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*Research Paper*

# ASSESSMENT OF THE EFFECT OF FREEZING ON THE SURVIVAL OF SOME PATHOGENIC BACTERIA IN ICE CREAM

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**Abstract**

Ice cream is a frozen dairy product Produced by freezing pasteurized mixture of milk and other components, but when pasteurized ice-cream mixture was inoculated with food borne pathogenic bacteria as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E.coli*) and *Yersinia enterocolitica* (*Y.enterocolitica*), they could survive at -20c° for sixty days as we noticed in our work and so we must be sure that preventive measures have to be applied not only in production steps, but also for ice cream after production up to the consuming especially at the selling point at which the chance of ice cream contamination and the probability of survival of the contaminant microorganisms increase and causing public health hazards.

Key words: survival, ice cream**,** *Staphylococcus aureus*, *Escherichia coli*, *Yersinia enterocolitica*.

## INTRODUCTION

Ice cream is one of the widely accepted dairy products among children and adults all over the world [1]. It is a delicious, highly nutritious food and has a therapeutic value for persons suffering from irritation and infection of the mouth and throat due to its coldness [2]. The high content of nutrients like lactose and proteins and its neutral pH make it an excellent growth medium for microbes, some of which may cause serious disease outbreaks like cholera, typhoid and bacillary dysentery in human beings [3].

The source of microbial contamination can be at various stages of manufacture from humans and environmental sources [4]. Inappropriate product handling, especially at the selling point will further increase the chance of ice cream to be contaminated with pathogens [3]. On the other hand the temperature at which it is kept and its manufacture from pasteurized milk provides eliminating conditions for a wide range of microorganisms [5]. Most pathogens don't multiply at freezer temperature because their enzymes don't work properly or deprivation of pathogen from water as it turned to ice. The slower the freezing process the larger the crystals become and the more cells they damage [6]. It shall be noted that there are contrary opinions stating that intracellular ice is protective in some cases, even though a large percentage of cells is inactivated during freezing, a high number of cells are surviving, making freezing and freeze-drying also a method for storage of microorganisms [7].

In addition to, ice cream has been incriminated as a transmitter of pathogenic microorganisms' as *Klebsiella*, *E.coli*, *Yersinia* and *Staphylococci* [8,9,10].

*S. aureus* is the third most common cause of food poisoning in the world [11] and the illness is due to the ingestion of preformed enterotoxins (SEs) produced in foods [12].

*Y. enterocolitica* is a common enteric pathogen in humans [13]. It has become increasingly important as a food contaminant and infection in humans is associated with significant death rates [14].

*E. coli*, is incriminated as an etiologic agent of food borne illness involving a variety of foods. The organism contaminates food through direct and indirect sources, as it is commonly found in the gastrointestinal tract of man and animals [15]. And so we applied this work to determine the viability of some important pathogenic micro organisms, including *S. aureus*, *Y.enterocolitica* and *E. coli* during different storage periods of vanilla ice cream, which were artificially inoculated in pasteurised mixtures during the freezing process.

## MATERIALS AND METHODS

### Preparation of ice cream mixture.

Ice cream mixtures were prepared acc. to [16] and then cooled to 20°C. After cooling to 20°C, vanilla flavour was added and stored for 24 h at 4°C [17]. In microbiological analyses bacterial strains were artificially inoculated to the each stored mixture and stored at –20°C for 2 months.

### Inoculation procedure and microbiological analysis:

Stock lyophilised cultures of *S. aureus* (ATTCC 6538), *E. coli* O157:H7 (ATTCC 10536) and *Y. enterocolitica* (RSKK 920) strains were received from Zagazig University, Faculty of veterinary medicine, Department of Microbiology. Cultures were grown at 37°C in Nutrient Broth (Oxoid) for 18-24 hours. For inoculation, the cell suspension was then diluted to a final estimated inoculation level of $10^6$ cfu per test with the same medium and plated by the spread plate technique on Baird Parker Agar for *S.aureus*, EMB Agar for *E.coli*, and Yersinia Selective Agar (CIN) for *Y.enterocolitica*. Each culture was artificially inoculated to 500 ml of melted ice cream mixture, filled in sterile flasks, and homogenized. The actual cfu / ml of the organism was confirmed by direct counts in appropriate selective media [18,19,20].

Samples of ice cream were stored at –20°C and analyzed for the survival characteristics of organisms in the 0., 7th, 20th, 40th and 60th day of the storage. A sample units were taken at random to ensure that a sample is representative of the lot [21]. For this purpose 10 g of each sample was transferred to sterile flasks containing 90 ml of 0.85% sterilized physiological saline solution and homogenized then make serial dilutions up to $10^{-6}$ and plated by the spread plate technique onto the selective mediums. Petri dishes were incubated for *S .aureus*, *E. coli* at 37°C for 24-48 hours, for *Y.enterocolitica* at 30°C for 24-48 hours. After incubation periods colonies were counted on the plates containing 30-300 colonies [19,20].

## RESULTS AND DISCUSSION

The results showed in table (1) reported that when pasteurized ice-cream mixture was inoculated with food borne pathogenic bacteria they could survive at -20c° for sixty days. The initial inoculated level of *E.coli* O157: H7 at ($4\times10^6$ cfu/g.) was decreased to $1.6\times10^5$ cfu/g. in the day 40 and did not change up to the end of the storage period.

These results are somewhat different to those recorded by [22] who recorded that cultures were significantly more susceptible to freezing injury, and survival rates were ranged from 21% to 0.001%, from the start of freezing till the end of storage, while the greatest lost in viability was observed during the freezing operation step, while [16] gave nearly similar results.

The presence of some organisms in ice cream samples may be due to their resistance to cold. *E.coli* proved to be moderately resistant, Coliforms were somewhat more sensitive [23].

*E. coli* is a reliable marker organism of the hygienic conditions maintained in food processes and primary matters. Nevertheless, in frozen foods, such as ice creams, the concentration of Gram negative bacteria is not easy to ascertain since a part of the viable population loses the ability to form colonies on selective media because of sublethal injuries that freezing treatment caused to the cells [24,25,26]. Therefore the direct enumeration in plates causes an underestimation of the real number of *E. coli* in a frozen sample. The consistence of the cell damage, as percent of the viable population, is similar to that reported in other works [27,28].

The results summarized in table (1) also showed that the initial inoculated level of *S. aureus* ($7.5\times10^6$) did not fall during the period of two months but began to decrease at the end of the storage period.

These results were similar to those recorded by [16,29] as they reported that *S. aureus* was shown to have high resistance in ice cream.

*Staphylococci* are rather resistant to inactivation by the temperature of freezing, particularly when compared to Gram –ve organisms. Freezing and thawing have very little effect on viability. For this treatment to cells causes some injury, but not death. Multiplication of germs and toxin formation are completely inhibited below 7 c° [30]. The survival rate of the organisms depended both on the specific peculiarities of the individual strains and on the composition of the ice cream mixture where *Staphylococci* were found to survive longer in milk ice cream [29].

**Table (1): Growth profile of some pathogenic organisms in ice cream at -20 for two months**

| **Pathogenic organisms** | **DAYS** | | | | |
|---|---|---|---|---|---|
| | **0** | **0** | **0** | **0** | **0** |
| ***E. coli*** | $4\times10^6$ | $4\times10^6$ | $4\times10^6$ | $4\times10^6$ | $4\times10^6$ |
| ***S. aureus*** | $7.5\times10^6$ | $7.5\times10^6$ | $7.5\times10^6$ | $7.5\times10^6$ | $7.5\times10^6$ |
| ***Y.enterocolitica*** | $30\times10^6$ | $30\times10^6$ | $30\times10^6$ | $30\times10^6$ | $30\times10^6$ |

From table (1) also we concluded that growth patterns for *Y. enterocolitica* were similar. Whoever counts of the bacteria decreased slowly during the storage period, these bacteria are regarded as resistant to freezing.

These results were similar to those recorded by [31] while [32] reported that *Yersinia* organisms remained viable up to the eighth month according to microorganism concentration.

*Yersinia* can withstand freezing and survive for extended periods in frozen food, even after repeated freezing and thawing. Destruction of viable cells under freezing and thawing and constant freezing conditions at -20C° was more rapid in distilled water than in milk. Presumably proteins and fats in the food matrix provide a protective effect for Yersinia [33].

Hazards bacteria that grow at refrigeration temperature include *Y.enterocoliti*-ca, Is nearly recognized as food borne disease organisms. These bacteria are apoteimtial threat for food held at refrigeration temperature and they grow much faster at a higher abuse temperature [34]. Although, psychotrophic bacteria could be destroyed by Freezing, many vegetative cells can survive freezing. Thus, some psychotrophic bacteria will grow in frozen foods as ice cream, if the food is subjected to temperature abuse [35,36].

Bacteria differ in their resistance to freezing; usually survival of gram-positive cocci (e.g. *Staphylococcus*) is higher than of gram-negative rods (e.g. *E.coli*). Stationary phase cells are more resistant than log-phase cells. The survival is also varies considerably among different strains of one species. Many kinds of food constituents like protein and carbohydrates increase freezing viability, whereas low pH decreases survival [37].

During freezing the same mechanism of ice formation is working as in cellular food, even though bacteria are smaller by several orders of magnitude. Most water freezes outside the cells, causing dehydration of the intracellular space. Intracellular freezing is occurring at higher freezing rates [38] summarized that the effect of freezing on microorganisms is largely determined by solute concentration and intracellular freezing, which is deadly [39,40].

The main effects of microorganisms adaptation to the cold was summarized by [41,42] as major effect is the adjustment of the fatty acid composition of the lipid bilayer membranes, making them shorter and incorporating more unsaturated chains. In this 24 way they prevent lipid solidification and membrane breakage.

Based on the results of the present study, we conclude that preventive measures have to be specifically targeted not only in production steps, but also in post- production period up to the consuming, especially at the selling point as increase the chance of ice cream contamination to

minimize the contamination with these microorganisms as if they were present they could be survive the freezing temperature causing many health problems.

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