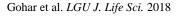
#### Microbiological Analysis of Ice cream









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# Microbiological Assessment of Branded and Local Ice Cream Samples

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ABSTRACT: Ice cream is the commonly used frozen milk product containing several nutrients, serving as media for the growth of microorganisms, potential to cause illness in human especially children. The study was planned to determine and compare the microbiological quality of branded (Movenpick, Walls and Omore) and Local ice cream samples collected from five towns of Lahore city. Total n=30 samples (n=15 from each brand) were collected and subjected to standard microbiological techniques for total viable counting (TVC), total coliform count (TCC) and identification of bacteria. Results showed that mean values of TVC and TCC was nil since no contamination was observed in branded ice cream samples while samples collected from local markets were harboring potential pathogen (100%) with mean value of  $1.5 \times 10^{5}$  CFU/g (TVC) and  $1.1 \times 10^{3}$  (TCC) ranging from  $1.6 \times 10^{5}$  to  $2.2 \times 10^{3}$  CFU/g. The confirmed bacteria isolated from samples (Local) were Staphylococcus aureus, Salmonella, Shigella and E. coli with percentage positivity of 43%, 33%, 49% and 52% respectively. Present study revealed that the local ice cream was contaminated with bacteria i.e. Staphylococcus aureus, Salmonella, Shigella and E. coli posing a potential hazard to human health while branded ice cream (Movenpick, Walls and Omore) was contamination free. Hygienic measures, pasteurization and HACCP implementation can reduce the risk in ice cream.

*Key words:* Ice cream, Total viable count, Total coliform count, Staph. aureus, Salmonella, Shigella and E. coli.

### **INTRODUCTION**

Milk and milk products are major source of minerals (calcium, phosphorus and potassium), vitamins (riboflavin) and high

quality proteins (Kahraman andKolanciyan, 2016) which are helpful in building bones and teeth(Barmanet al., 2017). Ice cream is the most commonly used and desired dairy product among the people (Jadhav and Raut, 2014). It contains sugar, milk, fats, milk solids,

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colors, flavors, emulsifying agents and stabilizers, all supporting potential pathogens. Being a dairy product, it serves as fine medium for the growth of microorganisms as per the neutral pH and long preservation periods(Naimet al., 2014). The contaminated product could then cause various food borne illnesses i.e. cholera, dysentery, food poisoning and typhoid in consumers(Ahmedet al., 2009).Ice cream may be infected with bacteria through raw material, stabilizers and during air processing, resisting the pasteurization processes (Ojokoh, 2006).

Several reports have been published signifying the prevalence of microorganisms such as*Listeria monocytogenes, Staphylococcus spp, Salmonella spp, Yersinia spp* and *Bacillus cereus*in ice cream (Kokkinakiset al., 2008). In Asia, Europe and Africa, multiple outbreaks were reported due to the consumption of ice cream causing gastrointestinal infections. In 1990 and 1995, two outbreaks of *Salmonella enteridis* were reported due to the use of frozen milk products(Ahmed et al., 2009).

It is a principal health concern to ensure food quality and safety hence; there is a need to impose strict legislation on quality of ice cream. It could be brought upon by maintaining the hygienic conditions during processing and manufacturing of frozen dairy products. The estimation of microbial contentplays a significant part in this regard and could not just preserve food quality standards but also strengthen them (Barman et al., 2017).

Present study entitled "Microbiological analysis of Branded and Local ice cream" was carried out to assess the quality of ice cream samples collected from Lahore city.

## MATERIALS AND METHODS

#### Sample collection

Ice cream samples (n=30) were collected from 3 branded (Movenpick, Walls and Omore) and 3 local shops of five towns of Lahore city. The samples were immediately transported in sterile ice jars to Biology laboratory, Lahore Garrison University for further processing.

#### Sample preparation

10 gram ice cream (10g) was transferred in sterile beaker and melted in water bath at 37°C. Melted ice cream (1mL) was added in test tube containing 9ml sterile normal saline(N.S) and serial dilution(10-fold) were prepared upto 10<sup>th</sup> dilution(Hossain et al., 2012).

#### Estimation of bacterial load

Total viable count was performed by spread plate method. 100µl sample from each dilution was inoculated using L-shaped glass spreader on nutrient agar followed by incubation at 37°C for 24 hours. Plates with negative results were further incubated to give 48 hours incubation and confirmation for no growth. Total number of colonies (30-300CFU) were counted using colony counter using following formula and documented(Jadhav andRaut, 2014).

TVC = No. of colonies×Dilution factor×Correction factor

#### **Estimation of Total coliforms**

Total coliform count was performed on MacConkey agar using spread plate technique. Agar plates were inoculated with 100µl sample from respective dilutions and incubated at 37°C for 24-48 hours(Naim et al., 2014). Total coliform count was calculated by following formula.

TCC = No. of colonies×Dilution factor×Correction factor

#### **Identification of Bacterial Isolates**

Isolated colonies were identified using standard microbiological techniques. was to observe Gram staining done microscopic characters. Macroscopic characterization of each isolate was performed usingbasal, differential and selective agars (macConkey, EMB, SS, MSA, Staph-110, and loeffler'sslaunt). The characters were observed and noted for identification(Naim et al., 2014).

Biochemical tests (catalase, coagulase, oxidase, motility, Indole, methyl red, vogesprausker, citrate utilization, nitrate reduction,  $H_2S$  production and triple sugar iron test) were carried out for the confirmation of bacterial isolates (Ahmed et al., 2009) Results were observed and documented.

# **RESULTS AND DISCUSSION**

Total viable count (TVC) and Total coli form count(TCC) of ice cream samples (Local and branded) was determined using standard microbiological techniques. The distribution patterns, ranges (minimum and maximum) with mean values are described in Fig.1 and Fig. 2. Mean value of TVC and TCC was nil since no contamination was observed in branded ice cream samples. A previous study conducted in Greece was concurrent with present study showing presence of microorganisms (CFU/mL) in samples(Kokkinakis et al., 2008). In another study conducted in Nigeria showed least bioburden in marketed ice cream samples as compared to locally produced ice cream (Yusuf et al., 2013). Previous study carried out in Gaza city revealed that collected samples were harboring bacteria responsible for food poisoning(Abou-El Khair et al., 2014). In a previous study in Dhaka city, Bangladesh the microorganism were less prevalent in industrially produced ice cream samples (Jannatet al., 2016). Past study revealed the incidence of bacteria in branded ice cream (igloo, polar, savoy and kwality) samples collected from Mymensingh but TVC was less(Hossain et al., 2012). The minor differences in samples may be due to poor hygienic measures and implementation of food safety andHazard analysis of critical control points HACCP standards during manufacturing.

Ice cream samples collected from local markets were harboring potential pathogen (100%) with mean value of 1.5×10^5CFU/g (TVC) and 1.1×10^3 (TCC) ranging from 1.6×10<sup>1</sup> to 2.2×10<sup>3</sup> CFU/g. The past study conducted in Istanbul showed total mesophillic aerobic count with mean 1.5 ×10^4 CFU/g while total enterobacteriacae count was reported as  $3.0 \times 10^{2}$  (mean) CFU/g(Kahraman and Kolanciyan, 2016). In Kauntan, the study on industrially and locally produced ice cream samples showed concurrent results (range10<sup>2</sup>- 10<sup>4</sup>) with current study (Yusuf et al., 2013). A previous study held at Akure, Nigeria determined the plate count was ranged  $1.8 \times 10^{3} - 2.0 \times 10^{10}$ 10^4 CFU/g(Ojokoh, 2006) which was almost parallel to present study. Another study conducted in India showed high TVC in samples ranging from  $1.2 \times 10^{4}$  to  $8.2 \times 10^{4}$ CFU/g(Jadhav and Raut, 2014).In Kolkata, study showed the standard plate count was ranging from 10<sup>2</sup> to 10<sup>7</sup> showing greater

prevalence than present study(Barman et al., 2017). The differences may be due to environmental condition suitable for microbial proliferation, unhygienic measures practiced in local premises, poor quality of raw material and no pasteurization treatment during mixing and manufacturing.

In current study, bacteria were identified using standard microbiological (microscopic, techniques macroscopic characters and biochemical profile). The above techniques confirmed the prevalence of various bacteria in local ice cream samples i.e. Staphylococcus aureus, Salmonella, Shigella and E. coli with percentage positivity of 43%, 33%, 49% and 52% respectively (Fig. 3) while ice creams samples collected from branded stores showed no growth. Study conducted in Nigeria showed similar

microorganism with varying percentages in samples collected ice cream from vendors(Yusuf et al., 2013). Another study conducted in India showed approximately similar results, harboring E. coli (40%), Salmonella (33%), Shigella (53%) and Staphylococcus aureus (40%)(Jadhav andRaut, 2014). In Gilgit, the prevalence of E. coli was high (100%) while Salmonella was less in percentage (15%) among ice cream samples(Ahmed et al., 2009). A study conducted in Iran showed incidence of Staph. aureus (28%) while the positive percentage of entrobacteriacae was 70% (Salehian et al., 2013). The prevalence of microorganism was parallel to current data, the variations in percentages might be the physiochemical factors and contamination of organisms during manufacturing.

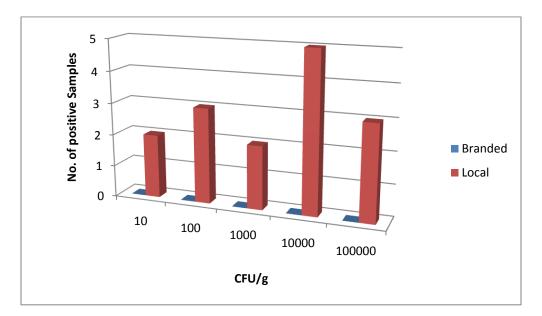
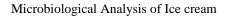


Fig. 1. Distribution of Total Viable count (CFU/g) of Ice Cream Samples (Branded and Local)



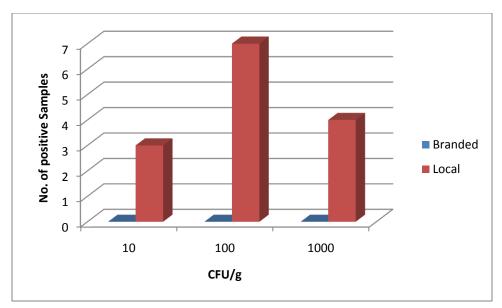


Fig. 2. Distribution of Total coliform count (CFU/g) of Ice Cream Samples (Branded and Local)

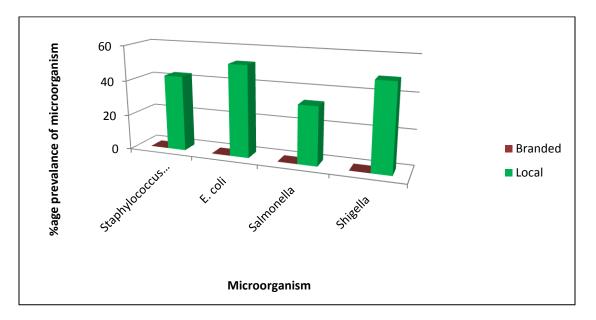


Fig. 3. Percentage Prevalence of microorganism in Local and Branded Ice cream Samples

### CONCLUSION

Present study revealed that the local ice cream was contaminated with various bacteria i.e. Staph. aureus, Salmonella, Shigella and E. coli posing a potential hazard to human health while branded ice cream (Movenpick. Walls and Omore) was contamination free. These bacteria can be introduced in ice cream through raw materials used for manufacturing, during processing and mixing etc. Hygienic practices, pasteurization and HACCP implementation can reduce the risk in ice cream. This study is consistent to limited number of sample, further research is required to get complete results.

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