

Original Research Article

Bacteriological Quality Analysis of Ice Cream Produced by the Small Factories of Dhaka City

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ABSTRACT

In summer season, mainly the children of lower socioeconomic class usually consume ice creams which are locally sold by the street vendors. It is important to analyze the microbial quality because it is directly related to the health of the children. This study the bacteriological profile of two types of ice cream, kulfi and lolly, collected from different factories of four different zones of Dhaka city. The factories were located in zone 1 (Shamoli), zone 2 (Sadarghat), zone 3 (Shankar) and zone 4 (Khilghao). It was found that, in all factories, the microbial load was above the cut off level (2×10^5 CFU/ml). For kulfi, the highest count was found in zone 2 (2.4×10^7 CFU/ml) and lowest in zone 4 (3.0×10^5 CFU/ml). For lolly the highest count was found in zone 2 (8.1×10^7 CFU/ml) and lowest count was found in zone 4 (2.6×10^5 CFU/ml). Pathogenic bacteria like *Salmonella*, *Shigella*, *Vibrio*, *E. coli*, *Enterobacter* along with other bacteria were found in all the samples tested. These results suggest that consumption of these ice creams might cause GI disturbances, stomach abscess, diarrhea and other diseases. As children are the main consumers of these types of low grade microbially contaminated ice cream, careful monitoring is necessary before the marketing of the products.

Key Words: Bacteriological quality, Ice cream, Gram negative organism, *Salmonella*, *E. coli*

INTRODUCTION

Ice cream is a congealed dairy product made by milk fat (about 10-16%), sugar (9-12%), non-fat milk solids (about 9-12%), 0.20- 0.50% stabilizer and/or emulsifier, flavoring agents, coloring materials and thickeners. [1-3] It is a popular dessert item all over the world especially in summer season. It is a very good medium for microbial growth as it has high nutritive value with a long storage and neutral pH. Ice cream quality depends on the extrinsic factors like manufacturing procedure and also on intrinsic factors such as the ingredients used and their proportions. [4] Ice-cream may become contaminated during

production, transportation and/or storage with a number of microorganisms. [5,6] The contamination might cause during pasteurization period, during the addition of other ingredients or from crack plants, faulty packaging process and storage. [7] Primary contamination sources include water, raw milk and secondary contamination sources include flavoring agents, utensils and handling of the ice creams. [8] Post-pasteurization microbial contamination could be identified by the presence of coliform. [9-11] Silliker et al. (1980) and Buckner et al. (1993) stated that microorganisms cannot grow in frozen mixes and it is only when there is delay

between pasteurization and freezing that spoilage by microorganisms can occur. [1,12,13] Some contaminant organisms may become harmful as they might be responsible for cholera, typhoid, bacillary dysentery. [5,13] Several reports have been known already due to the outbreak of gastrointestinal diseases by contaminated ice creams in Asia, Europe and North America. In 1990 and 1995, two outbreaks of *Salmonella* were informed in England and Wales because of contaminated ice cream consumption. [14,15]

The domination of psychrotrophic bacteria in the total microbial population is more pronounced when milk is produced in poor hygiene conditions and/or contains increased numbers of somatic cells. [16,17] For these reasons, psychrotrophic bacteria usually account for more than 90% of the total microbial population in cooled raw milk. Generally, psychrotrophic microorganisms can cause spoilage of milk and dairy product because they are able to produce extracellular or intracellular thermoresistant enzymes (proteases, lipases and phospholipases). [18]

In developed countries, quality control measures are taken very carefully to improve the shelf life of ice cream and also to prevent public health issues. In Bangladesh, quality control measures are not followed strictly and due to improper standard hygiene practices ice cream is affected. Additionally, poverty, lack of education and lack of awareness, they get poor nutrition and suffer from many types of diseases. It also affects our economy, which is not expected.

The present work was conducted to-

- a) Assess the quality of the small ice cream factories and personal hygiene of the factory workers
- b) Estimate the total viable bacterial count of the ice cream sample
- c) Identify the presence of pathogenic organisms in the samples.

MATERIALS AND METHODS

Questionnaire preparation

A questionnaire was developed for

collecting the data about environmental hygiene of the factory and the production procedure. Questions were asked to the workers and also observations were done on the factory condition. Data was collected about the raw materials, its source, and personal hygiene of the workers, storage condition and backup system.

Sample collection

Two different types of ice creams (kulfi and lolly) were collected from the small factories of four different zones as zone 1 (Shamoli), zone 2 (Sadarghat), zone 3 (Shankar) and zone 4 (Khilghao) to perform the qualitative and quantitative analysis of microbial flora. The ice creams were collected during the period of July and August. All those ice creams were carried by using a sterile ice box container and transported to the laboratory within 30 minutes of collection. The purchasing time was in between 9.00am to 10.00am.

Sample Preparation

The microbiological tests were performed in the microbiological laboratory of Institute of Nutrition and Food Science, University of Dhaka. One ice cream was taken from the box and allowed to melt in a sterile petridish. After melting, 1ml liquefied ice cream was pipetted and transferred into 9ml of 0.9% sterile sodium chloride solution in a sterilized test tube. The mixture was shaken thoroughly to mix it well and allowed to stand for 10 minutes. Initial diluted sample was prepared by following this procedure and further serial dilution were done after that.

Cultural, morphological and biochemical test:

Streak and spread plate techniques were performed for bacterial isolation. Plate Count Agar (PCA), MacConkey, SS (*Salmonella-Shigella*) and TCBS (Thio-sulfate Citrate bile Salts) agar were used and all of them were prepared according to instructions provided by the manufacturer company.

Plate count agar medium comprising glucose and yeast extract which was used

for bacterial enumeration. MacConkey agar was used for gram negative bacterial isolation. SS agar, selective and differential medium, was used to isolate *Salmonella* and *Shigella*. TCBS agar is an extremely selective media for isolating *Vibrio* spp.

For enumeration of the colony forming unit, 1 ml sample was cultured in PCA and MacConkey agar media and incubated for 24 hour at 37°C. On the following day, bacterial colonies were counted and morphology was analyzed for

identification. For further analysis, these were collected and maintained in Nutrient slant agar. Gram staining, KIA (Kligler's Iron Agar), MIU (Motility Indole Urea) tests were carried out according to the standard procedures for the biochemical properties of the isolates (manual of ICDDR) appeared on MacConkey, TCBS (Thio-sulfate Citrate bile Salts) and SS (Salmonella-Shigella) media. Catalase and oxidase tests were done also.

RESULTS AND DISCUSSION



Figure 1: Ice Cream Collection Zones

The samples (kulfi and lolly) were collected from 4 different zones of Dhaka city (figure 1). Silliker (1980) mentioned that contaminated ice cream mix may proliferate because of the temperature abuse during storage. In previous studies, it was reported that ineffective frozen storage became a reason for food poisoning outbreak. Because such condition is helpful for the activation of psychrotropic organisms. [6,11,19]

The average number of bacteria from kulfi and lolly on PCA and MacConkey agar is given in table 1. PCA agar were used for total bacterial count. For

kulfi, four different types of media were used for the total bacterial count and for identifying the specific types of microorganisms. According to BIS 1998, the maximum acceptable count of organisms is 2.5×10^5 CFU/g and no coliform bacteria can present in the ice cream. [20] However table 1 shows that all the zones crossed the acceptable limit of total viable count whereas condition was worst for zone 2 as 2.4×10^7 CFU/ml.

In case of lolly, the bacterial count was highest in zone 2 (8.1×10^7 CFU/ml) and others were also beyond the limit. The number of total average count of bacteria in

zone 1, zone 3 and zone 4 were 1.6×10^6 CFU/ml, 2.6×10^6 CFU/ml and 2.6×10^5 CFU/ml, respectively.

The scenario for the samples on MacConkey agar plate was similar to PCA media and the average counts 5.2×10^5

CFU/ml and 5.1×10^7 CFU/ml for zone 4 and zone 2, being the lowest and the highest respectively. The number of average count of bacteria in zone 3 and 1 were 6.5×10^5 CFU/ml and 7.8×10^5 CFU/ml respectively.

Table 1: Total bacterial count for Kulfi and Lolly on Plate count and MacConkey agar media in different zones of Dhaka city.

Zone no.	No. of samples	Kulfi		Lolly	
		PCA (CFU/ml)	MacConkey (CFU/ml)	PCA (CFU/ml)	MacConkey (CFU/ml)
1	60	2.1×10^6	7×10^3	1.6×10^6	7.8×10^5
2	60	2.4×10^7	4.3×10^3	8.1×10^7	5.1×10^7
3	60	2.5×10^6	5.7×10^5	2.6×10^6	6.5×10^5
4	60	3.0×10^5	2.3×10^5	2.6×10^5	5.2×10^5

Based on cultural, morphological and biochemical characteristics of the isolates with the available literature, the isolates were identified. [21]

Table 2 shows the identified bacteria from kulfi and table 3 represents the identified bacteria from lolly. For Kulfi, all the zones in Dhaka city contained *E.coli*, *Salmonella* and *Proteus*. *Vibrio* was found in all three zones except zone 4.

Similarly, *E. coli*, *Aeromona* and *Salmonella* were found in all four zones. *Shigella* was identified in zone 2 and zone 4. From these findings, it can be concluded that pathogenic organisms are present in these ice cream samples which may produce various kinds of fatal and non-fatal diseases.

Many researchers in various countries investigated the microbiological profile of ice cream by. Marino (1954), Keller (1987), Ikenebomeh (1993) and other researchers found bacterial contamination of ice cream at the level of 1.0×10^5 , 2.5×10^5 and 10^0 - 10^4 CFU/g. [19,22 ,23] Elahi and Rahman made a study in 2002 in Bangladesh on sanitary quality of commercially produced ice cream in 2002. They found the total bacterial count to be 3.2×10^3 to 1.5×10^4 CFU/ml. [7]

Table 2: Comparison among different types of Gram negative bacteria identified from kulfi from four different zones of Dhaka city

Zone 1	Zone 2	Zone 3	Zone 4
<i>Enterobacter</i>	<i>Enterobacter</i>	<i>Enterobacter</i>	
<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
<i>Salmonella</i>	<i>Salmonella</i>	<i>Salmonella</i>	<i>Salmonella</i>
<i>Virbio</i>	<i>Virbio</i>	<i>Virbio</i>	
	<i>Pseudomonas</i>	<i>Aeromonas</i>	<i>Aeromonas</i>

Table 3: Comparison among different types of Gram negative bacteria identified from lolly from four different zones of Dhaka city

Zone 1	Zone 2	Zone 3	Zone 4
<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
<i>Aeromonas</i>	<i>Aeromonas</i>	<i>Aeromonas</i>	<i>Aeromonas</i>
<i>Salmonella</i>	<i>Salmonella</i>	<i>Salmonella</i>	<i>Salmonella</i>
<i>Shigella</i>	<i>Enterobacter</i>	<i>Pseudomonas</i>	<i>Shigella</i>

All the Gram negative organisms, such as *Salmonella*, *Shigella*, *E. coli*, should be removed after pasteurization. But their presence indicates that there is a problem in the pasteurization process or there are possible sources contaminations in post-pasteurization period. This result is similar to the findings of other researchers from Costa Rica, India, Spain and Ireland. They also reported that contamination may come from packaging, transportation and selling of the products. [27-31]

Pathogenic bacteria contribute to globally important diseases, such as typhoid, which can be caused by bacteria such as *Salmonella* and food borne illnesses, which can be caused by such *Shigella*, *Campylobacter* and *Salmonella*. [22-27] Therefore, the presence of pathogenic microorganisms in considerably high level in the analyzed samples indicates threat to public health. As ice creams are mostly consumed by the children who are also a vulnerable group of population, the infection outcome can be worse.

CONCLUSION

The present study showed that the microbiological quality of the ice cream is below standard level. All of these organisms are fatal to health. As these low priced ice

creams were mainly consumed by the small children of lower socio-economic class, it is very important to monitor the quality to ensure their better health. This contamination may be a result of the improper practices of ice cream production, bad hygiene practices, faulty packaging and storage. Proper training about the hygiene practices, knowledge about the production for the workers can be an effective measure to improve the situation. Sufficient investigations of quality control measures are necessary for long term effect. HACCP practices should continue to overcome the contamination problems. This measures will directly affect the health of the nation positively and the overall economy in broader aspects.

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