# Microbial Contamination of Traditional Ice Creams in Rafsanjan, Iran

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**ABSTRACT:** Ice cream is a frozen dairy product that is made of suitable milk, sugar and flavoring agents. It easily contaminated and causes food poisoning. Since it harbors many bacterial pathogens, its microbial quality has always been crucially important to public health. The aim of this study, conducted to screen some selected national and international brands of ice cream include *Staphylococcus aureus* and *Escherichia coli* in Rafsanjan city, Iran. All producing and distributing of traditional ice creams were screened. The samples were collected in hygienic conditions. 40 samples of traditional ice creams were obtained randomly from the retail stores. All the samples were analyzed for microbial contaminations such as *Escherichia coli* and *Staphylococcus aureus* according to the Iran national standard No. 2406. The results showed that 30 Samples (75%) with Enterobacteriaceae, 6 cases (15%) with *Escherichia coli* and 2 cases (5%) with *Staphylococcus aureus* were contaminated and thus contamination of traditional ice creams was notable. However, pasteurization of ice-cream raw materials with sufficient heat, regarding health principles in several stages of producing and preparing health materials and controlling ice cream producing and distributing centers are necessary to decrease contamination.

**Keywords:** Microbial contamination; *Escherichia coli*; *Staphylococcus aureus*; Traditional ice cream.

## 1. INTRODUCTION

Production of non-pasteurized traditional ice cream that is made by conventional methods, have a long history in Iran and its consumption because of the sweet taste and cool in the summer is very common especially among children [1]. Ice cream has a high nutritional value. Its ingredients include milk, cream, non-fat solids, water, sweeteners, food emulsifiers, flavoring and color and thickener [2]. In addition to providing a large portion of nutritional needs, ice cream neutralizes the free radicals, prevent cancer and increase various microorganisms [3]. Primary contamination sources of ice cream include raw milk, water and secondary sources; include equipment, flavoring combinations and other additives. Production in open and contaminated environments can be a source of microbial pollution in the product [2]. Since there are no pasteurization facilities for manufacturers of traditional ice cream, microbial contamination after production is likely. On the other hand, its manual sales and inappropriate storage conditions can also increase the problem of microbial

contamination. Microbial contamination ice cream produced by industry methods, because of the pasteurization of milk and the final product production technology, is low, and the risk of entering contaminated factors to this type of ice cream mostly arising from additives, transportation resources, hands, pollution of devices, containers and environment, packaging and lack of sanitation by workers after pasteurization. However, in traditional ice cream, due to lack of pasteurization consumables and poor hygiene during the various stages of the production and maintenance, contamination is more likely and higher bacteriological evaluation becomes more important [4]. Despite the sanitary control in the food industry, there are still many cases of food poisoning that is one of the main problems even in more advanced societies. The increase in these diseases is considered very worrying [5]. According to the World Health Organization reports, about 1.5 billion diarrhea occurs in children less than 5 years, of which about 5 million cases lead to death. Contaminated food including milk and its products has an important role in this regard. In addition, in various investigations, isolation of pathogenic bacteria, including *Escherichia coli, Streptococcus, Micrococcus, Staphylococcus aureus, Salmonella*, *Yersinia enterocolitica* and *Listeria monocytogenes* has been reported in the ice cream Poiso [6]. Food poisoning and infections caused by *E. coli* are the most common types of diseases in developing countries and has been allocated 25 % of all cases of diarrhea. Poisoning caused by consumption of food contaminated with *E. coli* have been as acute gastroenteritis and is associated with clinical signs of diarrhea, sometimes fever and lethargy. *Staphylococcus aureus* is the main cause of staphylococcal food poisoning and wounds like infections, abscesses, pneumonia, meningitis, bacteremia, and toxic shock syndrome .The presence of Staphylococcus bacteria in milk products is an important significance in relation to consumer health because of the fact that some strains are highly pathogenic producing enterotoxins, which lead to food poisoning [7]. Since the most causes of ice cream contamination with two bacteria of *Escherichia coli* and *Staphylococcus aureus*, the same bacteria studied in this study.

## 2. MATERIALS AND METHODS

This study was a descriptive cross-sectional sampling, which was  performed over a period of one year in 2015 from all of the traditional ice cream production and sales centers in Rafsanjan, Iran. 40 samples of traditional ice cream, randomly in sterile containers and with the listed specifications on the container alongside ice packs, were transferred to the food control laboratory in the shortest possible time. Bacterial examination including evaluation of bacteria in traditional ice cream, including total count of bacteria, Enterobacteriaceae, *E. coli* and *Staphylococcus aureus* was carried out. The samples were serially diluted using quarter strength Ringer's solution and cultivated using pour plate method in Plate Count Skim Milk Agar (Merck, Company) [8]. To diagnose *Escherichia coli*, the samples were cultured in Brilliant double green broth (Merck, Company) medium. The other biochemical and diagnostic tests were done using Brilliant Green Bile Broth, Triple sugar iron agar, SIM and Simon citrate agar (Merck, Company). To identify *Staphylococcus aureus*, the samples were cultivated on double Giolitti-Cantoni-broth medium, Baird Parker agar (Merck, Company) and confirmation test such as coagulase test using rabbit plasma was used [8]. The all culture media incubated at 37 °C for 48 hours. Finally, the results compared with 2406 standard limit of milk [9].
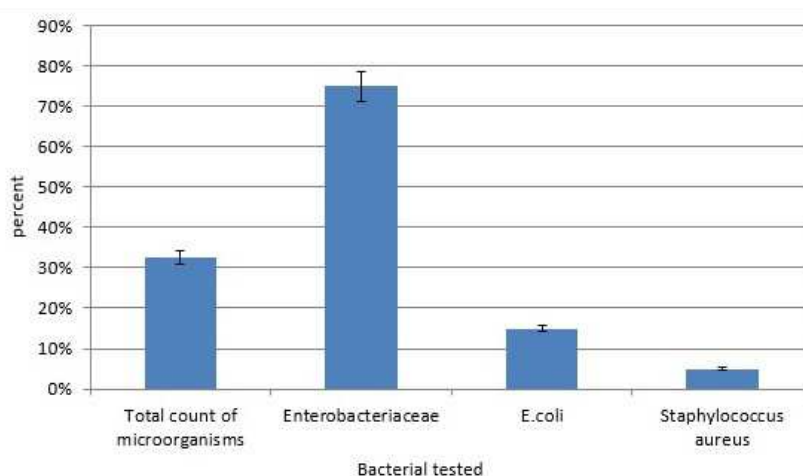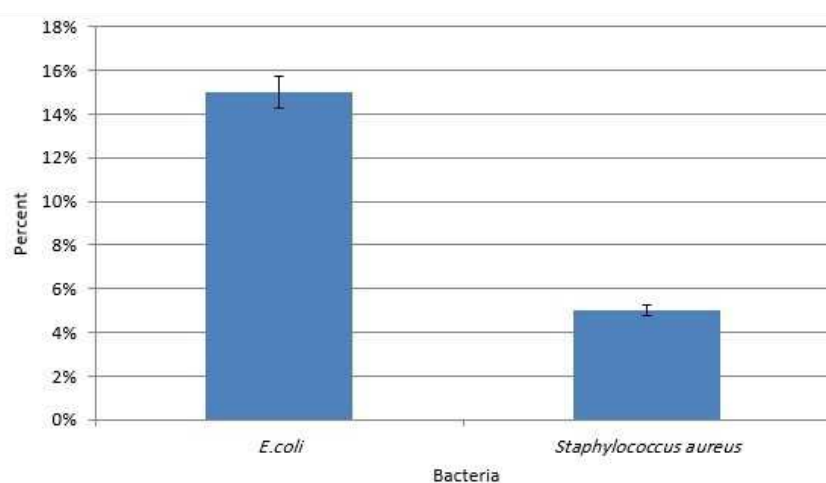
## 3. RESULTS

The quality of ice cream to total Enterobacteriaceae , *Escherichia coli* and *Staphylococcus aureus* were evaluated based on  National Standard, 2008 (Table 1). Gram-negative rod bacteria with negative oxidase test, Non-fermented lactose colonies, positive motility, Simon citrate negative were considered as *Escherichia coli*. Gram-positive cocci growing in Giolitti-Cantoni-broth medium and with dark colonies in Baird Parker agar were identified as *Staphylococcus aureus*.

**Table 1.** Microbial standard levels of ice creams.

| Type of microorganisms | Limit | Standard number |
|---|---|---|
| Total count of microorganisms | Maximum $5 \times 10^4$ | 5484 |
| Enterobacteriaceae | Maximum 10 | 2461-1,2 |
| *E. coli* | Negative | 5234 |
| *Staphylococcus aureus* | Negative | 6806-3 |
| *Salmonella* in 25 g | Negative | 10154 |
| Mold and yeast | $10^2$ | 4413 |

**Table 2.** The frequency contamination of ice cream according to season.

| Bacteria | Spring | | Summer | | Winter | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Number | Percent | Number | Percent | Number | Percent | Number | Percent |
| Mesophilic bacteria | 0 | 0 | 13 | 32.5 | 0 | 0 | 13 | 32.5 |
| Enterobacteriaceae | 8 | 20 | 15 | 37.5 | 7 | 17.5 | 30 | 75 |
| *E. coli* | 1 | 2.5 | 5 | 12.5 | 0 | 0 | 6 | 15 |
| *S. aureus* | 0 | 0 | 2 | 5 | 0 | 0 | 2 | 5 |



**Figure 1.** The rate of traditional ice cream contamination to bacterial tested.



**Figure 2.** The rate of traditional ice cream contamination to *S. aureus* and *E. coli.*

Based on obtained results, 30 samples (75%) showed Enterobacteriaceae, 6 samples (15%) *Escherichia coli* and 2 samples (5%) *Staphylococcus aureus* that were out of the standard quality. Most of contaminations have been shown in summer, which out of 75%, 37.5% were of Enterobacteriaceae in the summer and *E. coli* out of 15 percent, 12.5 percent were higher than limit in summer, and *Staphylococcus aureus* were out of 5 percent in the summer. The results of microbiological analysis have mentioned in Table 2, Fig. 1 and Fig. 2.

## 4. DISCUSSION

Ice cream as a nutrient is a fertile environment for the proliferation of microorganisms. The findings of this study and similar studies in Iran and abroad, confirm increasing the risk of microbial contamination in traditional ice cream. The present results showed that traditional ice cream in Rafsanjan, in some cases due to lack of sanitation, have inadequate hygiene levels. Because out of 40 samples of traditional ice cream, 13 samples (32.5%) of total count of microorganisms were above the limit and also 30 samples (75%) Enterobacteriaceae and 6 samples (15%) *Escherichia coli* and 2 samples (5%) of *Staphylococcus aureus* were out of permitted limit. Based on the results, the highest rate of contamination was related to Enterobacteriaceae, which is consistent with the studies in Iran and abroad. Hassanzadazar et al. showed 82.9% contamination with Enterobacteriaceae [10]. Salehian et al. mentioned 70% of Enterobacteriaceae [11]. Navidjoy et al. found that Enterobacteriaceae 92.5% was exceeded, the normal level [12]. In a study conducted Ekhtelat and colleagues in Ahvaz, Iran, 78.33% of samples were contaminated with Enterobacteriaceae [13]. In another studies, Enterobacteriaceae infection was 78.8% and 100% [14, 15]. Bazrafshan showed 70% of samples contaminated with Enterobacteriaceae [16]. Yaman reported 78% contamination with Enterobacteriaceae [17]. In terms of contamination with *E. coli*, 15% of the samples had levels higher than allowed, which in some cases are consistent with the results of other researchers, but also many others have obtained different results. During the review by Anuranjini in southern India, 19% infections revealed [14]. Nelshre reported 6% pollution [18]. Another researcher reported 10.6% pollution in Dakar [19]. Ekhtelat and colleagues in Ahvaz showed 10.83 % pollution [13]. In a study by Anvarinejad and his colleagues in Maragheh, 21.4% of the pollution was reported [14]. Rezaei in Arak confirmed 25% contamination with *E. coli* [20]. Salehian et al. showed contamination of 52% [11]. Hassanzadazar reported that 52.2% of samples were contaminated with *Escherichia coli* in Urmia [10]. *E. coli* is an indicator of fecal contamination, and high contamination to this bacterium can indicate a lack of proper washing of hands and avoiding the use of gloves in the supply and distribution centers [2]. Anuranjini showed 4% contamination with *Staphylococcus aureus* [21]. Joshi reported 2% contamination [22]. Hassanzadazar reported 2.8% pollution in Urmia [10]. Haeri Behbahani reported 4% pollution in Tehran [15] and higher rates have been reported in some studies. Ekhtelat in Arak 67.31 % [13], Navidjoy 45% in Urmia [12], Salehian in Sari, 28% [11], Anvarinejad in Maragheh 41.8 [14], Elsharef in Libya showed 38% of pollution [23]. *Staphylococcus aureus* is transferred through the polluted milk or through the hands, mouth and nose when preparing ice cream, so, observing personal hygiene standards and pasteurization of milk can have an effective role in the prevention of these products contamination. The possible source of staphylococcal entry into the dairy product may be unclean equipment, polluted water, unhygienic storage conditions, nose or septic lesions of persons involved. Thus, given the importance of traditional ice creams in the transmission of major bacteria causing infections and food poisoning to humans, in order to raise the quality of traditional ice cream and reduce tick-borne diseases the following are recommended: correct pasteurization of milk and ice cream mixture, checking proper pasteurization of milk,  provide health education to inform people, especially those who deal directly with the food, encourage the public to use pasteurized ice cream via mass media,  rigorous health monitoring by competent authorities on the work of traditional ice cream manufacturers and sellers,  use of healthy and pasteurized milk in making ice cream and create the right conditions for transport and storage of milk, bacterial contamination in industrial ice cream must also be examined, compared to traditional ice cream [16, 24].

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