EVALUATION OF MICROBIOLOGICAL QUALITY AND SAFETY OF LOCALLY PROCESSED ICE CREAM IN DHAKA CITY, BANGLADESH

Abu Hayat Mahmudun Nabi[1], Md. Nazmul Islam[2], Md. Sajib Al Reza[2,*], Md. Azizul Haque[2], Mesbah Uddin Talukder[2], Shamoli Akter[2], Md. Zainul Abedin[2], Md. Abu Zubair[2]
[1] Department of Microbiology, Stamford University, Dhaka, Bangladesh
[2] Department of Food Technology and Nutritional Science,
Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh
[*] E-mail: sajib.ftns2010@gmail.com

Abstract
Ice cream, a milk-based product, can be a good source for a microbial growth due to its nutrient content, optimal pH and long storage even though it is stored in a frozen state. A study was conducted to assess the microbiological quality and safety of locally processed ice cream in Dhaka city, Bangladesh. Seven different types of ice cream samples were collected from three local vendors in three different areas of Dhaka city. The APC was found ranging from 4.8 × 10^4 to 1.46 × 107 CFU ml^{-1} (log 4.68 and log 7.16 respectively) in all the samples. The Staphylococcus spp. counts were obtained from 9.0 × 10^2 to 2.1 × 10^4 CFU ml^{-1} (log 2.95 and 4.31 respectively). TFC was present in all samples. TFC was found ranging from 9.8 × 10^2 to 2.1 × 10^4 CFU ml^{-1} (log 4.32 and 2.99 respectively). Out of 21 samples 16 samples (76.19%) and 12 samples (57.14%) were contaminated with coliform and fecal coliform respectively. Coliform counts were fairly high (>10 MPN ml^{-1}), pointed to the poor hygienic practices. Four samples (19.04%) were confirmed to have contamination with Salmonella spp and two samples (9.52%) were contaminated with S. aureus. The biochemical tests clearly indicated that most ice cream samples were contaminated with pathogenic bacterial strains which are responsible for infectious diseases. The current study reveals that locally processed ice cream are not conforming with microbiological as well as biochemical specifications and thus conferred that locally processed ice cream in Dhaka city may not be safe for consumption.

Key words: Ice cream, Microbiological quality, *Salmonella*, *Staphylococcus aureus*, Biochemical tests

Submitted: 23.05.2016 Reviewed: 15.07.2016 Accepted: 02.09.2016

1. INTRODUCTION

A milk based sweetened frozen food named ice cream is widely popular among all groups of people irrespective of age and sex. Females and children are more interested in ice cream. Ice cream is manufactured mainly from milk other than fat, sugar, fruits, nuts, candies, syrup, flavoring and stabilizer with or without addition of color and after whipping allowed to freeze (Arbucle and Frandsen, 1966). It is vended in packages or in open containers at retail outlets/ice creams parlours. The open variety is handed out manually in cones, scoops or sundaes (Warke *et al.*, 2000). Children of vulnerable age groups are the major consumers of ice cream, so it is required to be microbiologically secure (Warke *et al.*, 2000). Different raw materials like raw milk and water considered the primary sources, whereas utensils, flavouring agents and handling considered the secondary sources of microbial contamination to ice cream. It may become infected with microorganisms during processing, transportation and storage (Hossain *et al*., 2012). During retail marketing, the microbiological safety and quality of ice cream principally depends upon the post manufacture handling of the commodity, as well as the sanitary conditions and efficiency of frozen storage.

Different local and international brands ice cream is sold in Dhaka city especially during the summer and also through the year. Ice cream quality mainly depends on two factors: extrinsic factors, i.e. manufacture procedure and intrinsic factors, i.e. ratio of ingredients used (Hankin and Hanna,, 1984). Milk is highly nutritious in nature and it is a main constituent of ice cream which serves as

favourable growth medium for microorganisms. Its moderate pH, high w_a (water activity) and available nutrients are favourable for microbial growth. Milk serves as a possible vehicle for pathogenic transmission and also permits these microorganisms to multiply, grow and produce certain toxins (Houghtby *et al*., 1992). Though freezing, pasteurization and hardening operations in ice cream production can remove most of the microbial hazards, but still some hazardous organism and their toxins are persistent due to different conditions.

Pasteurization can kill most of the microorganisms; but, they can be present in milk after pasteurization as post-pasteurization contaminants because of poor sanitation practices (Hankin and Hanna,, 1984).

Many psychrotolerant and psychrophiles microorganisms such as *Salmonella, S. aureus*, *Listeria* etc. are normally present in ice cream (Joshi *et al.*, 2004; Fuhr, 1986). These pathogenic microorganisms contaminated ice creams resulted in several disease outbreaks in some countries of Europe, North America and Asia (Chug, 1996).

Since Bangladesh does not have any structured food control authority to make sure safety of food supplies. It is very complicated to make certain whether the ice cream processed and marketed in Bangladesh is hygienically safe and sound and without any hazard related to public health. Therefore, this study designed to evaluate the microbiological quality and safety of locally processed ice cream in Dhaka city.

2. MATERIAL AND METHODS

2.1. Sample Collection

A total of 21 locally processed ice cream samples of 7 different types (Cone ice cream, Chockbar ice cream, Cup chockbar ice cream, Lolly ice cream, Kulfi (A), Kulfi (B) & Kulfi (C)) were collected randomly from 3 different area of Dhaka city. These ice cream samples were transferred aseptically in ice box. The experiments were carried out in the Food Microbiology Lab, Institute of Food Science & Technology (IFST), BCSIR. The temperature of the samples was maintained -20 °C until the experiment were carried out.

2.2. Sterilization

For effective sterilization or destruction of all organisms, all the glassware, most types of media cloths, rubber & others materials were subjected to Autoclave (ALP Co. Ltd., Tokyo, Japan) for pressure sterilization using steam.

2.3. Sample processing for microbial analysis

The ice cream was kept in water bath at 45 °C before taking samples, as per recommendation of Harrigan and McCance (1976); Rahman (1997). After thawing & when the liquefaction is completed. The top is so opened that the sterile pipette could be introduced for collection of samples. About 100 ml of liquid ice cream was pipette out from different depths and transferred into a sterile glass bottle fitted with a screw capped stopper. Hundred milliliter samples were taken in the measuring cylinder and then tenfold dilution was used to dilute the samples serially. Selected dilution for samples preparation was sterile by 0.1% peptone water at pH 6.8-7.0. When require, decimal dilutions were prepared according to standard method given by APHA (APHA, 1958; APHA, 1960).

2.4. Total Aerobic Plate Count (APC)

Enumeration of APC, were performed according to BAM (1995). The prepared samples of appropriate concentrations were used for inoculation by using pour plate technique. One millilitre of each of this dilution was inoculated onto nutrient agar (Plate count agar medium) (Oxoid, UK). The plates were allowed to incubation at 37 °C for 48 hours. The number of mesophillic aerobic bacteria per ml of sample was obtained in selected petridishes at level of dilutions dividing by obtained value. All colonies were counted using a colony counter (Yc-2A, Prama optical words Ltd, Tokyo). The actual numbers of bacteria were estimated as colony forming unit (CFU ml^{-1}).

The bacteria plate counts per ml per dilution were recorded using $\frac{n}{V \times R}$

Where,
n = Number of colonies
R = Dilution factor
V = Value of the particular dilution being put on the medium.

2.5. Detection and enumeration of *Staphylococci*

Spread plate method was used to identify *Staphylococci*. So 1 ml samples were inoculated in Mannitol salt agar (MSA) (Oxoid, UK) and incubated at 37 °C. Colonies were identified by morphological characteristics and biochemical properties. Then the TSC (Total Staphylococcal count) was calculated. The results of the TSC were expressed as the number of colony forming units per ml (CFU ml^{-1}).

2.6. Enumeration of Fungi

Total Fungal Count (TFC) was enumerated using potato dextrose agar by BAM (1995). 0.2 ml of raw sample of the homogenate was spreaded over each Petri dish containing Potato Dextrose Agar (PDA) (Oxoid, UK). The sample was homogenously distributed on the plate using a glass spreader in a backward and forward movement while rotating the plate. Then the plates were incubated properly.

2.7. Conformation and enumeration of Coliform and Fecal Coliform bacteria

Coliform bacteria were identified by MPN method by Lauryl Tryptose Broth (LTB) (Oxoid, UK) (Andrews, 1992). Gas formation in the tube containing LTB (Oxoid, UK) incubated at 37 °C for 24-48 hours indicate the positive presence of Coliform bacteria. Negative tubes were re-inoculated for 24 hours. Then tubes showing gas production were recorded. All tubes of the presumptive test producing gas at end of the 48 hours were submitted to the confirmation test. One loopful of presumptive positive broth was transferred to the Brilliant Bile Green Broth (BGBB) (Oxoid, UK) in the fermentation tubes and then incubated for 48 hours at 35 °C. Gas production within these hours indicated positive confirmation test. One or more plates containing Eosine Methylene Blue (EMB) agar media (Oxoid, UK) from presumptive positive test tubes were streaked in such a way so that discrete colonies may appear. Then the plates were incubated at 35 °C for 24 hours. Typical colonies are nucleated, with or without metallic sheen. Atypical colony was Opaque unnucleated mucoid after 24 hours incubated and pink colored. API-20E strips (BioMerieux S. A., Marcy-1'Etoile, France) and API-20E catalogue were used to confirm presence of *E. coli.* Faecal contamination was identified by the presence of *E. coli.*

2.8. Detection of *Salmonella spp.*

For detection of *Salmonella,* sample was inoculated for 24 to 48 hours at 37 ºC in Lactose Broth (LB) (Oxoid, UK). As there was no color or gas formation then 1 ml of pre-enriched culture was inoculated with Selenite broth (SB) (Oxoid, UK) at 37 ºC for 24 hours. The enriched culture was streaked on Bismuth sulfite agar (BSA) (Oxoid, UK) plate and incubated at 37 ºC for 24 hours. On BSA plate usual *Salmonella* colonies appear brown or gray to black in color sometimes with a metallic sheen. Some strains generate green colonies with little or no darkening of the surrounding. Two or more of each suspect colony from BSA plate was picked to slants of Triple Sugar Iron (TSI) agar (Oxoid, UK) and incubated at 37 ºC for 24 hours. *Salmonella* suspected cultures on TSI show alkaline (red) slants and acids (yellow) butts, with or without H_2S production (Blacking of the agar).

2.9. Detection of *Staphylococcus aureus*

For all ice cream samples, 25 ml was added to 225 ml of Ringers solution (Oxoid, UK). One millilitre from this dilution was transferred to 3 test tubes of Tryptone Soy Broth (TSB) (Oxoid, UK) with 10% sodium chloride (NaCl) and 1% sodium pyruvate (Andrews, 1992). The tubes were incubated at 37 °C for 48 hours. From tubes that were turbid and showing gas production after incubation, a loopful was streaked on Baird Parker Agar (BPA) (Oxoid, UK) supplemented with Egg Yolk Tellurite Emulsion (FD 046) (HiMEDIA). The plates

were incubated at 37° C for 24-48 hours. Colonies that were jet black with entire edges on BPA agar which are suspected to be *S. aureus* were transferred to tubes with 3 ml Brain Heart Infusion broth (BHI) (Oxoid, UK) for further verification. 0.5 ml of Rabbit coagulase plasma (Pro-Lab Diagnostics) was added to the tubes and incubated at 37 °C for 6 hours.

After incubation tubes were observed for clot formation. The cultures with *S. aureus* were stored on Tryptone Soy Agar (TSA) (Oxoid, UK) slants for identification using API-STAPH strips (BioMerieux S. A., Marcy-l'Etoile, France).

2.10. Biochemical test

Indole test, Methyl-red test (MR), Voges-Proskaur test (VP), Citrate utilization test, Urease test were performed to confirm the identification and differentiate the microbial population isolated from the ice cream samples (Prescott *et al*., 1999). In this purpose 24 hours old cultures were used.

3. RESULTS AND DISCUSSION

RESULTS

Twenty one locally produced ice cream samples belonging to seven types were collected from different retail stores located at Dhaka city.

All the samples were transported in an air tight ice box and kept in the deep freezer of the laboratory until analysis. APC, TSC, Coliform count, Fecal coliform count, Fungi, *Salmonella spp, S. aureus* and biochemical analysis were performed for the microbiological quality analysis of ice cream samples.

3.1. Aerobic plate count and *Staphylococcal* count

The APC was found ranging from 8.2×10^4 to 2.57×10^6 CFU ml^{-1} in cone ice cream, 2.9×10^6 to 4.8×10^4 CFU ml^{-1} in chocobar ice cream, 4.4×10^6 to 1.72×10^7 CFU ml^{-1} in cup ice cream, 4.3×10^6 to 1.20×10^7 CFU ml^{-1} in lolly ice cream, 8.4×10^4 to 1.9×10^6 CFU ml^{-1} in kulfi (A), 5.3×10^4 to 1.12×10^6 CFU ml^{-1} in kulfi (B) & 1.43×10^6 to 9.2×10^6 CFU ml^{-1} in kulfi (C) respectively (Table 1).

The *Staphylococcal* count was found range from 2.84×10^3 to 7.69×10^3CFU ml^{-1} in cone ice cream, 1.325×10^4 to 1.8×10^4 CFU ml^{-1} in chocobar ice cream, 1.55×10^3 to 2.88×10^3 CFU ml^{-1} in cup ice cream, 9.0×10^2 to 2.1×10^4 CFU ml^{-1} in lolly ice cream, 5.55×10^3 to 1.68×10^4 CFU ml^{-1} in kulfi (A), 8.5×10^3 to 1.46×10^4 CFU ml^{-1} in kulfi (B) & 1.05×10^3 to 1.845×10^4 CFU ml^{-1} in kulfi (C) correspondingly (Table 1).

3.2. Total fungal count

The result presented in Table 2 showed the TFC of 21 ice cream samples of seven different types.

The fungal loads found were not uniform and varied quite considerably. The average counts per ml of Cone ice cream, Chockbar ice cream, Cup chockbar ice cream, Lolly ice cream, Kulfi (A), Kulfi (B) and Kulfi (C) were log 4.4, log 3.69, log 3.99, log 3.92, log 4.14, log 3.99 and log 3.98 respectively.

The maximum and minimum range of total fungal load per ml of ice cream samples belonging to Cone ice cream, Chockbar ice cream, Cup chockbar ice cream, Lolly ice cream, Kulfi (A), Kulfi (B) and Kulfi (C) varied from log 3.89 to log 4.32, log 2.99 to log 4.14, log 3.67 to log 4.31, log 3.71 to log 4.08, log 3.85 to log 4.32, log 3.79 to log 4.18 and log 3.69 to log 4.22 respectively (Table 2).

The highest count was (2.1×10^4 CFU ml^{-1}) observed in NKA ice cream sample collected from Newmarket and lowest count (9.8×10^2 CFU ml^{-1}) was in SCH ice cream sample collected from Saydabad.

3.3. Total coliform and fecal coliform count

The highest coliform counts were found 28 MPN ml^{-1} in NCO, GCH, GCC, SCC, NL, GKA, NKA, GKB, SKB, GKC, NKC and SKC samples (Table 3). 76.19% of the locally produced ice cream sample was contaminated with coliform. Coliforms were detected in 16 samples of the 21 experimental samples. Most of the Samples that collected from Gulistan contaminated with highest number of coliform.

Table 1. Total number of aerobic and *Staphylococcal* count of ice cream samples

Type of Samples	Sample Code number	Name of Sampling Site	Aerobic plate count (cfu ml^{-1})	Log_{10} per ml	*Staphylococcus spp.* count (cfu ml^{-1})	Log_{10} per ml
Cone ice cream	GCO	Gulistan	8.2×10^4	4.91	4.1×10^3	3.61
	NCO	New Market	1.41×10^6	6.15	7.69×10^3	3.88
	SCO	Saydabad	2.57×10^6	6.41	2.84×10^3	3.44
Chockbar ice cream	GCH	Gulistan	3.76×10^6	6.58	1.8×10^4	4.25
	NCH	New Market	2.9×10^6	6.46	1.32×10^4	4.12
	SCH	Saydabad	4.8×10^4	4.68	4.25×10^3	3.62
Cup ice cream	GCC	Gulistan	1.46×10^7	7.16	1.79×10^3	3.25
	NCC	New Market	4.4×10^6	6.64	2.88×10^3	3.45
	SCC	Saydabad	1.72×10^7	7.24	1.55×10^3	3.19
Lolly ice cream	GL	Gulistan	1.20×10^7	7.07	9.0×10^2	2.95
	NL	New Market	4.3×10^6	6.63	2.1×10^4	4.31
	SL	Saydabad	9.6×10^6	6.98	9.25×10^3	3.97
Kulfi(A)	GKA	Gulistan	8.4×10^4	4.92	5.55×10^3	3.74
	NKA	New Market	1.56×10^6	6.19	7.6×10^3	3.88
	SKA	Saydabad	1.9×10^6	6.28	1.68×10^4	4.22
Kulfi (B)	GKB	Gulistan	1.12×10^6	6.04	1.46×10^4	4.16
	NKB	New Market	8.9×10^4	4.95	1.26×10^4	4.10
	SKB	Saydabad	5.3×10^4	4.72	8.5×10^3	3.93
Kulfi (C)	GKC	Gulistan	9.2×10^6	6.96	1.71×10^4	4.23
	NKC	New Market	2.56×10^6	6.41	1.05×10^3	4.02
	SKC	Saydabad	1.43×10^6	6.16	1.845×10^4	4.26

Note. GCO: Cone ice cream from Gulistan; NCO: Cone ice cream from New Market; SCO: Cone ice cream from Saydabad; GCH: Chockbar ice cream from Gulistan; NCH: Chockbar ice cream from New Market; SCH: Chockbar ice cream from Saydabad; GCC: Cup ice cream from Gulistan; NCC: Cup ice cream from New Market; SCC: Cup ice cream from Saydabad; GL: Lolly ice cream from Gulistan; NL: Lolly ice cream from New Market; SL: Lolly ice cream from Saydabad; GKA: Kulfi (A) from Gulistan; NKA: Kulfi (A) from New Market; SKA: Kulfi (A) from Saydabad; GKB: Kulfi (B) from Gulistan; NKB: Kulfi (B) from New Market; SKB: Kulfi (B) from Saydabad; GKC: Kulfi (C) from Gulistan; NKC: Kulfi (C) from New Market; SKC: Kulfi (C) from Saydabad

The lowest counts were 17 MPN ml^{-1} found in SL sample collected from Saydabad. It was found that highest fecal coliform counts were 20 MPN ml^{-1} found in NKA sample and lowest counts were 6.1 MPN ml^{-1} found in GKC sample collected from Gulistan. 57.14% of the ice cream sample was contaminated with fecal coliform. Fecal coliforms were detected in 12 samples of the 21 studied samples.

3.4. Occurrence of pathogenic bacteria in ice cream samples

The pathogenic bacteria *Salmonella spp.* was found in GKA, GKB**,** NKB and SKC ice cream sample (Table 4). Out of 21 samples, 4 samples (19.04%) were contaminated with *Salmonella spp*. Most samples that collected from Gulistan were contaminated with *Salmonella spp.* In this study, among 21 samples *S. aureus* was found in 2 samples. 9.52% sample was contaminated with *S. aureus.* The pathogenic bacteria *S. aureus* was found in only two samples such as NKA & SKB collected from Newmarket & Saydabad respectively.

Biochemical tests results revealed the pathogens presented in the ice cream samples which are responsible for infectious diseases are summarize in Table 5.

DISCUSSION

It has been well known that ice cream is a favorite food item for all groups of people. But this is also very good source of microorganisms. Ice cream might have different groups of microorganisms including spoilage and pathogenic ones.

Table 2. Total fungal count from ice cream samples

Type of Samples	Sample Code number	Total fungal count (cfu ml^{-1})	Log_{10} per ml
Cone ice cream	GCO	8.2×10^3	3.91
	NCO	7.88×10^3	3.89
	SCO	4.70×10^3	4.31
Chockbar ice cream	GCH	9.03×10^3	3.96
	NCH	1.376×10^4	4.14
	SCH	9.8×10^2	2.99
Cup chockbar ice cream	GCC	2.037×10^4	4.31
	NCC	4.72×10^3	3.67
	SCC	1.036×10^4	4.01
Lolly ice cream	GL	5.15×10^3	3.71
	NL	1.21×10^4	4.08
	SL	9.69×10^3	3.99
Kulfi(A)	GKA	7.1×10^3	3.85
	NKA	2.1×10^4	4.32
	SKA	1.82×10^4	4.26
Kulfi (B)	GKB	1.013×10^4	4.00
	NKB	6.13×10^3	3.79
	SKB	1.502×10^4	4.18
Kulfi (C)	GKC	1.102×10^4	4.04
	NKC	4.9×10^3	3.69
	SKC	1.669×10^4	4.22

Table 3. Total coliform and fecal coliform count by MPN method

Type of Samples	Sample Code number	Coliform count (MPN ml^{-1})	Fecal coliform count (MPN ml^{-1})
Cone ice cream	GCO	Nil	Nil
	NCO	28	Nil
	SCO	Nil	Nil
Chockbar ice cream	GCH	28	12
	NCH	20	17
	SCH	Nil	Nil
Cup chockbar ice cream	GCC	28	17
	NCC	Nil	Nil
	SCC	28	14
Lolly ice cream	GL	Nil	Nil
	NL	28	Nil
	SL	17	11
Kulfi (A)	GKA	28	14
	NKA	28	20
	SKA	24	Nil
Kulfi (B)	GKB	28	17
	NKB	24	Nil
	SKB	28	10
Kulfi (C)	GKC	28	6.1
	NKC	28	12
	SKC	28	10

Note. MPN: Most probable Number

Table 4. Occurrence of pathogenic bacteria in the ice cream samples

Type of Samples	Sample Code number	*Salmonella spp.*	*Staphylococcus aureus*
Cone ice cream	GCO	Not found	Not found
	NCO	Not found	Not found
	SCO	Not found	Not found
Chockbar ice cream	GCH	Not found	Not found
	NCH	Not found	Not found
	SCH	Not found	Not found
Cup chockbar ice cream	GCC	Not found	Not found
	NCC	Not found	Not found
	SCC	Not found	Not found
Lolly ice cream	GL	Not found	Not found
	NL	Not found	Not found
	SL	Not found	Not found
Kulfi(A)	GKA	Found	Not found
	NKA	Not found	Found
	SKA	Not found	Not found
Kulfi (B)	GKB	Found	Not found
	NKB	Found	Not found
	SKB	Not found	Found
Kulfi (C)	GKC	Not found	Not found
	NKC	Not found	Not found
	SKC	Found	Not found

Table 5. Summary of Biochemical tests performed

Type of Samples	Sample Code number	Indole	MR	VP	Citrate utilization	Suspected Microorganisms
Chockbar ice cream	GCH	+	+	-	-	*E.coli*
	SCH	-	+	-	-	*Shigella spp.*
Cup Chockbar ice cream	GCC	+	+	-	-	*E.coli*
	NCC	-	-	+	+	*Enterobacter spp.*
Lolly ice cream	SL	-	+	-	-	*Shigella spp.*
Kulfi(A)	GKA	-	-	+	+	*Enterobacter spp.*
	SKA	-	+	+	+	*Hafnia spp.*
Kulfi (B)	GKB	+	+	-	-	*E.coli*
	SKB	-	+	+	+	*Hafnia spp.*
Kulfi (C)	GKC	-	+	+	+	*Hafnia spp.*
	NKC	+	+	-	-	*E.coli*
	SKC	-	-	+	+	*Enterobacter spp.*

Note. +: Positive; - : Negative

This study aimed to find out different spoilage and pathogenic bacteria from locally produced ice cream sold in different place of Dhaka city and to characterize them for assessment. Depending on the availability of water, bacterial growth could be rapid in melted ice cream. If melted ice cream were contaminated and permitted to stay at elevated temperatures would make the product hazardous (Joshi *et al*., 2004).

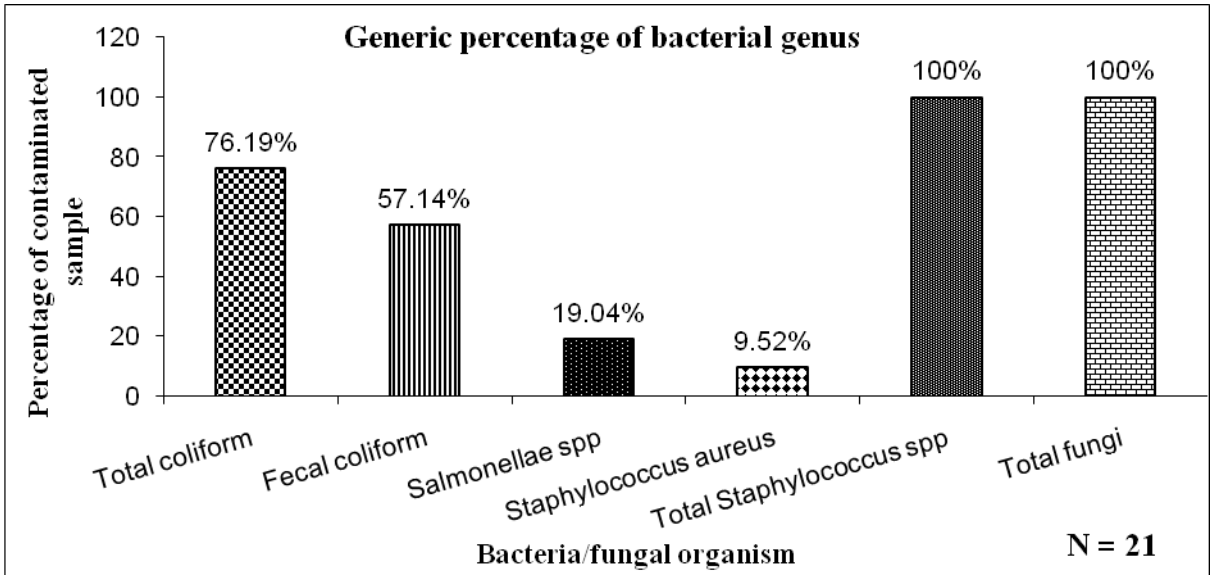

Figure 1. Percentage of sample contaminated with different types of microorganisms

All analyzed ice cream samples showed positive growth on Plate count agar indicating the presence of psychrophilic microorganisms (Joshi *et al*., 2004). In present study, total APC ranging from 4.8×10^4 to 1.72×10^7 CFU ml^{-1}. From these findings, it was revealed that most of the brands of ice cream samples were not within acceptable limit of public health safety because the samples exceeded the total viable count (1,00,000 CFU ml^{-1}) permitted under regulation (Hankin and Hanna, 1984; Marion, 1954; Vietoris *et al*., 2016).

Staphylococcus spp. was present in all the samples. Similar findings were reported by Shahriar *et al*. (2015). Most samples were not acceptable in Staphylococcal count according to FDA (2013) standard. The possible sources of these organisms in ice cream could be from coughing, talking and sneezing produce droplets which could settle on ice cream during transportation, storage and retailing (Ojokoh, 2006).

As per Bangladesh Standard and Testing Institute (BSTI) standard, the coliform count in ice cream should not be more than 10 MPN ml^{-1} **(**Rahman, 1997). The highest coliform counts were found 28 MPN ml^{-1}. In the present study, 76.19 % of the ice cream samples were contaminated with coliform. However, 57.14% ice cream samples were exceeded BSTI standard for coliform. It was found that highest fecal coliform count were 20 MPN ml^{-1}. 57.14% of the ice cream sample was contaminated with fecal coliform. The coliform standards for ice cream should not be over 10 MPN ml^{-1} (Frazier and Westhoff, 1958; James and Jay, 1978).

The present investigation however showed significantly high coliform counts in most ice cream samples. Hence, it could be taken into consideration as quality of ice cream was not good. It is known that coliforms play role in determining the hygienic quality index of food. The possible reasons behind these high counts were unhygienic processing. Besides this, most of samples might be contaminated during raw material processing, manufacturing, packaging and distribution (Hossain *et al*., 2012). The presence of coliform bacteria indicated the presence of fecal contamination in ice cream. Because these microorganisms are usually transmitted via fecal, oral route (Joshi *et al*., 2004).

The results also suggested the poor sanitation during the preparation and/or storage of these products. The occurrence of coliform and fecal coliform indicated presence of other harmful and pathogenic microorganisms such as

*Listeria monocytogens, S. aureus, Bacillus spp., Salmonella spp., Shigell*a *spp., Streptococus spp., Pseudomonas spp., Campylobacter spp., Brucella spp.* (Joshi *et al*., 2004; Omar *et al*., 2007).

The pathogenic bacteria *Salmonella spp* and *S. aureus* were found in four (19.04%) and two (9.52%) ice cream samples respectively. The counts for *Salmonella* and *S. aureus* were generally low relative to coliform, fecal coliform and *Staphylococcus spp*. As per the standards, *Salmonella* should be absent in 25 ml^{-1} of the sample (Omar *et al*., 2007). According to BSTI *S. aureus* should be less than 10 ml^{-1} of the sample **(**Rahman, 1997).

In this study 2 (9.52%) samples out of 21 were positive for *S. aureus* but exceeded the BSTI standard. The factors that contribute mostly to Staphylococcal food-borne outbreaks may be due to inadequate refrigeration, infected persons practicing poor personal hygiene, inadequate heat processing and holding food in warming devices at bacterial growth temperature.

The presence of Staphylococcus, mainly S. aureus when transmitted from animal and man, organism grows in number and release enterotoxin in the food results in staphylococcal food poisoning.

Production and secretion of enterotoxin occurs when ice cream products are not appropriately prepared and stored. Starch and proteins present in food also encourages enterotoxin production by microorganisms (Joshi *et al*., 2004).

In this study, total fungal count was observed in all samples. The highest count was 2.08×10^4 CFU ml^{-1} and lowest count was 9.8×10^2 CFU ml^{-1}. This finding suggested that there was a chance to have mycotoxins in these ice creams (Carlsson, 2006).

Escherichia coli, *Salmonella spp*., *Shigella spp*., *Enerobacter spp*. and *Hafnia spp*. were isolated and identified from the samples by various biochemical tests. Coliforms being non-spore formers should be susceptible to pasteurization.

Their post pasteurization presence in ice cream may be due to faulty heat process or to post pasteurization contamination by handlers with poor sanitary practices. The level of presence of these organisms in food has been described as index of food hygiene (Frazier and Westhoff, 1978). The presence of the organisms has great health concern as some of them may be capable of causing various ailments of man which may be fatal (Ojokoh, 2006).

Present work indicated that ice creams sold in small portions from bulk containers, exposed to the open air, have high microbial load, indicating low hygienic quality of the products. This finding also supports by previous studies (Shahriar *et al*., 2015; Hossain *et al*., 2012; Das *et al*., 2015).

These high counts may originate from the initial microflora of raw milk, the other ingredients, the environment, insufficient heat treatment and poor personal hygiene (Dubravka et al., 2012). It has been stated that production of ice cream locally on a small scale rather than industrially is a major factor associated with contamination of ice cream.

4. CONCLUSIONS

The current investigation has showed a poor level of hygiene in locally produced ice cream in Dhaka city, Bangladesh.

In this study, the count of microorganisms was exceeding the suggested criteria and the existence of some groups of pathogenic bacteria might impose a threat for public health, such pathogens may add to the risk of causing food borne illness principally for children and vulnerable elderly people.

This study has given overall bacterial load and quality of some particular ice cream products at a look. It is now crucial to focus on food safety and the food production with special care in order to eliminate almost entirely the attendance of any pathogen contamination.

Implementations of GMP and HACCP are mandatory especially at all post pasteurization steps will improve quality of ice cream. Staff training and transfer of knowledge especially in relation to food safety, good hygiene and sanitation practices, handling, maintaining and

cleaning of machine and tools may improve the situation.
Further improvements should be sought in personnel hygiene and the general hygiene conditions of post-pasteurization and at retail level. These steps will be effective in assuring quality.

5. ACKNOWLEDGEMENT

The authors would like to thank the Food Microbiology Lab, Institute of Food Science & Technology, Dhaka, Bangladesh for support to carry out this research work.

6. REFERENCES

[1] Arbucle, W. S., and Frandsen, J. H. M. S., 1966, Study on ice cream and related products. 3rd Ed. 2nd Revised printing. New York.12, 543-547.
[2] Warke R., Kamat A., Kamat M., Thomas P., 2000. 'Incidence of pathogenic psychrotrophs inice creams sold in some retail outlets in Mumbai, India. Food Control. 11(2), 77-83.
[3] Hossain K. M. H., S. M. Lutful Kabir, M. Mufizur Rahman, M. Bahanur Rahman, Khair Ahmed Choudhury, 2012. Organoleptic and Microbial Quality of Ice Cream 90 Sold at Retail Stores in Mymensingh, Bangladesh. Journal of Microbiology Research, 2(4): 89-94. DOI:10.5923/j.microbiology.20120204.05
[4] Hankin, L., & Hanna, J. G., 1984. Quality of ice cream and ice milk. Bulletin Connecticut Agricuiturai Experiment Station. 818, 6
[5] Houghtby G. A, Maturn L. J and Koeing E. K., 1992. Microbiological count methods. In: Marshall TR. (editor), Standard Methods for the Examination of Dairy Products, 16th edition., Washington DC.
[6] Joshi, D. R., Shah, P. K., Manandhar, S., Sharma, S., Banmali. P., 2004. Microbial quality of ice cream sold in Kathmandu. Journal of Nepal Health Research Council, 2(2) 37-40.
[7] Fuhr, R., 1986. Bacteriological quality of different types of ice cream and milk shake during 1980-1985.
[8] Chug, K., 1996. Salmonelia outbreak from ice cream. Indian Pediatrics. 33, 976-977.
[9] Harrigan, M. F., and McCance, M. E., 1976, Laboratory methods in food and dairy microbiology. Academic Press, London, England. pp. 193
[10] Rahman, M. M., 1997, Practical food microbiology, Bangla Academy, Dhaka Bangladesh, pp. 70
[11] American Public Health Association, 1958, Recommended methods for the Microbiological Examination of Food, APHA Inc., New York.
[12] American Public Health Association, 1960, Standard Methods for the Examination of Dairy Products. 11th ed. APHA Inc., New York
[13] BAM, 1995. Compendium of methods for the microbiological examination of foods. Food and Drug Administration Bacteriological Analytical Manual, USA.
[14] Andrews W., 1992. Manual of Food Control. 4 Rev. 1. Microbiological Analysis. Food and Agriculture Organisation of the United Nations (FAO): Rome.
[15] L. Prescott, M. Harley, P. John and K. Donald, Microbiology 3rd Edition, 1999.
[16] Marion, V., 1954. Maximum limits of bacterial count in ice cream. Annali della Sanita Pubblica. 13(4), 1075-82
[17] Vietoris V, Zajac P, Zubrická S, Čapla J, Čurlej J,, 2016. Comparison of total acterial count (tbc) in bulk tank raw cow's milk and vending machine milk. Carpathian Journal of Food Science and Technology. 8(1), 184-191
[18] FDA Circular No. 2013-010. 27 February 2013. Revised Guidelines for the Assessment of Microbiological Quality of Processed Foods.
[19] Shahriar Alam, Anis Alam Siddiqui, Tasmia Bari and Shafi Ahmed, 2015. Microbiological Quality Assessment of Industrially Produced and Street Vended Ice-Cream in Dhaka City. Bangladesh Research Publication Journal. 11(1), 87-92. Retrieve from
[20] Ojokoh, A.O., 2006. Microbiological Examination of Ice Cream Sold in Akure. Pakistan Journal of Nutrition. 5 (6): 536-538
[21] Frazier, W. C., and Westhoff, D. C., 1958. Pasteurized milk in Milk Ordinance and Code, USPHS, Food Microbiology. 1st ed., McGraw-hill Inc.; New Delhi, pp. 112.
[22] James, M., and Jay, J. M., 1978, Indices of Food Sanitary quality and microbiological standards and criteria. Modern Food Microbiology, 2nd ed. p. 311.
[23] Omar H. Baraheem, Hoda A. El-Shamy, Wafaa M. Bakr and Naglaa F. Gomaa, 2007. Bacteriological Quality of Some Dairy Products (Kariesh Cheese and Ice Cream) in Alexandria. Journal of the Egypt Public Health Association. 82(5-6) 491-510.
[24] Carlsson, A., 2006. HACCP project on toffee ice cream production. Food microbiology. Swedish University of Agricultural Sciences, Uppsala
[25] Frazier, W.C. and Westhoff, D.C., 1978. Food Microbiology. 3rd edition. pp 540 McGraw-Hill Book Company, New York.
[26] Das S., G. M. M. Anwarul Hasan, S. Parveen, 2015. Evaluation of Microbial load and quality of milk & milk based dairy products. Octa. Journal of Bioscience. 3(1), 1-4
[27] Dubravka Samaržija, Šimun Zamberlin and Tomislav Pogačić, 2012. Psychrotrophic bacteria and milk and dairy products quality: Psychrotrophic bacteria and milk quality, Mljekarstvo. 62 (2), 77-95.