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Original Article

Comparative Analysis of the Microbiological Quality between Industrially Produced and Street Vended Ice Creams Offered for Public Consumption in Dhaka City, Bangladesh

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ABSTRACT: Ice cream is a frozen food usually consumed throughout summer. Because it is nutritionally enriched food, it may harbors several potential pathogenic microorganisms. Its microbiological quality has continuously been crucially vital in aspect of public health concern. Therefore, this study was conducted to determine the bacteriological quality of industrially produced and street-vended ice-creams sold out in different areas of Dhaka city, Bangladesh. Randomly collected 49 ice-cream samples were analyzed for total viable count (TVC) (cfu/ml), coliform count (cfu/ml) and detection of pathogenic microorganisms e.g. *Staphylococcus* spp., *Escherichia coli and Salmonella* spp. Total viable count ranged from 2.3×10^3 to 1.7×10^4 cfu/ml in industrial brands whereas in street vended samples it was high in the range of 7.0×10^3 to 2.7×10^5 cfu/ml. 76 % of industrially produced and 100% of the street vended ice-cream were contaminated with total Coliform whereas 12% industrially produced and 75% street vended ice cream exceeded the limit of Bangladesh Standards and testing Institution (BSTI) standard for Coliform count. Four samples (16%) from industrially produced and 15 samples (63%) from street vendors were confirmed the presence of contamination with *E. coli.* No *Salmonella* spp. was found in industrially produced ice-cream and 6 samples (25 %) from street vendors were contaminated with *Salmonella* spp. 52% of industrially produced and 71 % of street vended samples were contaminated with *Staphylococcus aureus*.

The present study demonstrates that industrially produced ice creams are more hygienic contrast with locally produced ice creams. Locally produced ice-creams are not following the microbiological specifications of BSTI. It indicates that lack of maintenance of hygienic conditions during preparation, preservation and serving of ice cream. The presence of these pathogenic microorganisms in the samples should be seen with worry by the buyers, creating organization and the administration as food borne illness by *Escherichia coli, Staphylococcus* spp., *Salmonella* spp., is possible through consumption of contaminated ice cream. It is essential to educate local ice cream producers on good hygiene practices and good manufacturing practices to improve the quality of the product.

KEYWORDS: Ice cream, bacteriological quality, *Escherichia coli, Staphylococcus aureus*, *Salmonella* spp., Bangladesh

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INTRODUCTION

Ice cream is a nutritious food. The major component of ice cream is milk which contains significant quantity of saturated fat, protein and calcium as well as vitamin C[1]. Ice cream is consisting of milk, sweeting and stabilizing agents together with flavoring and coloring matter[2-3]. Ice cream is a nutritious frozen dairy food consumed by different age groups especially kids in the summer season. The ingredients of ice cream may be different combinations of milk, cream, evaporated or condensed milk, dried milk, coloring materials, sweetening agents, eggs and egg items, flavors, fruits, nuts, and stabilizers. However any of those ingredients may contribute microbial development and adversely affect the microbiological quality of the product which is judged by its bacterial load[4] . Also the high content of nutrients like lactose and proteins and its neutral pH make it an excellent growth medium for microorganisms some of which may cause serious disease outbreaks like Cholera, typhoid, bacillary dysentery in human beings[1]. Many psychrophiles and psychrotolerant microorganisms like *Listeria monocytogens*, *Staphylococcus aureus*, *Bacillus* species, *Salmonella* species, *Shigella* species, *Streptococus* spp., *Pseudomonas* spp., *Campylobacter* spp., *Brucella* spp. and coliform bacteria are generally present in ice cream[5-6]. The presence of these microorganisms in pasteurized ice cream indicates their capacity to survive the pasteurization process as in the case with spore formers[4] and they may persist in ice cream product thereafter. Coliforms were generally used as indicator microorganisms which demonstrate potential fecal contamination of food and water[7]. Possible sources of these microorganisms in ice cream have been documented to include raw materials used for the preparation of ice cream mix, such as milk and milk powder, cream, flavoring and coloring substances and stabilizer[1,8] and from contaminated air during processing[9-10]. The consumption rate of ice-cream is higher among children of vulnerable age groups therefore it is essential to maintain a high microbiological safe standard[9]. The likelihood of acquiring food borne illness by infant, kids, elderly individuals and immunosuppressed patients from this item is in all probability[11-12].

Therefore the aim of this research is to determine the bacteriological quality of ice cream; to understand the difference in the microbiological qualities between local and industrially produced ice creams and to assess the potentiality of ice cream to pose risk to public health.

MATERIAL AND METHODS

Sample Collection

A total of 49 samples, 24 are locally produced and 25 are industrially produced ice cream samples were purchased from hawkers and retail outlets in 6 different areas (Tejgaon, Banani, Mohakhali, Farmgate, Mirpur, Nowabgong) of Dhaka city. The samples were collected in a sterile container and brought to the laboratory immediately for proper analysis. The ice cream samples collected are analyzed twice.

Preparation of Serial Dilution

1ml of each sample transferred into a sterile test tube containing 9 ml of peptone water to give a dilution of 1: 10, homogenized by vortex and dilutions up to 10^5 were made[1, 13].

Primary Isolation of Bacteria from Samples

Total viable count (TVC), total coliform count, and Staphylococcal count were determined in nutrient agar, MacConkey agar and Mannitol salt agar respectively as per standard procedures. Eosin methylene blue agar and Bismuth sulphite agar were used for *E. coli* and *Salmonella* spp. count. Using pour plate technique, the prepared samples of concentration range from 10^{-1} to 10^{-5} were used for incubation. 1 ml of each of this dilution was inoculated on selective and differential media in duplicate and incubated at 37^{0}C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. Average of duplicate plates were counted and recorded as the numbers of colony forming unit (cfu/ml) of each ice cream sample for the observation of presence of pathogenic bacteria. The bacteria plate counts per ml were recorded using the following formula[11]:

$$\frac{n}{v \times r}$$

Where, n= Number of colonies developed on the media
r = Dilution factor
v = Volume of the particular dilution being put on the media

Purification and Storage of Isolates

Bacterial colonies that appeared on the primary isolation plates were subcultures onto fresh nutrient agar plates to obtain pure cultures of different isolates. The final cultures containing discrete colonies were transferred onto slants containing nutrient agar. The slants were stored in the refrigerator at 4˚C with transfers at intervals of 14 days for further studies outlined below.

Characterization and Identification of Isolates

The bacterial isolates from different samples were grouped on the basis of colonial morphology. The criteria used to categorized isolates were the size of the colony, color, surface, edge, slope and elevation as described by[11,14].

Microscopic Examination of the Isolates

Smears of each of the different bacterial isolates was made with a clean- grease free slide and air dried. These were directly observed under the microscope using the oil immersion objective (x100).The bacterial isolates were stained using Gram's Method of staining to study the morphological appearance of the cell as well as their Gram reactions (Whether they are Gram positive or Gram negative) and cell arrangement[15].

Biochemical Tests

Biochemical tests were performed to confirm the identity of the isolated bacteria. IMVIC test were done for coliform groups and *E. coli*, catalase, oxidase and coagulase for *Staphylococcus aureus,* Triple sugar iron (TSI), Lysin Iron agar (LIA) and Urease test for *Salmonella* spp. 24 hours old cultures were used to perform the biochemical test[2,11,16].

RESULTS AND DISCUSSION

The quality of most food is a function of the presence of total number of viable microorganisms which is revealed by the total bacterial count. However, the microbial load of food products is influenced by a variety of things for example the overall environment from which the raw materials were obtained, the environment in which it was processed, the sanitary conditions under which the food was handled and prepared, and the adequacy of processing procedures focused at reducing contaminants throughout the packaging, handling and storage of the food product[17]. Total Viable Count (TVC) was within the recommended limit of BSTI for all industrially produced ice cream samples with counts ranged from 2.3 x 10^3 to 1.7 x10^4 cfu/ml with an average of 6.19 x 10^3 cfu/ml whereas the street vendor samples exceeded the standard with counts ranged from 7.0 x 10^3 to 2.7 x 10^5 cfu/ml with an average of 6.42 x 10^4 cfu/ml. A study was directed concerning bacteriological quality of locally made open scoop ice creams sold by street vendors in various zones of Jalandhar city, Punjab, India which demonstrated heavy bacterial contamination ranging from 0.1x10^9 to 10.2x10^9 cfu/ml [18]. The microbial numbers in ice cream samples could have resulted from inadequate processing like initial inappropriate cooling of the hot ice cream mix, which can cause prompt multiplication of the microorganisms present in ice cream quickly after pasteurization[19]. It is likewise shown that high microbial count occurrence in virtually sterile mix could occur when cooled gradually at a temperature helpful for spore germination[20].

The presence of coliform is taken as a sign that other pathogenic microorganisms might likewise present in the sample[21-22]. According to BSTI standard for ice cream, the coliform count should not be more than 10/g of ice cream. In the present study, 12% of the industrially produced and 75 % of the street vended ice cream samples were contaminated with coliform. The average number of coliform count in branded samples is 4 cfu/ml and vendor sample is 15 cfu/ml which crossed the limit. Coliform isolates were further confirmed by IMViC test. Non-spore former coliforms should be susceptible to pasteurization. Their post pasteurization presence in ice cream indicates faulty heating method throughout preparation. Additionally, alternate reasons might either originate from polluted water, lack of personal hygiene of the manufacturer, utensils used for ice cream. Similar findings were also reported by[23] that the incidence of high coliform in ice creams marketed in Mangalore town, India.

The isolation and confirmation of *E. coli* was done by IMViC test. Food Safety and Standard Authority of India (FSSAI) stipulates that *E. coli* should be absent in one gram of ice cream. In the present study *E. coli,* indicative of fecal contamination, was found in 63% street vended ice cream with maximum *E. coli* count 19 cfu/ ml and 16% industrially produced ice cream with maximum *E. coli* count 3 cfu/ ml. A study directed by[1,24] reported that 640-683 colonies of *E. coli* present in 100 ml of drinking water. Such water is often the major source of contamination when used for preparation of ice cream. Once the ice creams become contaminated, freezing temperature later could not make the product safer for human consumption[25]. Comparatively low *E. coli* count is observed in branded samples, present in 4 samples out of 25 and relatively high count is observed in vendor samples, present in 15 samples out of 24.

Table 1. Enumeration of microorganisms of branded and street vended ice cream samples

Ice Cream Sample	Type of Ice cream	No. of sample analyzed	TVC cfu/ml	Coliform count cfu/ml	*E. coli* cfu/ml	*S.* aureus cfu/ml	*Salmonellaspp* cfu/ml
Branded	Brand 1	5	$5.8\times10^{3+}$	3^+	0^+	1^+	0^+
	Brand 2	5	$4.1\times10^{3+}$	4^+	0^+	2^+	0^+
	Brand 3	5	$6.2\times10^{3+}$	4^+	1^-	3^+	0^+
	Brand 4	5	$3.9\times10^{3+}$	1^+	0^+	0^+	0^+
	Brand 5	5	$1.1\times10^{4+}$	9^+	1^-	5^+	0^+
Street vended	Street vendor 1	3	$1.0\times10^{4+}$	21^-	4^-	9^+	7^-
	Street vendor 2	3	$1.6\times10^{4+}$	19^-	8^-	3^+	0^+
	Street vendor 3	5	$1.4\times10^{5-}$	18^-	5^-	10^-	0^+
	Street vendor 4	4	$1.1\times10^{5-}$	15^-	5^-	10^-	1^-
	Street vendor 5	5	$1.3\times10^{4+}$	14^-	8^-	4^+	2^-
	Street vendor 6	4	$1.7\times10^{4+}$	9^+	1^-	20^-	5^-
	Bangladesh standard		1.0x10^5 cfu/ml	10 cfu/ml	-	-	-
	Indian standard		-	-	0 cfu/ml	10 cfu/ml	0 cfu/25ml

+: complying to standard; **- :** not complying to standard

According to FSSAI *Staphylococcus aureus* should be less than 10/g of the ice cream sample. *Staphylococcus aureus* was present in 52 % of branded samples but all the count was within the prescribed limit of FSSAI. Whereas 71 % of road vended ice cream samples contained *Staphylococcus aureus* but 42 % exceeded the FSSAI standard. *Staphylococcus* isolates were further confirmed by biochemical test, Catalase and Oxidase. [26]Found that *Staphylococcus aureus* can survive better in frozen products like ice creams and can elaborate enterotoxin leading to food poisoning outbreaks. The presence of starch and protein are accounted for to support enterotoxin production. It is assumed that the potential sources of this organism in ice creams are hands, skin and covering of handlers where it is ordinarily found[11,27]. There are also some alternative sources such as coughing, talking and sneezing which produce droplets. These droplets could settle on ice cream throughout transportation, storage and retailing[1,19].

Salmonella spp. were absent in all branded ice cream but present in 25 % samples of road vendor ice cream. Three confirmatory biochemical tests such as Triple Sugar Iron (TSI) agar test, Lysin Iron Agar (LIA) test and the Urease test were performed for identification of *Salmonella* isolates. According to FSSAI standard, *Salmonella* spp. should be absent in 25 gram of the sample. Salmonella still the most important agent causing acute food borne illness[2,28]. Consumption of ice cream contaminated with *Salmonella* spp. has been the reason behind several disease outbreaks[5].

Table 2. Incidence and Percentage Infestation of Bacteria Isolated from Ice Cream.

Sample	Parameters	Average Count(cfu/ml)	Incidence	Percentage (%) Infestation
Branded Ice cream	Total Viable Count	6.2×10^3	0/25	0
	Coliform Count	4	3/25	12
	S. aureus	2	13/25	52
	E. coli	1	4/25	16
	Salmonella spp.	0	0/25	0
Street Vendor Ice cream	Total Viable Count	6.4×10^4	6/24	25
	Coliform Count	1.6×10^1	18/24	75
	S. aureus	9	17/24	71
	E. coli	5	15/24	63
	Salmonella spp.	2	6/24	25

CONCLUSION

In conclusion, results obtained in this study showed that the presence of potential pathogenic microorganisms within the analyzed ice cream samples should be viewed with concern by the customers and also the public health authorities. The presence of *Escherichia coli* and *Salmonella* sp. proposed that ice cream could be a source of infections to human by the members of the enteric pathogenic microorganism, such as *Klebsiella* sp., *Shigella* sp. and *Proteus* sp. Several steps during production can cause bacteriological hazards. Though pasteurization of milk can destroy most of the pathogens posing risk to public health, yet, the potential bacteriological hazards can still be found in the final products after pasteurization through cross contamination or improper handling. To improve quality of ice cream, Good manufacturing practice (GMP) is obligatory particularly at all steps of post pasteurization. The use of good quality raw materials and automatic machines to reduce handling will be effective in assuring good quality of ice cream. Adoption of good sanitation practices and application of the HACCP principles within the system along with education of workers on personal hygiene will definitely improve the quality of ice cream.

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