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Bacteriological Examination of Locally Prepared Ice cream in Lahore, Pakistan

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Abstract

Ice cream is a milk-based product which is consumed throughout the year in Pakistan but maximum in the summer season. The ice cream is prepared with different method and ingredients are introduced at different stages which can make ice-cream contaminated by bacteria either by unhygienic conditions or usage of unsterilized mixing utensils, dirty handlers, packing materials and utensils. This study was planned to reconnoiter the bacteriological count of ice cream prepared and sold locally in Lahore, Pakistan. Total sixty samples were collected of three different flavors (mango, vanilla, kulfa) from the different areas which include Johar Town, Mall Road, Thokar Niaz Baig, Liberty market and Raiwind road of Lahore, Pakistan. Twenty samples of each flavor were collected. Mean total bacterial count (CFU/g) in mango ice cream were $2.4x10^3$, $4.47x10^3$, $6.62x10^4$, $7.62x10^3$, $8.47x10^3$, in vanilla ice cream were $2.3x10^3$, $4.5x10^3$, 6.6x104, $7.67x10^3$, $8.5x10^3$ and in kulfa ice cream were $2.27x10^3$, $4.48x10^3$, $6.5x10^4$, $7.62x10^3$ and 8.57x103, respectively. All analyzed ice-cream samples (n=60) were found contaminated with bacteria (*E. coli*, Klebsiella and *Staphylococcus aureus*). The isolation of the probable potential pathogen from the ice cream samples analyzed is of public health significance. Strict measures to control unregistered ice-cream makers and to ensure quality assurance from production to consumer is required.

Introduction

Ice cream is a nutritious frozen desert that is usually made from dairy products and is available to people of all ages especially in the summer [1]. The ingredients used to make ice cream are milk, cream, evaporated or condensed milk, milk powder, dyes, spices, fruits, nuts, sweeteners, eggs, and egg stabilizers. Each of these species can have different types of specific bacteria [2].

During ice cream production, heating greatly reduces the nutritional form of milk. On another hand, spore-bearing microorganisms may pose a risk by using this type of dairy products. The presence of pathogens in ice cream is mainly due to the transportation and distribution tools, equipment, water, labor, environment, packaging materials and other contaminants [3]. Ice cream is milk based healthful food for human. It is excellent media for microbial growth due to high nutrient value, approximately neutral pH and long storage duration. These conditions are an excellent medium for the growth of many microorganisms, including some microorganisms that can cause disease in humans i.e. Cholera, typhoid fever, bacterial dysentery, worms and hookworm infections in Guinea [4].

Many of the psychrophilic and psychologically tolerant microorganisms in their present form are due to gradual and temporal changes. *Listeria monocytogenes*, *Staphylococcus aureus*, Bacillus species, Salmonella, Shigella, Streptococcus species, Pseudomonas sp, Campylobacter sp, Brucella sp, and coliforms are commonly found in ice cream [5]. The presence of pathogenic microorganisms in ice creams such as Salmonella, *Staphylococcus aureus*, *Escherichia coli*, etc. is well known, but the detection of pathogenic bacteria such as Bacillus cereus, *Yersinia enterocolitica*, *Listeria monocytogenes*, *E. coli* O157: H7 was rarely studied. Therefore, the bacterial count of ice cream samples was observed in this research, allowing a quantitative study of the main health parameters, including total oxygen demand, *Escherichia coli*, other members of Enterobacteriaceae, Enterococcus, yeast, and molds [6].

Ice cream is one of the main products of the dairy industry and continues to dominate the interest of most people [7]. Since most ice cream consumers are children of disadvantaged groups, they must have microbiological safety [8]. In the past, *E. coli* has been used as an indicator of microbes as a measure of fecal contamination and may be the presence of intestinal pathogens in food. Although some of these *E. coli* are non-pathogenic, their presence suggests the presence of fecal contamination and the presence of intestinal pathogens that cause various diseases. *E. coli* that occur in food is meaningful, suggesting that recent corruption is likely to be associated with intestinal pathogens [9]. In developed countries, ice cream adopts quality control measures to increase its shelf life to prevent potential public health threats. The microbial status of ice cream for public health is known in Germany, but such surveys are not known in developing countries [10].

Hence, the current study was piloted to check the presence of microorganisms in the ice cream available in Lahore city of Pakistan, which ultimately provided the information about the quality of raw ingredients and the sanitary nature of processing and storage processes.

Materials and Methods

Sample Collection

Sixty ice cream samples were randomly collected from different locations of Lahore city and transported to the Microbiology Laboratory of Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan by placing into the cold box. Distribution of samples collected from different areas/location of Lahore city is given in **Table 1**. The samples were immediately processed for analysis.

Table 1: Distribution of sample collected from different areas/location of Lahore city

Sr #	Flavor	Rewind road	Thokar Naiz Baig	Johar Town	Liberty market	Mall road	Total samples
1	Vanilla	4	4	4	4	4	
2	Mango	4	4	4	4	4	60
3	Kulfa	4	4	4	4	4	

Sample processing

One gram of ice cream was aseptically transferred into 9 ml of normal saline and homogenized by vertex. Three dilutions of each sample were prepared by thorough mixing under sterile conditions to obtain 10^{-3} dilution.

Microbial count

One ml sample taken from the 3rd dilution was poured on the surface of Nutrient agar, MacConkey's agar, and Blood agar. The plates were placed in sterilized culture hood for 20 minutes to allow water to be absorbed. The total bacterial count analysis was performed after 24hrs of incubation at 37°C in the bacterial incubator. Colonies with different color and

morphological characters were selected and sub-cultured onto media plates i.e. Nutrient agar, MacConkey's gar, and Blood agar to obtain pure cultures.

Biochemical analysis

From each of the isolated pure culture, Gram's staining was performed to know whether the bacteria is Gram +ve or Gram -ve. Briefly, a drop of sterile water was placed in the center of grease-free glass slide and a loopful bacterial culture was placed, mixed in water and spread thoroughly to make a thin film of bacteria. is the slide with culture was allowed to dry and heat in the air by sliding over the Bunsen burner flame for 2-3 times. The culture was stained with crystal violet for one minute and rinsed with tap water. The iodine solution (mordant) was added to the solution for one minute and washed again with tap water. The culture was de-stained with 90% ethanol by just rinsing the slide with 90% ethanol. Excessive ethanol was removed by washing with tap water. The slides were then stained with counter stain for one minute and washed with tap water. The stained slides were examined under an oil immersion objective (100X bright field connected microscope). The staining color and micromorphological characteristics of individual bacteria culture were noted. All the isolated bacterial cultures were furthered confirmed by biochemical test according to Barge's manual of determinative bacteriology, 7th Edition [11].

Results

Bacterial count in different ice cream samples

For microbial analysis, ice cream sample was collected from five different locations of the Lahore city which were further categorized into three different flavors of ice cream (vanilla, mango, and kulfa). For the bacterial count, the colonies were counted only for the plates in which 30-300 colonies were observed. The mean CFU/g values calculated were shown in **Table 2**.

Table 2: Mean CFU/g of bacteria in mango, Vanilla, and Kulfa flavor ice cream

Sr #	Name of area	Sample size	Mean CFU/g Mango	Mean CFU/g Vanilla	Mean CFU/g Kulfa	Total samples
1	Johar town	4	2.4x103	2.3x103	2.27x103	
2	Mall road	4	4.47x103	4.5x103	4.48x103	
3	Thokar niaz baig	4	6.62x104	6.6x104	6.5x104	60
4	Liberty market	4	7.62x103	7.67x103	7.62x103	
5	Raiwind road	4	8.47x103	8.5x103	8.57x103	

Percentage of bacteria isolated from ice cream samples

Out of 20 samples of mango ice cream, 3 samples were positive for the presence of E. *coli* collected from Raiwind road, 2 from Thokar Niaz Baig, 3 from Johar town, 1 from Liberty market and no sample was observed with *E. coli* contamination from Mall road (**Table 3**). Overall, E. *coli* was present in 45% mango ice cream samples. Out of 20 samples of vanilla ice cream, *E. coli* was isolated in 2 samples taken from Raiwind road, 1 from Thokar Niaz Baig, 1 from Johar Town, 2 from Liberty market and 1 of the samples from Mall road. Collectively, 35% samples of vanilla ice cream were found contaminated with *E. coli.* Out of 20 samples of kulfa ice cream, *E. coli* was found in 4 samples collected from Raiwind road, 3 from Thokar Niaz Baig, 1 from Johar town, 3 from Liberty market and 1 of the samples from Mall road. Overall 60% kulfa ice cream samples were positive for *E. coli* presence.

Out of 20 samples of mango ice cream, Klebsiella was found in 2 samples obtained from Raiwind road, 1 from Thokar Niaz Baig, 3 from Johar Town,1 from Liberty market and 1has been found in samples from Mall road. 40% samples of mango ice cream were positive for Klebsiella. Out of 20 samples of vanilla ice cream, Klebsiella was found in 2 samples taken from Raiwind road, 1 from Thokar Niaz Baig, 1 from Johar Town, 2 from Liberty market and 1 sample from Mall road was contaminated with Klebsiella. Collectively, 35% of vanilla ice cream samples were contaminated with Klebsiella. In kulfa ice cream samples (20 samples) Klebsiella was found in 2 samples collected from Raiwind road, 2 from Thokar Niaz Baig, 2 from Johar Town,0 from Liberty market and 1 sample was positive from Mall road. 35% of kulfa ice cream samples were contaminated with Klebsiella, collectively.

In mango ice cream samples (20 samples) *S. aureus* was found in 1 sample collected from Raiwind road, 1 from Thokar Niaz Baig, 1 from Johar Town, no sample was positive for *S. aureus* from Liberty market and 2 samples from Mall road were found positive for *S. aureus*. Altogether, 25% of *S. aureus*-positive samples in mango ice cream were observed. Out of 20 samples of vanilla ice cream, *S. aureus* was isolated in

2 samples collected from Raiwind road, 1 from Thokar Niaz Baig, 1 from Johar Town,1 from Liberty market and 1 from Mall road. Collectively, out of total 20 vanilla ice cream samples 30% samples were positive for *S. aureus*. Out of 20 samples of kulfa ice cream 2 samples taken from Raiwind road, 1 from Thokar Niaz Baig, 1 from Johar Town and no sample from Liberty market and Mall road were contaminated with *S. aureus*. 20 % of total kulfa ice cream samples were contaminated with *S. aureus*.

Table 3: Percentage of different bacteria isolated from ice cream samples

Sr #	Bacteria isolated	Flavor	Raiwind Road	Thokar Niaz Baig	Johar Town	Liberty Market	MALL road	%age
		Mango	3(4)	2(4)	3(4)	1(4)	0(4)	45
		vanilla	2(4)	1(4)	1(4)	2(4)	1(4)	35
1	*E. coli*	Kulfa	4(4)	3(4)	1(4)	3(4)	1(4)	60
		Mango	2(4)	1(4)	3(4)	1(4)	1(4)	40
		vanilla	2(4)	1(4)	1(4)	2(4)	1(4)	35
2	Klebsiella	Kulfa	2(4)	2(4)	2(4)	0(4)	1(4)	35
		Mango	1(4)	1(4)	1(4)	0(4)	2(4)	25
		vanilla	2(4)	1(4)	1(4)	1(4)	1(4)	30
3	*S. aureus*	Kulfa	2(4)	1(4)	1(4)	0(4)	0(4)	20

Discussion

Ice cream is a dairy product which is a good medium for the growth of microorganisms, some of which cause human diseases. Contaminated ice cream has led to multiple outbreaks of gastrointestinal disease in many countries in Asia, Europe and North America [12]. Possible sources of these microorganisms in ice cream are reported to be ingredients in ice cream mixes such as milk and milk powder, butter, flavoring and coloring agents, as well as disinfectants [13], [14]. The results obtained in this study represent the current state of microbial quality of ice cream sold in Lahore, Pakistan. All examined ice cream samples (n = 60) showed significant contamination of different bacteria (E. coli, Klebsiella and Staphylococcus aureus) [15].

The microbiological quality of ice cream delivered from June to July 2008 in the Chittagong region of Bangladesh was investigated. Ice cream of each brand was collected in the retail outlets in Chittagong City, Bangladesh. The average SPC numbers for the ice cream samples Kwality, Igloo and Sub Zero were 2×103, 3 × 103 and 4 × 103 CFU / g, respectively. Coliforms were detected in all ice cream samples and the average number of E. coli in different samples from Kwality, Igloo and Sub Zero was 12, 18 and 42 CFU / g, respectively. Coliforms exceed the standard values and can cause serious health problems. Hosein et al. (2012) [16] studied the microbial quality of ice cream samples of traditional chocolate cream from dairy stores were collected in Tabriz and Khoy from June 2009 to October 2009. Tests were performed for total bacterial and coliform load in collected samples. The total bacterial counts of Tabriz and Khoy were 8.77 ± 0.25 log CFU/g and 6.28 ± 0.25 log CFU/g, respectively. 73.33% of the coliform contamination in all samples was higher than the Iranian standard limit (50/g). These studies showed that the overall risk of consuming traditional ice in northwestern Iran is very high [17].

Ice cream samples which we collected from Raiwind road have a high level of contamination as compared to Johar Town, Mall road and Thokar Niaz Baig. Ice cream prepared on small scales and lower levels contained more contamination than nationally and internationally produced ice cream. Results suggested that ice cream produced on a local level have higher bacteriological counts due to high negligence about hygiene during the preparation and/or storage of final products. These include dirty spots and equipment used to make products by hand (personal communication with the handlers), coughing, talking, sneezing and use of contaminated dry milk, sugar and water are also a source of contamination.

Conclusions

The results obtained from present study demonstrated that locally produced ice cream has a higher level of contamination. It is recommended to take strict actions to monitor the preparation and storage of the products to control the infections caused by contaminated food. Regular sterilization of equipment, cleaning tools, and pasteurization/cooking of milk prior to use in preparation procedure of dairy products is required.

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