

# Assessment of Microbiological Contamination of Branded and Street Vended Ice-Cream: A Comparative Study in Tangail Municipality, Bangladesh

Afroza Khatun<sup>1</sup>, Masuma<sup>1</sup>, Md Younus Mia<sup>1\*</sup>, and Kamal Kanta Das<sup>2</sup>

<sup>1</sup> Department of Environmental Science and Resource Management, Mawlana Bhashani Science and Technology University, Bangladesh

<sup>2</sup>Department of Microbiology, Stamford University Bangladesh, Bangladesh.

\* E-mail: [mdmia1998@gmail.com](mailto:mdmia1998@gmail.com)

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## ABSTRACT

Ice cream is a popular dessert consumed by people of all ages, and its consumption can pose a risk of exposure to various microorganisms, including pathogenic bacteria and viruses. Assessment of the microbiological contamination status of branded and street-vended ice cream is crucial to ensure the public health safety. To identify potential sources of contamination, evaluate the effectiveness of the hygiene practices different microbiological and physicochemical analysis was done. Microbial analysis revealed that total viable bacteria in branded ice-cream ranged from  $4.8 \times 10^3$  to  $1.10 \times 10^5$  cfu/ml and in street vended ice-cream ranged from  $7.5 \times 10^4$  to  $1.6 \times 10^8$  cfu/ml. Total coliform bacteria present upto  $9.20 \times 10^3$  cfu/ml in branded ice-cream and  $5.3 \times 10^3$  to  $9.6 \times 10^6$  cfu/ml observed in street vended ice-cream. In case of specific pathogen most of the samples contaminated with *E. coli* and *Staphylococcus aureus* were found to be present in branded samples up to  $10^4$  cfu/ml and  $10^6$  cfu/ml on street samples. The pH of both type of ice-cream showed acidic to neutral condition. The range of Total soluble solids in several branded ice-creams were 26 to 29% and the value of TSS obtained in street vended ice-creams were ranging from 5 to 10%. These results indicated that, the microbial quality in all street ice-creams exceeded the BSTI standard and exhibited the lower quality than the industrially produced branded ice-creams due to comparatively faulty manufacturing process and poor hygiene practice.

**Keywords:** Food safety, physicochemical, Ice-cream, foodborne pathogens

## INTRODUCTION

Most of the people throughout the world, due to their hectic life style are now increasingly depend on ready-to-eat food. Milk considers as a complete food but as milk is a perishable food so it require immediate consumption but processed into other products like ice-cream, yoghurt, cheese, butter which can also contribute to human

nutrition (Rahman *et al.*, 2016; Moshood *et al.*, 2013). Among these processed dairy products, ice-cream is undoubtedly the most popular dairy product in whole world and continues to be a dominant interest of large segments of the population (Rahman *et al.*, 2016). Ice-cream is a congealed and nutritionally enriched dairy product combined of 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 (Armnianos *et al.* 2017; Aaku *et al.*, 2004; Potter and Hotchkiss 1995).

Physical, chemical and microbial quality of products are mainly affected by the methods of handling and storage conditions of ice cream (Akter *et al.*, 2019; Rahman *et al.*, 2016; Park *et al.* 2020). Most of the ice-creams become contaminated with microbes during production, transit, and preservation (Rahman *et al.*, 2016; Marjan *et al.*, 2014; Banik *et al.*, 2014; Akter *et al.*, 2019). Primary sources of contamination include the use of contaminated raw material like defiled water, contaminated milk and milk powder and secondary sources of contamination may include flavoring, coloring agents, utensils, stabilizers, handling and also from air during processing (Velázquez-Ordoñez *et al.*, 2019; Rahman *et al.*, 2016; Marjan *et al.*, 2014; Banik *et al.*, 2014; Akter *et al.*, 2019). Generally, sterilization method like, pasteurization followed by freezing and hardening can eliminate the most of pathogenic organisms; however a handful of hazards can still retain in the finished products (Rahman *et al.*, 2016; Akter *et al.*, 2019). Normally microorganisms cannot grow in frozen mixes and it occurs only when there is prolongation between pasteurization and freezing process responsible for that spoilage by microorganisms (Rahman *et al.*, 2016; Akter *et al.*, 2019). In the last few decades, several reports have been exhibited the manifestation of gastrointestinal diseases by contaminated ice creams in Asia, Europe and North America (Rahman *et al.*, 2016; Marjan *et al.*, 2014; Banik *et al.*, 2014; Akter *et al.*, 2019). In other prior reports, high microbial loads in ice-cream samples found from different city of Nigeria (Edward *et al.*, 2017; Mohammed *et al.*, 2019). Studies conducted in Iran investigated that the main bacteria which causes food poisoning in this

country include *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Staphylococcus aureus*, which are transferred to humans through the consumption of milk and dairy products, thereby leading to zoonotic diseases with a high rate of mortality (Ghorbani-Ranjbari *et al.*, 2011; Rahman *et al.*, 2016; Marjan *et al.*, 2014; Banik *et al.*, 2014; Akter *et al.*, 2019).

The consumption of ice-cream is higher in vulnerable age groups like children and these are also consumed by hospital patient in case of throat and mouth operation which raises the necessity to maintain a high microbiological safe standard of ice-creams throughout the world (Rahman *et al.*, 2016; Jadhav and Raut, 2014). The Centre for Food Safety (CFS) betokened the findings of a targeted food surveillance project to assess the microbiological quality of ice-creams and frozen confections today. In developed countries, quality control measures are taken to increase the shelf life of ice-creams as well as to prevent potential threat of public health now a days (Rahman *et al.*, 2016; Akter *et al.*, 2019). Unfortunately, Bangladesh is still behindhand in this respect. Due to improper standard hygiene practices, non- enforcement of routine inspection act and lack of awareness the standard quality of ice-cream is badly affected (Kells and Gilmour 2004; Rahman *et al.*, 2016; Akter *et al.*, 2019). Thus, the consumers are deprived of getting standard quality ice-cream and suffer from several food borne diseases in our country. Especially, street vended ice-creams are most popular among school children in rural area as it is the cheapest but pose great threat to public health. Being a popular ready-to-eat food among all age groups, ice-creams warrant the maintenance of sound microbiological quality to ensuring the public health safety (Rahman *et al.*, 2016; Akter *et al.*, 2019).

Considering all these facts, the present study was conducted to determine the

bacterial load in branded and street vended ice-creams in our country to ensure the public health safety and find out which one is more microbiologically safe.

## **MATERIALS AND METHODS**

### **Study area, duration, collection of sample**

The study was conducted at Tangail municipality, Dhaka, Bangladesh. In total 24 ice-cream samples were randomly taken out from three stations namely New bus stand, Old bus stand, Santosh at Tangail. Among them 15 samples were of branded ice-creams and 9 samples were of street ice-creams. All samples were transported in an insulated container packed in ice. After bringing the samples into the laboratory, these were moved into refrigerator immediately for analyzing and subsequent studies. The experimental study was carried out during the month of January-August in 2019.

### **Preparation of samples**

1mL of each sample will be taken aseptically with a sterile micropipette and transferred carefully into the test tube having 9mL sterile saline. Thus 1:10 dilution of the samples will be obtained. The mixture will be mixed properly and serially diluted up to  $10^{-5}$  according to the standard method. Thus, the samples will be studied in quantitative and qualitative method (Rahman *et al.*, 2016; Marjan *et al.*, 2014; Banik *et al.*, 2014; Akter *et al.*, 2019).

### **Microbial analysis**

The Spread Plate technique was performed for bacterial total plate count with serial dilution by following the standard procedure (APHA, 2004; Cappuccino and Sherman, 2001; Rahman *et al.*, 2016; Marjan *et al.*, 2014; Banik *et al.*, 2014; Akter *et al.*, 2019). Plate count agar, MacConkey agar, Eosine Methylene Blue agar, Mannitol salt agar were used for the growth of total viable bacteria, total coliform, total *E. coli*,

*Staphylococcus aureus* respectively. Colonies formed in the plates were counted by using digital colony counter after incubation at 37 °C for 24 hours (Rahman *et al.*, 2016; Marjan *et al.*, 2014; Banik *et al.*, 2014; Akter *et al.*, 2019). The actual numbers of bacteria were estimated as colony forming unit (cfu/ml) and the counted results were recorded by the standard equation.

### **Physico-chemical analysis**

pH meter was used for determination of pH of ice-cream and Total soluble solids content of ice-cream was determined by using a refractometer whereby a drop of solution was placed on its prism.

### **Statistical analysis**

MS Excel and SPSS were used for the statistical analysis of data. Results were presented in tabular form. Findings of the analyzed data were represented by different column charts.

## **RESULTS AND DISCUSSION**

### **Microbial analysis**

The range of total viable count (TVC) among different brands of ice-cream samples ranged from  $4.8 \times 10^3$  cfu/ml to  $1.10 \times 10^5$  cfu/ml at different locations (Figure 1). Among different brands of ice-cream samples, the highest value of TVC found in sample 5 which was  $1.10 \times 10^5$  cfu/ml (log value 5.04 cfu/ml) at Santosh and the lowest value of TVC found in sample 1 which was  $4.8 \times 10^3$  cfu/ml (log value 3.6 cfu/ml) at New bus stand.

According to Farah *et al.*, 2010, the highest microbial load was observed in Cornetto cone ice-cream ( $1.6 \times 10^3$  cfu/g) and lowest value found for Mango cup ice-cream  $6.3 \times 10^2$  cfu/g in Dhaka city which value is lower than the present study. Moshood *et al.*, 2013, found that the total bacteria count per ml of the industrially produced ice cream sample range between  $3.0 \times 10^3$  -  $8.8 \times 10^3$  cfu/ml with a mean count of  $5.3 \times 10^3$  in

Bauchi, Nigeria which indicates the lower value from this study.

According to Bureau of Indian Statistics (BIS) 1998, acceptable range of total viable count in ice-cream is  $2.5 \times 10^5$  cfu/g. In the present study, all brands of ice-cream did not exceed the cutoff level of standard value but all brands of ice-cream sample were more or less contaminated with pathogenic bacteria. But in different stations the value of branded ice-cream varied due to the contamination during transportation and the source of contamination could be occurred from retailers, most of them do not store ice-cream at the appropriate storage temperature. This unappropriated temperature may favors and encourage bacterial growth which eventually causes spoilage.

On the other hand, the range of TVC in street vended ice-cream was  $7.5 \times 10^4$  cfu/ml to  $1.6 \times 10^8$  cfu/ml at different locations (Figure 2). The highest value of total viable count was  $1.6 \times 10^8$  cfu/ml (log value 8.2 cfu/ml) which was found in lolly ice-cream at New bus stand and the lowest value was  $7.5 \times 10^4$  cfu/ml (log value 4.8 cfu/ml) found in mango ice-cream at Santosh.

According to Jannat *et al.*, 2016, Total viable count in street vended ice-cream samples was in the range of  $7.0 \times 10^3$  cfu/ml to  $2.7 \times 10^5$  cfu/ml in different locations of Dhaka city. This value was comparatively lower than our findings. Moshood *et al.*, 2013, found that the locally produced ice-cream sample had the higher mean count of  $2.0 \times 10^4$  cfu/ml and industrially produced ice-cream with a mean count of  $5.3 \times 10^3$  cfu/ml in Bauchi.

From this finding, it was confirmed that all the street vended ice-creams crossed the acceptable limit of BIS. The value of TVC of street vended ice-creams was comparatively higher than the branded ice-cream products. These are mainly due to

faulty manufacturing process, improper pasteurization and storage temperature and poor environmental hygiene practice in production and handling process. So, it is observed that, comparatively branded ice-creams were more hygienic than the street ice-creams (Rahman *et al.*, 2016).

According to Food Safety and Standard Authority of India (FSSAI), Total Coliform Count (TCC) of ice-cream should not exceed 100cfu/ml. According to Bureau of Indian Statistics 1998, no coliform bacteria should be present in ice-cream. From this finding, the mean value of all branded of ice-cream except sample 1 exceeded the Indian Food Safety Standard and regulation (2011).

According to Jannat *et al.*, 2016, the average number of coliform count in branded ice-cream samples was 4 cfu/ml in Dhaka city which value was lower than this study.

On the other hand, the range of TCC in street vended ice-cream was  $5.3 \times 10^3$  cfu/ml to  $9.6 \times 10^6$  cfu/ml at different locations (figure 4). The highest value of total coliform count was  $9.6 \times 10^6$  cfu/ml (log value was 6.9 cfu/ml) found in lolly ice-cream at new bus stand and the lowest value was  $5.3 \times 10^3$  cfu/ml (log value was 3.7cfu/ml) observed in mango ice-cream at Santosh.

Mokbul *et al.*, 2016, investigated bacteriological profile of two street of ice-cream, kulfi and lolly, collected from four different zones of Dhaka city. For kulfi, the highest coliform count was found in zone 3 ( $5.7 \times 10^5$  CFU/ml) and lowest in zone 1 ( $7 \times 10^3$  CFU/ml). For lolly, the highest count was found in zone 2 ( $5.1 \times 10^7$  CFU/ml) and lowest count was found in zone 4 ( $5.2 \times 10^5$  CFU/ml). Comparing this result with the previous study, it was found that the value of both studies almost similar or there is a slight higher extent of coliform found in their study. Elango *et al.*, 2010, studied 24 samples of Kulfi ice-cream sold in Chennai. In their study, the highest level of coliform count was found in local vendor samples ( $1.7 \times 10^3$  cfu /

g) followed by that of small-scale producer ( $7.7 \times 10^1$  cfu /g) and organized sector ( $2.4 \times 10^1$  cfu / g). From this investigation of street ice-creams, it was confirmed that all the studied ice-cream samples exceeded the standard value (BSTI 2005, Rahman et al., 2016).

Coliform organisms are the main microorganisms which reflect hygienic status of final product and effectiveness of hygienic practices in ice-cream production and their presence in any product indicates the bad hygiene practice. In both branded and street vended ice-cream exceeded the standard limit for total coliform count and comparatively street vended ice-cream showed higher total coliform count than branded ice-cream. These mainly occurred as coliform contamination were susceptible to pasteurization and other reasons for contamination might be the lack of personal hygiene of the workers of ice-cream factories.

In this research work, Total *Escherichia coli* count (TEC) among different brands of ice-cream samples were ranging from 0cfu/ml to  $4.5 \times 10^3$  cfu/ml at different locations (Figure 5). The highest value of TEC was found in sample 5 which was  $4.5 \times 10^3$  cfu/ml (log value 3.6cfu/ml) at Santosh and the lowest value found in sample 1 which was 0 cfu/ml (log 0) at New and Old bus stand.

The presence of *E. coli* in food indicated fecal contamination. Food Safety and Standard Authority of India (FSSAI) stipulates that *E. coli* should be absent in one gram of ice cream. In the other hand according to BSTI, acceptable limit of *E. coli* is 0 cfu/ml. The obtained value of the research work clearly revealed that most of the branded ice-cream samples exceeded the standard limit for TEC except sample 1.

Hassan *et al.*, 2015, the average *E. coli* counts obtained from the study was in Igloo  $9.26 \times 10^3$  CFU/ml (log 4.0), in Polar

$1.14 \times 10^3$  CFU/ml (log 3.0) and in Kwality  $7.95 \times 10^2$ CFU/ml (log 2.9) in Dinajpur district. In comparing with this study, it is shown that present study result is quite similar with their study. In another study of Edward *et al.*, 2017, found zero count of *E. coli* in industrially produced ice-cream.

On the other hand, the range of TEC in street vended ice-cream was  $4.1 \times 10^2$  cfu/ml to  $7.5 \times 10^4$  cfu/ml at different locations (figure 6). The highest value of Total *Escherichia coli* count was  $7.5 \times 10^4$  cfu/ml (log value 4.8cfu/ml) found in lolly ice-cream at New bus stand and the lowest value was  $4.1 \times 10^2$  cfu/ml (log value 2.6 cfu/ml) observed in mango ice-cream at Santosh.

Edward *et al.*, 2017, found *Escherichia coli* in locally produced ice-cream ranged from  $4.00 \times 10^3$ , to  $1.20 \times 10^4$  cfu/g which is almost closure to this study. Moshood *et al.*, 2013, studied locally produced ice-cream where the presence of *Escherichia coli* found in 7 samples. *Escherichia coli* had the highest frequency with the percentage occurrence of 35%.

The result of TEC found in analysis of street ice-cream clearly showed that the obtained value was much higher than the standard limit. In comparison with the industrially produced ice-creams the value of studied street ice-cream was fairly high. The use of comparatively more contaminated water may be from polluted river for production of ice-cream, improper heating and storage and poor hygiene practice are responsible for such lower quality of street ice-cream.

The range of *Staphylococcus aureus* count (SC) among different brands of ice-cream samples were ranging from  $1.8 \times 10^2$  cfu/ml to  $2.9 \times 10^4$  cfu/ml at different locations (Figure 7). The highest value of SC found in sample 5 which was  $2.9 \times 10^4$  cfu/ml (log value 4.5 cfu/ml) at Santosh and the lowest value of SC found in sample 1 which



was  $1.8 \times 10^2$  cfu/ml (log value 2.2 cfu/ml) at New bus stand.

In the other hand according to BSTI, the acceptable limit for *Staphylococcus aureus* count for ice-cream product is 10 cfu/ml. From the present findings, the mean value for each branded ice-cream showed that they exceeded the standard value for SC and lowest bacterial count observed in sample 1 and highest count observed in sample 5.

Study conducted by El-Ansary & Maria in Egypt, the average *Staphylococcus aureus* count was  $1.10 \times 10^3 \pm 2.45 \times 10^2$  cfu/ml, which could be associated with potential food poisoning hazards. Edwar *et al.*, 2017, studied the industrially and locally produced ice-cream sample in Nigeria. Where the least Staphylococcal mean count of  $2.00 \times 10^3$  cfu/g was recorded in the industrially produced samples.

On the other hand, the range of SC in street vended ice-cream was  $3.9 \times 10^4$  cfu/ml to  $7.9 \times 10^6$  cfu/ml at different locations (Figure 8). The highest value of *Staphylococcus aureus* count was  $7.9 \times 10^6$  cfu/ml (log value 6.8 cfu/ml) found in lolly ice-cream at New bus stand and the lowest value was  $3.9 \times 10^4$  cfu/ml (log value 4.6 cfu/ml) observed in mango ice-cream at Santosh.

Jannat *et al.*, 2016, studied that 71 % of street vended samples were contaminated with *Staphylococcus aureus* in Dhaka city. Edwar *et al.*, 2017, recorded in the locally produced ice cream samples ( $1.50 \times 10^4$  cfu/g). The absence of *S. aureus* has been rarely reported in several studies. In this study the value is much higher than the both study.

In this research work, it was observed that the value of SC in all street ice-creams were fairly high than the standard value of SC and also from industrially produced branded ice-creams. Staphylococcal species are widespread in the environment where the

human nasal cavity and skin is the largest reservoir of this bacterium. Comparatively poor maintenance of proper hygienic environment during production system of street ice-cream was the main reason for higher contamination of such type of bacteria than the industrially produced branded ice-creams.

### **Physico-chemical parameter analysis**

In this study, the concentration of pH for different branded ice-cream in different locations ranged from 5.5 to 6.9 (figure 9). The highest pH value was observed in sample 5 that was 6.9 and the lowest value of pH observed in sample 1 which was 5.5. In this study almost all the brands showed quite similar pH and the values are closer to pH value 7 which indicated the neutral condition according to pH scale. Commonly the pH value in ice-cream ranges from 6-7 which indicates neutral value of pH according to pH scale (Naim *et al.*, 2014). According to Marshall *et al.*, 2003, the pH of ice-cream varies with the composition of the product, in general it is in the range of 6.3 to 6.5. All the value of pH of branded ice-creams that were studied are quite similar as the value of pH depends on the composition of ice-cream and also within the range of neutral value. In comparison with the previous literatures the values obtained from this study were much closer to them.

On the other hand, the value of pH in street ice-cream that found from this research were ranged from 6.0 to 7.0 at different locations (figure 10). The highest value of pH was 7 observed in Kulfi ice-cream and the lowest concentration of pH observed in mango ice-cream which was 6. All the values were close to neutral value of pH.

The TSS in different branded ice-cream were ranging from 26 to 29% (Figure 11). The highest value of TSS was 29% observed in sample 5 and the lowest value of TSS was 26% observed in sample 1. The



values in different brands of ice-cream were almost closer to them.

On the other hand, the value of TSS observed in street vended ice-cream were ranging from 5% to 10% (Figure 12). The highest value was 10% observed in Lolly ice-cream and the lowest value was 5% observed in mango ice-cream. The highest average found in lolly which was 8.6% and the lowest average observed in mango which was 6%.

Findings from this work showed that though ice-cream is high fat and sugar content, it enhances the microbial growth also serves as a vehicle for transmission of pathogens. The results obtained from this study showed that both industrially manufactured branded and locally produced street vended ice-cream samples were contaminated with potential pathogenic microorganisms. Among them the industrially produced branded ice-creams offered comparatively better-quality products in respect of production system and sanitary condition than the street ice-creams. But in the case of branded ice-creams, the microbial quality was not satisfactory at all since every sample indicated the bacterial proliferation. Several steps during production can cause bacteriological hazards. Though pasteurization of milk can destroy most of the pathogens posing risk to public health, yet, potential bacteriological hazards can still be found in the final products after pasteurization through cross contamination or improper handling. At retail shops, improper storage temperature and prolonged storage time affects the microbiological quality of ice-cream. The high level of bacterial contamination can be attributed to poor hygienic conditions under which street ice-creams are produced, stored, transported and distributed, as it is largely in the hands of the roadside/ small local vendors. In this study work, some chemical parameters like pH and TSS were also observed. In both

categories of ice-creams, the pH and TSS were in satisfactory level.

## CONCLUSION

Considering the bacterial load in both studied ice-cream, it can be recommended that much attention is still needed to apply in aspects of microbiological quality control at each level of the production, handling, processing, distribution and storage of ice cream for attaining desired safety margins and giving assurance that the ice-cream product received by the consumer will be pure, healthful and of the quality claimed. The government authorized institute (like BSTI) should take intensive investigation to control the microbial and chemical quality of the ice creams as well as the public awareness about the adulterated ice creams should be increased.

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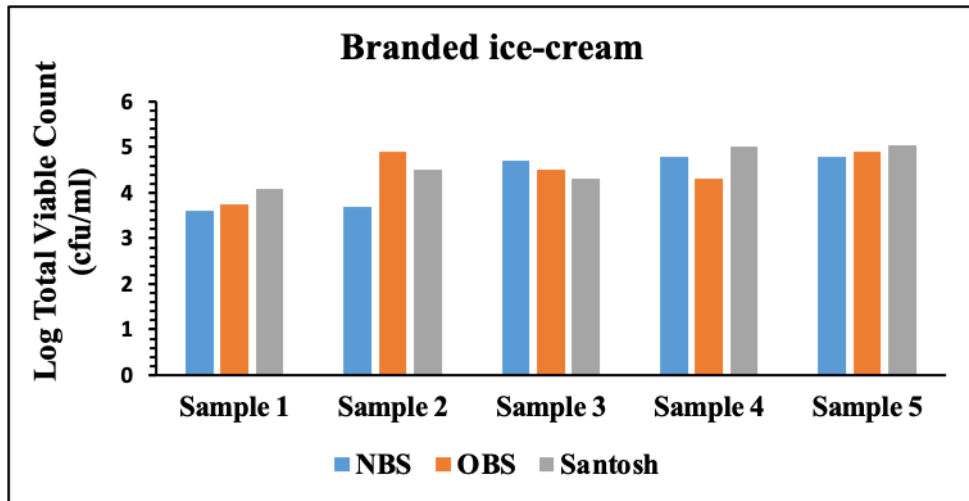


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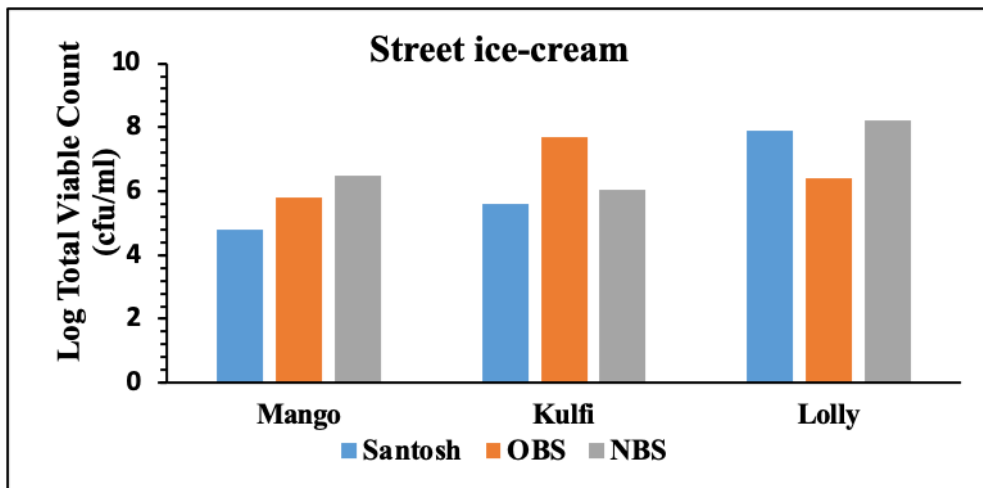


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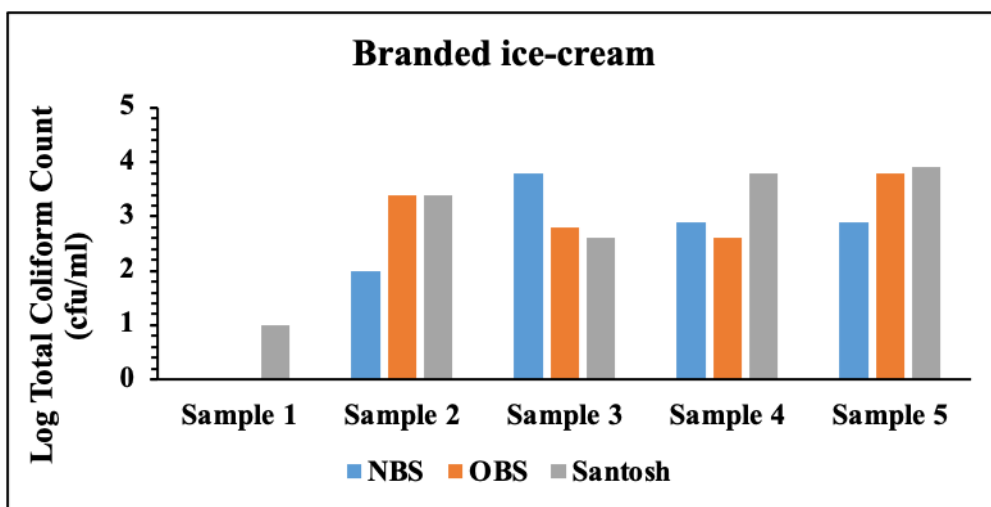




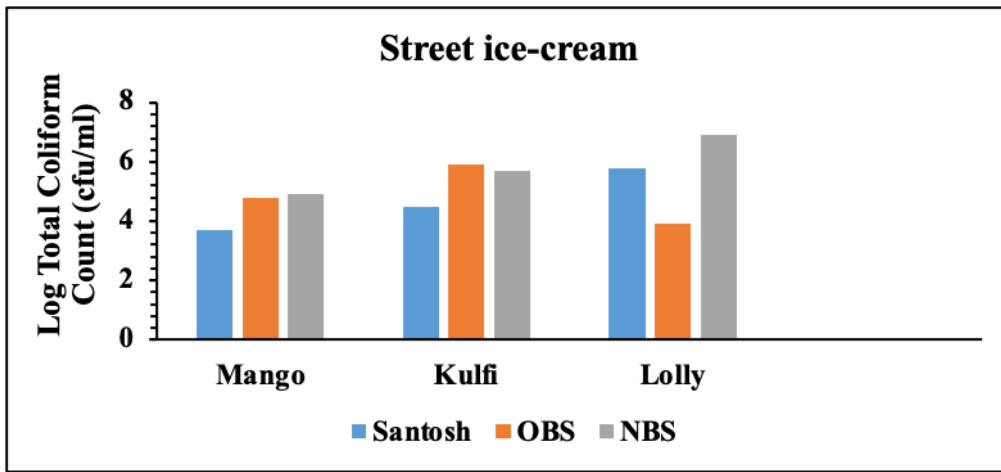
**Figure 1.** Status of Total Viable Count (TVC) in different branded ice-creams and locations. [Note: NBS= New bus stand, OBS= Old bus stand]



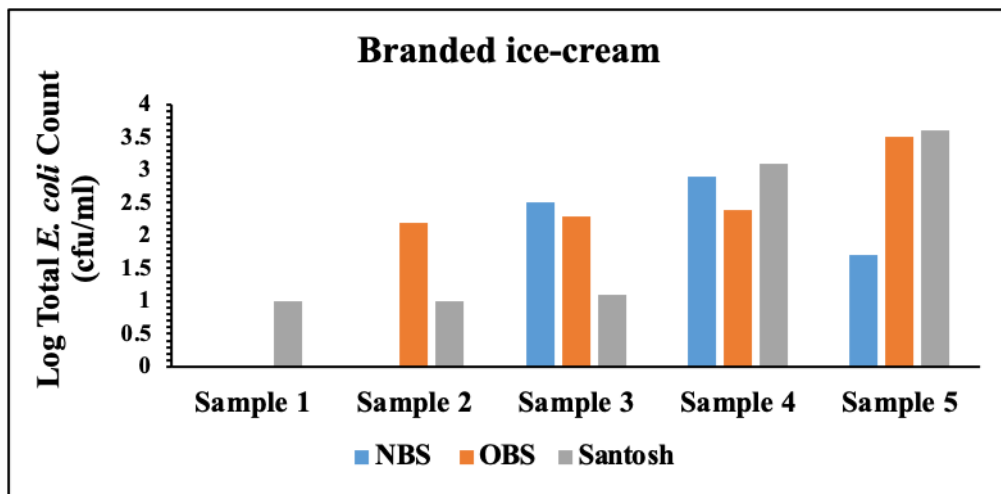
**Figure 2.** Total Viable Count of bacteria in different street vended ice-cream. [Note: NBS= New bus stand, OBS= Old bus stand]



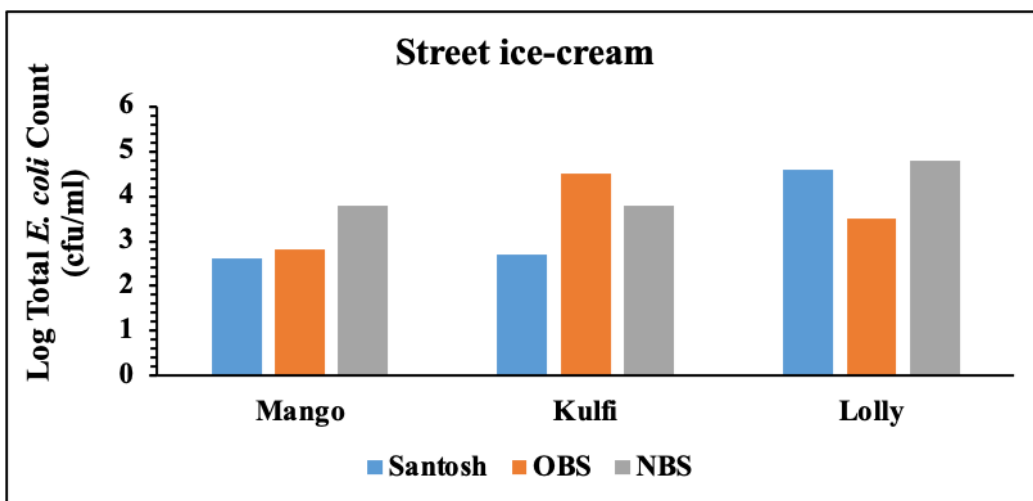
**Figure 3.** Total coliform count in different branded ice-cream in different locations. [Note: NBS=New bus stand, OBS=Old bus stand]



**Figure 4.** The concentration of Total Coliform count in different street vended ice-cream in different locations. [Note: NBS=New bus stand, OBS= Old bus stand]



**Figure 5.** The concentration of Total *E. coli* Count in different branded ice-cream in different locations. [Note: NBS= New bus stand, OBS= Old bus stand]



**Figure 6.** Total *E. coli* count in different street vended ice-cream in different locations. [Note: NBS= New bus stand, OBS= Old bus stand]

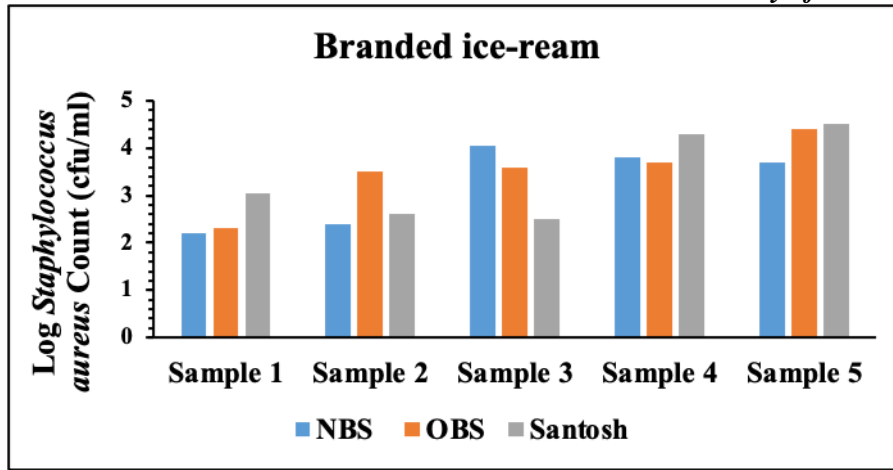


Figure 7. The concentration of *Staphylococcus aureus* count in different branded ice-cream in different locations. [Note: NBS= New bus stand, OBS= Old bus stand]

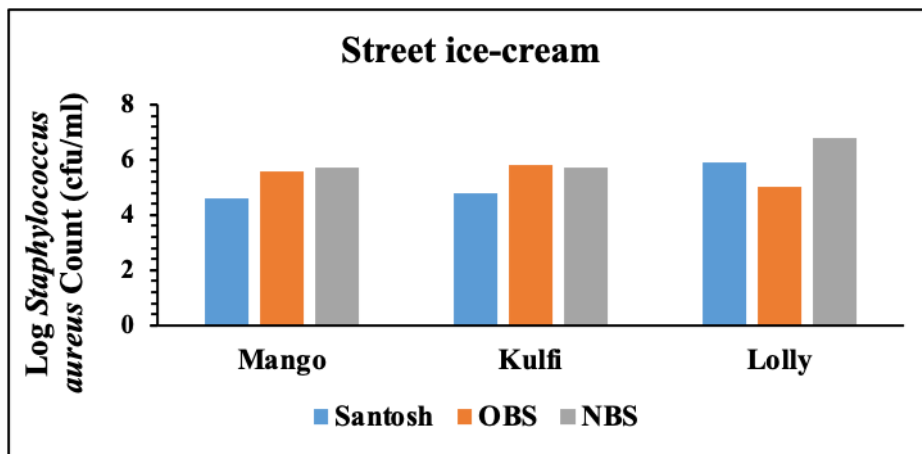


Figure 8. The concentration of *Staphylococcus aureus* count in different street vended ice-cream in different locations. [Note: NBS= New bus stand, OBS= Old bus stand]

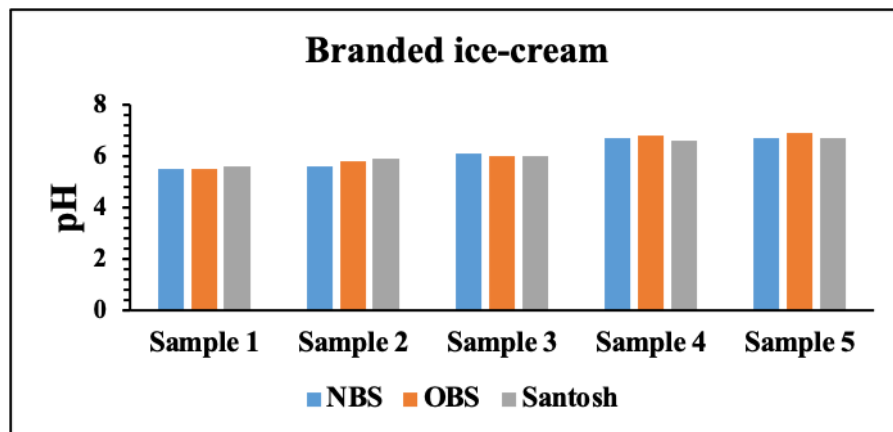
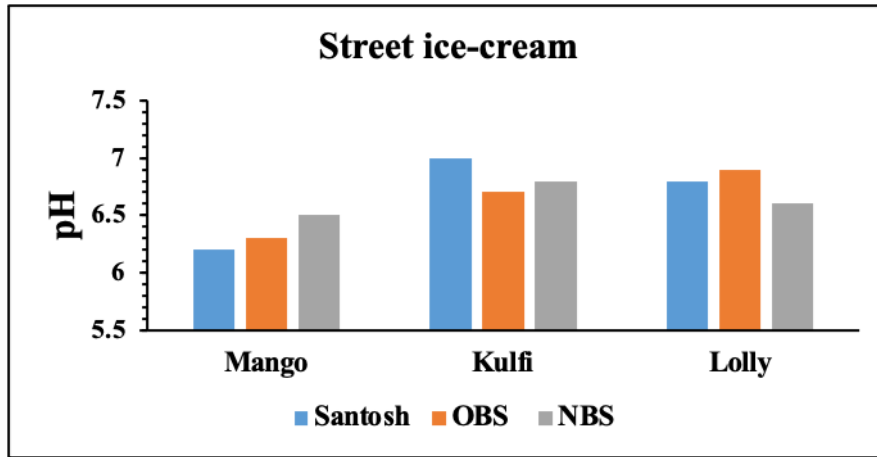
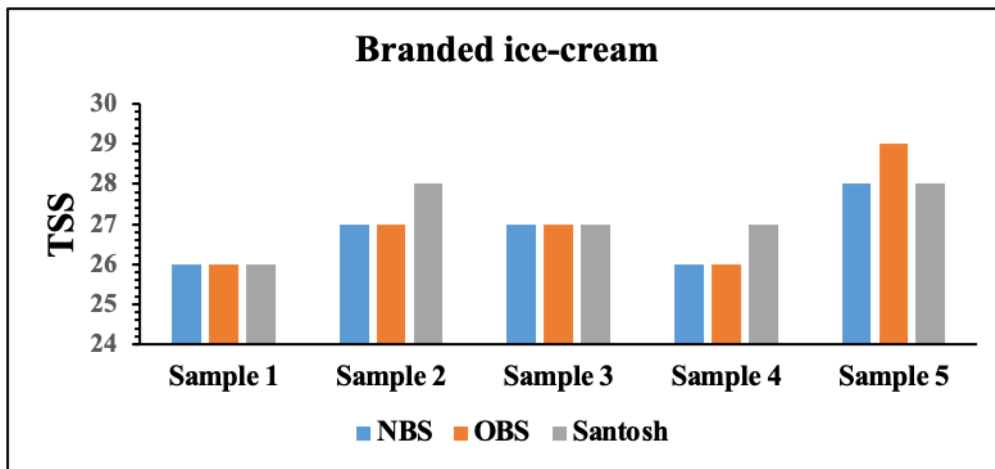


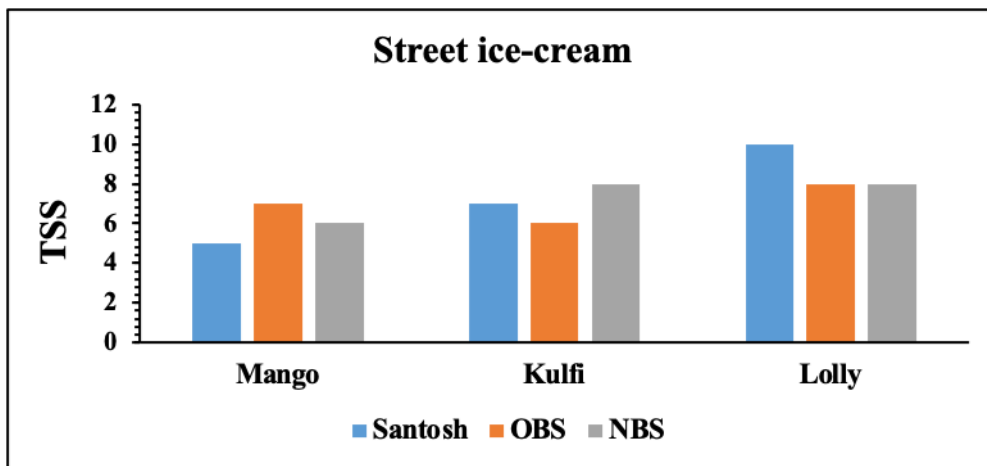
Figure 9. Concentration of pH in different branded ice-cream and locations. [Note: NBS=New bus stand, OBS= Old bus stand]



**Figure 10.** pH in different street vended ice-cream and locations. [Note: NBS=New bus stand, OBS= Old bus stand]



**Figure 11.** Total soluble solid in different branded ice-cream and locations. [Note: NBS=New bus stand, OBS= Old bus stand]



**Figure 12.** Total soluble solid in different street vended ice-cream and locations. . [Note: NBS=New bus stand, OBS= Old bus stand]