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# Survival Characteristics of Some Pathogenic Bacteria in Vanilla Ice Cream at Different Storage Periods (*)

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SUMMARY

Survival characteristics of Staphylococcus aureus (ATCC 29213), Escherichia coli (ATCC 25922), Enterococcus faecalis (ATCC 29212), Pseudomonas aeruginosa (ATCC 27853), Salmonella enterica serovar Enteritidis SZH (Nationales Referenzzentrum für Salmonellen und andere bakterielle Enteritis-erreger, Hygieneinstitut Hamburg, Germany) and Yersinia enterocolitica type 03 (RSKK 920) in pasteurised mixture at the different storage periods of vanilla ice cream were investigated. Organisms survived by the freezing process in either ice cream samples during the storage periods of two months. These results indicate the importance of the post-production steps in frozen dairy desserts.

Key words: Ice cream, pathogenic bacteria, hygiene, public health

ÖZET

Sade Dondurmada Bazı Patojen Bakterilerin Farklı Depolama Sürelerinde Canlı Kalma Özelliklerinin Araştırılması

Çalışmamızda dondurmanın hijyenik kalitesinin belirlenmesinde yaygın olarak incelenen mikroorganizmalardan Staphylococcus aureus (ATCC 29213), Escherichia coli (ATCC 25922), Enterococcus faecalis (ATCC 29212), Pseudomonas aeruginosa (ATCC 27853), Salmonella enterica serovar Enteritidis SZH (Nationales Referenzzentrum für Salmonellen und andere bakterielle Enteritis-erreger, Hygieneinstitut Hamburg, Germany) ve Yersinia enterocolitica tip 03 (RSKK 920) farklı dondurma gruplarına miskin dondurmaya işlenmesi sırasında mililitrede 10ˢ ile 10⁶ cfu olacak şekilde inoküle edilerek canlı kalma özellikleri dondurmanın farklı depolama sürelerinde (0., 7., 20., 40., 60. günler) araştırılmıştır. Bakteriler dondurmanın ilerleyen depolama sürelerinde canlılıklarını korumuşlar ve dondurma üretiminde üretim sonrasının önemi ortaya konulmuştur.

Anahtar Kelimeler : Sade dondurma, patojen bakteri, hijyen, halk sağlığı

ponent food system, which is consumed especially by children due to its flavoursome and freshness, among the dairy products (1,2,3).

Nevertheless, manufacturing of ice cream supports the growth of pathogenic bacteria like the members of Enterobacteriaceae (*E.coli*, *Y.enterocolitica* and *Salmonella species*), *S.aureus*, *E.faecalis*, and *P.aeruginosa*. Plants, manufacturer's hygienic conditions and microbial load of raw material (milk) and other ingredients are the leading contamination sources of the above mentioned and related organisms (4,5).

The main critical control point in ice cream processing is the microbiological quality of raw material. Raw milk may contain foodborne pathogenic bacteria, although it has been obtained from the healthy cows (6). International Dairy Federation (I.D.F.) has issued a standard for the raw milk's total viable counts (TVC). On the authority of the standard $10^5$ colony forming units (cfu) per millilitre TVC indicates the poor hygienic conditions of the raw milk production, whereas 2.0 x $10^4$ cfu/ml is a satisfactory result (7).

Recent years many food poisoning cases were reported due to the consumption of dairy products in many countries such as U. S. A and The Netherlands (8,9). In ice cream processing, pasteurisation of mixture eliminates non-sporeforming bacteria, which brings about a high microbiological quality and shelf life of milk and other products (10,11,12,7,13).

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Many researchers stated before processing and storing of food products at low temperatures (-20°C and –25°C) damage microorganisms because of the high content of cell water freezes, but mortality increase with storage time (12,14).

The objective of the present study was to determine the survival characteristics of some pathogenic bacteria at the 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 with the following composition: 2 litres UHT milk, 400 g sucrose, 100 g skim milk powder, 16 g butter, 10 g carrageenan as stabiliser and 0.5 g vanilla flavour. A part of sucrose and skim milk powder were dissolved in 2 litres of milk at 40°C and mixed together combined with the rest of the sucrose and stabiliser in a stainless steel pan. Then heated in a water bath. During this heating, butter was added and mixtures were pasteurised at 75°C for 1 minute and then cooled to 20°C. After cooling to 20°C, vanilla flavour was added and stored for 24 h at 4°C (15). In microbiological analyses bacterial strains were artificially inoculated to the each stored mixture and stored at –20°C for 2 months.

### Bacterial strains and inoculation procedure

Stock lyophilised cultures of *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212) and *Pseudomonas aeruginosa* (ATCC 27853) strains were received from Akdeniz University, Hospital of Medical Faculty, Department of Microbiology.

*Salmonella enterica* serovar Enteritidis SZH (Nationales Referenzzentrum für Salmonellen und andere bakterielle Enteritis-erreger, Hygieneinstitut Hamburg, Germany) and *Yersinia enterocolitica* type 03 (RSKK 920) strains were supplied from Istanbul University Istanbul Medical Faculty Culture Collections Research and Practices Center and Refik Saydam Hygiene Center, Ankara, respectively.

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 Nutrient Agar for enrichment and confirmation. XLD Agar (Merck) for *Salmonella enterica* serovar Enteritidis, Baird Parker Agar (Difco) for *S.aureus*, EMB Agar (Merck) for *E.coli*, Enterococcus Agar (Difco) for *E.faecalis*, Pseudomonas Agar Base (Merck) for *P.aeruginosa* and Yersinia Selective Agar for *Y.enterocolitica* were used. Each culture was artificially inoculated to 500 ml of melted ice cream mixture, filled in sterile flasks, and homogenised. Actual cfu/ml of the organism was confirmed by direct counts in appropriate selective media (16,17,18).

### Microbiological analyses

Samples of ice cream were stored at –20°C and analysed for the survival characteristics of organisms in the 0., 7th, 20th, 40th and 60th day of the storage. A sample unit consisted of a minimum of 100 g and, were taken sample units at random to ensure that a sample is representative of the lot (19). For this purpose 10 g of each sample was transferred to sterile flasks containing 90 ml of 0.85% sterilised physiological saline solution (Riedel-deHaën) and homogenized. The homogenate was prepared for serial dilutions up to $10^{-6}$ and plated by the spread plate technique onto the selective mediums. Petri dishes were incubated for *Salmonella enterica serovar* Enteritidis, *S .aureus*, *E. coli* and *E. faecalis* at 37°C for 24-48 hours, for *Y.enterocolitica* at 30°C for 24-48 hours and for *P. aeruginosa* at 25°C for 24-48 hours. After incubation periods colonies were counted on the plates containing 30-300 colonies (17,18).

### Statistical analyses

Each trial was repeated twice and duplicate samples were tested at each sampling time. The data on microbial count were analysed bys Randomised Parcel Design. Analysis consisted of analysis of variance and general linear model procedures, followed by determination of significance in the analysis of treatment effects using Duncan's multiple range test. Significance was based on a probability level of 0.05 ($p<0.05$) (20).

## RESULTS

Artificially inoculated pathogens survived during the freezing process and storage of the mixtures by two months (Table 1). Although it is recognized that microbial death may occur during the storage period, as well as during the freezing and thawing processes

Table 1. Level of significance and Duncan multiple range test for years' microbial counts ($p<0.05$)

| Response variable | Duncan multiple test range (means)[a] | | | | |
|---|---|---|---|---|---|
| | Days | | | | |
| | 0 | 7 | 20 | 40 | 60 |
| S.aureus[b] | 6.60 | 6.43 | 6.36 | 6.25 | 6.04 |
| E.coli[b] | 5.90 | 5.11 | 4.78 | 4.45[a] | 4.41[a] |
| E.faecalis[b] | 5.99[a] | 6.04 | 5.84 | 6.00[a] | 6.08 |
| P.aeruginosa[b] | 6.86[a] | 6.72[a] | 6.48[ab] | 6.36[b] | 6.08[ab] |
| S.enterica serovar Enteritidis[b] | 6.50[a] | 6.48[a] | 6.34 | 6.28[b] | 6.25[b] |
| Y.enterocolitica[b] | 6.08 | 6.04[a] | 6.04[a] | 5.82 | 5.66 |

[a] Means with the same letter are not significantly different

[b] Expressed as $\log_{10}$ cfu/g

## DISCUSSION

*E. coli* and *Salmonella enterica serovar* Enteritidis are the members of Enterobacteriaceae. The latter one is commonly present in meat and meat products, rarely in dairy products. Several researches indicated that both of the bacteria could not be resistant to pasteurisation applications in food processing (21,22,6). The counts of *E. coli* in raw milk samples, which were destroyed completely by heat treatment, were between $1.20x10^3$ cfu/g and $1.75x10^3$ cfu/g in ice cream during the manufacturing process (6). As stated in our experiment, if the pasteurised mixture was contaminated with foodborne pathogenic bacteria they could survive at –20°C for sixty days. The initial inoculated level of *E. coli* at $8.0x10^5$ cfu/g decreased significantly ($p<0.05$) to $2.8x10^4$ cfu/g in the 40th day and did not change significantly up to the end of the storage period ($p<0.05$).

The *Salmonella enterica serovar* Enteritidis load, present initially on ice cream, was reduced from $3.2x10^6$ cfu/g to $1.8x10^6$ cfu/g in a period of two months. However, the 0. and 7th, and also 40th and 60th days of the storage counts showed no significant differences between each pairs, respectively (Table 1). Among the Gram negative bacteria, *Salmonella spp.* are one of the causative organisms of significant morbidity and mortality in human beings and animals throughout the world. On the other hand, the Turkish regulator agencies require absence of *Salmonella* spp. in 25 g of ready-to-eat foods (23,16,21).

*S. aureus*, as an indicator of the good manufacturing processes, can be eliminated by pasteurising raw milk because of the heat labile properties of the bacteria. In any food $2.0x10^5$ colonies per gram indicates that bacteria may cause food poisoning (19,21). The initial inoculated level of *S. aureus* was $4.0x10^6$ cfu/g on ice cream.

The colony counts for *S. aureus* did not fall under the limit value (5.30 $\log_{10}$ cfu/g) during the period of two months, but significantly decreased at the end of the storage ($p<0.05$).

Species of the *Pseudomonas* may grow in raw milk and capable of producing heat resistant enzymes in ultrahigh-temperature which ends with the spoilage of stored milk (24). However, presence of the viable bacteria indicate the post heat processing contamination of the final product due to their heat sensitivity (25). The initial inoculated level of *P.aeruginosa* was $7.3x10^6$ cfu/g and $1.2x10^6$ cfu/g in the last day of the storage on ice cream.

Growth patterns for *Y.enterocolitica* in 7th and 20th day were similar (Table 1). However, counts of the bacteria decreased during the storage period was significant ($p<0.05$). These bacteria species are regarded as resistant to freezing, whereas sensitive to pasteurisation (21).

*E. faecalis*, survived without any significant decrease during the experimental period, in contrast the viable bacteria counts increased ($p<0.05$). *Enterococcus* strains may be present in the all forms of milk and dairy products (26) and are used widely as a starter culture for a large number of traditional cheeses (26,27). However, their pathogenic characteristics should be considered due to being stable in unfavourable conditions, especially heat and freezing treatments. Therefore, the milk, which is used in dairy products as a raw material has to meet special microbiological quality standards (e.g. International Dairy

Federation, Council of the European Union, Turkish Standards Institution) and have also processed with the other ingredients in suitable hygiene sanitation applications.

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.

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