The microbial load in traditional ice-creams collected from across the suburbs of Shiraz

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Abstract

Introduction:
Given their ingredients and a close-to-neutral pH, ice-creams provide a favorable environment for the proliferation of microorganisms that cause food poisoning and infections and present health risks. The present study was conducted to investigate microbial load and contamination with listeria monocytogenes and Escherichia coli in traditional ice-creams sold across the suburbs of Shiraz, Iran.

Methods and Materials:
A total of 240 ice-cream samples were randomly collected from the distribution centers located across the suburbs of Shiraz in spring and summer 2013 and were tested under standard conditions for total bacterial and Enterobacteriaceae count and form so as to determine their microbial load and potential contamination with listeria monocytogenes and E. coli.

Results:
The results obtained showed that 89 samples (37.08%) failed to meet the permissible limits of total bacteria determined by the Institute of Standards and Industrial Research of Iran. Moreover, 39 samples (16.25%) exceeded the permissible limits of Enterobacteriaceae. No significant differences were observed between the samples procured during the spring (5%) and those procured during the summer (6.25%) in terms of contamination with E. coli (P≤0.05). None of the samples were contaminated with listeria monocytogenes.

Conclusions:
The results obtained suggest a high level of contamination. A more extensive health monitoring and control of ice-cream production, distribution and supply centers and ensuring the use of pasteurized milk are essential for improving the quality of this food product.

Keywords: Microbial, Traditional, Ice-Cream, Listeria, E.Coli

Introduction
Ice cream is a complex mix of ingredients such as milk, sugar, non-fat solids and water. This dairy product is one of the main alternatives to milk in many diets and is always increasing in consumption, especially among children (1-3). Given its 12% fat, 11% non-fat milk, 15% sugar/sweetener content, pasteurizing the resulting mixture at a proper temperature so as to remove its pathogenic microorganisms is essential; however, pasteurization is not part of the making of traditional Persian ice-cream due to the lack of facilities in ice-cream shops (1 and
Ice-cream is likely to contain pathogenic bacteria, particularly Listeria monocytogenes, Escherichia coli and Staphylococcus aureus, if it contains contaminated milk that is not fully disinfected or if health codes are not observed in the stages of its production. Moreover, using contaminated equipment and failing to safely store the produced ice-cream can lead to the growth of micro-organisms in them (4&5). Studies conducted in Iran show that the main bacteria causing food poisoning in this country include Escherichia coli, Salmonella, Listeria monocytogenes and Staphylococcus aureus, which are transferred to humans through the consumption of milk and dairy products, thereby leading to zoonotic diseases with a high rate of mortality (6-8).

Listeria monocytogenes is a globally widespread pathogen that reproduces slowly at refrigerator temperature. This species of bacteria has been known for years as a foodborne pathogen that causes epidemic and sporadic cases (9). In 1983, a listeriosis outbreak occurred in Massachusetts that was caused by pasteurized milk. In another outbreak in California, Mexican cheese contaminated with Listeria monocytogenes contaminated a lot of people. Almost 30% of the infected died in both cases (10). Given the importance of the subject and the existence of shops and parlors producing and selling this type of ice-cream in the surrounding areas of Shiraz in Iran, the present study was conducted to examine the microbial burden and contamination of traditional ice-creams sold in the areas outside Shiraz with Listeria monocytogenes and Escherichia coli.

**Materials and Methods**

The present analytical cross-sectional study was conducted in the spring and summer of 2013 in Shiraz, Iran. In line with the number of parlors and shops producing and selling this type of ice-cream, 240 traditional ice-cream samples were randomly collected from the suburbs and surrounding areas of Shiraz and were then sent to the Food Lab of Islamic Azad University, Kazeroun Branch (11). Once the ice-creams were melted and diluted through serial dilution, the bacteria belonging to the Enterobacteriaceae family were prepared using the MPN method (12) and the testing for coliform bacteria was carried out using the standard reference method of 1-5486, Iran (13). From the 1-10, 2-10 and 3-10 dilutions, 1 mL was cultured through the mixed culture method and in double layers in select Violet Red Bile agar plates and was incubated for 24 hours at 37°C. The colonies suspected of coliform, i.e. the purple or red colonies with a diameter of 0.5-2 mm, were counted and the potential or hypothetical number of coliforms per mL of the ice-creams was calculated based on the following equation (13 & 15).

\[
\text{Dilution factor reverse } \times \text{Colony count} = \text{Potential or hypothetical number of coliforms per mL}
\]

In the next step, to confirm contamination with coliform bacteria, five of the suspected colonies were cultured in five tubes containing Brilliant Green Bile Lactose Broth with Durham tube and were then placed under 37°C for 24-48 hours. Contamination with coliform was confirmed if gas accumulation occurred in the Durham tube as a result of lactose fermentation (13 & 16). To detect Escherichia coli contamination, the positive gas tubes yielded in the previous step were cultured in Pepton, Methyl Red - Voges Proskauer and Simmon Citrate, and if the indole- methyl red test came out positive and the Voges Proskauer and Simmon Citrate tests came out negative, Escherichia coli contamination was confirmed. To detect Listeria monocytogenes, 25 g of the ice-cream samples were cultured in 225 mL of listeria enrichment broth. Two cultures were prepared for each sample; one of the tubes was kept incubated at 35±2°C for 48 hours and the other was refrigerated at 4°C for 15 days. The
content of the tubes was then cultured in Palcam agar base (procured from Danesh Azma Co.) as a select solid culture medium for Listeria using the linear culture technique and was then incubated at 35°C for 48 hours. The black colonies with a protruding center and a transparent halo were selected as suspects and a pure culture was prepared in TSA plates. The catalase test and the Sulfur Indole Motility test (SIM media) were then performed (15 & 17). The suspected colonies were then gram stained and studied after examining the morphology of the bacteria. Salicin sugar fermentation, rhamnose, arabinose, maltose, mannitol, xylose and nitrate tests were then carried out. In the final step, to finalize the detection, the CAMP test was performed with S. aureus in a blood agar plate. The data obtained were analyzed in SPSS-17 using the t-test and at a significance level of P<0.05. In this study, the researchers used the same methods as Rezaei et al. in 2011 (18).

Results:
A total of 89 (37.08%) of the ice-cream samples tested exceeded the maximum permissible limits of the Iranian National Standards Organization (INSO) in terms of coli bacteria count and 39 (16.25%) in terms of enterobacteriaceae count. The mean overall rate of enterobacteriaceae contamination was estimated as 104×1.7 cfu/g, while 62.29% exceeded this limit in the spring and 37.70% in the summer. The mean count of coli bacteria was 106×3.9 and the mean count of enterobacteriaceae 104×1.7 per gram in the traditional ice-cream samples tested. The comparison of the mean enterobacteriaceae counts using the t-test revealed a significant difference of 102 per gram between the enterobacteriaceae counts in traditional ice-creams sold in the surrounding areas of Shiraz and the standard permissible limits (P≤0.05). E. coli contamination was 5% in the spring and 6.25% in the summer. No statistically significant differences were observed between the seasons examined (P≤0.05; Table 1).

As for listeria contamination in the traditional ice-creams sold in the surrounding areas of Shiraz, no contamination with Listeria monocytogenes was observed in any of the samples in either of the two seasons examined; however, seven samples (2.91%) were contaminated with other species of listeria. The results of complementary biochemical tests performed on these seven suspects showed that they were consistent with listeriosis (Table 2).

Table 1: Bacterial contamination in traditional ice-cream samples sold in the surrounding areas of Shiraz

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample Count</th>
<th>Maximum Permissible Limits of the INSO</th>
<th>Mean (cfu/g)</th>
<th>Standard Deviation</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae Count</td>
<td>240</td>
<td>10^2×1</td>
<td>10^6×1.7*</td>
<td>10^3×4.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Coliform Count</td>
<td>240</td>
<td>10^5 ×5</td>
<td>10^5×3.9*</td>
<td>10^5×5.6</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*The asterisk shows significant differences with the INSO at the P<0.05 level.

Discussion
The results of the present study showed that 37.08% of the ice-creams tested were not safe in terms of their coli bacteria count. Studies conducted in other countries where hygiene is not strictly observed have yielded similar results with respect to the contamination and unsafety of traditional ice-creams. For example, in Turkey, 55% of the ice-creams sold are unsafe; in Pakistan, this rate was reported as high as 72% (19 & 20). In a study conducted in 1997 in Penompen, Vietnam, 83.3% of traditional ice-creams sold in the city were contaminated (21). In European...
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and north American countries, all the stages of the production and distribution of traditional ice-creams contain a certain risk of bacterial contamination (22 & 23). It is presumed that traditional ice-creams are equally unsafe in all regions of the world; however, their causes of contamination are different. It can be concluded that, in addition to contaminated milk, other substances such as sugar and vanilla are among the main causes of contamination and unsafety in traditional ice-creams sold around Shiraz. Studies conducted in other countries have identified the contamination of raw substances and the poor compliance with hygiene as the main factors contributing to the outbreak of foodborne diseases (24 & 25). The storage of salep, sugar and vanilla in open containers and using unwashed hands instead of sterile cups can also result in contamination (26 & 27).
The findings also revealed 11.25% of the traditional ice-cream samples to be contaminated with E. coli. In a study conducted in 2002 in Yasuj, Iran, Pourmohammadi et al. reported 17% of the traditional ice-creams sold across this city to be contaminated with E. coli (28). In a study conducted in 2000 in Sangal on 313 samples of ice-cream sold on the street, Idari et al. reported 45% of the samples to lack a favorable quality in terms of bacterial contamination, 214 to exceed the maximum permissible limits of coliform bacteria and 10.6% to be contaminated with E. coli (29). In another study conducted in 1996 in Cameroon, 71.3% of the country’s ice-creams were contaminated with coliform bacteria, caused by the use of undrinkable water and poor hygiene (30). The contamination of this food product with E. coli can be attributed to the use of contaminated water.
Listeria bacteria are widely present around humans in soil, feces, stagnant waters and river water and foods from plants and animals. These bacteria can easily contaminate food products, including milk. Given the bacteria’s ability to grow in 4ºC and since the main ingredient of ice-creams is milk, the growth of these bacteria in ice-cream is not unexpected (31-32).

None of the samples examined in the present study were contaminated with Listeria monocytogenes, although seven were contaminated with listeriosis. In 2004, Denz et al. conducted a study in Adana, Turkey, and could not find any Listeria monocytogenes to isolate from the ice-cream samples sold across the city; however, 37.9% of the samples they tested were contaminated with listeriosis and 1.7% with Listeria innocua and Listeria welshimeri (33). This finding is consistent with the results of the present study. Overall, the present findings are indicative of the low health standards and the high bacterial contamination of ice-creams produced and sold in the surrounding areas of Shiraz.

Conclusion
Given the significant role of food health and safety in establishing food security and since ice-creams are among the most consumed food products among children and adolescents, the proper health monitoring and control of the parlors and shops that produce, distribute and sell traditional ice-creams and ensuring their use of pasteurized milk for preparing this food product appear essential.

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Conflicts of Interest
The author has no conflicts of interest to declare.
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