

Assessment of Pathogenic Bacteria from Ice Cream and Ice Pop Sold in Gaborone, Botswana

S. Mathews¹, L. Ngoma¹, B. Gashe² and S. Mpuchane²

¹*Department of Biological Sciences, School of Environmental and Health Sciences, Faculty of Agriculture, Science and Technology, North-West University, Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa*

²*Department of Biological Sciences, Faculty of Science, University of Botswana, 4775 Notwane Rd. Gaborone, Botswana Private Bag UB 0022 Gaborone, Botswana*

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ABSTRACT This study was intended to isolate the following pathogenic bacteria; *Yersinia enterocolitica*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* species. Out of these four organisms, *L. monocytogenes* was not found in all samples. Pathogens were isolated from 31.4% of the 150 samples of open ice cream and 12.0% was isolated from packed ice cream. The targeted pathogens were not found in ice pop. *St. aureus* was found to have the highest percentage from both samples (18.7% and 53.0% respectively) followed by *Salmonella* species (8.0%) and *Yersinia* (4.7%). Coliforms and faecal coliforms were also isolated and characterised. Open ice cream was found to have a large number of coliforms (88%) and faecal coliforms (34.8%) of the total samples tested.

INTRODUCTION

Different dairy products provide different nutrients and allow a certain group of the microorganisms to grow on them (Osamwonyi et al. 2011; Gucukoglu et al. 2013). The products also vary in pH and water activity (aw) thus providing different growth environments (Frank 1997; Aaku 2000; Caglayanlar et al. 2009). Ice cream though it is a dairy product, the temperature at which it is kept and its composition provides eliminating conditions especially for those organisms that cannot tolerate low temperatures. Possible sources of these microorganisms in ice cream have been reported to include raw materials used for the composition of ice cream mix such as separated milk and milk powder, cream, flavouring, colouring substances, stabilizers (Caglayanlar et al. 2009) and from air during processing (Osamwonyi et al. 2011). The presence of these organisms in pasteurized ice cream could be due to their ability to survive the pasteurization process as in the case with spore formers, (Aaku, 2000; Osamwonyi et al. 2011) and they may persist in ice cream product thereafter. Psychrotrophic microorganisms are therefore the major contaminants and pathogens associated with ice cream and other foods that are served in frozen or chilled state (Arnaut-Rollier et al. 1999; Caglayanlar et al. 2009). Psychrotrophic organ-

isms are microbes that strive best in low temperatures (refrigeration temperatures as well as freezing temperatures) (Schroder 1984). The major psychrotrophic bacteria found in milk and milk products include species of *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Chromobacterium*, *Citrobacter*, *Clostridium*, *Corynebacterium*, *Flavobacterium*, *Lactobacillus*, *Microbacterium*, *Moraxella*, *Serratia*, *Streptococcus*, *Pseudomonas*, *Aeromonas*, *Enterobacter*, *Klebsiella*, *Staphylococcus*, *Micrococcus*, *Yersinia*, *Listeria* and *Escherichia* (Schroder 1984; Sinell 1989; Eneroth et al. 1999). Some of the psychrotrophic yeasts that have been found in association with refrigerated foods include *Candida*, *Cryptococcus*, *Rhodotorula*, *Saccharomyces*, *Hansenula*, *Debaryomyces*, *Kluveromyces*, *Torulopsis*, *Trichosporon*, and *Pichia* (Sinell 1989) and the moulds include *Alternaria*, *Aspergillus*, *Cladosporium*, *Mucor*, *Penicillium*, *Rhizopus*, *Fusarium* and *Trichothecium* (Alexopoulos et al. 1996). Fungi predominate in refrigerated food spoilage when water activity, acidity, processing, or packaging conditions select for their growth over bacteria (Alexopoulos et al. 1996). The emergence of psychrophilic foodborne pathogens and the rising level of foodborne diseases have raised a lot of concerns about the safety of refrigerated food (Sinell 1989; Szabo et al. 2000). The pathogenic psychrotrophs that grow at or below 5°C include *Aeromonas hydrophila*, *Clostridium botulinum*, *Listeria monocytogenes*,

Address for correspondence:
E-mail: 24014028@nwu.ac.za

Vibrio cholerae, *Yersinia enterocolitica* and some strains of *Escherichia coli* (Walker et al. 1990; Cotton and White 1992). Other foodborne pathogens such as *Bacillus cereus*, *Clostridium perfringens*, *Salmonella* species, and *Staphylococcus aureus* have minimal growth temperatures between 5°C and 15°C and their growth is dependent on the ecology of food, competitive microflora, storage temperature, time and other conditions such as pH (Rivas et al. 1984; Baylis et al. 2000; Szabo et al. 2000). Psychrotrophic bacterial pathogens isolated from desserts and other dairy products such as ice creams and sherbets have been found to be the cause of food poisoning related outbreaks in different parts of the world (Warke et al. 2000). Though psychrotrophic organisms have been found to cause spoilage to milk, they have not been associated with spoilage of ice cream (Kraft 1992) if done rapidly but delay or temperature abuse may occur, the possibility for bacterial growth particularly in soft serve products exists (Schroder 1983; Kraft 1992). As most of the ice cream consumers are children of vulnerable age groups, it is required to be microbiologically safe (Warke et al. 2000; Caglayanlar et al. 2009). Therefore, the objective of this study was to isolate, quantify and identify coliforms, faecal coliforms and pathogens present in ice creams and ice pops sold in Gaborone.

MATERIAL AND METHODS

Coliform and Faecal Coliform Count

The Most Probable Number (MPN) method was used to determine total coliforms, faecal coliforms and *E. coli* for all the three food commodities. The samples were subjected to Lauryl Tryptose broth (LT) (OXOID) following the 3 x 3-tube method (Andrews 1992). The tubes were incubated for 48 hours at 35°C. A loopful of cultures showing gas production from the tubes was streaked on Eosin Methylene Blue agar (EMBA) (OXOID) to confirm presence of coliforms. Colonies characterised by a metallic green sheen on EMBA were further characterised and identified using API-20E strips (BioMerieux S. A., Marcy-1'Etoile, France) and API-20E catalogue to confirm presence of *E. coli*. The presence of *E. coli* was an indication of faecal contamination. The MPN table was used to determine the number of coliforms present per ml of sample.

Isolation of Pathogens

Four pathogenic microorganisms were targeted. These were *Y. enterocolitica*, *L. monocytogenes*, *St. aureus* and *Salmonella* species. These were targeted as they are some of the common human pathogenic microorganisms found in dairy products of which ice cream is one of them.

St. aureus

For all ice cream and ice pop samples, 25 ml was added to 225 ml of Ringers solution (OXOID). From the dilution 1ml was transferred to 3 test tubes of Tryptone Soy Broth (TSB) (OXOID) 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) supplemented with Egg Yolk Tellurite Emulsion (FD 046) (HiMEDIA). The plates were incubated at 35°C for 24 - 48 hours. Colonies that were jet black with entire edges on BPA agar which are suspected to be *St. aureus* were transferred to tubes with 3 ml Brain Heart Infusion broth (BHI) (OXOID) for further verification. 0.5 ml of Rabbit coagulase plasma (Pro-Lab Diagnostics) was added to the tubes and incubated at 37°C for six hours. After incubation tubes were observed for clot formation. The cultures with *St. aureus* were stored on Tryptone Soy Agar (TSA) (OXOID) slants for identification using API-STAPH strips (BioMerieux S. A., Marcy-1'Etoile, France) and API-STAPH catalogue.

Salmonella

For all the samples enrichment was done by adding 25 ml of the sample into 225 ml Tetrathionate broth (TT) (OXOID) (Beumer et al. 1991). This was incubated at 37°C for 24 hours. Following incubation a loopful was streaked on plates of Hektoen Enteric (HE) (OXOID) and Brilliant Green Agar (BGA) (OXOID) (Andrews, 1992). The plates were incubated at 35°C for 24 hours and colonies were observed. Blue-green colonies in HE agar and pinkish colonies in BGA that represented *Salmonella* were subcultured and subjected to Triple Sugar Iron (TSI) (OXOID) slants and Urea agar

(OXOID) slants and incubated at 37°C for 24 hours (Andrews 1992). The positive TSI slants, alkaline (red) slant and acid (yellow) butt and negative Urea agar slants, no colour change, after incubation were subcultured and kept on TSA slants at 4°C for further characterisation and identification. API-20E strips (BioMerieux S. A., Marcy-1'Etoile, France) were used for identification. Isolates confirmed by this test were subjected to serological testing using polyvalent antiserum containing agglutinins for *Salmonella* O antigens (Mast Assure) and *Salmonella* H antigens (Mast Assure) for verification that they are *Salmonella* isolates (Kelly et al. 1985; Gray 1995).

Yersinia

Enrichment was done by adding 25 ml of each of the test sample in 225ml of Peptone Sorbitol Bile Broth (PSBB) (Andrews 1992) in a conical flask. The flasks were incubated at 10°C for 20 days. After incubation, a loopful was transferred into 1 ml of 0.5% saline from which a loopful was streaked on plates of *Yersinia* Selective Agar base (YSA) (OXOID) supplemented with *Yersinia* Selective Supplement (HiMEDIA, FD 034). The plates were incubated at room temperature (about 25°C) for 24 hours (Andrews 1992). The plates were observed and colonies that had colourless entire edges with deep red center which are suspected to be *Yersinia* were subjected to Triple Sugar Iron agar (TSI) (OXOID) slants. Those that showed an alkaline/acid reaction with no production of H₂S on the slants were taken as presumptive colonies (Schiemann and Wauters 1992) and were subcultured on TSA agar. Cultures were stored on Tryptone Soy Agar (TSA) slants for further characterisation and identification and kept at 4°C. API-20E strips (Bio-Merieux S. A., Marcy-1'Etoile, France) were used for final identification of the organisms.

Listeria

Enrichment was done by adding 25 ml of each of the test sample in 225ml of *Listeria* Selective broth (LSB) (OXOID) (Curtis and Lee 1995) with *Listeria* Selective Supplement (PALCAM, FD 061) (HiMEDIA) in a conical flask. The flasks were incubated at 10°C for 20 days. After incubation, a loopful was streaked on plates of

Listeria Selective Agar base (LSA) (OXOID) supplemented with Modified *Listeria* Selective Supplement (OXFORD, SR 206E) (OXOID) (Curtis and Lee 1995). The plates were incubated at 30°C for 24 hours (Andrews, 1992). Dark brown and black colonies suspected to be *Listeria* were sub-cultured in TSA agar for further characterisation and identification. Colonies were stored on TSA slants at 4°C. API-List strips (Bio-Merieux S. A., Marcy-1'Etoile, France) were used for identification.

Physiochemical Analysis

pH Measurement

The pH of samples was measured using an Accumet/Fisher Scientific model 50 pH meter (London, U.K.) with a miniature combination glass electrode.

Temperature Measurement

The temperature of samples was determined using U.K 76mm Immersion thermometer.

RESULTS

Microbial Analysis

Coliforms Counts

The total coliform count ranged from 4 colonies of bacteria/g to 1500 colonies of bacteria/g for both open ice cream (sample A) and packed ice cream (sample B). Faecal coliform count ranged from 4 colonies of bacteria/g to 1500 colonies of bacteria/g and 4 - 460 colonies of bacteria/g for open ice cream and packed ice cream, respectively

Total Coliform Counts for Open Ice Cream (Sample A)

The results showed high coliform counts from open ice cream. Coliforms were found in 132 (88%) of the 150 samples tested. Coliforms were not detected from 18 (12%) of the tested samples. The frequency for the different ranges of counts was as shown in Figure 1. Of the 132 positive samples, 84 (63.6%) had counts above the microbiological standards for ice cream which is 100 bacteria/g.

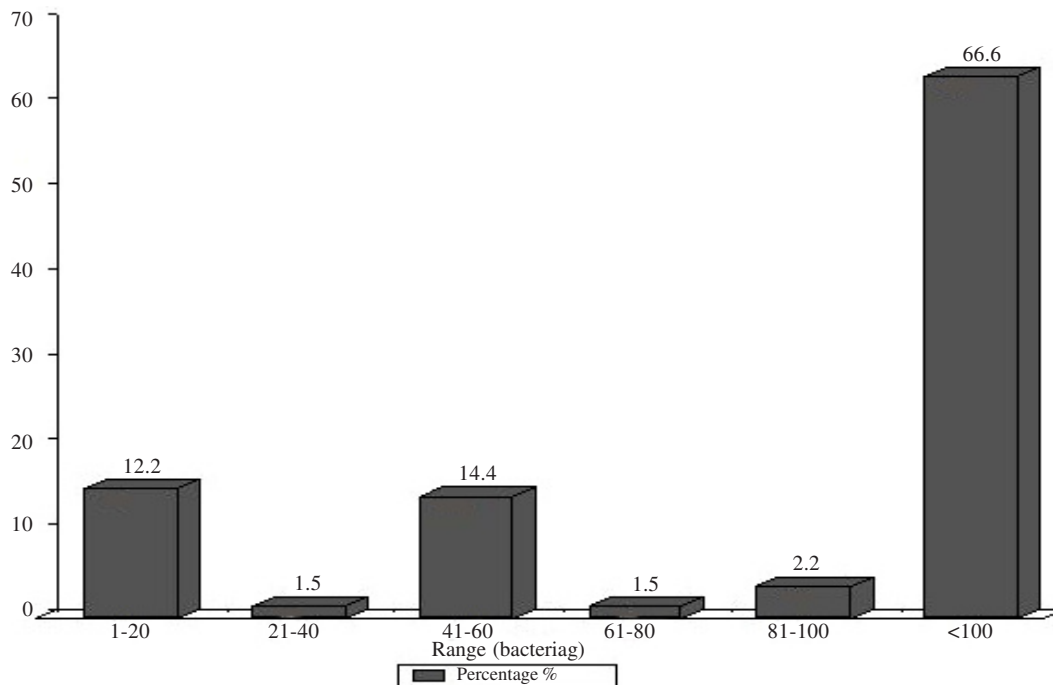


Fig. 1. Total coliform count (bacteriag) for open ice cream (sample A)

Total Coliform Count for Packed ice cream (sample B)

Packed ice cream showed low coliform counts (Fig. 2). Coliforms were detected from 16 (10.7%) samples of the 150 tested. Of all the 16 samples, only two (12.5%) had counts above 100 bacteria/g and 87.5% of the samples was within the microbiological standards (Fig. 2).

Faecal Coliform and *E. coli* Isolated from Sample A

The results shows a high degree of faecal contamination with 46 (34.8%) of the 132 samples being positive for faecal coliforms. All the faecal coliforms were also positive for *E. coli*. The frequency on the bacterial counts was presented in Figure 3. Of the 46 samples, 37% and 32.6% had counts above 100 colonies of bacteria/g for faecal coliform counts and *E. coli*, respectively.

Faecal Coliform and *E. coli* Isolated from Sample B

Faecal coliforms were isolated from 9 (6.0%) only. All the samples that were found to contain

faecal coliforms were also positive for *E. coli* (Fig. 4). All samples that did not have faecal coliform or *E. coli* had counts above the microbiological specifications for ice cream (100 colonies of bacteria/g). Out of the 150 samples tested, 141 (94%) did not have faecal coliforms nor *E. coli*.

Study on Targeted Pathogens

The targeted pathogens were *St. aureus*, *Salmonella* species, *Yersinia* species and *L. monocytogenes*. Of the 300 samples of ice cream tested, 47 of the open ice cream and 18 of the packed ice cream had some pathogens isolated but no *L. monocytogenes* was isolated though.

Pathogenic Micro-organisms Isolated from Open Ice Cream (Sample A) and Packed Ice Cream (Sample B)

Pathogens were isolated from 47 (31.4%) of the 150 samples of open ice cream tested. *St. aureus* constituted the highest percentage of these (18.7%). *Salmonella* constituted 8% and *Yersinia* was 4.7% of the total pathogens isolated from sample A (Fig. 5). Of the *Salmonella* isolates

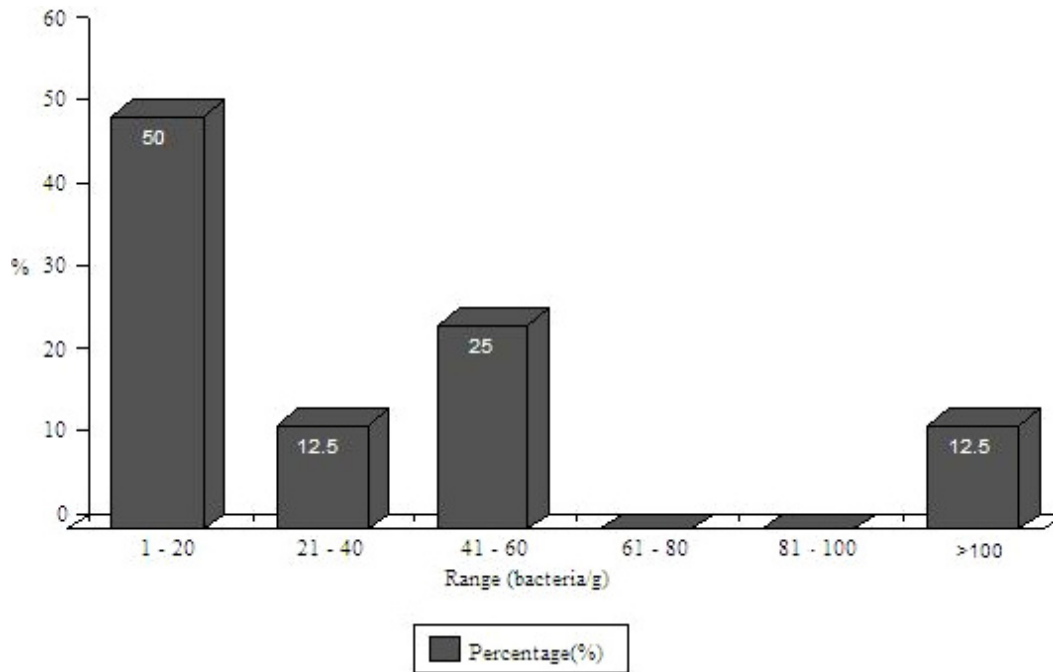


Fig. 2. Total coliform count (bacteri/g) for packed ice cream (sample B)

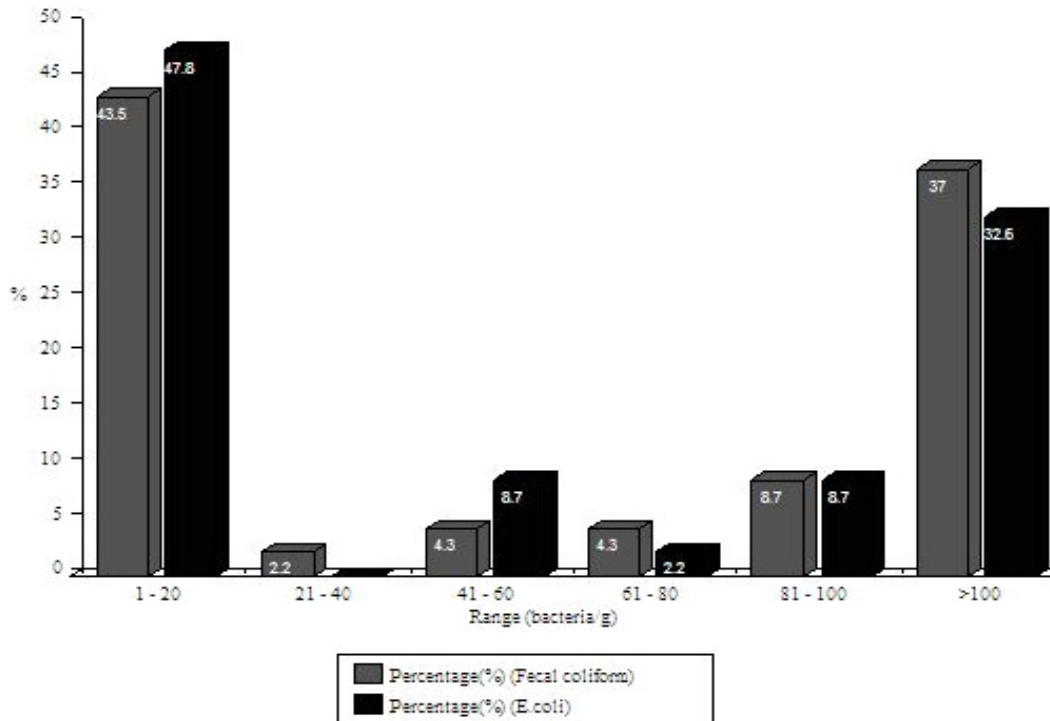


Fig. 3. Faecal coliform count and E. coli (bacteri/g) for open ice cream (sample A)

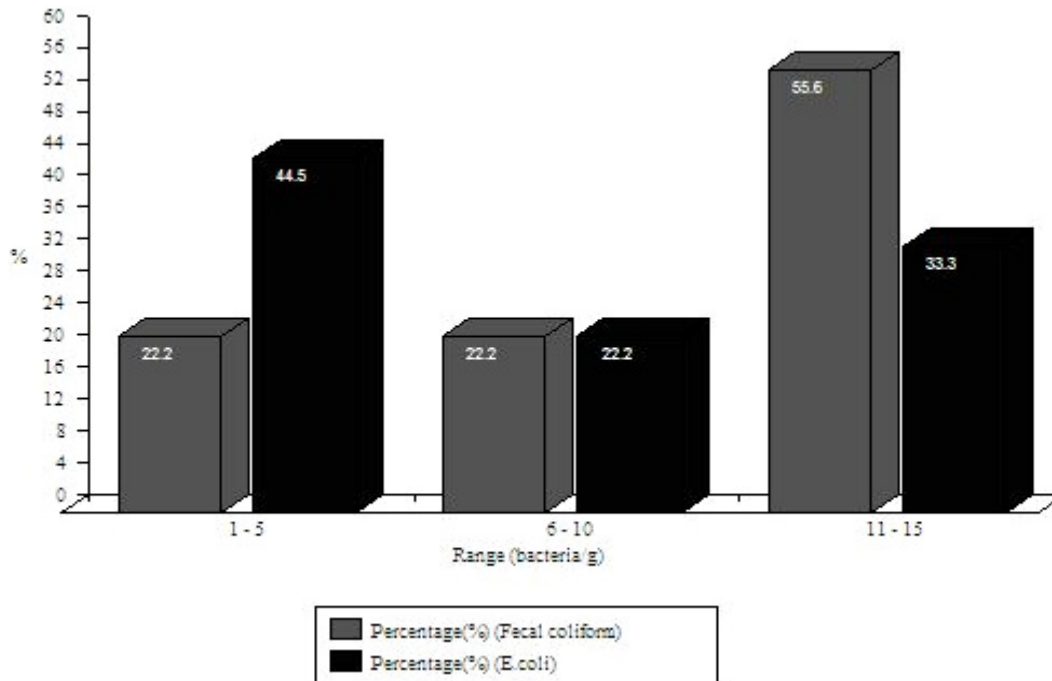


Fig. 4. Faecal coliform count and E. coli (bacteri/g) for packed ice cream (sample B)

belonged to *Salmonella* group O. Of the 150 samples of packed ice cream tested, pathogens were isolated from 18 (12.0%) samples. From this sample *St. aureus* constituted 5.3 % followed

by *Yersinia* with 4.0% and *Salmonella* was only 2.7% of all the pathogens isolated from sample B (Fig. 5). The *Salmonella* species isolated from packed ice cream, all belonged to group O.

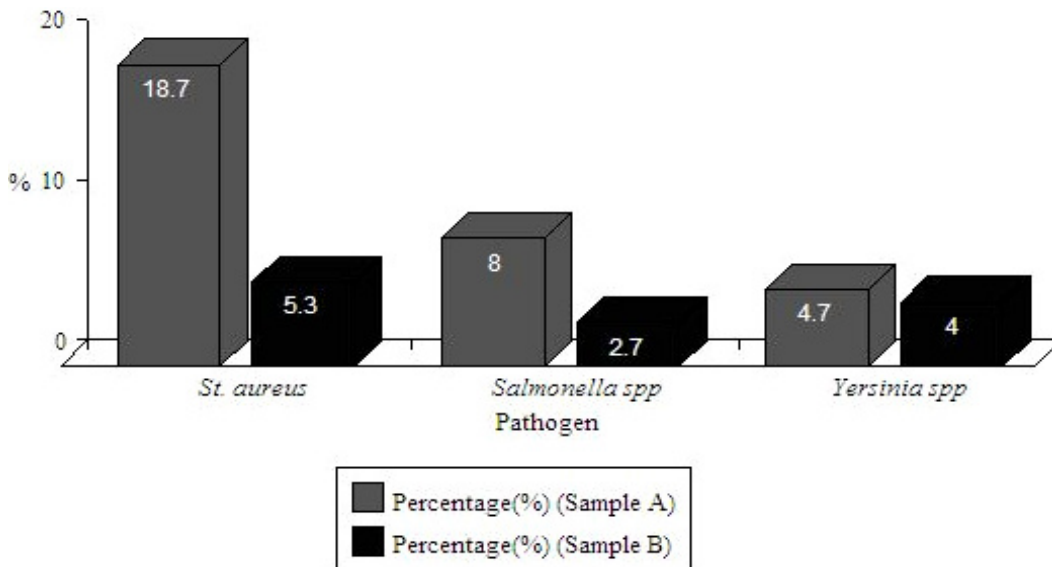


Fig. 5. Pathogens isolated from open ice cream (sample A) and packed ice cream (sample B)

DISCUSSION

The microbiological standards for ice cream as according to International Dairy Federation proposal in 1982 are as follows; Mesophilic count $n = 5, c = 2, m = 2.5 \times 10^4, M = 2 \times 10^5$. Coliforms $n = 5, c = 2, m = 10, M = 100$, *Salmonella* $n = 10, c = 0, m = 0$ (Adams and Moss 1995). The results show that 20% of the open ice cream samples exceeded the microbiological standards. The results indicate that open ice cream is prone to post pasteurisation contamination during handling and/or in the serving machine. This shows that the quality of ice cream is sold to the consumer is of poor quality. A similar study done in Mumbai, India showed higher bacterial load of 10^5 to 10^6 CFU/ml and was 10 - 100 fold higher than the ISI standards of 5×10^4 (Warke et al. 2000).

The results for total coliforms and faecal coliforms for open ice cream shows high contamination of up to 63.6% and 37% of samples, respectively, (Figs. 1 and 3) which exceed the standard for ice cream of which the acceptable is between 10 and 100 cells for every 5 samples tested. This is an indication of poor sanitation practices on the food commodity. The results showed that 88% of all the tested samples of open ice cream were positive for total coliforms while 30.7% were positive for faecal coliforms. The results were similar to those found by Maifreni et al. (1993), Wilson et al. (1997) and Warke et al. (2000). The high incidence of coliforms in these studies was mainly associated with poor hygienic practices by the handling personnel.

The high incidence of indicator organisms in ice cream has been highly associated statistically with high counts in the same product (Wilson et al. 1997). The accepted standard for coliforms in ice cream is 10 colonies of bacteria/ml (Richter et al. 1992; Adams and Moss 1995). Of all the positive samples for total coliforms from open ice cream, 63.6% exceeded the accepted standard by the Food and Drug Administration (FDA) and the U. S. Department of Agriculture (USDA) for manufactured milk products. In a similar study carried out in Cameroon, 71.3% were found to be positive for total coliforms (Wouafo et al. 1996). This has some health implications.

The presence of faecal coliforms and *E. coli* poses a major health concern as far as microbiological safety is concerned. This is due to the fact that their presence implies the possibility of

having pathogens. Due to the fact that coliforms cannot withstand pasteurisation (Jay 1996), their presence in ice cream being a pasteurised product, indicate contamination either by the selling personnel or improperly cleaned the vending machines (Austin and Bergeron 1995; Lunden et al. 2000). For the packed ice cream, only one (6.7%) of the samples had counts above 100 colonies of bacteria/g for total coliform counts. The samples containing faecal coliform and *E. coli* had counts which were below 100 colonies of bacteria/g. This showed less contamination of packed ice cream but implies poor hygiene practices. The coliform in packed ice cream might have been mainly due to post pasteurisation contamination due to prolonged storage under inadequately clean freezers. This is due to that it was observed that some of the packed ice cream remained in the freezer in supermarkets until the containers get damaged and some were found covered in dust, which indicate poor sanitation and hygienic practices by personnel or management. Incidence of *St. aureus*, *Salmonella* species and *Yersinia* species was observed in both open and packed ice cream (Fig. 5).

The pathogens were more frequent in open ice cream (31.4%) than packed ice cream (12.0%) which shows a strong association between indicator organisms and presence of pathogens as well as increased counts. *L. monocytogenes* was not detected in any of the samples for both types of ice cream. This was unlike other studies done on the same commodity in Costa Rica and Mumbai, whereby *L. monocytogenes* was found to be the major contaminant (Warke et al. 2000; Windrantz and Arias 2000) although in low numbers (Greenwood et al. 1991; Harrison et al. 2000; Kamat et al. 2000). Incidence of *S. aureus*, *Salmonella* species and *Yersinia* species was 18.7%, 8.0% and 4.7%, respectively, in open ice cream and 5.3%, 2.7% and 4.0%, respectively, in packed ice cream (Fig. 5). The results are in concurrence with similar studies performed on the same food commodity by Wouafo et al. (1996), Wilson et al. (1997) and el-Sherbini et al. (1999). The presence of these pathogens implies that contamination does occur and as a result leave the food commodity not very safe to eat (Sandhya et al. 2012). Due to the fact that pathogens are not supposed to be present in ice cream as per the microbiological specifications for ice cream ($n = 10, c = 0$) the product is of poor quality. This therefore means that hygienic

practices have to be employed and emphasized regarding these food commodities to ensure microbiological safety. These pathogenic organisms have been known for causing a lot of gastroenteritis related outbreaks in different parts of the world (Jay 1996; el-Sherbini et al. 1999; Windrantz and Arias 2000). Their presence in food therefore poses a serious health threat (Wouafo et al. 1996; Gucukoglu et al. 2013).

CONCLUSION

The psychrotrophic counts were high which implies that the possibility of spoilage is high as they are the potential spoilage organisms in frozen food products. The presence of mesophiles as well as psychrotrophs in packed ice cream indicates that post pasteurisation contamination does occur during storage in the supermarkets. The presence of faecal coliforms in some samples implies that hygienic and sanitation practices need to be improved to ensure safety of the ice creams. The presence of yeasts and moulds also implies poor quality of open ice cream.

REFERENCES

- Aaku EN 2000. Microbiological Quality of raw, pasteurised milk and 'madila' (cultured fluid milk) marketed in Botswana. *Botswana Notes and Records* (Unpublished).
- Adams MR, Moss MO 1995. *Food Microbiology*. London: Chapman and Hall.
- Alexopoulos CJ, Mims WC, Blackwell M 1996. *Introductory Mycology*. 4th Edition. New York: John Wiley and Sons, Inc.
- Andrews W 1992. *Manual of Food Control. 4 Rev. 1. Microbiological Analysis*. Food and Agriculture Organisation of the United Nations (FAO): Rome.
- Arnaut-Rollier I, Lieven De Zutter, Jan Van Hoof 1999. Identities of the *Pseudomonas* spp in flora from chilled chicken. *International Journal of Food Microbiol*, 48: 87-96.
- Austin J W, Bergeron G 1995. Development of bacterial biofilms in dairy processing lines. *Journal of Dairy Research*, 62: 509-519.
- Baylis CL, MacPhee S, Betts RP 2000. Comparison of methods for the recovery and detection of low levels of injured *Salmonella* in ice cream and milk powder. *Letter Applied Microbiology*, 30(4): 320-324.
- Buchanan RL 2000. Acquisition of microbiological data to enhance food safety. *Journal of Food Protection*, 63(6): 832-838.
- Cotton LN, White CH 1992. *Listeria monocytogenes*, *Yersinia enterocolitica* and *Salmonella* in Dairy plant environments. *Journal of Dairy Science*, 75(1): 51-57.
- Caglayanlar GE, Kunduhoglu B, Coksoyler N 2009. Comparison of the Microbiological Quality of Packed and Unpacked Ice Creams Sold in Bursa, Turkey. *Journal of Arts and Sciences*, 12: 93-102.
- Dalu JM, Feresu SB 1996. Survival of *Listeria monocytogenes* in three Zimbabwean fermented milk products. *Journal of Food Protection*, 59(4): 379-383.
- de Centorbi OP, de Guzman AC, de Cuadrado AA, Laciari AL, Alcaraz LE, de Milan MC, de Pederiva N B 1989. Determination of the sanitary quality and detection of *Salmonella* spp and *Yersinia enterocolitica* in ice cream. *Revista Argentina De Microbiologia*, 21(2): 63-69.
- Djuretic T, Wall PG, Nicholas G 1997. General outbreaks of infectious intestinal disease associated with milk and dairy products in England and Wales: 1992 to 1996. *Communicable Disease Report CDR Rev*, 7(4): R54.
- Doyle MP 1988. *Yersinia enterocolitica*. *Food Technology*, 42(4): 8.
- El-Kest SE, Yousef AE, Marth EH 1991. Fate of *Listeria monocytogenes* during freezing and frozen storage. *Journal of Food Science*, 56(4): 1068-1071.
- el-Sherbini M, al-Agili S, el-Jali H, Aboshkiwa M, Koga M 1999. Isolation of *Yersinia enterocolitica* from cases of acute appendicitis and ice cream. *Eastern Mediterranean Health Journal*, 5(1): 130-135.
- Enorothe A, Ahrne S, Molin G 2000. Contamination of milk with Gram-negative spoilage bacteria during filling of retail containers. *International Journal of Food Microbiology*, 57: 99-106.
- Farber J, Peterkin PJ 1991. *Listeria monocytogenes*: A foodborne pathogen. *Microbial Review*, 55: 476-571.
- Frank JF 1988. Enteropathogenic *Escherichia coli*. *Food Technology*, 42(4): 12-13.
- Frank JF 1997. Milk and dairy products. In: Mp Doyle, LR Beuchat, TJ Montville (Eds.): *Food Microbiology: Fundamentals and Frontiers*. Washington, DC: ASM Press, pp. 101-116.
- Garcia-Armesto MR, Sutherland AD 1996. Temperature characterization of psychrotrophic and mesophilic *Bacillus* species from milk. *Journal of Dairy Research*, 64: 261-270.
- Greenwood MH, Roberts D, Burden P 1991. The occurrence of *Listeria* species in milk and dairy products: A national survey in England and Wales. *International Journal of Food Microbiology*, 12: 197-206.
- Gucukoglu A, Cadira Q, Terzia G, Kevenk TO, Alisarli M 2013. Determination of enterotoxigenic and methicillin resistant *Staphylococcus aureus* in ice cream. *Journal of Food Science*, 78(5): 738-741.
- Harrison WA, Peters AC, Fielding LM 2000. Growth of *Listeria monocytogenes* and *Yersinia enterocolitica* colonies under modified atmospheres at 4 and 8°C using a model food system. *Journal of Applied Microbiology*, 88: 38-43.
- Hennessy TW, Hedberg CW, Slutsker L, White KE, Besser-Wiek JM, Moen ME, Feldman J, Coleman MW, Edmonson LM, MacDonald KL, Osterholm MT 1996. A national outbreak of *Salmonella enteritidis* infections associated with ice cream. *New England Journal of Medicine*, 334(20): 1281-1286.
- Indar-Harrinath L, Daniels N, Prabhakar P, Brown C, Baccus-Taylor G, Comissiong E, Hospedales J 2001. Emergence of *Salmonella enteritidis* Phage Type 4 in the Caribbean: Case-Control Study in Trinidad and Tobago, West Indies. *Clinic Infectious. Disease*, 32(6): 890-896.
- Kamat A, Warke R, Kamat M, Thomas P 2000. Low-dose irradiation as a measure to improve microbial quality

- of ice cream. *International Journal of Food Microbiology*, 62: 27-35.
- Kleer J, Hildebrandt G 2000. Microbial contamination of frozen foods. *Archiv Fur Lebensmittelhygiene*, 51(2): 42-45.
- Knight MT, Newman MC, Benzinger Jr MJ, Agin JR, Ash M, Hughes D 1996. TECRA Listeria Visual Immunoassay(TLVIA) for detection of *Listeria* in foods: Collaborative study. *JAOAC Int*, 79(5): 1083-1094.
- Kraft AA 1992. *Psychrotrophic Bacteria in Foods: Disease and Spoilage*. CRC Press: Florida. 147-236.
- Little CL, Knochel S 1994. Growth and Survival of *Yersinia enterocolitica*, *Salmonella* and *Bacillus cereus* in Brie stored at 4, 8 and 20°C. *International Journal of Food Microbiology*, 24: m137-145.
- Luisjuan-Morales A, Alaniz-De La RO, Vazquez-Sandoval ME, Rosas-Barbosa BT 1995. Prevalence of *Listeria monocytogenes* in raw milk in Guadalajara, Mexico. *Journal of Food Protection*, 58(10): 1139-1141.
- Lunden JM, Miettinen MK, Autio TJ, Korkeala HJ 2000. Persistent *Listeria monocytogenes* strains show adherence to food contact surface after short contact times. *Journal of Food Protection*, 63(9): 1204-1207.
- Maifreni M, Civilini M, Domenis C, Manzano M, Di Prima R, Comi G 1993. Microbiological quality of artisanal ice cream. *Zentralbl Hyg Umweltmed*, 194(5-6): 553-570.
- Miettinen MK, Bjorkroth KJ, Korkeala HJ 1999. Characterization of *Listeria monocytogenes* from an ice cream plant by serotyping and pulsed-field gel electrophoresis. *International Journal of Food Microbiology*, 46: 187-192.
- Monge R, Utzinger D, Arias ML 1994. Incidence of *Listeria monocytogenes* in pasteurized ice cream and soft cheese in Costa Rica, 1992. *Revista de Biologia Tropical*, 42(1-2): 327-328.
- Nastasi A, Mammina C, Fantasia M, Pontello M 1997. Epidemiological analysis of strains of *Salmonella enterica* serotype *Enteritidis* from foodborne outbreaks occurring in Italy, 1980-1994. *Journal of Medical Microbiology*, 46(5): 377-382.
- Newsome RL 1988. *Staphylococcus aureus*. *Food Technology*, 42(4): 14-15.
- Ombui JN, Kaburia HF, Macharia JK, Nduhiu G 1994. Coliform counts and *Escherichia coli* in raw commercial milk from dairy farmers in Kiambu District, Kenya. *East African Medical Journals*, 71(10): 635-639.
- Osamwonyi OU, Obayagbona ON, Olisaka F, 2011. Evaluation of the bacteriological quality of ice creams sold in some locations within Benin City. *Journal of Food Science and Technology*, 5(3): 6-11
- Ravishankar S, Harrison MA 1999. Acid adaptation of *Listeria monocytogenes* strains does not offer cross-protection against an activated lactoperoxidase system. *Journal of Food Protection* 62(6): 670-673.
- Richter RL, Ledford RA, Murphy SC 1992. Milk and milk products. In: C Vanderzant, DF Splittoesser (Eds.): *Compendium of Methods for the Microbiological Examination of Foods*. 3rd Edition. Washington DC: American Public Health Association, pp. 837-853.
- Rivas M, Cordal de Bobbi ME, Raffo Palma M, Moro AA 1984. Ice cream of Industrial manufacture: Incidence of *Staphylococcus aureus* and enterotoxigenicity. *Revista Argentina de Microbiologia*, 16(4): 225-228.
- Sandhya AM, Chodhury R, Bhattacharya R, Nagaran AG, Chakraborty D 2012. Direct detection of *Salmonella* without pre-enrichment in milk, ice-cream and fruit juice by PCR against h1a gene. *Journal of Food Control*, 23: 559-563
- Schroder MJA 1984. Origins and levels of post pasteurisation contamination of milk in the dairy and their effects on keeping quality. *Journal of Dairy Research*, 51: 59-67.
- Sinell HJ 1989. The hygiene of refrigerated and frozen foods. *Zentralbl Bakteriol Mikrobiol Hyg [B]*, 187(4-6): 533-545.
- Slavchev G 1986. Development and survival of *Yersinia enterocolitica* in pasteurised milk and ice cream. *Veterinary Medicine Nauki*, 23(6): 77-85.
- Szabo EA, Scurrah KJ, Burrows JM 2000. Survey for psychrotrophic bacterial pathogens in minimally processed lettuce. *Letters in Applied Microbiology*, 30: 456-460.
- Toora S, Budu-Amoako E, Ablett RF, Smith J 1992. Effect of high-temperature short-time pasteurisation, freezing and thawing and constant freezing, on the survival of *Yersinia enterocolitica* in milk. *Journal of Food Protection*, 55: 803-805.
- Uljas HE, Schaffner DW, Duffy S, Zhao L, Ingham SC 2001. Modelling of combined processing steps for reducing *Escherichia coli* O157: H7 populations in apple cider. *Applied Environment Microbiology*, 67(1): 133-141.
- Vought KJ, Tatini SR 1998. *Salmonella enteritidis* contamination of ice cream associated with a 1994 multistate outbreak. *Journal of Food Protection*, 61(1): 5-10.
- Walker J, Archer P, Banks JG 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. *Journal of Applied Bacteriology*, 68: 157-162.
- Wang G, Zhao T, Doyle MP 1997. Survival and growth of *Escherichia coli* O157: H7 in Unpasteurized and Pasteurized milk. *Journal of Food Protection*, 60(6): 610-613.
- Warke R, Kamat A, Kamat M, Thomas P 2000. Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India. *Food Control*, 11: 77-83.
- Wilson IG, Heaney JC, Weatherup ST 1997. The effect of ice-cream-scoop water on the hygiene of ice cream. *Epidemiology Infectious*, 119(1): 35-40.
- Windrantz P, Arias ML 2000. Evaluation of the bacteriological quality of ice cream sold at San Jose, Costa Rica. *Archivos Latino Americanos De Nutrition*, 50(3): 301-303.
- Wouafo MN, Njine T, Tailliez R 1996. Hygiene and microbiologic quality of ice creams produced in Cameroon. A public health problem. *Bulletin de Societe Pathologie Exotique*, 89(5): 358-362.