Microbial quality of ice cream sold openly by retail outlets in Turkey

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SUMMARY

This study aimed to investigate microbiological quality of ice cream samples sold openly (in small portions from bulk containers exposed to the open air) by retail markets in Turkey. The samples (n=73) were analyzed to determine the hygienic status of the ice creams and also for the presence of some Gram positive and Gram negative pathogenic bacteria. Hygienic parameters included the total aerobic mesophilic bacteria (TAB), Enterobacteriaceae, coliforms, Enterococcus spp., yeast and mould counts. The pathogens included Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus), Escherichia coli (E. coli), E. coli O157: H7, Salmonella spp., Yersinia spp. Of the 73 ice creams examined, 4.1 % of the samples had unacceptable hygienic quality according to the criterion (105 cfu/g) recommended in Turkish Food Codex (TFC), 78 % of the samples contained the Enterobacteriaceae which had a higher detection rate than coliforms (52 %), ranging from 102 to 106 cfu/g. The numbers of coliforms were not in accordance with the standard of TFC (95 MPN cfu/ml). Considering the percentage distribution of Enterococcus spp. in samples, 47 % of the ice cream samples were within the range of 102 to 104 cfu/g. Total yeast and mould counts varied between 102-106 cfu/g and 18 % of samples would not be acceptable, based on TFC level. The counts regarding the yeast and mould ice cream samples suggest the need for adequate heat treatment and appropriate storage conditions. E. coli was found in 15 out of 73 ice cream samples, but interestingly there was no indication for the presence of E. coli O157 : H7. Out of 73 ice cream samples, 9.6 % gave positive result for Yersinia spp. Five (6.8 %) ice cream samples were found to be positive for the presence of Salmonella spp. while, 24 samples yielded positive for S. aureus which is in the range of 102 to 106 and 15 % of the samples do not comply with the TFC standards (104 cfu/g). B. cereus was present in 19 % of ice cream samples. The detection level for B. cereus (+) samples in this work cannot be regarded as potential hazard according to TFC (No : 24511) which is recommended to be lower than 1.0x104 cfu/g. This study has indicated that the hygienic quality of the investigated open ice creams samples were inadequate. Considering the Enterobacteriaceae, coliforms, Enterococcus spp., yeast and mould counts, and the presence of S. aureus, E. coli, Salmonella spp., ice creams sold in open may pose potential risk for public health especially for vulnerable people. This study supports the necessity of providing hygienic precautions by producers and retailers and their control periodically in Turkey.

KEY WORDS : Ice cream, Food Safety, Pathogens, Microbiological Quality, Public Health

RÉSUMÉ

Qualité microbiologique de glaces vendues en vrac dans les épicerie en Turquie

L’objectif visé par cette recherche est l’analyse de la qualité microbiologique d’échantillons de glaces vendues ouvertement (les glaces sont vendues en de petites portions dans des grands récipients exposés en plein air) par les épiceries en Turquie. Les 73 échantillons ont été analysés pour les bactéries pathogènes à Gram positif et à Gram négatif. Les paramètres hygiéniques recherchés ont été : la flore totale aérobie thermophile, les Enterobacteriaceae, les coliformes, les Enterococcus spp., le dénombrement des levures et moisissures. Les pathogènes recherchés sont les Staphylococcus aureus (S. aureus), les Bacillus cereus (B. cereus), les Escherichia coli (B. coli) et les Yersinia spp. Sur les 73 échantillons analysés, 4.1 % de ceux-ci ont dépassé les normes hygiéniques établies par le codex de l’alimentation turc (TFC). 78 % des échantillons contenaient des Enterobacteriaceae. Le pourcentage observé des coliformes ne correspond pas aux normes du Codex de l’Alimentation Turc (95 MPN cfu/ml). Lorsqu’on prend en considération le pourcentage de la dispersion du Enterococcus spp dans les échantillons, 47 % des échantillons de glace avaient les valeurs comprises entre 102 et 104 cfu/g. Le dénombrement des levures et moisissures était compris entre 102 et 104 cfu/g et, selon les normes du Codex de l’Alimentation Turc, 18 % des échantillons ne seraient pas acceptables. Nous avons noté la présence d’E. coli dans les 15 échantillons des 73 analysés mais il n’y avait aucun signe de la présence d’E. coli O157 : H7. 9.6 % des 73 échantillons ont donné un résultat positif pour Yersinia spp. Pour la présence de Salmonella spp, 5 échantillons (6.8 %) de glace ont donné un résultat positif. 24 échantillons ont donné un résultat positif pour la présence de S. aureus ; cette bactérie a été observée à des teneurs allant de 102 à 104 cfu/g et 15 % des échantillons n’étaient pas conformes aux normes (104 cfu/g) du Codex de l’Alimentation Turc (TFC). B. Cereus était présent dans 19 % des échantillons de glace mais a un niveau inférieur aux recommandations sanitaires. Cette recherche a démontré que les qualités hygiéni- ques des échantillons de glaces vendues ouvertement étaient insuffisantes. La présence d’Enterobacteriaceae, de coliformes, d’Enterococcus spp, et de certaines bactéries pathogènes peut représenter un risque potentiel pour la santé publique, surtout pour les enfants et pour les personnes sensibles. Ce travail montre la nécessité d’améliorer les pratiques hygiéniques chez les producteurs en Turquie et d’effectuer des contrôles réguliers.

MOTS CLÉS : glaces (crème glacée), sécurité alimentaire, bactéries pathogènes, qualité microbiologique, santé publique, hygiène.

Introduction

Ice cream is a popular product consumed particularly in summer as well as throughout all year and continues to be a dominant interest of large segments of the population [18]. The ingredients of ice cream may be various combinations of milk, cream, evaporated or condensed milk, dried milk, coloring materials, flavors, fruits, nuts, sweetening agents, eggs and eggs products, and stabilizers. Any of these may contribute microorganisms and affect the quality of the product as judged by its bacterial load or its content of various specific species of bacteria [9,26]. Time-dependent heating during the ice cream making reduces largely the vegetative forms of the microorganisms. On the
other hand, spore bearing microorganisms may well pose risks through consumption of this kind of milk products. Furthermore, the presence of pathogens in ice cream samples is mostly by means of tools and equipments, water, workers, environment, packaging materials and contaminations during the transportation and distribution of ice cream [4,6,22].

The presence of pathogenic microorganisms in ice cream such as *Salmonella* spp. [5,8], *Staphylococcus aureus*, *Escherichia coli* [5,8,12,14,22,25] have been well documented, but examination of pathogenic bacteria like *Bacillus cereus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Brucella* spp. and *E. coli* O157 have been rarely studied. These studies indicated the necessity for a more steady microbial examination of ice cream since ice cream is particularly consumed by children of vulnerable age groups [15,28]. Ice cream samples in this work were therefore examined in terms of microbial counts that allow the quantitative checking of principal hygienic parameters including the total aerobic counts, coliforms, *Enterobacteriaceae*, *Enterococci* spp. and yeast and mould. Additionally, examination of the pathogenic bacteria like *E. coli*, *E. coli* O157: H7, *Yersinia* spp., *Salmonella* spp., *S. aureus*, *B. cereus*, were also performed.

**Materials and methods**

A total of 73 ice cream samples (100 g each) were collected and examined during July and August, 2004 in Kars, Turkey. The samples were transported to the laboratory in a cool box and stored at -20 °C prior to examination. The preparation of the samples was carried out in accordance with the method of WARKE ET AL. [28]. Traditional microbiological methods and media were used for the isolation and enumeration of total aerobic mesophile bacteria, coliforms, *Enterobacteriaceae*, *Enterococci* spp., yeast-mould and the pathogenic bacteria like *E. coli*, *E. coli* O157: H7, *Yersinia* spp., *Salmonella* spp., *S. aureus*, *B. cereus* (Table 1).

The number of *E. coli* was determined by the most probable number (MPN) technique with three tubes using Brilliant Green Bile 2 % Broth (Oxoid, CM31). The presence of *E. coli* in tubes was confirmed by streaking on Endo Agar (Oxoid, CM435). Colonies with characteristic greenish metallic color were confirmed as *E. coli* with the indole, methyl-, catalase, oxidase, urease, motility in SIM (Oxoid CM435) medium, growth on Kligler Iron Agar (KIA) (Oxoid, CM33).

For *E. coli* O157: H7, colourless and sorbitol negative presumptive colonies were streaked on Fluorocult Violet Red Bile agar (Merck 1.04030) and incubated at 42 °C for 24-48 h. Then Gram stain, catalase, and IMVIC tests were performed to the isolates. Those confirmed with biochemical tests were then checked with Dryspot *E. coli* O157 latex agglutination (Oxoid, 200075) test. In parallel, isolates identified as *E. coli* O157 were also tested with antisera O157 and H7 (Oxoid, Denka Seikan 210753 and 211057) as described by the manufacturer.

For *Yersinia* spp., suspected colonies were subjected to Gram stain and further biochemical characterization tests of catalase, oxidase, urease, motility in SIM (Oxoid CM435) medium, growth on Kligler Iron Agar (KIA) (Oxoid, CM33).

**Results and Discussion**

The results of this study are presented in Figure 1 and 2. The aerobic mesophile bacteria (TAB) ranged between 10^2-10^6 cfu/g. Ice cream is considered having unacceptable hygienic quality when the TAB exceeds 10^5 cfu/g according to the Turkish Food Codex (TFC) for ice cream (No : 24511) [2] (Table 2). Only 4.1 % of the samples in the current study exceeded this value. In previous studies, low levels of TAB were reported by SAGDIC ET AL. [22] and Kanbakari et al. [13] in the range of 2.1x10^1-2.6x10^4 cfu/g (Isparta, Turkey) and 1.7x10^1-1.7x10^5 cfu/g (Denzi, Turkey), respectively. On the contrary, unsatisfactory detection rate found for TAB in the present work was lower than the results reported as 33.3 %, 63.3 %, 44 %, and 49.27 % in the cities of Kars, Ankara, Aydn and Antalya of Turkey, respectively. [5,8,16,25]. Unsatisfactory levels of TAB have also been reported in many other countries. In the study of Masud [19] from Pakistan, 72 % of the samples had TAB counts over 10^6 cfu/g. From the city of Bangalore in India, Sarada and Begum [23] found 53 % of the samples had counts of 10^10 cfu/g. MAIFRENI ET AL [17] from Udine in Italy reported that 8 % of the samples had TAB counts exceeding 10^3 cfu/g. WARKE ET AL. [28] from India also reported 33 % of the samples having excessive counts of 10^6 cfu/ml, which did not comply with the Indian standard. The results found in those studies and present work indicate that ice creams sold in small portions from bulk containers, exposed to the open air, have high microbial load, indicating low hygienic quality of the products in many countries. These high counts may originate from the initial microflora of raw milk and the other ingredients and their quality, the environment, insufficient heat treatment and poor personal hygiene. It has been previously stated that production of ice cream locally on a small scale rather than industrially is also a major factor associated with contamination of ice cream [4,8,13].

Seventy-eight percentage of the samples contained the *Enterobacteriaceae* which was a higher rate than faecal coliforms. Incidence of *Enterobacteriaceae* (53 %) in ice cream samples was also reported by EROL ET AL. [8]. *Enterobacteriaceae* in food products indicates faecal contamination. Therefore, microbiological quality of the samples in this study seems to be low. Poor microbiolog-
Coliforms were detected from 52% of the samples in the range of $10^2$ to $10^6$ cfu/g and their numbers were not in accordance with the TFC standard of 95 MPN/ml (Table 2). Many reports dealing with the occurrence of coliforms in ice cream have been accumulated. In those studies, various rates of coliforms, which did not comply with the TFC standard in Turkey, were reported as 13.3%, 40%, 88.4%, 96% and 83% by SAGDIC ET AL. [22], LELOGLU ET AL. [16], TOKLU AND YAYGIN [25], KANBAKAN ET AL. [13], and KIVANC ET AL. [14], respectively. Likewise, in some developing countries, such as Pakistan and India, several rates of coliforms were also reported as 66% and 100% [28], respectively. The presence of coliforms in ice cream samples may indicate insufficient heat treatment, unhygienic or low hygienic materials or tools used, water being contaminated or good manufacturing practice being not followed.

![Table 1](image)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Media Used</th>
<th>Incubation Temperature (°C)</th>
<th>Incubation Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerob mesophile bacteria</td>
<td>Plate Count Agar (Oxoid, CM463)</td>
<td>30°C</td>
<td>2-3</td>
</tr>
<tr>
<td>Enterococci spp.</td>
<td>Kanamycine Aesculin Azide Agar (Oxoid, CM591)</td>
<td>37°C</td>
<td>1-2</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>Violet Red Bile Glucose Agar (Oxoid CM485)</td>
<td>37°C</td>
<td>1-2</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Violet Red Bile Lactose Agar (Oxoid CM107)</td>
<td>37°C</td>
<td>1-2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Brilliant Green Bile 2% Broth (Oxoid, CM31)</td>
<td>37°C</td>
<td>1-2</td>
</tr>
<tr>
<td>ES. coli O157:H7</td>
<td>Modified Novobiocin EC Broth (mEC+n, Merck 1.10765)</td>
<td>42°C</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CT-SMAC (Oxoid CM 813 with SR 172 E)</td>
<td>42°C</td>
<td>1-2</td>
</tr>
<tr>
<td></td>
<td>Flurocult Violet Red Bile (Merck 1.04030)</td>
<td>42°C</td>
<td>1-2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Baird Parker Agar (Oxoid CM275)</td>
<td>37°C</td>
<td>1-2</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>Cereus Selective Agar (Merck 1.05267)</td>
<td>30°C</td>
<td>1-2</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>Buffered Peptone Water (Oxoid CM 509)</td>
<td>37°C</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rappaport Vassiliadis Broth (Merck 1.07700)</td>
<td>43°C</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Salmonella Shigella Agar (Merck 1.07667)</td>
<td>37°C</td>
<td>1-2</td>
</tr>
<tr>
<td>Yersinia spp.</td>
<td>Tryptone Soya Broth (Oxoid CM 129)</td>
<td>4°C</td>
<td>7,14,21</td>
</tr>
<tr>
<td></td>
<td>Cefsuladin Irgasan Novobiocin Agar (Oxoid CM653)</td>
<td>25°C</td>
<td>2</td>
</tr>
<tr>
<td>Yeast-mould</td>
<td>Rose Bengal Chloramphenicol Agar (Oxoid, CM 549)</td>
<td>25°C</td>
<td>4-5</td>
</tr>
</tbody>
</table>

![Figure 1](image)

**Figure 1.** The percentage and viable count ranges of bacteria and the presence of some pathogens in ice cream samples sold openly.

**Figure 2.** The percentage (%) of enteric pathogenic microorganisms in ice cream samples.
**Table 2. The Turkish Food Codex microbiological standards for ice cream**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Max. Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerob mesophile bacteria (cfu/ml)</td>
<td>1.0 x 10^5</td>
</tr>
<tr>
<td>Coliforma</td>
<td>95</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Absent in 25 g</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>Absent in 25 g</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (cfu/ml)</td>
<td>1 X 10^2</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> (cfu/ml)b</td>
<td>1.0 x 10^4</td>
</tr>
<tr>
<td>Mould (cfu/ml)</td>
<td>1.0 x 10^2</td>
</tr>
<tr>
<td>Yeast (cfu/ml)</td>
<td>1.0 x 10^3</td>
</tr>
</tbody>
</table>

*a* Based on MPN Table (/ml), *b* Applies to flavored ice cream.

*E. coli* is an indicator for faecal contamination and the possibility of the presence of enteric pathogens. *E. coli* was found in 15 out of 73 ice cream samples. The count of *E. coli* was found to be higher than 1.1x10^5 MPN/g in all positive samples and was higher than that of TAMSUT AND GARCIA [24] and Erol et al. [8]. However, there was no indication for the presence of *E. coli* O157 : H7 in our study. This may be due to low incidence of *E. coli* in milk and milk products. On the contrary, higher incidence rates than our result were also reported. MASUD [19] found that 46 % of the samples (n=50) were contaminated with *E. coli* and various incidence rates of 3.33 %, 22 %, and 70 % were also reported by EROL ET AL. [8], KIVANC ET AL. [14], and TOKLU & YAYGIN [25] in different cities of Turkey. Considering the Enterobacteriaceae, coliforms and the presence of *E. coli* in samples may indicate a lack of good manufacturing practice during the production and ice cream produced in domestic or catering premises may be relatively important vehicles for the causes of gastrointestinal diseases. These results, therefore, reveals a need for implementing regulatory measures like good manufacturing practices, hygienic distribution and retail storage practices for ensuring microbiological safety of ice cream sold in open containers. Likewise, personal hygiene should be emphasized as KANBAKAN ET AL. [12] stated that coliform contamination on the hands of personnel in sales department was higher than on the hands of factory workers and hand washing with soap only may not be enough for the cleaning of hands.

Of the 73 ice cream samples examined, 9.6 % gave positive result for *Yersinia* spp. The detection rate was extremely lower than the findings of WARKE ET AL. [28], who reported that 100 % of their samples were contaminated with *Yersinia* spp., but higher than the rate of 2.5 % reported by PEDERIVA AND GUZMAN [21]. Experimental studies have shown that the growth of *Yersinia* spp. are temperature-dependent and this is explained by the selective effect of refrigeration temperature which is more favorable for the growth of *Yersinia* spp. than other pathogens like *E. coli*, *S. Typhimurium*, *S. aureus* [1]. The low incidence of *Yersinia enterocolitica* in this study, in comparison with other pathogens, may presumably be due to the inefficient storage conditions which may favor the overgrowth of other microorganisms including pathogens. Transmission of this psychotrophic bacterium via food chain has not been reported [28] but PEDERIVA AND GUZMAN [21] stated that ice cream may constitute a possible transmission path for *Y. enterocolitica*, and might lead to gastroenteritis outbreaks among children consuming great amounts of ice cream, particularly during the hot season. Therefore, contaminated ice cream samples may be a risky type of food for public health in terms of carrying the *Yersinia* spp.

As shown in Figure 2, five (6.8 %) samples were found to be positive for the presence of *Salmonella* spp. The detection rate for *Salmonella* spp. was not in agreement with the results of MAIFRENI ET AL. [17], KIVANC ET AL. [14], WARKE AL [28], ASLANTAS [3], and KANBAKAN ET AL. [13] who reported no *Salmonella* spp. in their ice cream samples. However, comparing to our results, lower frequency of *Salmonella* spp. contamination has been also reported in Venezuela and Camerun, ranging from 1 % to 5 % [24,30], respectively. Likewise, detection rate of *Salmonella* spp. in our study was significantly lower than the rate of 53 % reported by DIGRAK AND OZCELİK [5]. The presence of *Salmonella* spp. in ice cream may possibly be due to the either fresh eggs or egg powder used in the ice cream production as being stated in previous works. The presence of *Salmonella* spp. may pose a great risk for public health since *Salmonella* outbreaks from ice cream have been reported previously [10, 20,27].

Considering the percentage distribution of *Enterococcus* spp. counts in the samples, 47 % were within the range of 10^2 and 10^3 cfu/g, with the most being detected at 10^2 cfu/g. In previous works, the counts of 1<1.0x10^3 cfu/g in some (89-94 %) ice cream samples were reported [17]. In a recent work, EROL ET AL. [8] found *Enterococcus* spp. counts ranging from 10^2 to 10^3 cfu/g, with the most being detected at 10^2 cfu/g in Ankara, Turkey. Their incidence frequency of 87 % for *Enterococcus* spp. were comparatively higher than our result. The count for *Enterococcus* spp. is being considered as one of the parameters used for food hygiene quality. Low hygienic quality of the samples regarding *Enterococcus* spp. occurrence in the previous and current work may have been resulted from insufficient heat treatment exposed to milk during the production or from post contamination.

In the present study, 24 samples yielded positive for *S. aureus* ranging from 10^2 to 10^3 cfu/g and 15 % of the samples do not comply with the TFC standard of 10^2 cfu/g. The absence of *S. aureus* in ice cream samples has been rarely reported in several studies [4,17,22,29]. On the other hand, lower or higher incidence rates of *S. aureus* than our result in this study have been reported. In Ankara and Elazig, Turkey, *S. aureus* was present in 10 % of the open ice cream samples at the counts of 10^2 cfu/g. In Aynid, Turkey, LELOGLU ET AL. [16] found this rate as 20 % at the counts above the safe limit level of 10^2 cfu/ml. Higher detection rates of 62.3 % and 100 %, at the counts above the safe limit level were also reported by KIVANC ET AL. [14] and WARKE ET AL. [28], respectively. The presence of *S. aureus* may be resulted from either insufficient pasteurization of milk, or human exposure. In humans, the main reservoir of *S. aureus* is the

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