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Microbiological Quality of Ice Cream Sold in Gilgit Town

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Abstract: Ice cream is a food commonly consumed during summer. It harbors many potent pathogens, its microbial quality especially bacteriological quality has always been crucially important to public health. This study aims to assess bacteriological quality of ice cream sold in different areas of Gilgit town. Randomly collected ice cream samples were studied to determine the microbiological load or colony forming units per gram (CFU/g) using the standard aerobic plate count method. All samples showed positive growth of bacteria ranging from 2.2×10^3 to 8.2×10^4 CFU/g. Seven bacterial species were isolated. The highest frequency of isolation was *Escherichia coli* 20 (100%) and least isolation was *Salmonella spp.* 3 (15%). For confirmation the colonies were tested biochemically and were identified up to species level.

Key words: Ice cream, Gilgit town, microbiological load, *Escherichia coli*

INTRODUCTION

Ice cream is a nutritionally enriched congealed dairy product consumed by all age groups particularly children, during summer (Sharif *et al.*, 2005). The ingredients of ice cream may be various combinations of milk, cream, evaporated or condensed milk, dried milk, colouring material, flavors, fruits, nuts, sweetening agents, eggs and eggs products stabilizer. Any of these may account for the various specific species of bacteria (Yaman *et al.*, 2006).

Ice cream is a nutritious food for human and also an excellent medium for the growth of many microorganisms some of which may cause diseases in human beings e.g. Cholera, typhoid, bacillary dysentery. Contaminated ice cream causes several outbreaks of gastrointestinal diseases in a number of countries in Asia, Europe and North America (Dijuretic *et al.*, 1997; Dijuretic and Wall, 1996; Chug, 1997).

In England and Wales two outbreaks of *S. enteritidis* phage type 4 infections were reported in 1990 and 1995 due to consumption of ice cream (Hennessy *et al.*, 1996).

Quality of ice cream depends on extrinsic factors that include manufacture procedure, as well as intrinsic factors that include the proportion of ingredients used. Primary sources of microbial contamination of ice cream include water and raw milk, whereas secondary sources include flavoring agents, utensils and handling.

Possible sources of these microorganisms in ice cream have been reported to include raw materials used for the composition of ice cream-mix, such as milk and milk powder, cream, flavouring and colouring substances and sanitizer (Verma, 1972; Bathla and Rao, 1973) and from contaminate air during processing (Gomez, 1969). In Gilgit town ice cream is manufactured on small scale by using the dried and raw milk and sale in the city at retail outlets.

The aim of this study was to determine the bacteriological quality of commercially soled ice creams in Gilgit town and their potential risk to public health.

MATERIALS AND METHODS

Collection of samples: Twenty ice cream samples (cups) were randomly collected from eight different areas/location of Gilgit town (Fig. 1). The collected samples were immediately shifted to the laboratory of Department of Biological Sciences, Karakoram International University, Gilgit in a cold box.

Microbiological analysis: Serial dilutions: In serial dilutions 1 gram of ice cream was aseptically transferred into 9 ml of distilled water and homogenized by vortex. Subsequent serial dilutions were made up to 10^5 .

Culture of samples: For aerobic enumeration of colonies 1 ml sample was cultured on Nutrient agar (Oxoid) and incubated at 37°C for 24 h. The following day the total number of colonies were counted and read morphology, for their identification. Separated colonies were sub culture on freshly prepared Nutrient agar, MacConkey (Oxoid) and Salmonella Shigella agar (Oxoid) plates. Further identification of the Gram negative colonies was performed by biochemical tests such as production of urease, utilization of sugars, carbon and production of indole, H₂S gas, oxidase and on the basis of motility of the bacteria (WHO Manual, 1987) and the gram positive bacteria colonies were identified by coagulase and catalase tests.

RESULTS

The total viable counts in samples of the eight locations of Gilgit town are presented in Table 1. The samples of all the areas show heavy contamination of bacteria

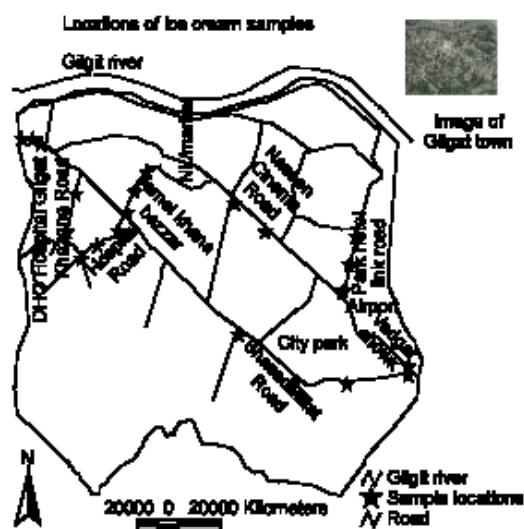


Fig. 1: Site collection area of Ice cream samples in Gilgit city

ranging from $> 2.2 \times 10^3$ CFU/g to 6.8×10^4 CFU/g. The mean results indicate that the highest contamination was found in Punial Road ice cream samples 6.6×10^4 CFU/g followed by Park Hotel link Road 3.6×10^4 CFU/g, Nasim Cinema Bazaar 2.5×10^4 CFU/g, Hospital Road 8.5×10^3 CFU/g, Jamat Khana Bazar 7.7×10^3 , Yadgar chowk 4.5×10^3 CFU/g, Shaheed Milat road 3.4×10^3 CFU/g and Khazana Road ice cream samples had 2.4×10^3 CFU/g.

Table 2 show the occurrence of different bacteria in the investigated ice cream samples. *Escherichia coli* is the commonest organism and was isolated from all the samples of all the areas, *Klebsiella sp.* was isolated from 17 samples except sample numbers PRIS₂, PRIS₃ of Punial road and sample no. NCBIS₁ of Nasim Cinema Bazar. Overall isolation of *Proteus* was from 09 samples i.e. in all the samples of hospital road, in one sample of each of Punial road, Khazana road, Jamat khana Bazar, Park hotel link road Yadgar Chowk and Shaheed Millat road. *Bacillus sp.* were isolated from 4 samples, i.e. in one sample each of Punial road, Khazana road, Jamat Khana Bazar and yadgar chowk. *Salmonella* was isolated from 3 sample i.e. one each from Punial road, Nasim Cinema road and Park hotel link road. *Staphylococcus sp.* was in 10 samples i.e. in two samples of Punial road, one sample each of Hospital road and Park hotel link road, in 02 samples each of Jamat Khana bazar, Nasim Cinema bazar.

Table 3 shows incidence of different bacteria isolated during the investigation.

The infestation ratio were: *Escherichia coli* 20 (20) 100%, *Klebsiella sp.* 17 (20) 85%, *Proteus* 9 (20) 45%, *Bacillus sp.* 04 (20) 20%, *Salmonella* 03 (20) 15%, *Staphylococcus sp.* 10 (20) 50%.

Table 1: Total Viable count (CFU/g) of various bacteria isolated from ice creams samples

serial No.	Name of Area	Sample No.	Total viable Bacterial count cfu/g	
			Individual	Mean
1	Punial Road	PRIS ₁	6.8×10^4	6.6×10^4
		PRIS ₂	6.3×10^4	
		PRIS ₃	6.7×10^4	
2	Khazana Road	KRIS ₁	2.4×10^4	2.3×10^4
		KRIS ₂	2.2×10^4	
3	Hospital Road	HRIS ₁	8.4×10^3	8.5×10^3
		HRIS ₂	8.7×10^3	
		HRIS ₃	8.4×10^3	
4	Jamat Khana Bazar	JKBIS ₁	7.5×10^3	7.7×10^3
		JKBIS ₂	7.8×10^3	
		JKBIS ₃	7.6×10^3	
5	Nasim Cinema Bazar	NCBIS ₁	2.8×10^4	2.5×10^4
		NCBIS ₂	2.2×10^4	
		NCBIS ₃	2.2×10^4	
6	Park Hotel Link Road	PHLRIS ₁	2.9×10^4	3.6×10^4
		PHLRIS ₂	3.2×10^4	
		PHLRIS ₃	3.7×10^4	
7	Yadgar Chowk	YCIS ₁	4.4×10^3	4.5×10^3
		YCIS ₂	4.6×10^3	
8	Shaheed Millat Road	SMRIS ₁	3.5×10^3	3.4×10^3
		SMRIS ₂	3.2×10^3	

DISCUSSION

The results obtained in this study represent the current status of microbiological quality of ice cream being sold in Gilgit town. All the analyzed ice cream samples ($n = 20$) showed heavy contamination of notable bacteria (*E. coli*, *Klebsiella*, *Proteus*, *Salmonella* and *Staphylococcus*) which indicates fecal contamination. The presence of this high level of fecal coliforms contamination represents a public health risk due to the possible presence and transmission of pathogens such as enteropathogenic *Escherichia coli*, Hepatitis A virus, poliomyelitis virus, while *Entamoeba histolytica* may also be present in the ice cream (Arias and Windrantz, 2000). The mode of transmission of all these bacteria is fecal-oral route and or via common house flies.

The results suggest negligence such as poor sanitation during the preparation and/or storage of these products. These include the observed dirty premises and utensils used, the use of bare hands in preparing the products (personal communication with the handlers).

In this study three most important genera *Escherichia coli*, *Salmonella sp.* and *Staphylococcus sp.* were also isolated. *Escherichia coli* strains EPEC, ETEC, EHEC and invasive strains are pathogenic to children of < 5 years. *Salmonella* is still the most important acute agent causing food borne diseases (Tood, 1997). Consumption of ice cream contaminated with enteropathogenic bacteria such as *Salmonella* has been the cause of several outbreaks (Hennessy et al., 1996). In the Northern Areas of Pakistan Gilgit Ahmed et al. (2005) isolated twenty one *Salmonella typhi* from 585 suspected gastroenteritis patients. *Staphylococcus*, which is commonly *Staphylococcus aureus* when

Table 2: Bacterial Species isolated from different ice cream samples from areas

S. No.	Name of Area	Sample No.	Bacteria isolated from ice cream
1	Punial Road	PRIS ₁	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Bacillus sp.</i> ; <i>Proteus sp.</i> ; <i>Salmonella sp.</i>
		PRIS ₂	<i>E. coli</i> ; <i>Staphylococcus sp.</i>
		PRIS ₃	<i>E. coli</i> ; <i>Staphylococcus sp.</i>
2	Khazana Road	KRIS ₁	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Bacillus sp.</i>
		KRIS ₂	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Bacillus sp.</i>
3	Hospital Road	HRIS ₁	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Staphylococcus sp.</i> ; <i>Proteus sp.</i>
		HRIS ₂	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Proteus sp.</i>
		HRIS ₃	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Proteus sp.</i>
4	Jamal Khana Bazar	JKBIS ₁	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Proteus sp.</i>
		JKBIS ₂	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Bacillus sp.</i> ; <i>Staphylococcus sp.</i>
		JKBIS ₃	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Staphylococcus sp.</i>
5	Nasim Cinema Bazar	NCBIS ₁	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Proteus sp.</i>
		NCBIS ₂	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Styphalococcus sp.</i>
6	Park Hotel Link Road	PHLRIS ₁	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Salmonella sp.</i>
		PHLRIS ₂	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Proteus sp.</i>
		PHLRIS ₃	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Styphalococcus sp.</i>
7	Yadgar Chowk	YCIS ₁	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Bacillus sp.</i>
		YCIS ₂	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Proteus sp.</i>
8	Shaheed Millat Road	SMRIS ₁	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Styphalococcus sp.</i>
		SMRIS ₂	<i>E. coli</i> ; <i>Klebsiella sp.</i> ; <i>Proteus sp.</i> ; <i>Staphylococcus sp.</i>

Table 3: Incidence and percentage infestation of bacteria isolated from Ice cream

Name of bacteria isolated	Incidence	Percentage infestation
<i>Escherichia coli</i>	20 (20)	100
<i>Klebsiella sp.</i>	20 (17)	85
<i>Proteus sp.</i>	20 (09)	45
<i>Bacillus sp.</i>	20 (04)	20
<i>Salmonella sp.</i>	20 (03)	15
<i>Staphylococcus sp.</i>	20 (10)	50

transmitted from man and animal, can lead to staphylococcal food poisoning as a result of growth of the organism and release of enterotoxin into the food. Enterotoxin production and secretion occurs especially when ice cream products are not hygienically prepared and stored. The presence of starch and protein encourage enterotoxin production by the microorganisms (Wistreich and Lechtman, 1980). The possible source (s) of this organism in ice cream could be from human nose where it is commonly found; hands, skin and clothing of handlers (Hobbs and Golbert, 1982). Coughing, talking and sneezing produce droplets, which could settle on, ice cream during transportation In Gilgit most of the population produce homemade ice cream for domestic use as well as commercial purposes by using the ingredients dry milk, sugar and water.

The presence of fecal coliform indicates post-treatment contamination which may either come from water, lack of personal hygiene of the ice cream manufacturer, utensils used for ice cream and distribution environment. A study conducted by Ahmed and Shakoory (2002) reported 640-683 *E. coli* colonies /100 of drinking water. The ice cream manufacturers use the same water

for the preparation of ice cream as well as for washing of their hands and utensils. Once the ice cream become contaminated, freezing temperature later could not make the product safer (Jay, 1996).

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