Studies on Microbiological Quality of Ice-Creams Sold in and Around Greater Hyderabad Municipal Cooperation

P. P. Sirisha*, P. Sravani and N. Krishnaiah

Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Rajendranagar, Hyderabad, India

*Corresponding author

A B S T R A C T

The microbiological quality of ice-cream samples collected from co-operative, branded private and non-branded sectors sold in and around Greater Hyderabad Municipal Co-operation, Telangana state was studied. The standard plate count, coliform count, faecal coliform count and yeast and mould counts were 2.51x10^5 CFU/gm, 8.05x10^2 CFU/gm, 2.57x10^2 CFU/gm, and 2.66x10^2 CFU/gm from cooperative sector, 3.08x10^5 CFU/gm, 2.2x10^3 CFU/gm, 9.57x10^2 CFU/gm and 3.87x10^3 CFU/gm from branded private sector, 4.99x10^7 CFU/gm, 9.68x10^5 CFU/gm, 4.32x10^4 CFU/gm and 4.28x10^4 CFU/gm from non-branded sector respectively. The incidence of Staphylococcus spp. was 60% from co-operative sector, 53% from branded private sector and 100% from non-branded sector. The incidence of Salmonella spp. was 40%, 73%, 90% from co-operative, branded private, and non-branded sectors respectively. The incidences of Escherichia coli, Listeria spp., and Bacillus spp. was 86%, 73%, 80% from branded private sector and 100%, 76%, and 93% from non-branded sector respectively. The microbiological quality of ice-creams was good from cooperative sector followed by branded sector and fair from non-branded sector.

Introduction

Ice cream is a delicious frozen western product containing milk, sweetening and stabilising agents together with flavouring and colouring matter (Graff-Johnson, 1974). It is consumed by large segments of population and more popular among children and inevitable during feasts. The production and consumption of ice creams and other frozen dairy products is increasing day by day all over the world.

The microbiological quality of ice cream sold in retail shops mainly depends on contamination during product handling as well as efficiency and sanitary conditions during frozen storage. The high content of
nutrients like lactose and proteins and its neutral pH make it an excellent growth medium for microbes, some of which may cause serious disease outbreaks (Ahmed et al., 2009). Problems in maintaining cold chain during marketing and inadequate pasteurisation are the main sources of contamination in India, apart from contaminated raw materials, improper handling and storage (Verma et al., 1992). Microbiological quality of ice cream is determined by TVC, coliform count, faecal coliform count, yeast and mould count and certain pathogen like Staphylococcus spp., Salmonella spp., Escherichia coli, Listeria, Bacillus spp. etc.

During last few decades, it has been observed that consumption of contaminated ice creams was responsible for disease outbreaks in many countries (Djuretic et al., 1997). Since the consumption of ice cream was higher among children of vulnerable age groups, there is need to maintain high microbial safety standards (Champagne et al., 1994). Presently, majority of ice creams sold in and around GHMC especially brand less was not up to mark with reference to microbial quality. So an attempt was made to study the microbial quality and presence of certain pathogens in the ice creams from different sources in and around GHMC.

Materials and Methods

Sample Collection

For study purpose, the samples were divided into three categories: co-operative, branded and non-branded sources. Samples were collected from the sales centres, packed in thermocol box with ice, transported and stored at -20°C in the department of Veterinary Public Health and Epidemiology, College of veterinary science, Rajendranagar, Hyderabad till further testing.

Preparation of dilutions

For serial dilutions preparation, 10 gm of ice cream was aseptically transferred into 90 ml of sterile distilled water and homogenised by vertex. Subsequent serial dilutions were prepared up to 10^6.

Media preparation

Various media like nutrient agar for Total viable counts, Mc Conkey agar for coliform and faecal coliforms and potato dextrose agar for yeast and mould count were used as per the methods described in IS:2802 (1964). For studying the incidence and counts of pathogenic organism Mannitol salt agar for Staphylococcus spp., Selenite F broth for Listeria spp., XLD for Salmonella spp., Mannitol yeast extract polymixin agar for Bacillus spp., were used. One ml of dilutions serially from 10^5 and 10^6 for total viable count, 10^1 to 10^4 for coliforms and specified dilutions for different microorganisms are transferred to petri dishes and respective liquid media was added and allowed to set. Petridishes for total viable count, coliforms and other pathogens were incubated at 35°C for 24 to 48 hours, whereas for faecal coliforms incubated at 44.5°C for 24 to 48 hours and counts were taken with the help of colony counter. The incubation period for yeast and moulds was 3 to 5 days. The pathogenic microorganisms were confirmed with various specified biochemical tests.

Results and Discussion

The total viable count, coliform counts, faecal coliform counts, yeast and mould counts of ice cream samples collected from different sources were presented in table 1. The total viable cell count in the ice cream for co-operative sector were least (2.51 x 10^5 CFU/gm) which was within the limits
specified under BIS (2.5 lakhs /gm), almost similar to the findings of Nelapati et al., (2009) who reported counts of 2.62 x 10^5 in the sample from co-operative sector. The total viable cell Count in the samples from unbranded sources was very high (4.99 x 10^7 CFU/gm).

Counts of 4.5x10^5 CFU/gm, 3.07x10^5 CFU/gm and 1.2x10^3 CFU/gm reported by Warke et al., (2000), Nelapati et al., (2009) and Jadav and Raut (2014) respectively were less than the counts observed in the present study (3.08 x 10^6 CFU/gm) for the samples collected from branded private sector. The SPC count in the samples from unbranded source was 4.99 x 10^7 CFU/gm in present study, was less than the counts (0.1x 10^9 to 1.02x10^9 CFU/gm) reported by Kumar et al., (2011) and higher than the counts 1.2x10^5 to 7x10^5 CFU/gm) reported by Jadav and Raut (2014).

The coliform counts in the present study was 8.05x10^2 CFU/gm, 2.2x10^3 CFU/gm and 9.68x10^5 CFU/gm from co-operative, branded and non-branded respectively, which were higher than counts reported by Nelapati et al., (2009) from the three sources. Singh et al., (1976) reported coliform count similar to the counts from co-operative sector, where as Patel and Vyas (1971) reported counts similar to branded private sector samples in present study, Thattil et al., (1972) reported low count (442/gm). Warke et al., (2000) reported coliform count of 9.72 x 10^1 to 6x10^3 CFU/gm in the samples from branded private sector, which was almost similar to counts (2.2x10^3 CFU/gm) observed in the present study.

Coliform count of 9.68x10^5 CFU/gm was observed in present study from unbranded samples, which was almost similar to the count (4.5x10^5 CFU/gm ) reported by Harsh Kumar et al., (2011) and more than the counts of 2.3x10^3 CFU/gm and 2.48x10^4 CFU/gm reported by Warke et al., (2000) and Ambily and Bena (2012) respectively .

The yeast and mould counts in the sample from branded private sector was 3.87x10^3 CFU/gm in the present study, was almost similar to counts of 4x10^3 CFU/gm and 3.87x10^2CFU/gm reported by Ojokoh (2006) and Nelapati et al., (2009) respectively . A count of 4.28x 10^4 CFU/gm in the sample from unbranded sources in the present study was more than the count of 1x10^3 CFU/gm observed by Yaman et al., (2006) and almost similar to the counts of 1.03x10^3 CFU/gm reported by Nelapati et al., (2009). The yeast and mould counts in the sample from co-operative sector was 2.66x10^2 CFU/gm in the present study,was less than the counts of 2.66x10^3 CFU/gm reported by Nelapati et al., (2009).

**Incidence of pathogenic microorganisms**

The incidence of *Staphylococcus spp.*, *Salmonella spp.*, *Escherichia coli*, *Listeria spp.* and *Bacillus spp.* in ice-cream samples from different sources was presented in table 2.

The incidence of *Staphylococcus spp.* in the present study was 100% from non-branded sector, which was similar to the findings of Ikenebometi and Ogaguria (1993) and higher than the incidence (40%) reported by Jadav and Raut (2014). The incidence of *Staphylococcus spp*. In the ice-cream samples from branded private sector was 53% in the present study, which was less than incidence (100%) reported by Ojokoh (2006). The incidence of *Staphylococcus spp*. in co-operative sector samples in present study was 60%. The incidence of *Salmonella spp*. in branded private ice-cream samples in the present study was found to be higher (73%) than the incidence (60%) observed by Ojokoh
Salmonella spp. incidence in co-operative was 40% which was less than branded private sector where as, for non-branded sector the incidence was 90%, which was higher than other two sources.

The incidence of *Escherichia coli* in the ice-cream samples from non-branded category was 100% in the present study, which was very high compared to the incidence (40%) reported by Jadhav and Raut (2011). The incidence was 63% and 86% in co-operative and branded private ice cream samples respectively in the present study. The *Escherichia coli* incidence in co-operative and branded private samples was also high and alarming for public health.

Incidence of *Listeria spp.* in the present study was 53% in co-operative sector ice-cream samples. Warke *et al.*, (2000) reported 53% incidence of *Listeria spp.* in branded private sector ice-cream samples, which was less than the incidence of 73% observed in the samples from branded private sector, but similar to the incidence in co-operative sector samples observed in the present study. The incidence of *Listeria spp.* in the ice-cream samples from non-branded sector was 76%, which was lower than the incidence (100%), reported by Warke *et al.*, (2000).

The incidence of *Bacillus spp.* in the samples from co-operative sector was less (43%), which was less than the incidence in the samples from branded private and non-branded sectors in the present study. Warke *et al.*, (2000) reported 26.6% incidence of *Bacillus spp.* in the ice-cream samples from branded private sector, which was very less than the incidence of 80% observed the present study. Yaman (2006) reported very less incidence (19%) of *Bacillus spp.*, in the ice-cream samples from non-branded sector compared to higher incidence (93%) observed in the present study.

**Pathogenic microorganisms counts**

The pathogenic counts of *Staphylococcus spp.*, *Salmonella spp.*, *Escherichia coli*, *Listeria spp.* and *Bacillus spp.* in ice-cream samples from different sources was presented in table 3.

The *Escherichia coli* counts in the ice-cream samples from non-branded sector was high (9.6x10^4 CFU/gm), least in co-operative sector (4.8x10^3 CFU/gm), and in between in branded private sector (3.52x10^3 CFU/gm).

The counts of *Staphylococcus spp.* in the present study from co-operative sector ice cream samples was 1.2x10^3 CFU/gm, which was higher than the counts of 1.07x10^2 CFU/gm reported by Ambily and Been (2012). The counts of *Staphylococcus spp.* in the samples from non-branded sector was 3.7x10^4 CFU/gm, which was higher than the counts of 1x10^2 CFU/gm, reported by Yaman *et al.*, (2006). The counts in the samples from branded private sector was 5.82x10^3 CFU/gm, which was in between the counts of co-operative and non-branded sector in the present study.

The counts for *salmonella spp.* was 5.8x10^1 CFU/gm in branded private sector, which was higher than the counts of 1x101 CFU/gm reported by Jadav and Raut (2014). The counts of 3.8x10^2 CFU/gm was observed in the samples from non-branded sector in the present study, was less than the counts of 8x10^1 CFU/gm reported by Jadav and Raut (2014).

The counts of *salmonella spp.* from co-operative sector was 1.8x10^1 CFU/gm, was less compared to the counts from branded private and unbranded sectors in the present study. The *Listeria spp.* counts from non-branded sector was high (6.2x10^2 CFU/gm), intermediate in branded private sector.
(8.5x10^1 CFU/gm) and least in co-operative sector (2.5x10^1 CFU/gm) in the present study. The counts for *Bacillus spp.* in the present study from non-branded sector was 3.56x10^3 CFU/gm, which was less than the counts of 1x10^4 CFU/gm reported by Yaman *et al.*, (2006). The counts from branded private sector (5.2x10^1 CFU/gm), was intermediate and the counts from co-operative sector (1.8x10^1 CFU/gm) and was least in the present study.

**Table.1** Total viable counts, coliform count, faecal coliform count and yeast and mould counts in ice-cream samples from different sources (CFU/gm)

| Source          | SPC/ml        | Coliform/ml  | Faecal coliform | Yeast and moulds |
|-----------------|---------------|--------------|-----------------|------------------|
| Co-operative    | 2.51 x 10^5   | 8.05 x 10^2  | 2.57 x 10^2     | 2.66 x 10^2      |
| Branded         | 3.08 x 10^5   | 2.2 x 10^3   | 9.57 x 10^2     | 3.87 x 10^3      |
| Non-Branded     | 4.99 x 10^7   | 9.68 x 10^3  | 4.32 x 10^4     | 4.28 x 10^4      |

**Table.2** Incidence rates of microorganisms in the ice-cream samples from different samples

| Incidence       | *Staphylococcus* spp. | *Salmonella* spp. | *Escherichia coli* | *Listeria* spp. | *Bacillus* spp. |
|-----------------|-----------------------|-------------------|-------------------|-----------------|-----------------|
| Co-operative    | 60%                   | 40%               | 63%               | 53%             | 43%             |
| Branded         | 53%                   | 73%               | 86%               | 73%             | 80%             |
| Non-branded     | 100%                  | 90%               | 100%              | 76%             | 93%             |

**Table.3** Pathogenic micro-organisms counts in ice-cream samples from different sources (CFU/gm)

| Incidence       | *Staphylococcus* spp. | *Salmonella* spp. | *Escherichia coli* | *Listeria* spp. | *Bacillus* spp. |
|-----------------|-----------------------|-------------------|-------------------|-----------------|-----------------|
| Co-operative    | 1.2 x 10^3            | 1.8 x 10^1        | 4.8 x 10^2        | 2.5 x 10^1      | 1.8 x 10^1      |
| Branded         | 5.82 x 10^4           | 5.8 x 10^1        | 3.52 x 10^4       | 8.5 x 10^1      | 5.2 x 10^1      |
| Non-Branded     | 3.7 x 10^4            | 3.8 x 10^2        | 9.6 x 10^4        | 6.2 x 10^2      | 3.56 x 10^3     |

The general microbial quality, incidence and counts of certain pathogenic microorganisms like *Staphylococcus* spp., *Salmonella* spp., *Escherichia coli*, *Listeria* spp. and *Bacillus* spp. was very high in the samples from non-branded sector, good from cooperative sector and optimum from branded private sector. Infants and children, the lovers of ice-cream may consume more amount of ice-cream mostly from non-branded sector due to cheaper price, so, care should be taken while consuming ice-creams from brand less or road side sellers.
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