × 10^6/gm was found in unpacked butter collected from the shops at Anarkali, Lahore. The colonies were off-white, gram -ve and samples of rods. The minimum contamination 1.5 × 10^5/gm was found in the samples of blue band butter collected from Cantt area. The colonies were off-white, gram +ve and round.

Spore formers: The aerobic spore formers ranged from 0-280/gm while anaerobic spore formers ranged from 0-170/gm in different samples of butter. The maximum aerobic and anaerobic spore formers were found to be present in the unpacked sample of Iqbal Town and in Lyallpur butter of Shadbagh, respectively (Table 1).

Mould and yeast: The total count of mould in butter sample varied in between 0-280/gm and yeast cultures were only

Table 1: Microbial examination of butter

Brands	Locality	Replicates	Total viable Count/gm	Characteristics of predominant colonies			Spore formers/gm		Coliform/ml			Mould/ gm	Yeast/gm
				Colour	Morphology	Gram's Staining	Aerobic	An aerobic	MPN	Fecal	Non fecal		
							80	-	3	+	-	40	-
	Iqbal Town	3	1.6×10^6	Off white	Round	+	150	30	9	+	+	80	-
Blue Band	Station	3	2.4×10^6	"	Rod	-	59	-	0	-	-	18	-
butter	Samanabad	3	3.0×10^5	"	Round	+	100	15	.	-	-	10	-
	Cantt. Area	3	1.5×10^5	"	"	+	10	2	0	-	-	12	-
	Cantt. Area	3	6.0×10^5	"	"	+	60	-	0	-	-	3	-
	Iqbal Town	3	8.0×10^5	"	"	+	10	-	0	-	-	12	-
Milkpak	Muslim Town	3	1.0×10^6	"	"	-	5	2	3	+	-	10	-
butter	Daroghawala	3	1.5×10^6	"	"	-	-	-	3	+	+	0	-
	Cantt. Area	3	2.0×10^6	"	"	+	-	-	0	-	-	2	-
	Iqbal Town	3	4.0×10^5	"	"	+	20	-	3	+	-	5	-
	Iqbal Town	3	2.0×10^6	"	"	+	17	-	11	-	+	-	-
Nurpur	Anarkali	3	1.3×10^6	"	Rod	-	30	1	4	+	+	55	-
butter	Yateem Khana	3	1.4×10^6	"	Round	+	20	-	3	-	-	30	-
	Samanabad	3	8.0×10^5	"	"	+	1	2	0	-	-	5	-
	Cantt. Area	3	5.0×10^5	"	Rod	+	6	2	7	+	-	-	-
	Shadbagh	3	1.5×10^6	"	Rod	-	10	-	4	+	+	30	-
Punjab	Wahdat Rd	3	7.0×10^5	"	Rod	-	40	10	0	-	-	8	-
butter	Muslim Town	3	1.0×10^6	"	Round	+	100	-	15	-	+	20	-
	Iqbal Town	3	2.5×10^6	"	Rod	-	80	20	0	-	-	1	-
	Iqbal Town	3	9.0×10^5	"	Rod	+	40	1	4	+	-	50	-
	Anarkali	3	$2.0 \times * 10^6$	"	Round	+	170	40	0	-	+	80	-
Pakpur	Samanabad	3	3.8×10^6	"	Round	+	80	-	7	+	-	29	-
butter	Iqbal Town	3	1.8×10^6	"	Rods	-	180	30	4	+	+	80	-
	Station	3	2.3×10^6	"	Rods	-	200	-	0	-	-	100	-
	Wahdat Rd	3	7.0×10^5	"	Round	+	18	3	4	+	-	-	-
	Station	3	1.3×10^6	"	Round	+	30	17	9	+	+	-	-
Sunrise	Anarkali	3	2.5×10^6	"	Round	-	50	8	0	-	-	30	-
butter	Iqbal Town	3	1.7×10^6	"	Round	+	100	14	7	-	+	15	-
	Samanabad	3	2.3×10^6	"	Rod	-	50	-	0	-	-	50	-
	Shadbagh	3	1.4×10^6	"	Round	-							
	Gharishahu	3	1.46×10^6	"	Round	+	2	-	0	-	-	37	20
Lyallpur	Station	3	2.8×10^6	"	Rod	-	50	6	9	+	+	146	-
butter	Anarkali	3	2.5×10^6	"	Rod	-	150	50	15	-	+	20	4
	Shadbagh	3	4.0×10^6	"	Rod	-	200	170	4	+	-	130	-
	Yateem Khana	3	1.5×10^6	"	Rod	-	160	-	6	+	+	50	-
	Station	3	3.8×10^6	"	Rod	-	10	-	6	-	+	200	-
Rahatpur	Daroghawala	3	2.67×10^6	"	Rod	-	20	-	3	+	-	120	-
butter	Garhi Shahu	3	1.69×10^6	"	Rod	-	130	80	14	+	+	10	-
	Samanabad	3	2.59×10^6	"	Rod	-	100	70	9	+	-	-	-
	Shadbagh	3	2.09×10^6	"	Rod	+	90	-	7	-	+	80	-
	Shadbagh	3	2.0×10^6	"	Rod	-	80	50	20	-	-	20	-
	Station	3	3.7×10^6	Pale Yellow	Round	+	180	30	6	+	-	28	-
Kausar butter	Anarkali	3	4.5×10^6	"	Round	+	120	50	0	-	-	50	30
	Garhi Shahu	3	3.9×10^6	"	Rod	+	100	10	39	+	-	90	-
	Shadbagh	3	3.0×10^6	"	Rod	-	90	-	3	-	+	80	-
	Anarkali	3	6.6×106	Off White	Rod	-	130	-	3	+	-	8	-
Unpacked	Daroghawala	3	5.5×10^6	"	Rod	-	80	100	20	+	-	50	-
butter	Iqbal Town	3	3.8×10^6	"	Rod	-	280	30	7	-	+	280	-
	Samanabad	3	4.5×10^6	"	Rod	-	120	51	15	-	+	40	-
	Anarkali	3	5.0×10^6	"	Rod	-	100	80	39	+	-	190	-

present in 'Kausar and Lyallpur' butter. The maximum number of moulds was found to be present in the unpacked samples of butter collected from Iqbal Town (Table 1).

Coliforms: The total coliform count/ml in different varieties of butter were ranged from 0-39/ml (Table 1). It was maximum in 'Kausar butter' and 'unpacked butter' of Anarkali i.e.; both 39/ml. Both fecal and non-fecal coliforms were found in almost equal ratio in these samples. Coliform count was minimum in 'Milkpak' butter i.e., 0-3/ml. Fecal coliforms were present in 2 samples while non-fecal in one sample of 'Milkpak' brand.

Discussion

Food products serve not only as source of nutrition for humans and other animals but also as substrates for the growth of microorganisms. The uncontrolled growth of microorganisms in food causes spoilage, a serious problem accounting for sizeable losses of food products that are needed to meet global nutritional requirements. A major concern in food microbiology is the development of an understanding of the process involved in food spoilage. The present study describes the microbiological examination of 50 samples of butter collected from local market. The milk

products were analysed for (I) total viable count (ii) mould and yeast (iii) spore formers and (iv) coliforms. Salted butter is less linely to support microbial growth than unsalted butter (Frazier and Westhoff, 1978).

The moisture present in the butter is not a continuous phase but as droplets surrounded by fat therefore it does not favour the growth and transfer of bacteria. The bacterial content of the finished butter is of little consequence as long as the bacteria are non-pathogenic. Fresh butter may contain as many as 5.0×10^7 bacteria/gm. This number rapidly decreases until after a month or two only a few hundred thousands are present (Fred, 1978). The total viable count was higher in the unpacked sample of butter i.e., 6.6×10^6/gm. This shows that the hygienic conditions are not adequate for processing. This should be discouraged. The high the total viable count, the less is shelf life of the product. Mahfouz *et al.* (1985) reported that the total viable count of the butter ranged from 170 to 320 million/gm.

One of the factors for the growth of moulds, is the availability of the moisture and since the salted butter is low in moisture. Therefore, all the samples of butter showed lower mould count than viable count. So there was also a variation in microbial counts. Butter undergoes fungal spoilage rather commonly by species of following genera; *Cladosporium, Alternaria, Aspergillus, Mucor, Rhizopus* and *Geotrichum*, etc. Generally higher lipid content and low water content make the butter more susceptible to spoilage by moulds than by bacteria (James and Coulter, 1998). Milkpak and Punjab butter showed lowest mould count/gm i.e., 2-12/gm and 0-30/gm, respectively. Abdel-Rahman and El-Bassiony (1985) studied the psychrotrophic moulds in butter samples. Total mould counts and psychrotrophic moulds constituted 40.4 and 48.7 of mould counts on butter, respectively. Contamination of these processed milk products with mould spores during packaging is possible and such contamination can result in mould growth on the product before it reaches the consumer. Appropriate hygienic measures can minimize or eliminate this problem.

The primary taxonomic sub-division of the spore formers is based on the physiological property. The relation to free O_2, the anaerobic spore formers (genus *Clostridium*) are strictly anaerobic, rapidly killed by exposure to O_2. The aerobic spore formers (*Bacillus* and *Sporosareina*) are either strict aerobes or facultative anaerobes (Stanier *et al*., 1971). In case of butter sample aerobic spore formers were maximum in unpacked butter i.e., 280/gm. The primary habitat of *E. coli* is the intestinal tract of man and other animals (*E. coli* has 'coli' word for colon). Coliforms in butter are generally considered to be of animal origin and tend to indicate hygienic standards of products and its keeping quality rather than the presence of human pathogens. The more carefully the milk is produced and handled, lower the coliform count. Coliform group is of significance for causing spoilage in Dairy products. Coliforms are short, gram -ve, rods, that ferment lactose with the production of gas, within 48 hours at 37°C (James and Coulter, 1998). Haq *et al.* (1995) reported the presence of fecal and non-fecal coliform bacteria in raw milk samples of Khoa.

Fecal coliform are distinguished most readily from non-fecal coliforms by using the evaluated incubation temperature. This is accomplished by inoculating the plates usually at 45°C. Fecal coliforms are found in feces, others have been called non-fecal because they are normal inhabitants of soil. In 24 samples of butter fecal coliforms were present and in 20 samples non-fecal coliforms were found. For coliform detection Most Probable Number (MPN) system was used (De Man, 1977). In case of butter samples, unpacked sample showed maximum MPN i.e., 39/ml. Coliform bacteria are frequently present in butter. The presence of coliforms in all the above samples of butter probably due to poor sanitary condition. Contamination might be due to unhygienic condition of dairy utensils, food handlers, animal feed, interior cow's udder, soil and water which are primary sources of microorganisms. Butter may also be contaminated during the packing and probably due to rough wrapper.

Conclusively when pathogen-free milk, an active lactic starter culture, and good hygienic practices are used to produce milk products, a safe food results and if these products are then handled and distributed in a sanitary manner, this food will be safe for consumption.

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