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RECOVERY OF FREEZE INJURED PATHOGENIC BACTERIA FROM PACKED ICE CREAM

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Abstract:

This research was conducted to recovery and enumerates the Froze injured bacteria isolated from different brands of ice cream sold in Zakho – Duhok markets. A total of 100 samples of ice creams with different flavors (plain, Mix chocolate and Mix fruit) were analyzed. All samples showed aerobic mesophilic bacteria count within the standard. The injured Coliforms bacteria were recovered and the count was exceeded the limits in four brands (Chyaw, Amca, Twin and Mufid) while no coliforms bacteria had been detected in other brands (Adlin, PAK and Bernard) which indicate post-treatment contamination from water, and unpasteurized milk. All brand ice cream show recovery of injured *Staphylococcus aureus* ranged from (10 MPN/g) to (220 MPN/g). The results indicate that the highest contamination was found in Mix chocolate of PAK brand (220 MPN /g), Mix chocolate of Domino brand (210 MPN/g), Mix fruit and Mix chocolate of Mufid brand (201 MPN/g and 200 MPN/g), which indicate the contamination of flavorings and ingredients during production. None of the samples from Adlin, PAK and Bernard brands showed the presence of injured *Salmonella* spp. while in contrast of Chyaw, Amca, Twin and Mufid brands which showed heavy contamination ranged from (7 MPN/g) to (250 MPN/g), the highest contamination was found in all types of Twin and Mufid brands followed by Amca brand, which indicate post process contamination or contaminated the tank with raw milk. Results show ice cream is unsuitable for consumed and the need of observing the hygienic quality of markets. The presence of injured pathogenic bacteria in commonly consumed food should be concerned with the consumer, company and government.

Keywords: Ice Cream, Recovery, Injured, Bacteria

Introduction

Ice cream is a frozen dessert, mainly made of milk with the addition of flavorings such as chocolate and Fruit. The mixture is homogenized and pasteurized before freezing and storage at low temperature (Robert T. Marshall & Arbuckle 1996). Ice cream product is rich with nutrients such as lactose and proteins make it an excellent medium for the growth of various species of bacteria (Frazier & Westhoff 1992). Many pathogenic bacteria such as; *Staphylococcus aureus,* Coliforms*, Escherichia coli* and *Salmonella* spp. isolated from ice cream (Walker *et al.* 1990; Cotton & White 1992; Hennessy TW *et al.* 1996; Chugh 1997; Pooran *et al.* 2012; Gücükoğlu *et al.* 2013). The sources of bacterial contamination of ice cream include water, raw milk, flavoring agents, and poor personal hygiene (Vasavada 1988; Wilson *et al.* 1997). Most microbes do not grow under freeze temperature due to deactivation of their enzymes and deprivation of bacteria as water turn to ice. Slow freezing process causes more bacterial cell damage because of the formation of large ice crystal (Gill 2002). The damage action of slow cooling has been attributed to two general mechanisms: solute toxicity (Mazur 1984) and shrinkage of bacterial cell due to exceed the minimum critical volume of cells, leading to loss in plasma membrane material and shrinkage of bacterial cells (Meryman 1970; Steponkus *et al.* 1981). A recent hypothesis has report that during freezing salt bridges in cytoplasmic proteins opened; result in the cell swelling past the isotonic volume and lysis due to the dilution that accompanies thawing (Muldrew *et al.* 2000). Despite of a large number of bacteria cells are dying during freezing, some bacteria are surviving, making freezing and freeze- drying also a way for storage of microbes (Acker & McGann 2003). Third most common cause of food poisoning in the world is *Staphylococcus aureus* and the illness is due to the ingestion of food contaminated with enterotoxins (Dagnew *et al.* 2012). *Staphylococcus aureus* can survive longer in milk ice cream and their resistances to freezing process depend on specific individual strains and the composition of the ice cream mixture (Gogov *et al.* 1984). The presence of *Escherichia coli* in ice cream indicates the fecal contamination and poor hygiene (Hobbs & Golbert 1982). *Escherichia coli* contaminates food through direct and indirect sources, as it is commonly found in the gastrointestinal tract of man and animals (Frazier & Westhoff 1992; Jay 2000),

although it cannot survive the frozen and pasteurization processes, it could be presented in the product with some ingredients that have been added after pasteurization or improper sanitation of equipment and environments (Jay 2000; Zhang *et al.* 2007). The presence of *Salmonella* spp. in ice cream has been responsible for an epidemic of Salmonellosis in developing countries, where sanitation is poor and diarrheal diseases are endemic, national outbreaks of *Salmonella* Enteritidis infections from ice cream were most likely the result from contamination of pasteurized ice cream premix during transport in tanker trailers that had previously carried non-pasteurized liquid eggs containing *Salmonella* Enteritidis (AC. 1990; Hennessy *et al.* 1996). Although several international imported and Iraqi national brands of package ice cream with a variety of flavors have been marketed, no research work has been done on the recovery and detection of injured bacteria in ice cream. Microbiological quality of ice cream reflects the sanitary conditions during processing and packing stages and is an indication of food safety. Hence, the aim of this study was to recover and detect the pathogenic bacteria in ice cream sold in the market of the city and to assess the potential of this frozen product to pose risks to public health.

Materials and Methods

Collection of samples

This research work was carried out over a period of six months starting in July, 2015 to December, 2015**.** Hundred ice cream samples of different package branded were randomly collected from different markets of Zakho-Duhok town. Packed brand ice cream was divided to plain, mix fruit and mix chocolate. The samples comprised of eight packed industrial ice cream brands (Domino, Adlin, PAK, Bernard, Chyaw, Amca, Twin, and Mufid). Three samples each were collected for each brand over a period of six months. The collected samples were transported to the microbiology laboratory in an ice box within one hour of collection and kept frozen at -18°C till microbiological analysis.

Microbiological Analysis:

The nine tubes most probable number method was used to detect and recovery the stressed and injured Aerobic Mesophilic Bacteria, *Staphylococcus aureus*, Coliform and *Salmonella* spp. in packing brand ice cream.

Recovery and Enumeration of Total Aerobic Mesophilic Bacteria (TAMB)

25 mL was added to 225 mL of buffered peptone water as (pre-enrichment broth) and serial dilutions up to 10^{-3} then add 1 mL of each dilution to each of the three tubes containing 9 mL of nutrient broth (as enrichment broth) and incubate at 37°C for 24 hours. Following incubation, a loopful was streaked on plates of Nutrient Agar (Roberts & Greenwood 2003). The plates were incubated at 35°C for 48 hours and typical colony was selected and their identity was confirmed by standard biochemical reactions (Barrow 2003). The number of tubes that contain the target bacteria had been record to obtain the most probable number.

Recovery and Enumeration of Total Coliform Count (TCC)

25 mL was added to 225 mL of buffered peptone water as (pre-enrichment broth) and serial dilutions up to 10^{-3} then add 1 mL of each dilution to each of the three tubes containing 9 mL of Mac-Conkey broth (as selective enrichment broth) and incubate at 37°C for 24 hours. Following incubation, a loopful was streaked on plates of Mac-Conkey Agar (Roberts & Greenwood 2003). The plates were incubated at 35°C for 48 hours and pink colony was selected and their identity was confirmed by standard biochemical reactions (Barrow 2003). The number of tubes that contain the target bacteria had been record to obtain the most probable number.

Recovery and Enumeration of *Staphylococcus aureus*

25 mL was added to 225 mL of Ringer solution as (pre-enrichment broth) and serial dilutions up to 10^{3} then addition 1mL of each dilution to each of the three tubes containing 9 mL of Brain Heart Infusion broth (as selective enrichment broth) and incubate at 37°C for 24 hours. Following incubation, a loopful was streaked on plates of Mannitol Salt Agar (Roberts & Greenwood 2003). The plates were incubated at 35°C for 48 hours and yellow colony was selected and their identity was confirmed by standard biochemical reactions (G. I. Barrow 2003). The number of tubes that contain

the target bacteria had been record to obtain the most probable number.

Recovery and Enumeration of *Salmonella Spp.*

Making serial dilution up to 10^{-3} by adding 25 g of samples in 225 mL of buffered peptone water (as pre-enrichment broth), then add 1 mL of each dilution to each of the three tubes containing 9 mL of Tetrathionate broth (as selective enrichment broth) and incubate at 37°C for 24 hours. Following incubation, a loopful was streaked on plates of Salmonella Shigella Agar (Roberts & Greenwood 2003). The plates were incubated at 35°C for 48 hours and black center colony was selected and their identity was confirmed by standard biochemical reactions (Barrow 2003). Subculture on Triple Sugar Iron (TSI) slants and incubated at 37°C for 24 hours. The number of tubes that contain the target bacteria had been record to obtain the most probable number.

Statistical Analysis

The statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) software version 16 and compared by chi-square tests. To compare the mean counts of total aerobic plate, total Coliform, *Staphylococcus aureus* and *Salmonella* spp.

Results and Discussion

The results illustrate the current status of injured bacteria in brand ice cream sold in markets of Duhok city. A total of 100 ice cream samples marketed in Duhok province was examined for bacterial contamination. The total Aerobic count of all brand ice cream samples was shown in Figure 1. All samples show count within the limits of a standard which is 2.5×10^{5} MPU/g.

The results in Figure 2, shows the Coliforms count for brand ice cream was varying between heavy contaminated and not presence at all. Coliform count exceeded the limits in four brands (Chyaw, Amca, Twin and Mufid) while no Coliforms bacteria had been detected in other three brands (Adlin, PAK and Bernard). The Coliform counts were ranged from (7 MPN/g) to (350 MPN/g), the highest loads observed in Mix fruit ice cream of both Mufid brand (350 MPN/g) and Twin brand (300 MPN/g), while Domino brand samples were within the standard ranged between (7 MPN/g -28 MPN/g). Overall the results illustrated that the coliform contamination was more in mix fruit ice cream compared to plain and chocolate ice cream. Thus indicates the post-treatment contamination

from water, unpasteurized milk, and some packed ice cream remained in the freezer for a long time which affected by the frequent electricity shutting.

As shown in Figure 3, the count of *Staphylococcus aureus* ranged from (10 MPN/g) to (220 MPN/g). Each of Adlin, Bernard, Chywa, Amca and Twin Brands, show *Staphylococcus aureus* counts within the standard value which is 100 MPN/g, while The highest contamination was found in samples of chocolate ice cream of PAK brand (220 MPN/g), Domino brand (210 MPN/g) and Mufid brand (200 MPN/g). The sources of the contamination introduce via un-pasteurization flavorings and ingredients during production. The main source of *Staphylococcus aureus* is the skin and nasal cavity from these sources bacteria find their way into air and dust, into clothing and foods. Therefore, it is important to consider that the processing and handling of the food products should be so designed to minimize contamination and to make unfavourable medium for the growth of these organisms. When ice creams are produced with low numbers of *Staphylococci*, they will remain free of enterotoxin if kept either below 4℃ until consumed. The factors that contribute mostly

to staphylococcal food-borne outbreaks may be due to inadequate refrigeration due to frequent electricity shutting. *Salmonella* is able to survive for a long time in ice cream, but cannot survive the pasteurization of milk. None of the samples from Adlin, PAK and Bernard brands showed the presence of *Salmonella* spp. while in contrast of Chyaw, Amca, Twin and Mufid brands which showed heavy contamination (Figure 4). *Salmonella* should be absent in 25 g of sample, while in this study *Salmonella* spp. were detected in four brands of ice cream which incidentally had loads of Coliforms also. The *Salmonella* count ranged from (7 MPN/g) to (250 MPN/g), the highest contamination was found in Plain samples of Twin (250 MPN/g) and Mufid (245 MPN/g) followed by Amca (240 MPN/g), which suggest that the products are unsuitable for consume. These bacteria are able to survive for a long time in ice cream, but cannot survive the pasteurization of milk. Their presence indicates post process contamination or contaminated the tank with raw. Consumption of such product has been causes of food poisoning (Baird-Parker 1990; Hennessy *et al.* 1996; Zhang *et al.* 2007; Rastegar *et al.* 2013; Chugh 1996; Islam *et al.* 2014).
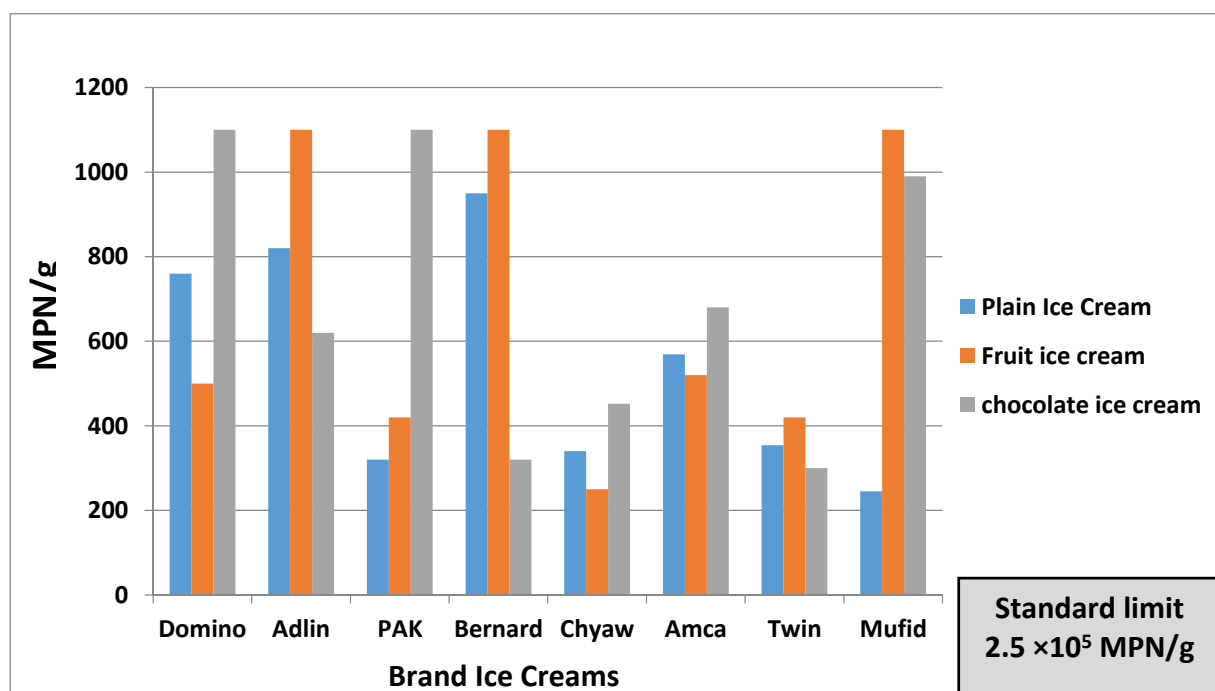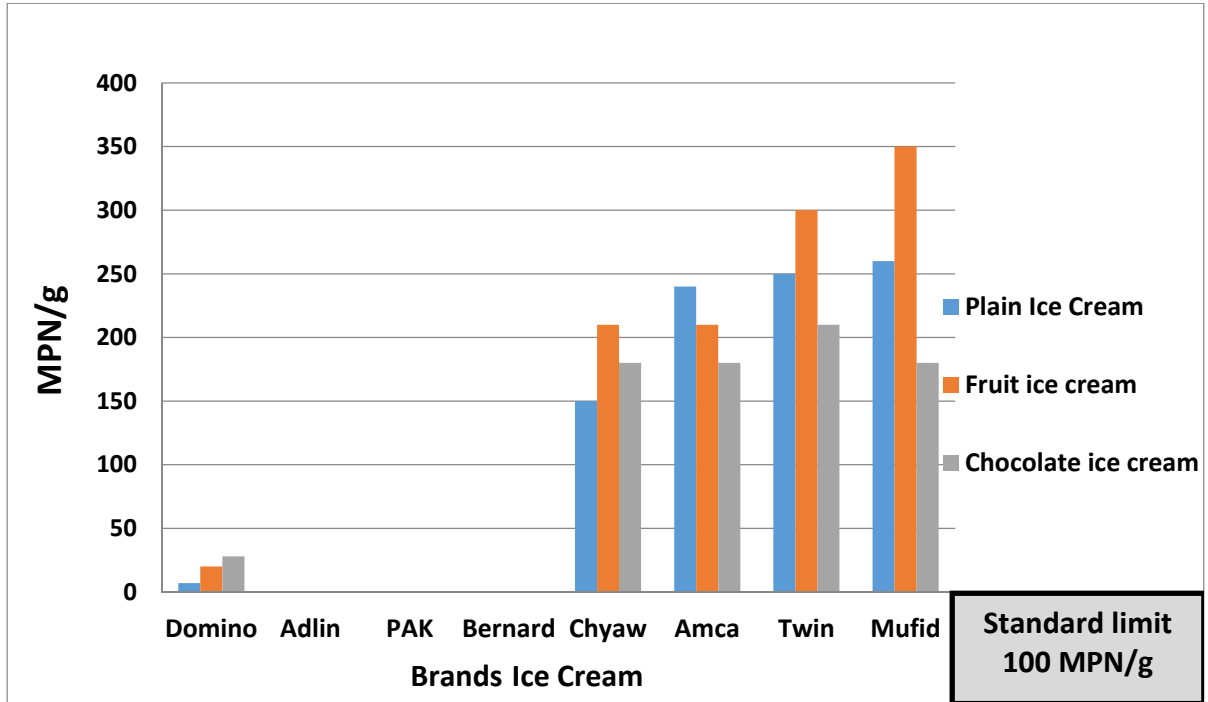

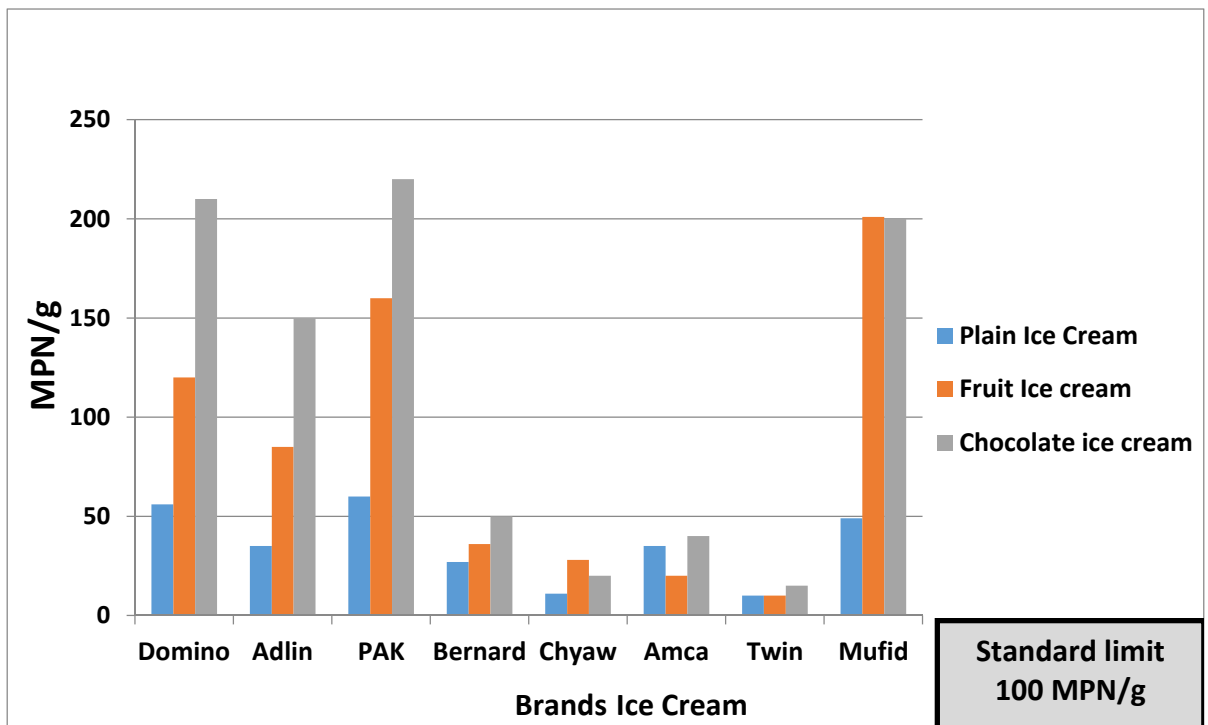**Figure 1.** Total Aerobic Mesophilic count

Figure 2. Total Coliforms count

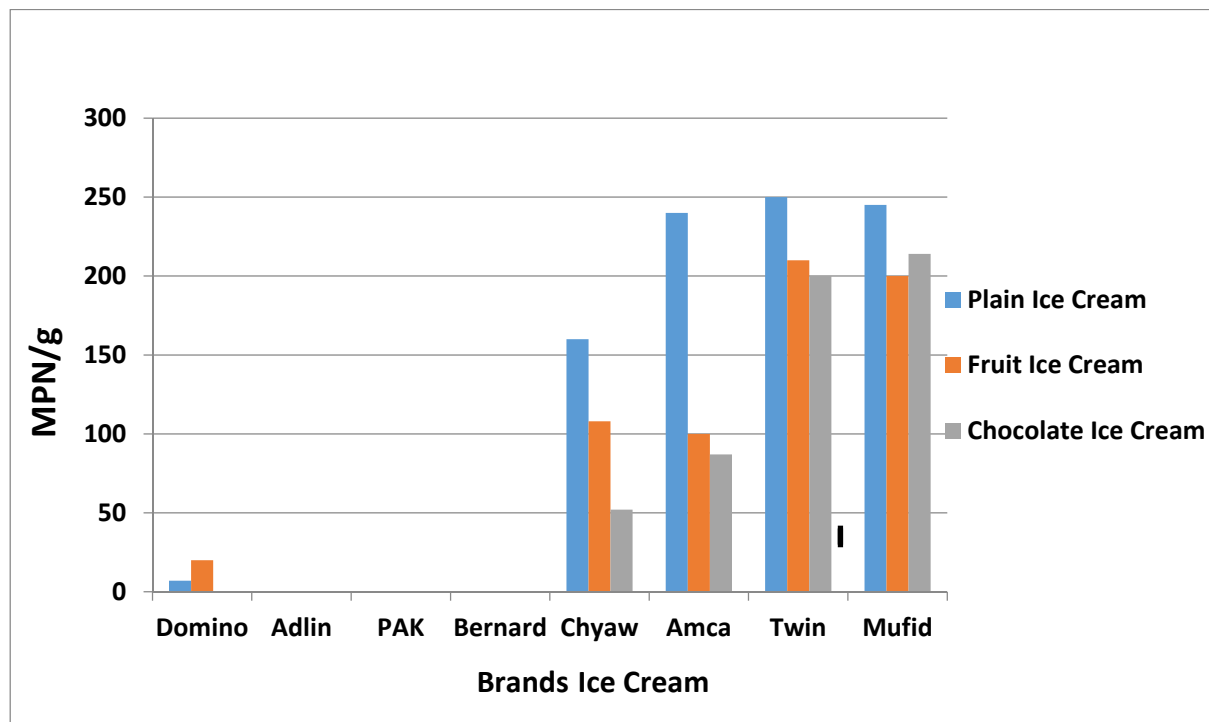**Figure 3.** Total *Stahpylococcus aureus* count

Figure 4. Total *Salmonella* spp. count

Conclusion

The current investigation illustrates a very poor level of hygienic quality in the service of brand ice creams sold in Duhok markets, Iraq. The high load of microorganisms exceeds the standard of food safety and standard regulation and it is necessary to develop hygienic statutes of ice cream made locally, In the country, Vanilla, Chocolate and fruit ice cream are the most popular flavor, had a poorest bacteriological quality. The aerobic bacteria count was within the standard. The heavy contamination of some local brand ice creams with Coliforms and *Salmonella* spp. illustrates the poor sanitation during preparation and storage of the products. The high load of *Staphylococcus aureus* in almost all samples represents the poor personal hygiene and used contaminated flavor and integrates. It is clear from the results that most of the ice cream is unsuitable for consume according to standard of food safety and standard regulation.

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