Microbiological Quality of Ice Cream Consumed in Istanbul

Tolga Kahraman1*, Ari Manuk Kolanciyan2

Abstract
Ice cream is a dairy product that is produced by freezing a mixture enriched with sugar, cream, stabilizers, emulsifiers and aroma materials. The present study was aimed at determining the microbiological quality of 150 ice cream samples (75 plain and 75 strawberry-flavored) collected from artisanal producers in Istanbul, Turkey. The samples were investigated for total mesophilic aerobic bacteria (TMAB), Enterobacteriaceae, Salmonella spp. and Listeria monocytogenes. Results showed that TMAB counts ranged from 2.0 x 10^1 - 2.5 x 10^3 cfu/g with a mean of 1.5 x 10^4 cfu/g, while Enterobacteriaceae count ranged from < 10^3 - 8.8 x 10^3 cfu/g with a mean of 3.0 x 10^2 cfu/g. Overall, 23.33% (35/150) of ice cream samples were of unacceptable quality based on recommended criteria by the Turkish Food Codex. Salmonella spp. was not determined in the samples. L. monocytogenes was detected in only one strawberry-flavored ice cream sample. The results indicated that ice cream might have been contaminated with pathogens, presenting a potential hazard for public health. Therefore, it is essential to ensure the safety of final products by improving the quality of production technology and sanitation strategies.

Keywords
Ice Cream — Microbiological Quality — Public Health — Salmonella spp. — L. monocytogenes

1 Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Istanbul University, Istanbul, Turkey
2 SGS Supervise Inspection Companies Association, Istanbul, Turkey
*Corresponding author: tolgakah@istanbul.edu.tr

Introduction
Dairy products are an important source of calcium, high-quality protein, potassium, phosphorus, and riboflavin (25). Among these products, ice cream is the most commonly consumed frozen milk product, which contains a variety of ingredients in addition to milk, cream, water, milk solids-not-fat, milk fat or other fat, sugar, emulsifying and stabilizing agents, flavors and colors (29). The manufacturing of ice cream mainly includes four steps: mixture preparation, ageing, freezing and hardening. The mixture preparation phase consists of mixing ingredients, pasteurization and homogenization steps. Then, the mixture is rapidly cooled to 2-4°C in order to preserve its bacteriological quality, and then stored for approximately 24h, which is known as the aging phase where all of the dry ingredients are hydrated and the fat is crystallized. After the aging, the product is passed to the ice cream freezer, where its temperature is reduced rapidly to -5°C. The final process is hardening, which freezes out most of the water at -40°C (12). Ice cream is very rich in nutrients that provide an ideal growth environment for many microorganisms. The mishandling, poor production process and the low quality of ingredients may lead to the increase of the number of microorganisms. These may cause considerable hygienic and quality problems and create a potential public health risk (5). Warke et al. (35) reported that most of the ice cream consumers were children of vulnerable age groups; that is why it must be microbiologically safe.

Microbiological quality of ice cream is determined by total mesophilic aerobic bacteria (TMAB), Enterobacteriaceae count and presence of pathogenic microorganisms. Several studies of the microbiological quality of ice cream were carried out by Massa et al. (24) in Italy, Windrantz and Arias (37) in Costa Rica, El-Sharef et al. (15) in Libya and Wilson (36) in Ireland. In Turkey, the quality has been determined by Celik et al. (10), Bostan and Akın (8) and Ozcan (30); but most of these studies did not extensively investigate TMAB, Enterobacteriaceae, Salmonella spp. and L. monocytogenes together.

The present study was undertaken to determine the microbiological quality of ice cream consumed in Istanbul, which has a geographical importance due to its location and represents a transit corridor between Europe and Asia.

Material and Methods
Sample Collection. Ice cream samples were randomly collected between July 2012 - June 2013 from artisanal producers in Istanbul, Turkey. A total of 150 (75 plain and 75 strawberry-flavored) ice creams were investigated for TMAB and Enterobacteriaceae counts as well as for presence of Salmonella spp. and L. monocytogenes. All samples were immediately transferred to the laboratory in cold boxes at 4 °C.

Microbiological Analysis. Microbiological analyses were carried out according to the methods specified by Turkish Standards (34). For this purpose, 10 g of the samples were aseptically taken and transferred into the sterile plastic bags containing 90 ml of Ringer solution (Oxoid, BR0052),...
Table 1. Total mesophilic aerobic bacteria (TMAB) and Enterobacteriaceae counts (cfu/g) found in ice cream samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>n</th>
<th>MIN</th>
<th>MEAN</th>
<th>MAX</th>
<th>MIN</th>
<th>MEAN</th>
<th>MAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain ice cream</td>
<td>75</td>
<td>2.0 x 10^1</td>
<td>2.0 x 10^4</td>
<td>1.6 x 10^5</td>
<td>1.0 x 10^4</td>
<td>8.8 x 10^4</td>
<td>3.5 x 10^2</td>
</tr>
<tr>
<td>Strawberry-flavored ice cream</td>
<td>75</td>
<td>3.0 x 10^1</td>
<td>2.0 x 10^4</td>
<td>1.6 x 10^5</td>
<td>1.0 x 10^4</td>
<td>8.8 x 10^4</td>
<td>3.5 x 10^2</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>2.0 x 10^1</td>
<td>2.0 x 10^4</td>
<td>1.5 x 10^4</td>
<td>1.0 x 10^4</td>
<td>8.8 x 10^4</td>
<td>3.0 x 10^2</td>
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and homogenized for 1–2 min. Following homogenization, 10-fold serial dilutions were made in sterile Ringer diluent and inoculated on specific culture media. TMAB was enumerated using Plate Count Agar (PCA, Oxoid CM 463) after incubation at 30 °C for 48 h (18). Enterobacteriaceae were examined by surface plating on Violet Red Bile Glucose Agar (VRBG, Oxoid CM 1082). Plates were incubated at 37 °C for 24 h (20).

For the isolation of Salmonella spp., pre-enrichment was done by suspending 25 g of sample in 225 ml buffered peptone water (BPW) (Oxoid CM0509) followed by incubation at 37 °C for 16-20 h. Then, 0.1 ml of mixture was transferred to Rappaport-Vassiliadis (RV) (Oxoid CM0866) and Muller-KaufmannTetrahionate Broth (MKTn) (Oxoid CM0343). MKTn and RV broths were incubated for 24h at 42°C. After incubation the samples were streaked on Hectoen Enteric Agar (HE) (Oxoid CM0419) and XLD Agar (Oxoid CM0469), and incubated for 24h at 35°C. The typical colonies were identified by Triple Sugar Iron Agar (TSI) (Oxoid CM0277), Lysine Iron Agar (LIA) (Oxoid CM0381), urease test (Oxoid CM0071) and confirmed with Salmonella antiserum (O (Oxoid R30858101) and H-Vi polyvalent antiserum (Oxoid R30163201)(21).

For detection of L. monocytogenes, 25 g of ice cream samples were added to 225 ml of One Broth Listeria Selective Supplement (Oxoid, SR0234) containing One Broth Listeria Base (Oxoid, CM1066). Samples were homogenized in a stomacher bag for 60 sec at normal speed and incubated at 32 °C for 24 h. A 0.1 ml portion of the enrichment broth was streaked onto Brilliance Listeria Agar (Oxoid, CM1080) supplemented with Brilliance Selective Supplement (Oxoid, SR227) and Brilliance Listeria Differential Supplement (Oxoid, SR228). After incubation (32°C for 24 h), typical colonies were transferred to Tryptic Soy Agar with Yeast Extract (TSYE) (Oxoid CM0131) and incubated for 24-48 h at 30°C. The typical colonies were verified by Gram staining, catalase reaction, tumbling motility at 20-25 °C, Methyl Red-Vogues Proskauer (MR-VP) reactions, CAMP test, nitrate reduction and fermentation of sugars (2).

**Statistical Analysis.** In order to verify if any difference exists between distributions of data from the two sampling sites, variances were tested for homogeneity by Shapiro – Wilk (P < 0.001). Statistical comparisons of the mean values, ranges and medians between the groups were performed using The Mann – Whitney U test for skewed distribution. Level of significance is P < 0.05. Variables were analyzed by the SPSS statistical software (31).

**Results**

The ranges, means and distribution of TMAB and Enterobacteriaceae counts found in the ice cream samples are given in Table 1, Figure 1 and 2. The mean load of TMAB in plain ice cream was estimated at 2.0x10^5 cfu/g ranging from 2.0x10^1 to 2.0x10^5 cfu/g, while the mean TMAB in strawberry-flavored ice cream was 1.0x10^5 cfu/g with the range from 3.0x10^1 to 1.6x10^5 cfu/g. There were no significant differences between the mean TMAB counts found in plain and strawberry-flavored ice cream (P > 0.05). According to the Turkish Food Codex (TFC) (33), ice cream is considered of unacceptable hygienic quality when the Enterobacteriaceae count exceeds 10^5 cfu/g. 34.67% (26/75) of plain ice cream samples and 12% (9/75) of strawberry-flavored ice cream samples exceeded this limit. No significant differences were observed between the mean Enterobacteriaceae counts found in plain and strawberry-flavored ice cream (P > 0.05). All of the samples were negative for Salmonella spp., while L. monocytogenes was detected only in one (0.67%) ice cream sample. According to TFC (33), the presence of Salmonella spp. and L. monocytogenes in 25 g of ice cream is not acceptable.
Discussion and conclusion

TMAB is used to measure sanitary quality of production process and food products, and often indicates contamination of raw materials, unsatisfactory sanitation and unsuitable storage temperature, or a combination of these (7). Our findings are similar to those of Ozcan (30). On the other hand, higher contamination results (4.6 x 10^7, 1.6 x 10^6, 1.6 x 10^5 and 2.2 x 10^4) were reported by Omurtag et al. (28), Boyukkara and Sagun (9), Celik et al. (10) and Mukan (26), respectively. Our results may indicate that the sampled ice cream had a low microbial load of ingredients and better storage conditions.

Enterobacteriaceae are indicators of fecal contamination. Quantification of these organisms provides an insight in the effectiveness of personal hygiene, production conditions and sanitation procedures (32). In this study, Enterobacteriaceae ranged from <10^1 cfu/g to 8.8 x 10^3 cfu/g with a mean of 3 x 10^2 cfu/g in all samples. According to TFC (33), 35 of 150 samples (23.33%) were unacceptable. The highest contamination levels were observed in plain ice cream samples, which can be related to the higher acidity of strawberry-flavored ice cream (11). Higher mean Enterobacteriaceae counts were previously reported by Omurtag et al. (28), Celik et al. (10), Mukan (26), Evrensel Gunes (17) and Mukan and Evliya (27). Differences between the findings obtained from several studies may be due to production techniques, contamination after production, preservation conditions or inadequate personal hygiene.

Salmonella spp. were not detected in this study. Similar results were reported by Arslan et al. (4), Bostan and Akın (8), Or (29) and Aydn (6). Contrary to this, Agaoglu and Alemdar (3), Erol et al. (16) and Korel et al. (23) reported higher results (17.3%, 2% and 14%, respectively). Such differences may be explained by different detection methods and sampling procedures.

Presence of L. monocytogenes in the ice cream samples was examined in several countries, and prevalence of the pathogen broadly varies: in Costa Rica 11.6% (37), Sri Lanka 33.33% (19), Chile 6.8% (13) and Libya 4.38% (15). The authors explained such high prevalence estimates with Enterobacteriaceae. However, other studies reported lower prevalence levels (6, 26, 28, 30). The highest contamination levels were observed in plain ice cream samples, which can be related to the higher acidity of strawberry-flavored ice cream (11). Higher mean Enterobacteriaceae counts were previously reported by Omurtag et al. (28), Celik et al. (10), Mukan (26), Evrensel Gunes (17) and Mukan and Evliya (27). Differences between the findings obtained from several studies may be due to production techniques, contamination after production, preservation conditions or inadequate personal hygiene.

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Ice cream is manufactured according to different protocols and recipes in Turkey, and, therefore, it may be contaminated with pathogens such as Salmonella spp. and L. monocytogenes thus causing great public health problems. The quality of the raw milk, heat treatment of the milk as well as personnel and production hygiene are the most important critical points for prevention of growth of undesirable microorganisms during the manufacturing. Therefore, it is essential to ensure application of good hygiene practices and to standardize production process, which would be beneficial for enhancing the safety of food.

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Mikrobiološki kvalitet sladoleda koji se konzumira u Istanbulu

Sažetak

Uvod i ciljevi
Sladoled je mlječni proizvod koji se dobiva smrzavanjem smjese obogaćene šećerom, vrhnjem, stabilizatorima, emul-fizikatorima i aromama. Sladoled je bogat hranjivim sadjevima koji predstavljaju idealnu sredinu za rast mnogih mikroorganizama. Loše rukovanje, neodgovarajući proces proizvodnje i slaba kvaliteta sastojaka mogu izazvati povećanje broja mikroorganizama. Ovo povećanje može uzrokovati znatne higijenske probleme i probleme kvalitete te stvoriti potencijalni rizik za javno zdravlje.

Mikrobiološka kvaliteta sladoleda je određena ukupnim brojem mezofilnih aerobnih bakterijama (TMAB), ukupnim brojem bakterija familije Enterobacteriaceae i prisustvom patogenih mikroorganizama. Ova studija ima za cilj odrediti mikrobiološki kvalitet sladoleda koji se konzumira u Istanbulu koji je od velikog značaja zbog svog geografskog položaja, predstavljaći tranzitni koridor između Evrope i Azije.

Materijal i metode
U ovoj studiji je metodom slučajnih uzoraka prikupljeno 150 uzoraka sladoleda (75 običnih i 75 sa okusom jagode) od zanatskih proizvođača. Uzorci su ispitivani na TMAB i Enterobacteriaceae kao i na prisustvo Salmonella spp. i L. monocytogenes. Svi uzorci su odmah po prikupljanju prebačeni u laboratorij u hladnim kutijama na 4 °C. Mikrobiološka analiza je izvršena metodama specificiranim prema turskim standardima. U ovu svrhu je na aseptičan način prikupljeno po 10 g od svakog uzorka koji je ubačen u sterilne plastične vreće koje sadrže 90 ml Ringerove otopine, i homogeniziran 1-2 minute. Nakon homogenizacije su napravljena desetostruka serija razblazenja u sterilnom Ringerovom razblazaivaču, i izvršena je inokulacija na specifične podloge.

Rezultati i interpretacija
Rezultati su pokazali da se broj TMAB kretao između 2.0 \( \times 10^1 \) - 2.5 \( \times 10^5 \) cfu/g, sa srednjom vrijednošću od 1.5 \( \times 10^4 \) cfu/g, dok se broj enterobakterija kretao od 10\(^1\) (i manje) do 8.8 \( \times 10^3 \) cfu/g, sa srednjom vrijednošću od 3.0 \( \times 10^2 \) cfu/g. Nije bilo signifikantnih razlika između srednje vrijednosti TMAB u običnom sladoledu u odnosu na sladoled sa okusom jagode (\( P > 0.05 \)). Prema Turškom kodeksu o hrani (TFC), sladoled se smatra neprihvatljive higijenske kvalitete ako broj enterobakterija prelazi 102cfu/g. U skladu s ovim standardom 34.67% (26/75) uzoraka običnog sladoleda i 12% (9/75) uzoraka sladoleda sa okusom jagode je bilo higijenski neispravno. Nisu uočene neispravne razlike između srednje vrijednosti broja enterobakterija u običnom sladoledu i u okusnom sladoledu (\( P > 0.05 \)). Najviše razine kontaminacije su uočene u uzorcima običnog sladoleda, što se može objasniti činjenicom da sladoled sa okusom jagode ima veću kiselost. Salmonella spp. nije pronađena u uzorcima, a L. monocytogenes je pronađena samo u jednom uzorku sladoleda sa okusom jagode (\( P > 0.05 \)). Najviše razine kontaminacije zahtijevaju interventionale mjere za prevenciju razmnožavanja patogena u proizvodnom procesu.

Glavni zaključak
Sladoled se u Turškoj proizvodi prema različitim protokolima i receptima, te stoga može biti kontaminiran različitim patogenuma, kao što su Salmonella spp. i L. monocytogenes, te može uzrokovati velike probleme javnom zdravstvu. Kvalitet sirovog mlijeka, tretman mlijeka toplotom i higijena osoblja i proizvodnje predstavljaju najvažnije kritičke tačke u prevenciji rasta neželjenih mikroorganizama u proizvodnom procesu. Stoga je neophodno osigurati dobru higijensku praksu uz standardizaciju proizvodnog procesa čime bi se povećala sigurnost hrane.