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# General Microbiological Quality of Ice Cream and Ice Pop Sold in Gaborone, Botswana

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**ABSTRACT** The study was aimed at investigating the microbial quality of ice cream and ice pop sold in Gaborone. From the study, a variety of microorganisms were isolated. The population of bacteria was found to increase from the open ice cream samples while the pre-packed ice cream had a lower population of bacteria. The types of microorganisms were both mesophilic and psychrotrophic. The population of mesophiles in open ice cream ranged between 3.30 $log_{10}$CFU/g and 6.78 $log_{10}$ CFU/g. The psychrotrophs in the same food samples ranged between 2.0 $log_{10}$ CFU/g and 6.70 $log_{10}$ CFU/g. These values were found to exceed the acceptable microbiological standard. The results for packed ice cream had lower counts of microbes (ranging between 2.48 and 5.48 $log_{10}$ CFU/g), with only one sample out of 150 having a log count above the microbiological standard of 4.0 $log_{10}$ CFU/g.

## INTRODUCTION

Ice cream and other edible ices can be referred to as frozen dairy desserts (Silliker et al. 1980). These include among others ice cream, *sherbet,* ices and ice milk. Ice cream contains mainly milk fat (about 10-16%; depending on the standard), non-fat milk solids (about 9-12%), sugar (sucrose) (9-12%), water (55 - 64%) and 0.20-0.50% stabiliser and/or emulsifier (Silliker et al. 1980; Pick et al. 1990; Potter and Hotchkiss 1995). Fruits, nuts, candies and syrups are optionally added into ice cream for flavour enrichment. It is sold in packages or in open containers at retail outlets/ice cream parlours, the open variety being distributed manually in scoops, cones, or sundaes across the counter (Çaðlayanlar et al. 2009). During production, transportation and storage, it may become contaminated with several microorganisms. Microorganisms are transmissible to humans through milk and milk products (Vasavada 1988; Tomislav et al. 2012). Some of the diseases associated with pathogenic bacteria associated with the dairy industry are tuberculosis, brucellosis diphtheria, scarlet fever, Q-fever and most commonly gastroenteritis (Vasavada 1988; Miettinen et al. 1998; Warke et al. 1999). According to the World Health Organisation (1988), national surveillance of milk and milk products conducted around the world have revealed that generally 5% of ice cream produced is contaminated. The pasteurisation temperatures that are currently used are sufficient to destroy the most heat-resistant of the non-spore forming pathogenic organisms such as *Mycobacterium tuberculosis* and *Coxiella burnetti* (Adams and Moss 1995). The micro-organisms that can stand pasteurisation are mainly the gram positive spore-forming bacteria and thermoduric non-spore-formers. The rest of gram negative and gram positive non-spore forming bacteria, yeasts and moulds are destroyed by pasteurisation (Jay 1996). The microflora of these frozen products before pasteurisation is mainly determined by ingredients used in making the products (Roberts et al. 1998). These usually include *Micrococcus* species, *Bacillus subtilis* and *Lactobacillus casei* that withstand pasteurisation (Roberts et al. 1998; Silliker et al. 1980). Under modern production conditions, thermoduric psychrotrophic microorganisms and particularly *Bacillus* species, determine the shelf life of pasteurised milk products during extended storage though (Frank et al. 1993; Garcia-Armesto and Sutherland 1996). This improved pre-packaging treatment has therefore reduced the microbiological load in dairy products. The microbial count usually goes down to below 80 per ml after the ice cream mix has gone through proper or adequate pasteurisation which is usu-

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ally more severe than the minimal requirements (Roberts et al. 1998) and this is mainly a result of the spores that survive the heat treatment (Roberts et al. 1998). Yeasts and moulds isolated from ice cream have been mainly associated with the use of cane sugar obtained from inadequately treated raw sugar cane (Silliker et al. 1980; Roberts et al. 1998). 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 pasteurisation and freezing that spoilage by microorganisms can occur. The microbiological quality of ice cream during retail marketing mainly depends upon the post production handling of the product as well as efficiency and sanitary conditions during frozen storage (Nelapati et al. 2009; Warke et al. 2000). The machines used to serve soft serve ice cream if not properly maintained can be the major sources of contamination due to biofilm formation (Hennessy et al. 1996; Dempsey et al. 2000; Kleer and Hildebrandt 2000; Nelapati et al. 2009). Organisms such as *Listeria monocytogenes* have been found to have an enhanced adherence to food contact surfaces in ice cream processing facilities and poultry plants (Austin and Bergeron 1995; Lunden et al. 2000) and as a result this may promote its survival in food processing facilities and initiate persistent plant contamination (Lunden et al. 2000). *L. monocytogenes* has been found to resist destruction at temperatures about -18oC (El-Kest et al. 1991) but it is destroyed by pasteurisation (Roberts et al. 1998; Silliker et al. 1980) and it can only be present when there is post pasteurisation contamination that it can be found in the products (Roberts et al. 1998; Nelapati et al. 2009). Low numbers of *L. monocytogenes* Scott A survived in ice cream for 5 months of frozen storage at -18oC with no indication of sublethal injury (Monge et al. 1994). It has also been isolated in several brands of ice cream and ice cream bars (Monge et al. 1994; Roberts et al. 1998; Silliker et al. 1980). Ice cream has not been associated with human listeriosis though (Miettinen et al. 1999; El-Kest et al. 1991). *Salmonella* outbreaks have been reported from ice cream in India (Chug 1996) and in the United States (Roberts et al. 1998; Hennessy et al. 1996). These have been isolated from ice cream (Greenwood et al. 1991; Schillinger 1999; Warke et al. 2000). Outbreaks of *Salmonella* have been reported in ice cream and other frozen desserts in different parts of the world such as USA, UK and India (Indar-Harrinauth et al. 2001; Hennessy et al. 1996; Dodhia et al. 1998; Mahon et al. 1999). Nationwide outbreak of salmonellosis was more likely the result of contamination of pasteurised ice cream premix during transport in tanker trailers that had previously carried non pasteurised liquid eggs containing *Salmonella enteritidis* (Hennessy et al. 1996). Pathogens if present may survive in ice cream for many months (Roberts et al. 1998; Okojoh 2006). Several factors are important in the production of high quality ice cream and are associated with the stages of production (Roberts et al. 1998; Little and De louvois 1999). These include cleaning and disinfection, hygiene of storage area, hygienic design and personnel training and failure to adhere to these practices may lead to high bacterial counts and potential public health problems (Roberts et al. 1998; US Department of Health Education and Welfare 1995c). Micro-organisms have been found to contaminate ice cream mix and proliferate as a result of temperature abuse; leading to food poisoning (Silliker et al. 1980; Buckner 1993; Warke et al. 2000; Tomislav et al. 2012). Food poisoning events have been reported from inefficient frozen storage chain under warm tropical climatic conditions leading to temperature abuse during transporting and distribution of ice cream (Champagne et al. 1994; Warke et al. 2000). This is because under such conditions the psychrotrophic bacteria present in the ice cream are activated (Tomislav et al. 2012). Other ice cream related out breaks have been found to occur as a result of post pasteurisation contamination especially in tankers that have previously transported unpasteurised raw eggs (Roberts et al. 1998; Mahon et al. 1999). The processing of these commodities involve pasteurisation; a process in which most micro-organisms are destroyed. Improper and insufficient heat treatment may lead to some organisms such as spore forming bacteria to survive and as a result making the product not safe to eat. This is due to the fact that the ingredients used to make ice cream such as milk fat may have some spore forming organisms that have survived heat treatment of milk prior to fat production (Jay 1996). The other ingredients such as sugar, emulsifiers and flavourants may have been contaminated by some heat resistant micro-organisms that include yeasts and moulds which may contaminate the product. Ice pops, the other ice cream related product, are mainly made from water, sugar and colourants. The dry ingredients if not properly stored may become contaminants of the resultant product. When the home-made ices are considered, no water treatment is employed prior to making of the products and thus could pose high risks of contamination to the products. Post-pasteurisation contamination may also occur making the products microbiological unsafe to eat. However, very little research work has been done on isolation, microbiological quality of ice cream and ice pop offered for public consumption in Gaborone. Hence, the principal goal of this present study was to assess the microbial quality of ice cream and ice pop sold in Gaborone. To achieve this objective, a series of bacterial species were isolated from ice cream and ice pop and identified using API-20E strips (BioMerieux S. A., Marcy-1'Etoile, France) and API-20E catalogue

## MATERIAL AND METHODS

### Sampling Area

The ice cream samples were obtained mainly from retail stores, street vendors and ice cream vending machines in different parts of Gaborone. All the samples were commercial made and were products of Dairymaid, South Africa. Open ice cream was bought at various vending machines at different serving points in Gaborone and ice cream vehicles. The packed ice cream was bought from different supermarkets in Gaborone. Ice pops were bought from street vendors at different places in Gaborone including at gates of schools. The two brands had preservatives sulphur-dioxide and sodium benzoate respectively.

### Samples Collection

Sampling took place from July 2000 through May 2001. Four hundred and ten (410) samples were analysed to determine microbiological quality. This number was distributed among the three food commodities that were studied, that is, open ice cream (sample A; n=150), packed ice cream (sample B; n=150) and ice pop (sample C; n=110). Samples of ice pop were collected and thawed by immersing in a hot water bath at 55°C for 30 seconds while the ice cream was thawed by standing at room temperature for 10 minutes (Andrews 1992).

### 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.

### Total Plate Count

Twenty- five (25) ml of the samples was added to 225ml of sterile Ringers solution (OXOID) and shaken to make a homogenous mixture (Andrews 1992). The samples were then serially diluted to 10-3 for open ice cream (sample A), 10-2 for packed ice cream (sample B) and ice pop (sample C). Total aerobic plate counts were estimated through the pour plate method by mixing 1 ml of the 10 folds dilutions with 15 ml of Plate Count Agar (PCA) (OXOID) (Andrews 1992; Swanson et al. 1992).The plates were made in duplicates for each dilution and were divided into two sets. The sets were incubated at 10°C for 10 days for psychrotrophic counts (Frank et al. 1993) and 30°C for 48 hours for mesophilic counts respectively (Andrews 1992; Frank et al. 1993). The plates with colonies ranging between 25 and 250 were counted. After incubation representative colonies from each plate were picked. The isolates were then purified by sub-culturing on Tryptone Soy Agar (TSA) (OXOID) for later identification. Strains were kept in Tryptone Soy Agar (TSA) slants at 4°C; a procedure which was followed to store organisms for other microbiological evaluations that follows isolation. 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 at species level.

### Yeast and Mould Counts

Yeasts and moulds were determined using Corn Meal agar (CMA) (OXOID) at pH 5.5 and Potato Dextrose agar (PDA) (OXOID) with 10% tartaric acid, respectively. Serial dilutions were made to 10-2 for all the samples and spread plate technique was employed for determination of moulds while for yeasts pour plate method was used. The plates were incubated at 30°C for 5 days and examined periodically for growth and colony count (Andrews, 1992). Representative colonies were isolated and sub-cultured in Potato Dextrose agar (PDA) (OXOID). The cultures isolates were stored at 4°C on PDA slants awaiting characterisation and identification.

### Statistical Analysis

Plate-count data were transformed into logarithms before statistical treatment. Statistical analysis was carried out using the SPSS software package for Windows 10.0. The t Test was performed for variation in mean microbial counts in open and packed ice cream.

## RESULTS

Thirty-nine species of bacteria were isolated from ice cream. The highest percentages of these organisms were isolated from open ice cream. The occurrence and identity of the various bacterial isolates are shown in Table 1. In general, most of the isolates were gram negative bacteria (66.7%) from both sample types. The Gram positives were 13 (33.3%). Of the 39 species, 18 were detected from both open and packed ice cream, 16 were detected from open ice cream only and 5 from packed ice cream.

Of the 34 species isolated from sample A, 26 (76.5%) were Gram negative bacteria while 8 (23.5%) were Gram positive bacteria. The Gram negative bacteria were dominated by bacteria that belong to the family Enterobacteriaceae (57.7%). The genera constituting this were identified as shown in Figure 1. Of these, the *Enterobacter* species made the highest percentage of occurrence (40%) followed by *Klebsiella* species making 26.7%. Other remaining species belonging to this family made 33.3% (Fig. 1)The other Gram negative bacteria isolated from open ice cream constituted 42.3%. This was made by organisms belonging to the genus *Aeromonas*, *Acinetobacter*, *Alcaligenes*, *Burkholideria*, *Buttiauxella*, *Flavobacterium*, *Pseudomonas, Kingella* and *Xanthomonas*. The Gram positive isolates from open ice cream included organisms from genera *Staphylococcus, Actinomycetes*, *Bacillus*, *Corynebacterium* and *Enterococcus* (Fig. 2). Of the 23 isolates from sample B, 13 (56.5%) were Gram negative bacteria with 11 (84.6%) of these belonging to the family Enterobacteriaceae. Gram positive species isolated from sample B were 8 (43.5%). The genus for the Gram negative isolates were distributed as shown in Figure 3. The Gram positive isolates included species of the genera *Enterococcus* (10%), *Staphylococcus* (10%), *Bacillus* (10%), *Leuconostoc* (10%), *Actinomycetes* (10%), *Corynebacterium* (50%),

**Table 1: Identity of bacteria isolated from open ice cream and packed ice cream**

| *Microorganisms* | *Open ice cream (Sample A)* | *Packed ice cream (Sample B)* | *Population range for the organisms ($log_{10}$ CFU/g)* |
|---|---|---|---|
| *Acinetobacter genospecies* | 15 | 3 | 2.3-3.78 |
| *Aeromonas haedrophila gr* | 2 | 4 | 2.6-2.78 |
| *Alcaligenes latus* | 3 | 5 | 2.3-3.3 |
| *Bacillus brevis* | 6 | 0 | 2.3-3.47 |
| *Bacillus megaterium* | 3 | 1 | 2.0-3.0 |
| *Burkholideria cepacia* | 2 | 0 | 1.0-2.47 |
| *Buttiauxella agrestis* | 1 | 2 | 1.0-2.0 |
| *Actinomycetes sp.* | 11 | 6 | 1.3-4.6 |
| *Corynebacterium species* | 0 | 7 | 1.3-2.7 |
| *Corynebacterium jeikeium* A | 4 | 3 | 1.6-2.47 |
| *Corynebacterium jeikeium* B | 0 | 3 | 1.47-2.85 |
| *Corynebacterium aquaticum*B | 0 | 2 | 1.0-2.0 |
| *Enterobacter aerogenes* | 4 | 0 | 1.3-3.7 |
| *E. agglomerans Biogroup* 2B | 2 | 0 | 1.0-2.3 |
| *E. cloacae* A | 18 | 7 | 2.47-6.78 |
| *E. cloacae* B | 12 | 0 | 2.47-4.78 |
| *E. gergoviae* | 4 | 1 | 2.3-2.18 |
| *E. sakazakii* | 4 | 2 | 2.70-3.95 |
| *Enterococcus casseliflavus* | 2 | 1 | 1.0-2.47 |
| *Flavobacterium breve* | 4 | 0 | 2.0-3.0 |
| *F. meningosepticum* | 2 | 0 | 1.0-2.0 |
| *Hafnia alvei* A | 1 | 0 | 1.0 |
| *Kingella denitrificans* | 3 | 0 | 1.3-2.47 |
| *Klebsiella oxytoca* | 4 | 0 | 1.47-3.6 |
| *K. planticola* | 7 | 2 | 2.47-3.47 |
| *K. pneumoniae ss ozaenae* | 7 | 2 | 2.47-4.3 |
| *K. pneumoniae ss pneumoniae* | 7 | 2 | 1.6-4.48 |
| *Kluyvera ascorbata* | 1 | 1 | 1.0-2.3 |
| *Leuconostoc paramesenteroides* | 0 | 2 | 2.0-2.6 |
| *Proteus penneri* | 2 | 1 | 1.3-2.7 |
| *Pseudomonas aeruginosa* | 3 | 0 | 1.3-2.47 |
| *Pseudomonas fulva* | 1 | 2 | 1.0-2.3 |
| *Serratia liquefaciens/grimesii* | 2 | 3 | 1.3-2.47 |
| *Serratia marcescens* | 1 | 1 | 1.47-1.7 |
| *Staphylococcus auricularis* | 5 | 0 | 1.0-3.85 |
| *S. epidermidis* | 6 | 0 | 2.48-4.6 |
| *S. lentus* | 16 | 4 | 1.85-5.78 |
| *Xanthomonas maltophilia* | 2 | 0 | 1.0-1.7 |

### Yeasts and Moulds

Few isolates of moulds were obtained from the samples. Most isolates were obtained from

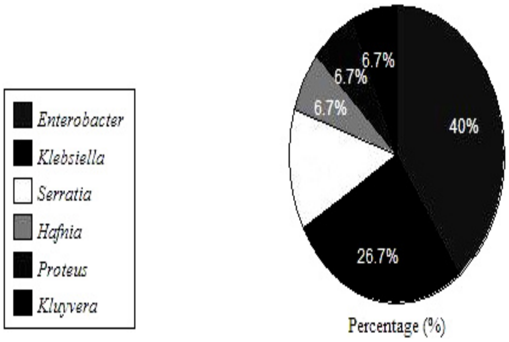


**Fig. 1. Percentage distribution of Enterobacteriaceae isolates from open ice cream**

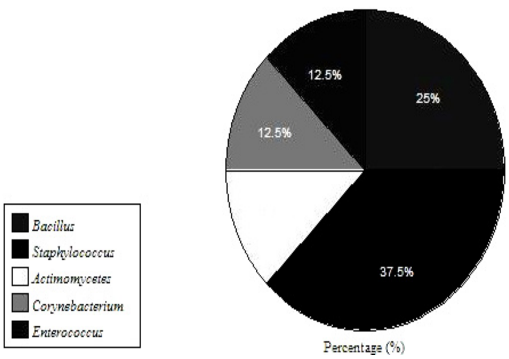


**Fig. 2. Percentage distribution of gram positive bacteria isolated from open ice cream**

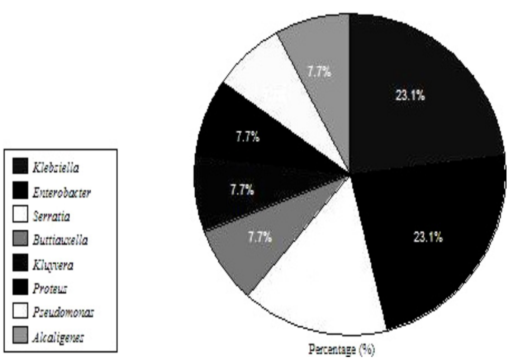


**Fig. 3. Percentage distribution of gram negative isolates from packed ice cream**

open ice cream. The yeasts isolated were *Rhodotorula* (23 from open ice cream and 5 from packed ice cream) and *Candida* (15 from open ice cream and 3 from packed ice cream) species. There were very few (2 from open ice cream) moulds isolated and were identified as belonging to the genera *Aspergillus.*

**Mesophilic, Psychrotrophic, Yeast and Mould Counts for Sample A**

The pH of ice cream was 6.8 and the initial temperature was -9°C. Results from the study show that open ice cream had high percentage of bacterial counts both mesophilic and psychrotrophic. The bacterial counts ranged from 3.30 log10 CFU/g to 6.78 log10 CFU/g for mesophiles, and 2.0 log10 CFU/g to 6.70 log10 CFU/g for psychrotrophs (Fig. 4). The frequency for each range of counts was as shown in Figure 4. Twenty (15.2%) of these positive samples had counts greater than 5.3 log10 CFU/g, which is the highest count accepted in ice cream as per microbiological standards, for mesophiles and fifteen (11.4%) for psychrotrophs. The presence of psychrophiles resulted in the counts for psychrotrophs within some ranges exceeding that of mesophiles (Fig. 4). The mean of mesophilic counts was 4.48 log10 CFU/g and that for psychrotrophs was 4.38 log10 CFU/g. The yeast population was low and were found in 38 (25.3%) of the 150 samples tested (Fig. 4). The mean count of yeasts was 2.9 log10CFU/g. Moulds were isolated from only two samples.

**Mesophilic, Psychrotrophic and Yeast and Moulds Counts for Sample B**

Bacteria were found in 16 (10.7%) of the packed ice cream samples (Fig. 5). Aerobic bacterial counts ranged from 2.48 log10 CFU/g to 5.48 log10 CFU/g for mesophiles. Psychrotrophic counts ranged from 2.48 log10 CFU/g to 5.48 log10 CFU/g. The distribution of the microbial counts was as shown in Figure 5. For the range 3.0 - 3.9 log10 CFU/g, the frequency of psychrotrophs exceeded that for mesophiles as a result of the presence of psychrophiles. For this sample only one sample had count above 5.3 log10 CFU/g. Of all the 150 tested samples, 134 (89.3%) had no bacterial growth after incubation. The mean for mesophilic counts and psychrotrophic counts were 3.44 and 3.4 log10 CFU/g respectively. Of all the 150 samples tested only 8 (5.3%) harboured yeasts. The mean count of yeasts was 2.44 log10 CFU/g. No moulds were isolated from packed ice cream.

**Ice Pop (Sample C)**

The pH of ice pop was found to be between 3.4 and 3.44 for the samples tested. Bacterial

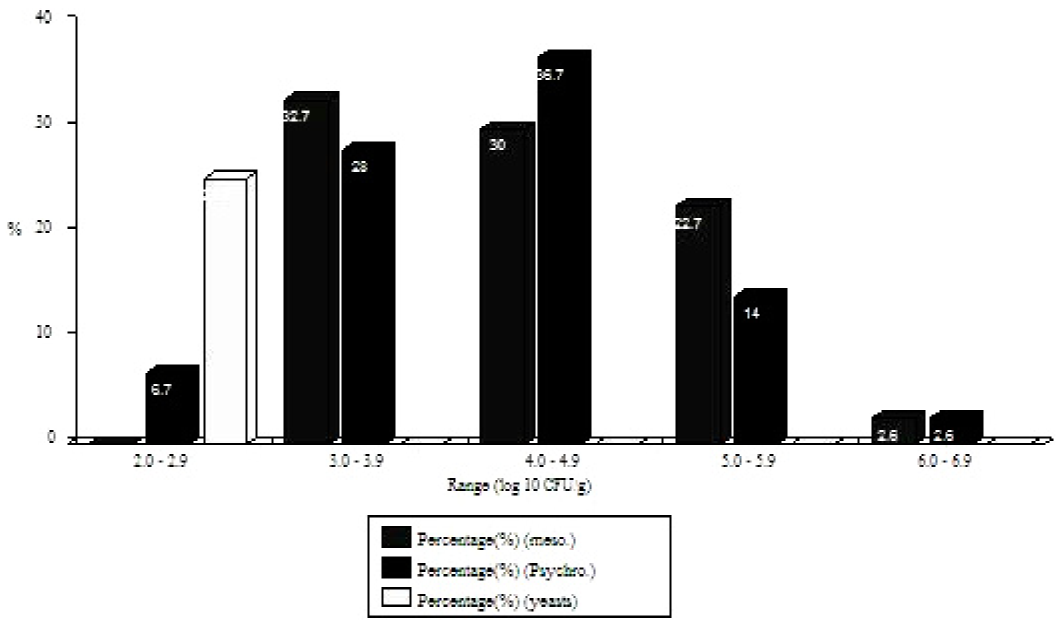


**Fig 4. Percentage value of Mesophiles, psychrotrophs and yeasts (log10 CFUg) for open ice cream (sample A)**

growth was observed from two of the 110 samples tested. The bacterial counts were 2.48 log10 CFU/g and 2.30 log10 CFU/g for both mesophiles and psychrotrophs. There were no yeasts or moulds that were isolated from this food commodity.

### Comparison of Bacterial Load for the Three (3) Sample Type

There were significant differences in bacterial counts between open ice cream, packed ice cream and ice pop samples analysed. This was

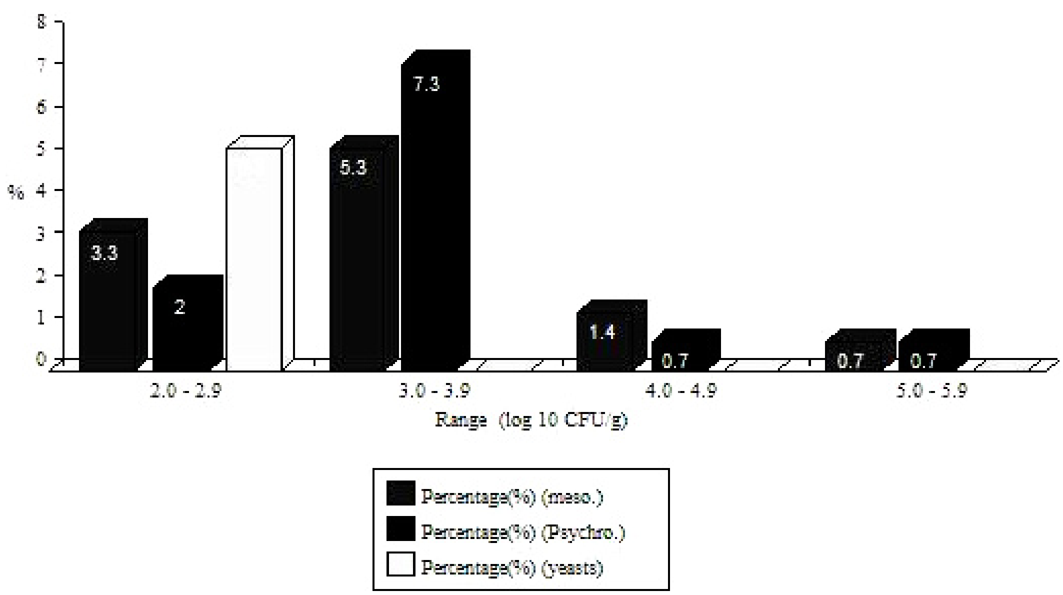


**Fig. 5. Percentage value of Mesophiles, psychrotrophs and yeasts (log10 CFUg) for packed ice cream (sample B)**

**Table 2: t-test to compare bacterial counts for the three (3) sample types**

| | *Mean count* | | | |
|---|---|---|---|---|
| | *Bacterial count log10 CFU/g* | *Yeast count log10 CFU/g* | *Total coliform (bacteria/g)* | *Escherichia coli (bacteria/g)* |
| Open ice cream *n=150* | $3.956^{a}$ + (0.133) | $0.690^{d}$ + (0.103) | $254.2^{f}$ + (16.628) | $36.553^{h}$ + (8.277) |
| Packed ice cream *n=150* | $0.304^{b}$ + (0.0787) | $0.130^{e}$ + (0.0449) | $3.880^{g}$ + (1.523) | $0.527^{i}$ + (0.196) |
| Ice pop *n=110* | $0.043^{c}$ + (0.0025) | 0 | 0 | 0 |
| t-test | -23.598(P<0.05) | -5.003(P<0.05) | -14.452(P<0.05) | -4.351(P<0.05) |

α.......*i* different letters for significantly different mean
*n* = number of samples
(SE) standard error of mean

**Table 3: Level of bacterial (mesophilic counts) contamination for the three samples**

| *Sample stype* | *Samples size* | *No of contaminated samples* | *Level of contamination(log10 values)* | | |
|---|---|---|---|---|---|
| | | | *1.0-2.9* | *3.0-4.9* | *5.0-6.9* |
| Open ice cream | 150 | 132 (88%) | 0% | 94 (63%) | 38 (25%) |
| Packed ice cream | 150 | 16 (11%) | 5 (3.4%) | 10 (6.6%) | 1 (0.7%) |
| Ice pop | 110 | 2 (1.8 %) | 2 (1.8%) | 0% | 0% |

Chi-square ($\chi^2$) test: $\chi^2 = (O-E)^2/E$ E = RT x CT/N
Where O = observed, E= Expected, RT = raw total, CT = column total and N = total of columns

indicated by the *p* value less than 0.05 ($p < 0.005$) (Table 2). Open ice cream was the highly contaminated sample with 88% of the samples collected being contaminated (Table 3).

The chi ($\chi^2$) was found to be 398.2. This therefore implies that there is a significant difference on the level of contamination among the three food samples. The chi square value was greater than the critical value ($p > 9.48$).

## DISCUSSION

### Microbiological Quality of Ice Cream (Open and Packed)

The microbial content of frozen dairy products that include ice cream largely reflects the quality of the ingredients used for their manufacture, handling during and after manufacturing and sanitation of equipment used. Due to the fact that ice cream mix has to undergo pasteurisation the microbial counts are expected to be low, less than 100 bacterial cells per ml (Richter et al. 1992; Tomislav et al. 2012)). The only organisms that might be present after pasteurisation are the spore-formers such as *Bacillus* species and other thermoduric bacteria that might have been part of some the ingredients. The microbiological quality of open ice cream and packed ice cream in terms of mesophilic, psychrotrophic and yeasts counts were different (Tables 1, 2 and 3). For packed ice cream only 10.7% of the tested samples had bacteria isolated compared to 88% of all the tested samples of open ice cream (Table 2). The yeasts were isolated from 5.3% of all the tested samples of packed ice cream while for open ice cream they were isolated from 25.3% of all the tested samples. The difference may have been brought about by the different conditions under which each of the food products is stored and distributed or served (Aleksieva and Mirkov 1983; Wouafo et al. 1996; Wilson et al. 1997; Little and De louvois 1999; Ahmed et al. 2009). Soft serve ice cream or open ice cream as is referred to in this study, is prone to higher levels of contamination (88%) as the vending machines used to serve the ice cream and the personnel involved in serving as well as the air may be sources of contaminants (Austin and Bergeron 1995; Lunden et al. 2000; Ahmed et al. 2009). The storage conditions for the premix may also result in contamination. The level of contamination of the packed ice cream was lower (10.7%) and may be this was a result of some of the organisms not surviving freezing temperatures for extended periods or being injured at these storage temperatures (Adams and Moss 1995; Nelapati et al. 2009). The microbiological standards for ice cream as according to Internatinal Dairy Federation proposal in 1982 is set for Mesophilic count $n = 5$, $c = 2$, $m = 2.5 \times 104$ , $M = 2 \times 105$. The results show that 20% of the open ice cream samples exceeded the microbiological standards. The results indicate or suggest 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 and Pakistan showed higher bacterial load of 105 to 106 CFU/ml and was 10 - 100 fold higher than the ISI standards of 5 x 104 (Warke et al. 2000; Ahmed et al. 2009).

**Occurrence of Organisms and Identification**

Ice cream has been found to be mainly contaminated by bacteria. The ratio of bacteria to yeasts and moulds isolated from ice cream was about 5:1. The most pre-dominant bacteria were found to be the Gram negative bacteria (66.7%) (Figs. 1-3). Due to that all the Gram negative bacteria are destroyed at high temperatures, their presence clearly indicates post processing contamination either during, packaging, transporting or selling of the commodity. These results are in concurrence with findings from other studies done in different parts of the world, including India (Kamat et al. 2000; Warke et al. 2000), Costa Rica (Windrantz and Arias 2000), Ireland (Wilson et al. 1997), Cameroon (Wouafo et al. 1996) and Spain (Jordan et al. 1995).

**Bacteria in Open Ice Cream**

The bacteria isolated from open ice cream were mainly the gram negative, making 68.5% of the total bacterial isolates. Of these a large and significant number belonged to the family Enterobacteriaceae (Fig. 1) and these results are similar to the findings in Hyderabad city (Rajalakshmi 1983). These are the organisms that are mainly found in the environment and some as part of the natural microflora of humans (Jay 1996). These can usually get into ice cream from sources such as dust, soil, equipment and hands of handling personnel either during storage or during filling of the vending machine and selling. The different groups of organisms isolated also suggests that soil, water, other food stuffs, and air might be the sources of contamination for open ice cream. Among these are some pathogenic organisms such as *Aeromonas hydrophila* that of recent has become one of the major psychrotrophic organisms of health concern together with *Yersinia enterocolitica* and *Listeria monocytogenes* (Kraft 1992; Merino et al. 1995; Jay 1996) and *Pseudomonas fulva.* The gram positive bacteria isolated from open ice cream were less (Fig. 2). The group was dominated by the genus *Staphylococcus*, results similar to those obtained by Okojoh (2006). This is due to the fact the organisms are commonly found in the nose, throat and on hair and skin of more than 50% of healthy individuals (Newsome 1988; Adams and Moss 1995) and as a result this might be the possible source of contamination. The organisms in this genus have also been found to withstand a variety of adverse conditions that include low temperatures (Jay 1996). Unhygienic practices may have led to contamination of the product by the other groups of organisms as most of them are found in the environment; and presence of *E. coli* and *Enterococcus casseliflavus* indicates faecal contamination as the organism is of faecal origin (Giraffa et al. 1997). Other organisms such as *Bacillus megaterium* and *Bacillus brevis* might have survived pasteurisation though (Jay 1996). This is due to that the organisms are spore-formers and as a result can survive pasteurisation.

**Bacteria in Packed Ice Cream**

There was no significant difference in the number of gram negative and gram positive bacteria isolated from packed ice cream (Fig. 3). In addition to the genera that were isolated from open ice cream, there were other organisms such as CDC group, *Kingella denitrificans* and *Leuconostoc paramesenteroides.* Others such as *Xanthomonas, Hafnia, Aeromonas, Acinetobacter, Burkholideria, Flavobacterium* were not isolated from open ice cream. The presence of these organisms that are sensitive to heat is an indication of post processing contamination. The organisms might have gained access to ice cream from soil, air and dust via the food handler and utensils as these are their main sources (Jay 1996; Tomislav et al. 2012). This implies that though there is contamination, the degree at which it occurs differs from contamination of open ice cream and these organisms are mostly psychrotrophs such as *Flavobacterium* and *Aeromonas*. This might be due to the fact that open ice cream become exposed to organisms during handling while packed ice cream is mainly stored in frozen state and once packed it is less frequently handled (Hennessy et al. 1996; Dempsey et al.

2000; Kleer and Hildebrandt 2000). Some of the organisms present are those that are likely to have survived pasteurisation such as *Bacillus* species and *Corynebacterium* species. Although *Xanthomonas* is a plant pathogen, it has been also found to be among the most common foodborne genera (Jay 1996) and its presence in ice cream might have been due to contamination by the food handlers after handling vegetables or fruits.

### Yeasts and Moulds in Open Ice Cream and Packed Ice Cream

The results showed that contamination of the two the commodities with yeasts and moulds, was very low. This might be due to the great diversity of bacteria present in ice cream. This may also suggests that the spores of yeasts and moulds in the air are favoured more by high temperatures than the low temperatures of the ice cream. The result being that the temperatures in Gaborone are about 25°C.

## CONCLUSION

The results of this study showed that the quality of open or soft serve ice cream was poor as compared to packed ice cream. This is due to the high microbiological counts of bacteria and presence of indicator organisms than is permitted. 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 yeasts and moulds also implies poor quality of open ice cream. Ice pop is safe to eat as far as the results from this research have shown but further studies on the commodity need to be done. The quality of ice cream should be improved by aiming at reducing contamination. This can be acquired through teaching the concerned personnel on hygiene and the importance of good hygienic practices. The people involved in the selling of ice cream (especially open or soft serve ice cream) and ice pops must be given regular refresher courses on handling of ice cream and related products and the importance of keeping frozen food commodities at the correct temperatures. The equipment must also be regularly checked to ensure their sterility so as to avoid contamination. The HACCP (Hazard Analysis Critical Control Point) method must be effectively employed in the ice cream manufacturing industry to check every production step to avoid errors and ensure products of good quality and acceptable microbiological standards. Seminars and workshops on the importance of good manufacturing and production practices and good hygiene towards good health should be conducted for the management personnel. Those selling in vendors should be encouraged to keep their selling places clean and should also be encouraged to buy refrigerators to keep their ice pops so as to avoid temperature abuse that may result in their commodities having negative health effects.

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