PRINT: ISSN 0972-0073 ONLINE: 2456-6802
Ethno Med, 7(3): 227-236 (2013)
DOI: 10.31901/24566772.2013/07.03.11

The Effect of Different Temperatures and Chemical Substances on Growth of Some Bacterial Species Isolated from Ice Cream and Ice Pop (Sorbates) Sold in Gaborone, Botswana

S. Mathews[1], L. Ngoma[1], B. Gashe[2] and S. Mpuchane[2]

[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

KEYWORDS Ice Cream. Ice Pop. Sodium Benzoate. Sulphur Dioxide. Psychrotrophic. Mesophilic Bacteria. Temperature

ABSTRACT: The study was intended to find out different temperatures affect growth of bacteria isolated from ice cream and the effects of sodium benzoate and sulphur dioxide on the growth of *Klebsiella oxytoca*. The preservatives were found to be effective in inhibiting the growth of this organism. Lowering pH to acidic also made the preservatives more effective. Bacterial growth of four selected isolates from ice cream and ice pop which are, *Salmonella typhimurium*, *Staphylococcus aureus, Escherichia coli* and *Yersinia* was found to vary from temperatures between $^{-}$5°C and 25°C with the organisms striving best at temperatures 4°C – 25°C. When the four organisms were subjected to $^{-}$5°C they did not grow. The population of organisms was declining.

INTRODUCTION

Ice cream and its related products have certain extrinsic factors related to storage environment. The different microorganisms in any environment have different temperatures at which they are active or they can grow and multiply. This result in the micro-organisms being classified into three broad groups based on temperature. These are psychrotrophs, mesophiles and thermophiles (Jay 1996). Psychrotrophs have optimum growth at 15°C - 30°C and are capable of growing at temperatures less than 7°C. Mesophiles have an optimum growth temperature of 30°C - 40°C and do not grow at refrigeration temperatures. The thermophiles have optimum growth between 55°C - 65°C with some that belong to this group that can grow at refrigeration temperatures like some *Bacillus* species (Sinell 1989; Garcia-Armesto and Sutherland 1996; Jay 1996; Montville 1997). Many of the problems associated with texture and body of ice cream are not of microbial origin (Potter and Hotchkiss 1995; Koxholt et al. 2001). Psychrotrophic bacteria are known to cause defects such as bitterness, rancidity and fruity by acting on proteins and fats in fluid milk (Frank 1997). Due to the fact that ice cream has milk fat and proteins, the same defects may occur as a result of spoilage by psychrotrophic organisms. The presence of microorganisms in ice cream implies that these may produce lipolytic enzymes that will act on the fat in ice cream (Frank et al. 1993). This may result in free fatty acids that may result in rancid flavour defects. Low temperatures that are lower than the range of temperatures for growth have been found to prevent production of proteases by psychrotrophic organisms and as a result no protease related defects are likely to occur (Frank 1997). This has been found to be the same for lipase production by psychrotrophic organisms. Another defect that might occur as a result of microorganisms is curdling (Silliker et al. 1980) but this may only occur with very high bacterial populations in ice cream (Silliker et al. 1980). Preservatives are usually added to food to improve their microbiological quality. This is due to the fact that the pH of foods influences their susceptibility to microbial growth (Gould 2000; Lund and Eklund 2000). The most commonly used preservatives in drinks which include ice pops are chemical compounds (Gould 2000). Of these organic acids (benzoic, sorbic and propionic acids) and inorganic acids (sulphites and nitrites) are the most commonly used (Gould 2000; Lund and Eklund 2000; Uljas et al. 2001). These preservatives are mainly weak acids and are affected by many factors that include pH of the food, the target microorganisms and storage environment (Piper 1999; Duffy and Schaffner

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

2001). In this study only sodium benzoate and sulphurdioxide were discussed as they are the ones that have been used as preservatives in ice pop. Sodium benzoate is preferred over benzoic acid as it is highly soluble in water (Gould 2000). Sodium benzoate has been generally recognised as safe (GRAS) at 0.1% in drinks with the antimicrobial activity residing in the undissociated molecule (Jay 1996). This preservative is most effective at the lowest pH values of foods and ineffective at neutral values and therefore is used in ice pops mainly as antifungal agents (Jay 1996; Lund and Eklund 2000; Uljas et al. 2001). To control bacteria a much higher concentration is required (Gould 2000) but adjustment of medium to acidic pH with HCL reduces amount of sodium benzoate required to inhibit bacterial growth (Lund and Eklund 2000). *Listeria monocytogenes* has been found to be inhibited at sodium benzoate concentrations between 1000 mg l^{-1} and 2500 mg l^{-1} (Razavilar and Genigeorgis 1998; Lund and Eklund 2000). Other organisms that have been found to be effectively inhibited at these concentrations are *E. coli*, *S. aureus*, *Lactobacillus* species, yeasts and moulds (Piper 1999; Lund and Eklund 2000; Duffy and Schaffner 2001). At lower concentrations of about 1000 mg l^{-1} growth of organisms was found to occur but at a reduced rate (Lund and Eklund 2000). The action of sodium benzoate is mainly pH related but it is also affected by temperature (Jay 1996). At temperatures of about 21°C and pH 5.0, growth of *L. monocytogenes* was inhibited at 2000 mg l^{-1} of sodium benzoate (Razavilar and Genigeorgis 1998; Lund and Eklund 2000) and reduced at lower temperatures while at 13°C and same pH 500 mg l^{-1} sodium benzoate prevented growth of *L. monocytogenes* (Razavilar and Genigeorgis 1998). Greater inhibition of bacterial growth by sodium benzoate is expected at lower temperatures (Brul and Coote 1999; Dock et al. 2000; Gould 2000). The concentration of sodium benzoate that is generally regarded as safe (GRAS) in non-alcoholic beverages or soft drinks that include ice pops is 1000 mg per litre (Lund and Eklund 2000). The antimicrobial action of sodium benzoate is based on its ability to block oxidation of glucose, disruption in membrane activity and amino acid transportation in bacterial cells as a result inhibiting growth (Jay 1996; Brul and Coote 1999). Sulphur dioxide is used either as a gas/liquid or in the form of its neutral or acid salts (Jay 1996; Gould 2000). Sulphur dioxide as a preservative is mainly used in foods to inhibit growth of yeasts and moulds in low pH and low water activity foods, growth of Enterobacteriaceae and other Gram-negative bacteria in higher pH and high water activity foods (Gould 2000). Unlike sodium benzoate, Sulphur dioxide is used in lower concentrations of about 20 to 200 microgram per litre (GRAS) in ice pops to inhibit bacteria, yeasts and moulds (Jay 1996; Gould 2000). According to Gould (2000), some organisms of the Enterobacteriaceae studied were found to be inhibited by free sulphite at different concentrations as follows; *Salmonellae*, 15 to 109 mg per litre; *E. coli* 50 to 195 mg per litre; *Citrobacter freundii*, 65 to 136 mg per litre; *Y. enterocolitica*, 67 to 98 mg per litre; *Enterobacter agglomerans*, 83 to 142 mg per litre; *Serratia marcescens*, 190 to 241 mg per litre and *Hafnia alvei*, 200 to 241 micrograms per millilitre. The antimicrobial action of sulphur dioxide is mainly on the disruption of metabolic actions of the microorganisms (Piper 1999). Some organisms have been found to develop resistance to these weak acids by inducing an acid tolerance response (ATR) or degrading the preservative using some specific enzymes (Brul and Coote 1999). Organisms such as *Pseudomonas aeruginosa, S. typhimurium, Shigella flexneri, Listeria monocytogenes* and *E. coli* have been studied and subjected to sodium benzoate, benzoic acid and other organic acids at very low pH of about 2.0 and found to develop acid tolerance response (Razavilar and Genigeorgis 1998; Brul and Coote 1999; Lund and Eklund 2000). Other mechanisms that the organisms use to survive in acid conditions as according to Lund and Eklund (2000) and Piper (1999) include log-phase ATR, a pH-dependent stationary-phase ATR and a pH-independent general stress resistance induced in the stationary phase. The objective of this study was to find out the effect of different temperatures and chemical substances on growth of some bacterial species isolated from ice cream.

MATERIAL AND METHODS

Inoculum Preparation

Cells of *E. coli*, *Yersinia, K. oxytoca, St. aureus* and *S. typhimurium* (isolated from ice cream) were grown in Nutrient Broth (NB) (OXOID) for 24 hours at 37°C so as to achieve

the log phase. A volume of 1.0 ml of each of the organisms at log phase was mixed with sterile distilled water to have an optical density of 0.02 at 420 nm (Spectronic 20D+) (Kleyn et al. 1999). From this suspension, 1 ml was plated in Nutrient Agar (NA) (OXOID). This was performed to determine the population of the organism at that optical density. The preparation was about 103/ml for each of the test organisms. The standardized inoculum was used to inoculate ice cream or be in the presence of certain preservatives.

Growth of *E. Coli, Yersinia* species, *St. aureus* and *S. typhimurium* in Ice Cream

Hundred (100) gram of melted ice cream was inoculated with 1 ml of 10^{5-6}/ml bacterial suspension of the pure culture of each test organism in a conical flask. The inoculated ice creams were incubated at 25°C, 10°C, 4°C and -5°C. During incubation, the ice creams were examined by transferring 1 ml from each flask into 99 ml of ringers' solution followed by 10 fold serial dilution 0.1 ml of each dilution factor was plated in respective selective medium such as Salmonella Shigella Agar (SS) (OXOID). Yersinia Selective Agar base (YSA) (OXOID) supplemented with Yersinia Selective Supplement (HiMEDIA FD 034), Baird-Parker agar (BPA) (OXOID) supplemented with Egg Yolk Tellurite Emulsion (HiMedia) and Eosin Methylene Blue Agar (EMBA) (OXOID). The ice cream was analysed periodically at 30 minutes intervals for the first 6 hours at 25°C and 10°C and then every 24 hours for twelve days at 4°C. At -5°C analysis was done for 15 days (Warke et al. 2000). Plating was done in duplicate and colonies were counted for growth (Warke et al. 2000).

Effect of Sodium Benzoate and Sulphur Dioxide (As Preservatives) on *K. oxytoca*

Different concentrations of sodium benzoate (0.005, 0.01 and 0.015 mg/ml) were prepared and Nutrient Broth (NB) (OXOID) (Lund and Eklund 2000). For sulphur dioxide, potassium metabisulphite salt was added to Nutrient Broth (NB) (OXOID) (Gould 2000) in the following concentrations; 0.01, 0.02 and 0.025 mg/ml. Two sets were prepared for each concentration for each preservative. For the other set the pH of the broth was adjusted to 3.4 using 0.1 N-hydrochloric acids while the other was left at pH 7.0. One ml of standard inoculum of the test bacteria (*K. oxytoca*) was introduced into 100ml of Nutrient broth containing the different concentrations of the two preservatives and the flasks incubated at 30°C. Controls were made by inoculating Nutrient broth that had no preservatives added. One of the controls had a pH of 7.0 and the other had a pH of 3.4 (same as that of ice pop). For the first 7 days 1ml transferred to 99 ml of ringers' solution followed by 10 fold serial dilution. 0.1 ml was then plated in duplicate in Nutrient agar (NA) (OXOID) plates every 24 hours and thereafter the same amount was plated every five days for 30 days to determine the population of the test organism

Physiochemical Analysis

PH Measurement

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

Temperature Measurement

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

RESULTS

Growth Kinetics and Survival of *S. typhimurium*, *St. aureus, E coli* and *Yersinia* in Ice Cream

The pH of ice cream was 6.8 and the initial temperature was -9°C. The organisms were found to behave almost the same at 25°C (Fig. 1). At 10°C and 4°C, for *S. typhimurium* and *E. coli* the population did not significantly increase (Figs. 2 and 3).

Short Term Growth Curves for *E. coli, St. aureus, Yersinia* and *S. typhimurium* in Ice Cream

The organisms were found not to increase in population for the first three hours in ice cream at four different temperatures (Figs. 1-4). At 25°C growth for all the organisms was observed after 3 hours of incubation. Significant growth was also observed after 6 hours of incubation at 10°C

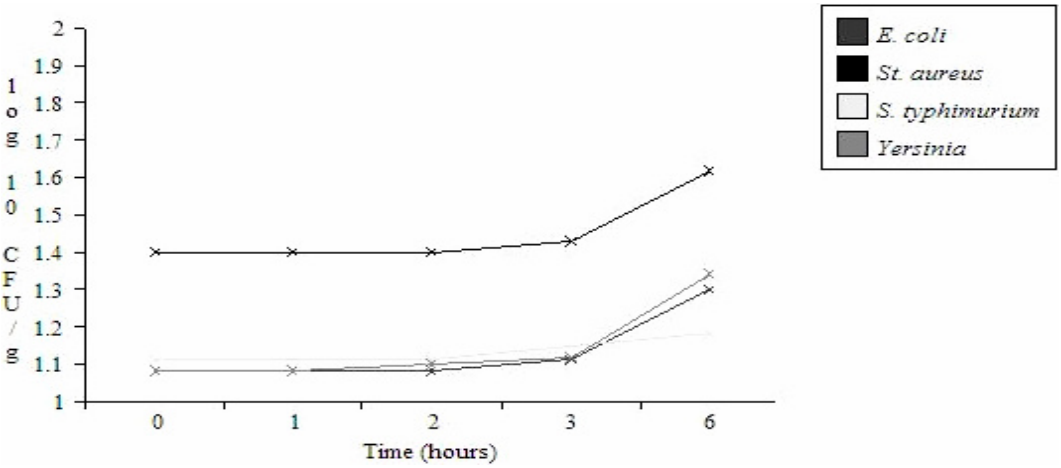

Fig. 1. Growth curve for E. coli, S. typhimurium, St. aureus and Yersinia at 25°C

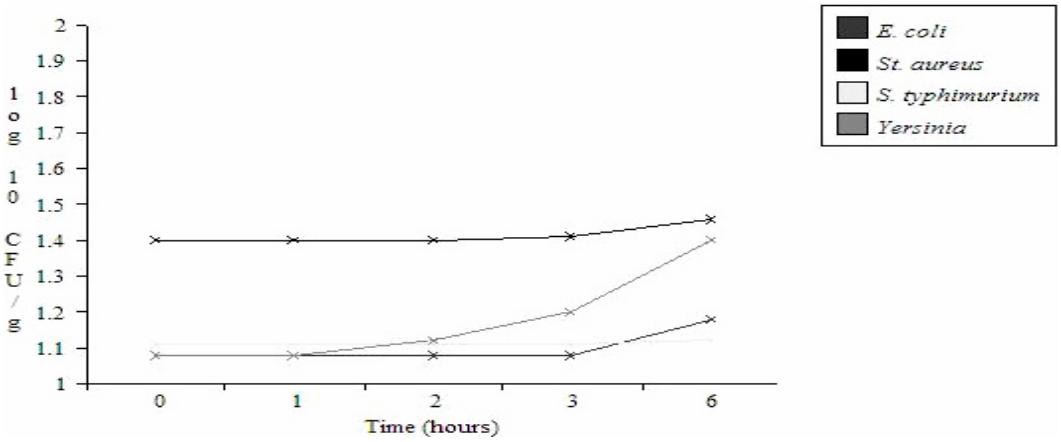

Fig. 2. Growth curve for E. coli, S. typhimurium, St. aureus and Yersinia at 10°C

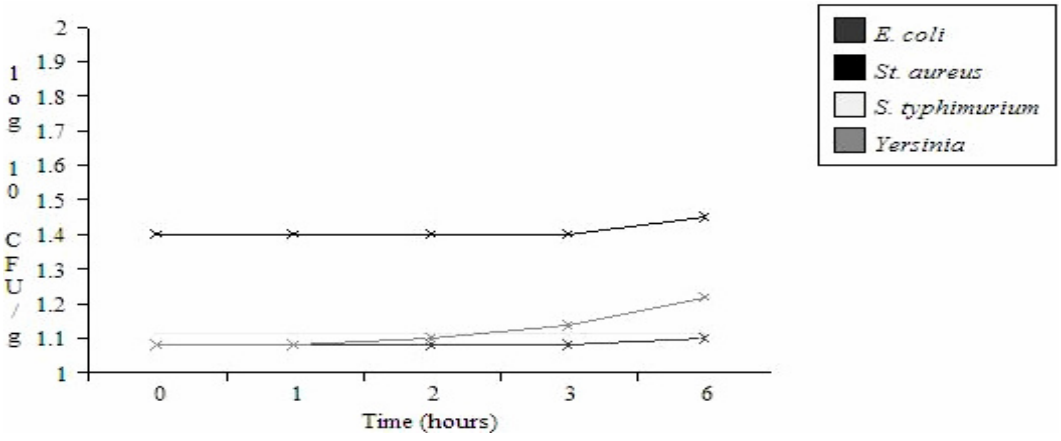

Fig. 3. Growth curve for E. coli, S. typhimurium, St. aureus and Yersinia at 4°C

and 4°C. At -5°C there was no growth nor decline in population was observed for the first 48 hours of incubation for all the four organisms (Fig. 4) and the population declined as from day four for *E. coli* and *S. typhimurium*. For the other two organisms decrease in population was observed after 6 days for *St. aureus* and 15 days for *Yersinia* (Fig. 4) As growth indicates living or viable cells, *S. Typhimurium*, *Yersinia, St. aureus* and *E. coli* were found to survive in ice cream at higher temperatures as well as low temperatures. At low temperatures the organisms were found to survive for a longer period in ice cream. *Yersinia* was found to also multiply significantly though slowly.

Ice Cream as a Growth Medium For *S. typhimurium*

At 25°C the population significantly increased from 10^1 to10^4 bacteria/g for the first two days (Fig. 5). The population then declined to 10^2 after eight days. At temperatures 10°C and 4°C, growth was observed though it was low (Fig. 5). From day 20 there were no detectable cells in the ice cream at these two temperatures.

Ice Cream as a Growth Medium for *E. coli*

At 25°C the population significantly increased from 10^1 to10^4 bacteria/g for the first two days (Fig. 6). The population then declined to 10^2 after day eight. At temperatures 10°C and 4°C, growth was also observed (Fig. 6). At 10°C and 4°C the population of *E. coli* increased but slowly (Fig. 6).

Ice Cream as a Growth Medium for *Yersinia*

At 25°C the population of *Yersinia* increased from 10^1 to10^4 bacteria/g for the first two days of incubation (Fig. 7). At 10oC and 4°C the

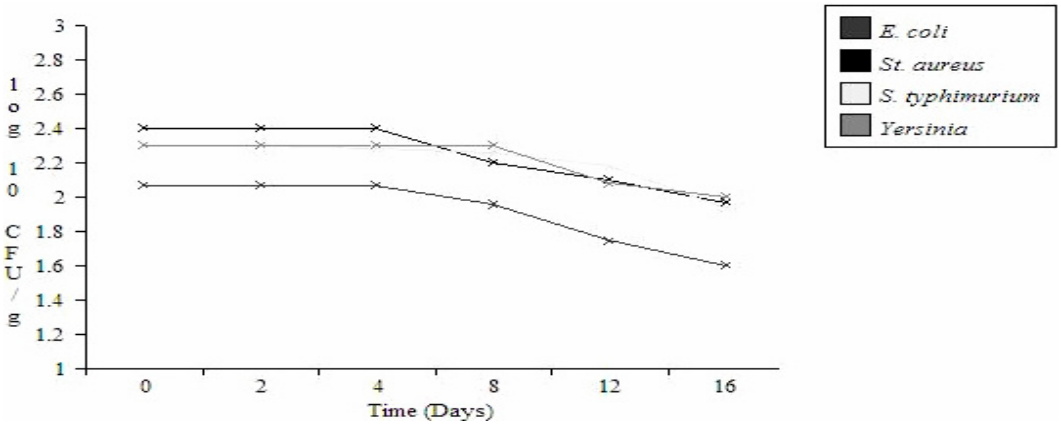

Fig. 4. Growth curve for E. coli, S. Typhimurium, St. aureus and Yersinia at -5°C

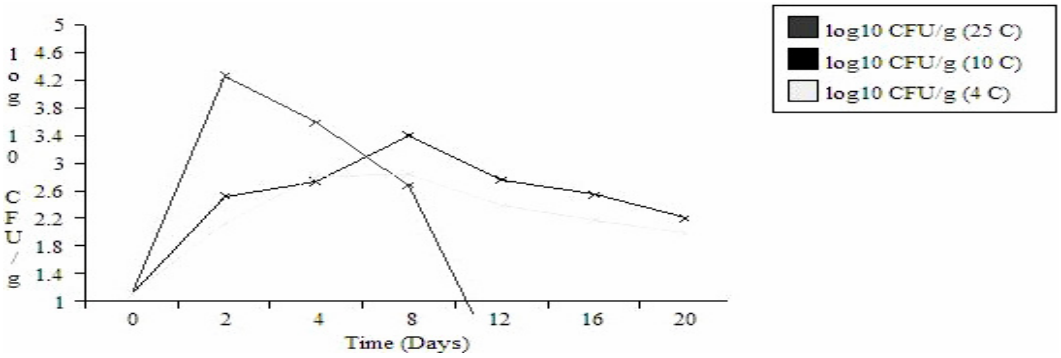

Fig. 5. Growth S. typhimurium in ice cream at three different temperatures

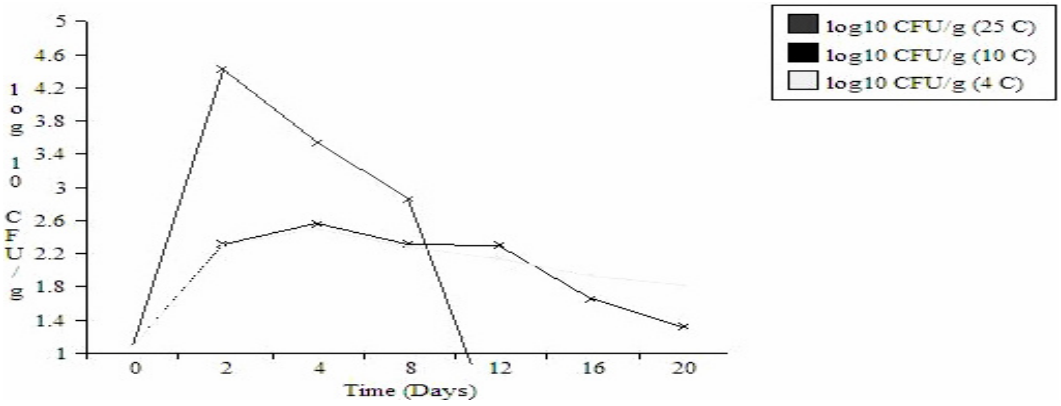

Fig. 6. Growth E. coli in ice cream at three different temperatures

population of *Yersinia* increased from 10^1 to 10^4 bacteria/g and 10^3 bacteria/g respectively (Fig. 7) for the first 16 days of incubation. The population of the organism then declined.

Ice Cream as a Growth Medium for *St. aureus*

At 25°C the population of *St. aureus* increased from 10^1 to 10^4 bacteria/g for the first two days of incubation (Fig. 8). At 10°C and 4°C the population of *St. aureus* increased from 10^1 to 10^3 bacteria/g and 10^2 bacteria/g. respectively (Fig. 8) for the first 12 days of incubation.

Effect of Sodium Benzoate and Sulphur Dioxide (SO_2) on *K. oxytoca* Isolated from Ice Pop

After *K. oxytoca* was subjected to different concentrations of SO_2 and sodium benzoate, the organism was found to behave as shown in figures (Figs. 9 and 10) at pH 7.0. The concentration of sodium benzoate that is used in ice pop is 1.0 mg/g. The results show that the preservative have inhibitory effects on the organism even though the rate is slow.

The results show that sulphur dioxide was more effective at inhibiting *K. oxytoca* than sodium benzoate even though incubation was done at same temperature of 30°C. This is clearly shown by the two figures (Figs. 9 and 10). In sodium benzoate, at 1.0 mg/g which is the concentration used in ice pop, the population of *K. oxytoca* was still 10^1 after 5 days of incubation while for sulphur dioxide at the concentration of 0. 2 mg/g which is used in ice pop after the same duration of incubation no viable cells were observed (Fig. 10). When the organism (*K. oxytoca*) was subjected to different concentrations of the two preservatives at pH 3.4 which is similar to that of ice pop, the organism behaved as shown

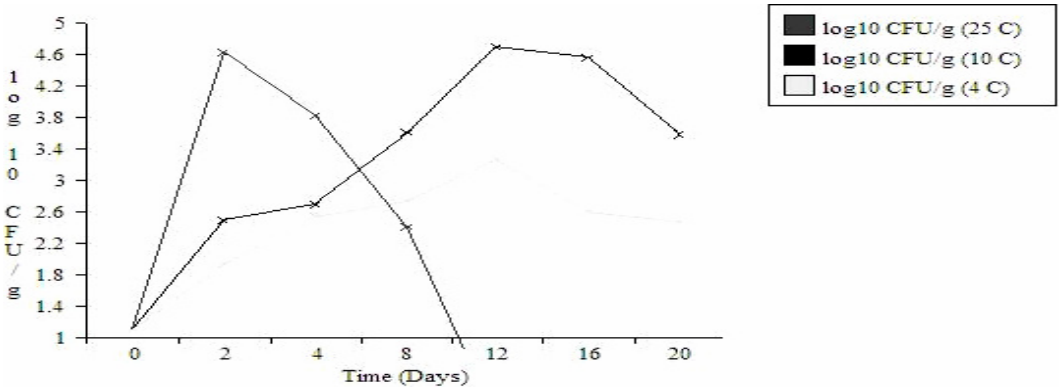

Fig. 7. Growth Yersinia in ice cream at three different temperatures

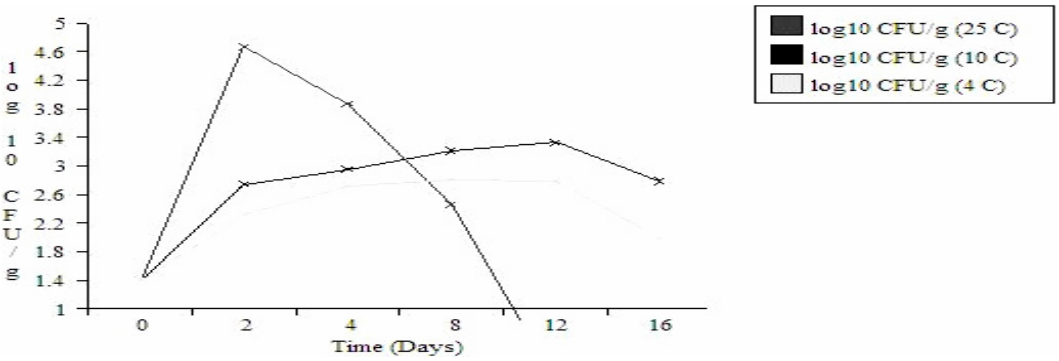

Fig. 8. Growth St. aureus in ice cream at three different temperatures

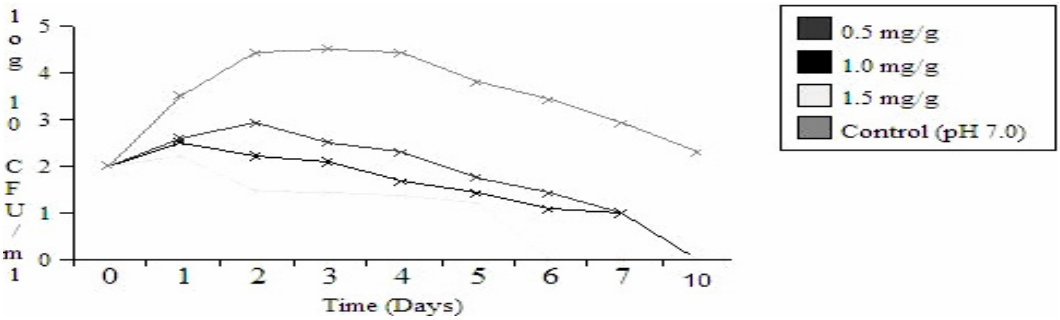

Fig. 9. Effect of sodium benzoate on K. oxytoca at pH 7.0

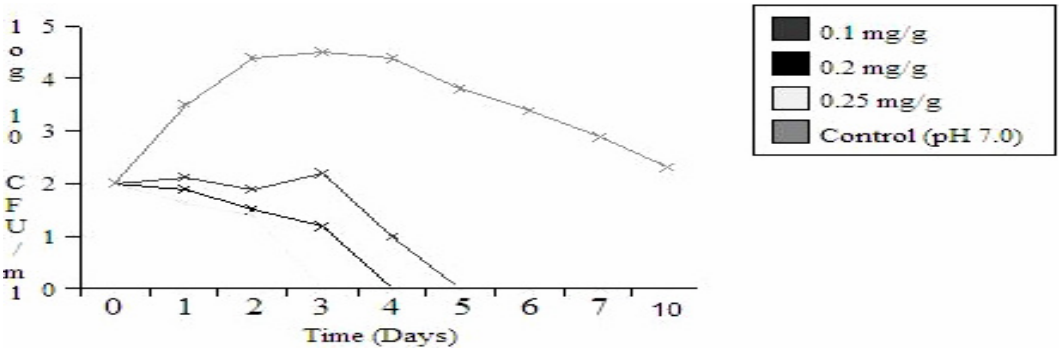

Fig. 10. Effect of sulphur dioxide on K. oxytoca at pH 7.0

in Figures 11 and 12. Both preservatives proved to be highly effective against the organism at pH 3.4. The two controls used shows that pH alone also have great effects on the organism.The minimum concentration of sodium benzoate at which both the isolate and standard culture of *K. oxytoca* were inhibited at pH 3.4 was 0.5 mg/g. In sulphur-dioxide at the same pH the organism (both isolate and standard culture) was inhibited in 0.1 mg/g.

DISCUSSION

Growth and Survival of *E. coli, S. typhimurim, St. aureus* and *Yersinia* at -5°C, 4°C, 10°C and 25°C

Some pathogens may also be contaminants and if temperature abuse occurs then they proliferate as was shown in the results by growth of *S. typhimurium, E. coli, S. aureus* and *Yersinia*

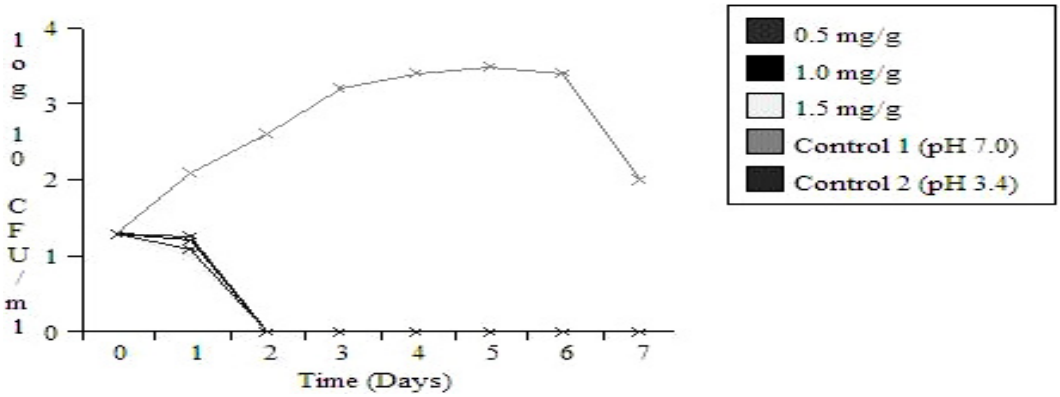

Fig. 11. Effect of sodium benzoate on K. oxytoca at pH 3.4

species (Figs. 1, 2, 3 and 4) at stimulated temperature abuse conditions. This can then result in spoilage or food poisoning. The results showed that ice cream can be an ideal medium for growth of these organisms (Figs. 5, 6, 7 and 8). This was more evident when the samples were incubated at 25°C which is equivalent to ambient temperatures here in Gaborone; even though due to high competition with other mesophilic organisms in ice cream led to survival time of the organisms being short. The exposure of the commodity to this temperature has to be for more than 3 hours (which is practical not possible) for organisms to start to multiply though, as in less than this time no increase in population was observed. The results found were similar to those found in a similar study performed in Mumbai (Warke et al. 2000) and another in UK (Little and Knochel 1994) in that the organisms were found to increase in population in ice cream at same temperatures. Growth was also observed at lower temperatures but was slow for *E. coli* and *S. typhimurium* as increase in population was observed after 8 days of incubation. Although the population for the two organisms increased, there was no significant increase observed at 4°C (Fig. 3).

Growth of *Yersinia* was slow but it was significant at both temperatures. In both cases the population started to increase after 2 days of incubation compared to 3 days for the other two organisms. At 10°C the population of *Yersinia* increased to about 10^5 while at 4°C the population increased to about 104 which was quite significant (Figs. 2 and 3). At 4°C and 10°C *Yersinia* seem to grow well as competition was reduced at these temperatures that led to the organism surviving for longer period of time. *Yersinia* have been reported to be a poor psychrotrophic competitor (Jay 1996) and when the population of competitors increases then its population decreases faster as was shown at 25°C, *St. aureus*

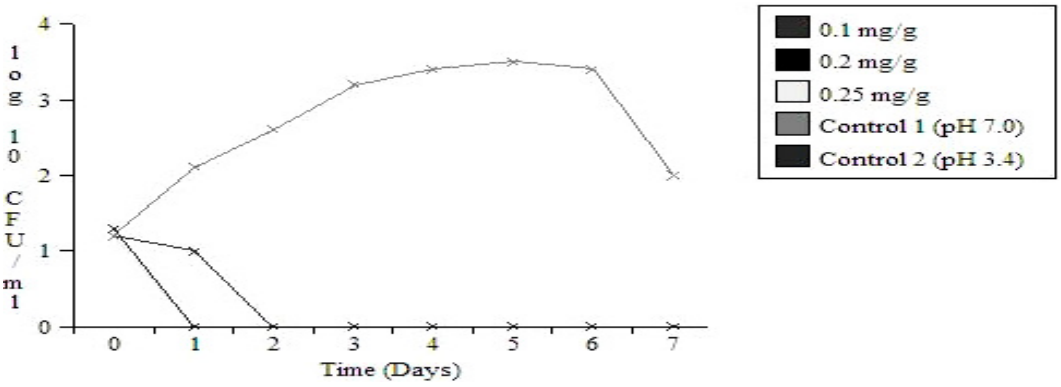

Fig. 12. Effect of sulphur dioxide on Klebsiella oxytoca at pH 3.4

was found to also grow in ice cream at 10°C and 4°C. Although growth was slow, it was significant reaching up to 10^4 from 10^1 after four days of incubation. The levels at which the organisms grew were effective to cause infection as the population exceeded 100 cells/g (ISI 1964; Warke et al. 2000). This is due to the fact that even small doses of pathogenic organisms in foods, such as ice cream, constitutes a potential risk particularly to children, pregnant women, immuno compromised adults and the elderly (Jay 1996; Warke et al. 2000). This shows that at refrigeration temperatures the organisms are capable of growing if the commodity is kept for prolonged time. Though ice cream is not kept at refrigeration temperatures, temperature abuse and inadequate freezing during storage of ice cream may result in the organisms proliferating (Figs. 1, 2 and 3). At -5°C the population of the organisms was found to decrease for the first 24 hours of incubation (Fig. 4). This shows that the organisms have experienced cold shock and as a result their metabolic activities inhibited (Jay 1996). The population continued to drop although slowly and this was more prominent with *E. coli* (Fig. 4). *Salmonella* was found to survive better than *E. coli*. *Yersinia* although it is psychrotrophic organism, it was found to be affected the same way as these other two organisms as well as *St. aureus* but even after 28 days of incubation, about 10^2 cells were observed which shows that it has a higher survival ability in stored frozen ice cream than *E. coli* and *Salmonella*.

The population of *St. aureus* and *Yersinia* remained constant for more than four days after incubation but due to their viability being affected, a decline in population was observed. This might be due the fact that at lower temperatures of about -10°C the degree of destruction done to microorganisms is lower and slow as compared to temperatures about -2°C to -4°C (Jay 1996). At these temperatures close to 0°C more microorganisms have been found to be destroyed and also at a faster rate. Ice cream is stored at temperatures about -9°C and as a result if microorganisms are in the commodity there are higher chances for them to survive and the little population that remains is capable of causing infection when consumed depending on the type of pathogen and the size of the infective dose for that particular microorganism. For example, the infective dose of *E. coli* O157:H7 is about 0.3 to 0.5 cells per gram in the contaminated food (Jay 1996). Temperature abuse may also give chance for the cells to proliferate.

The presence of the organisms makes the foods not safe to eat as the organisms have the ability to survive. Both preservatives managed to inhibit growth of the organism (*K. oxytoca*) which indicates that they are equally effective against microbial activity in the commodity. Effect of these preservatives has been found to be affected by both elevated temperatures and pH (Lund and Eklund 2000; Gould 2000). Similarly, at pH 7 the organism was found to survive longer than when the pH was 3.4 corroborating that of ice pop. The acidic pH on its own proved to have inhibitory effects on the organism. This clearly shows that acidic pH is necessary for the effectiveness of the preservatives. This therefore implies that the presence of the organism in the food product might have been due to inadequate processing or mistakes during processing. Due to the fact that various organisms have been found to develop some resistance to adverse conditions such as pH and temperature (Brul and Coote 1999; Lund and Eklund 2000) it means that there is likelihood of them surviving. Implementation of Hazard Analysis at Critical Control Points (HACCP) must therefore be emphasised to ensure safety of the food commodity and also assure the lowest possible risk by food-borne pathogens in ready to eat foods (Lammerding and Fazil 2000; Warke et al. 2000).

CONCLUSION

The increase in temperature of ice cream does lead to an increase in the population of the following; *S. typhimurium*, *St. aureus, E. coli* and *Yersinia* in ice cream. These organisms were targeted as their presence in food substances poses a health threat to humans. This implies that ice cream should not be allowed to thaw to temperatures above 0°C. The introduction of preservatives sulphur dioxide and sodium benzoate is appropriate as they help inhibit microbial organisms and thus keeps the product safe for consumption.

REFERENCES

Brul S, Coote P 1999. Preservative agents in foods: Mode of action and microbial resistance mechanisms; a Review. *International Journal of Food Microbiology* 50: 1-17.

Dock LL, Floros JD, Linton RH 2000. Heat inactivation of *Escherichia coli* O157: H7 in apple cider containing malic acid, sodium benzoate and potassium sorbate. *Journal of Food Protection,* 63(8): 1026-1031.

Duffy EA, Belk KE, Sofos JN, Bellinger GR, Pape A, Smith GC 2001. Extent of microbial contamination in United States pork retail products. *Journal of Food Protection*, 64(2): 172-178.

Duffy S, Schaffner, DW 2001. Modelling the survival of *Escherichia coli* O157: H7 in apple cider using probability distribution functions for quantitative risk assessment. *Journal of Food Protection,* 64(5): 599-605.

Garcia-Armesto MR, Sutherland AD 1996. Temperature characterization of psychrotrophic and mesophilic *Bacillus* species from milk. *Journal of Dairy Research,* 64: 261-270.

Gould GW 2000. Preservation: past present and future. *British Medical Bulletin,* 56(1): 84-96.

Jay JM 1996. *Modern Food Microbiology.* 5th Edition. New York: Chapman & Hall.

Kleyn J, Bicknell M, Gilstrap M 1999. *Microbiology Experiments: A Health Science Perspective* 2nd Edition. Boston: WCB McGraw-Hill.

Lammerding AM, Fazil A 2000. Hazard identification and exposure assessment for microbial food safety risk assessment. *International Journal Food Microbiology,* 58: 147-157.

Lund BM, Eklund T 2000. Control of pH and use of Organic acids. *The Microbiological Safety and Quality of Food*, 1: 175-199.

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 Food Microbiology,* 46: 187-192.

Montville TJ 1997. Principles which influence Microbial growth. Survival and Death in Foods. In: MP Doyle, LR Beuchat, TJ Montville (Eds.): *Food Microbiology: Fundamentals and Frontiers*. Washington DC: ASM Press, pp. 13-29.

Oxoid Manual 1973. *The Oxoid Manual of Culture media. Ingredients and other Laboratory Services*. 3rd Edition. London: Oxoid Ltd.

Pick DF, Hurford DP, Bair RC, Goepfert GL 1990. Ice cream butterfat content by volume as a possible predictor of taste preference. *Percept Mot Skills,* 70(2): 639-642.

Piper PW 1999. Yeast superoxide dismutase mutants reveal a pro-oxidant action of weak organic acid food preservatives. *Free Radical Biology Medicine,* 27(11-12): 1219-1227.

Razavilar V, Genigeorgis C 1998. Prediction of *Listeria* spp growth as affected by various levels of chemicals, pH, temperature and storage time in a model broth. *International Journal Food Microbiology,* 40(3): 149-157.

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 Microbiologia,* 16(4): 225-228.

Sinell HJ 1989. The hygiene of refrigerated and frozen foods. *Zentralbl Bakteriol Mikrobiol Hyg [B]*, 187(4-6): 533-545.

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.

Warke R, Kamat A, Kamat M, Thomas P 2000. Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India. *Journal of Food Control,* 11: 77-83.