



## Determination of Microbiological Quality of Ice Cream Sold in Istanbul and Their Evaluation in Terms of Public Health

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### ABSTRACT

**BACKGROUND:** Food safety has been defined by the World Health Organization and the Food Agriculture Organization as the application of rules and measures to ensure healthy food production during the production, processing, storage, transportation and distribution of food. Ice cream, which is a type of dessert consumed with many different kinds especially in summer. It is rich in nutrients, thus creating a favorable environment for the reproduction of microorganisms. Considering the hygiene rules applied during the COVID-19 process, it is aimed to evaluate the ice creams sold in the open in terms of food safety.

**METHODS:** In the present study, 75 ice cream samples flavored with plain, fruit, and nuts were

collected aseptically from 25 enterprises in Istanbul, Türkiye. Microbiological quality of ice cream samples were evaluated in terms of public health risks which may occur during the production and storage of ice creams. For these goals, the presence and enumeration of Total Mesophilic Aerobic (TMA) bacteria, Enterobacteriaceae, Staphylococcus aureus and Salmonella spp. were investigated. The results were assessed according to the Turkish Food Codex on Microbiological Criteria Regulation.

**RESULTS:** About a third of all ice cream samples (32%; n=8) from 25 enterprises were determined in accordance with the Turkish Food Codex on Microbiological Criteria Regulation in terms of hygiene and consumability. The TMA bacteria level was determined %88 (n=22) in plain ice creams, %64 (n=16) in fruit ice creams and %92 (n=23) in nuts ice creams. Enterobacteriaceae were detected at the level of 40% (n=10) in plain ice creams, 12% (n=3) in fruits and 40% (n=10) in nuts. Multiple antibiotic resistance was found for S. aureus in only one enterprises's nuts flavored ice cream. Salmonella spp. was not found in any of 75 ice cream samples.

**CONCLUSION:** Ice creams sold in the open was seen that the microbiological quality of less than half of the enterprises was not at the desired level. Due to the probably production, transportation and sales conditions were unhygienic which this flavour dessert may be a potential danger to public health.

**KEYWORDS:** *Ice cream, microbiological quality, hygiene quality, dairy product, food safety.*

## INTRODUCTION

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have defined food safety as “the application of rules and measures to ensure healthy food production during the production, processing, storage, transportation and distribution of food” (1). Disruptions that may occur in any of these stages may put the safety of food at risk (2). In the Regulation on the Inspection and Control of Food Safety and Quality in Türkiye, the food safety is the whole of the measures taken to eliminate physical, chemical, biological and all kinds of damages that may occur in foods (3). There are four basic principles of food safety which are to prevent the contamination of food with harmful and undesirable factors, to remove these factors (elimination), to stop the proliferation and spread of pests (inhibition) and to neutralize them with appropriate methods (4). It is very important to implement the legislation developed for food safety and security in Türkiye. It turns out that important problems can be encountered in access to nutritious foods, especially if the necessary precautions are not taken in the areas where problems are detected (3).

Foodborne diseases are a widespread and growing public health problem in developed and developing countries (5). It is stated that millions of people in the world suffer from food-borne diseases every year, and some of these diseases even result in death. They occur when any food or beverage containing harmful bacteria, parasites, viruses or chemicals is consumed, and it is reported that about 20% of them are due to inadequate hygiene (6). Within the COVID-19 pandemic whose effect still continues for the last few years has turned into one of the health problems where hygiene is the most important. This disease was declared as a global epidemic that emerged in Wuhan, China and spread

rapidly to the whole country and then to the whole world (7). Covid -19 has resulted in approximately 28 million cases in more than 218 countries. It has been reported that death rates are more than 900 000, resulting in quarantine of one third of the world's population. It is known that the COVID-19 disease, which still continues to spread, is caused by the SARS-CoV-2 virus (8). While it is known that the transmission routes of this virus are transmitted through respiratory droplets and human-to-human contact, it has been suggested that it can also be transmitted to food surfaces. WHO and the Center for Disease Control and Prevention (CDC) stated that there is no evidence that the disease is directly transmitted through food and water, but they stated that the virus may spread depending on the consumption of food (9). Following this widespread disease, ensuring hygienic conditions in our country has been prioritized. Although there is no evidence that the coronavirus is transmitted by food, the WHO has made several statements that more attention should be paid to food safety practices than ever before to reduce the risk (10). Washing hands regularly, having adequate facilities for disinfection, and storage conditions of foods are among the important points to be considered (11). It is expected that the hygiene awareness that has developed with this epidemic disease will reduce the contamination that occurs especially in various food groups.

Dairy products play an essential role in the transmission of many diseases in countries where hygienic standards are not implemented insufficiently. Contaminated milk and products made from it may contain many microorganisms that are involved in the formation of many foodborne diseases (12). Ice cream, which is one of the dairy products consumed dessert with many various types in large quantities in Türkiye. The fact that it has many different species and is rich in nutrients provides a favorable environment for the reproduction of microorganisms. The cooling process applied during the production of ice cream prevents the

reproduction of microorganisms and positively affects the structure of the ice cream (13). However, it should be evaluated considering the risky situations that may occur during the storage or distribution of ice cream. Although the number of Total Mesophilic Aerobic (TMA) bacteria was included as a general criterion to assess the risks that may occur during production and storage, there is no certain limits in the Turkish Food Codex on Microbiological Criteria Regulations (14, 15). At the same time, it is important to determine the level of *Staphylococcus aureus* (*S. aureus*) that is a food safety and public health problem in ice creams (2). Approximately, one in three healthy people *S. aureus* in their nose was found, and it often contaminate the hands, fingers and face (16). This contamination is an important source of cross contamination in food and beverage businesses. According to Turkish Food Codex on Microbiological Criteria Regulation, *S. aureus* being more than  $10^3$  cfu/g puts the food at risk, while more than  $10^6$  cfu/g can increase the human intoxication cases (15). In the Turkish Food Codex Microbiological Criteria Regulation dated 2009, limits are specified only for *E.coli* and *Salmonella* from the *Enterobacteriaceae* family (15). In another Codex dated 2011, limits are specified for *Enterobacteriaceae* and *Salmonella* (14). It is stated in the Turkish Food Codex on Microbiological Criteria Regulation that *Enterobacteriaceae*, which informs us about the hygienic quality of food, should not exceed  $10^2$  cfu/g (14).

In addition, according to the rules determined by the Turkish Food Codex, *Salmonella* bacteria must not be present in ice cream sample due to these dangerous results on human health (17). The gastrointestinal system of humans, animals, birds and rodents are the source of *Salmonella spp.* which are very common in the environment. Symptoms such as diarrhea, fever and abdominal cramps can be observed within a few hours following the consumption of foods containing *Salmonella spp.* (18).

## MATERIALS AND METHODS

This study was conducted in accordance with the guidelines in the Helsinki Declaration, and was approved by the Non-Interventional Ethical Committee at Bezmialem Vakif University (No: 2021/114). Plain, fruit and nut flavored ice creams, which are sold unpackaged in places such as patisseries, buffets, ice cream shops and cafeterias in Istanbul, are purchased as they are sold to the consumer. A total of 75 samples, 25 of each flavor, were obtained. Microbiological analyzes were performed in the Food Microbiology Laboratory of the Department of Nutrition and Dietetics, Faculty of Health Sciences, Bezmialem Vakif University.

Ten grams of the samples were weighed and placed with 90 ml of sterile peptone water (Conda, Spain) in a sterile stomacher bag for the homogenization in Stomacher (VWR, Italy). From the homogeneous sample, dilution series up to  $10^{-5}$  were prepared and inoculated into the peptone water (0.1%) (Merck, Türkiye). Seeding from dilutions for TMA bacterial presence and count was done using Plate Count Agar (PCA) (Diatek, Türkiye). It was incubated at  $37^{\circ}\text{C}$  for 48 hours. The presence and enumeration for *Enterobacteriaceae* were carried out by MacConkey agar (Merck, Türkiye) for 24-48 hours at  $37^{\circ}\text{C}$ . Baird Parker (BP) Agar (Diatek, Türkiye) was used for the identification and enumeration of *S. aureus*. 0.1 ml of sample placed in the medium from dilutions was incubated at  $37^{\circ}\text{C}$  for 48 hours. *S. aureus* identification and confirmation after the colony morphology was identified on BP agar, gram staining, catalase and coagulase testes were performed. Coagulase positive colonies were tested for antimicrobial susceptibility. Antibiotic susceptibility of *S. aureus* isolates was performed by disc diffusion method in accordance with the National Clinical Laboratory Standards Committee Guidelines (19). For this goal, Mueller-Hinton agar (Oxoid, England) was prepared and ampicillin (10 mcg), amoxicillin/clavulanic acid (30 mcg), gentamicin (30 mcg), tetracycline (30 mcg), methicillin (5 mcg), vancomycin (30 mcg),

pencillin G (10 mcg), and erythromycin (15 mcg) antibiotics (Bioanalyse, Türkiye) were marked at 3 cm intervals (20-23). Identification of *Salmonella spp.*, pre-enrichment was done with 0.1% peptone water. It was incubated at 37°C for 16-20 hours. Selective enrichment in Rappaport Vassiliadis media (Oxoid, England) of the pre-enriched samples was carried out at 42°C for 24 hours. From selective enrichment samples 0.1 ml were inoculated into XLD agar plates (Diatek, Türkiye) which were incubated at 42°C for 24 hours. After the planned incubation duration, the petri dishes with 25-250 bacterial colony growth were counted using the colony counter (Interscience, France) and the colony forming unit (cfu) per 1 gram of each ice cream sample was calculated. The obtained data was evaluated in terms of food safety in accordance with the Turkish Food Codex Microbiological Criteria Regulation (14,15).

## RESULTS AND DISCUSSION

TMA bacteria, *S. aureus*, *Enterobacteriaceae*, and *Salmonella spp.* were determined and counted as a result of the of plain, fruit and nut flavored ice creams taken from 25 enterprises. In order to measure the microbiological and hygiene qualities of food products and the production process, it has emerged in all

businesses where we have analyzed the TMA bacteria for the control of the cleanliness of the raw materials and the suitability of the storage temperature (24). According to our results, the TMA bacteria level was determined as  $6.8 \times 10^4$  cfu/g in plain ice creams,  $3.8 \times 10^4$  cfu/g in fruit ice creams and  $5.1 \times 10^4$  cfu/g in ice creams with nuts. In a study conducted in Kars, they found the number of TMA bacteria to be  $4.0 \times 10^7$  cfu/g (25). In another study carried out in Romania, this rate was determined as  $1.5 \times 10^2 - 3.0 \times 10^4$  cfu/g (26). These differences in the results of other researchers and in our study may be due to differences in storage conditions or hygiene points at the time of sale.

Especially *Enterobacteriaceae*, which is an indicator of fecal contamination, are encountered in production conditions where personal hygiene is not observed and in environments where sanitation procedures are not taken into account (27). It was revealed that 68% (n=17) of the businesses we recruited were not paying attention to these conditions. *Enterobacteriaceae* were detected at the level of 40% (n=10) in plain ice creams, 12% (n=3) in fruits and 40% (n=10) in nuts. The minimum and maximum values of bacteria appearing in ice creams are given in Table 1.

**Table 1:** Microbiological analysis results of ice creams (n=75) with different flavors (cfu/g)

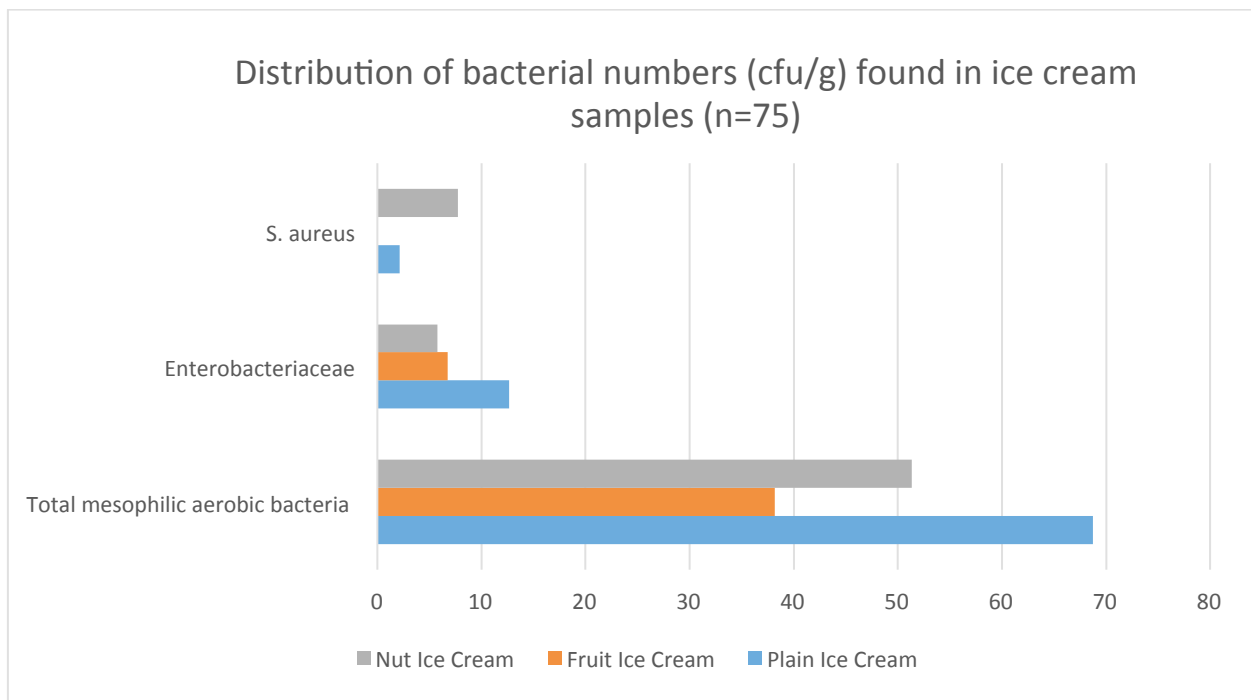
	Plain Ice Cream		Fruit Ice Cream		Nut Ice Cream	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
<b>TMA (cfu/g)</b>	0	$2.5 \times 10^4$	0	$2.50 \times 10^4$	0	$2.5 \times 10^4$
<b><i>Enterobacteriaceae</i> (cfu/g)</b>	0	$1.62 \times 10^4$	0	$1.13 \times 10^4$	0	$7.3 \times 10^3$
<b><i>S. aureus</i> (cfu/g)</b>	0	$3.20 \times 10^3$	0	0	0	$1.81 \times 10^4$

According to the Food Safety Criteria given in the Turkish Food Codex on Microbiological Criteria Regulation, it is known that ice creams

containing more than  $10^2$  cfu/g *Enterobacteriaceae* are not safe (15). Considering this criterion, 23 ice cream samples

(30.6%) we collected were not suitable for consumption in terms of food safety. Similar to our rates, 35 (23.3%) of 150 samples collected in another study conducted in Istanbul were found to be unacceptable in terms of *Enterobacteriaceae*. It has been suggested that the highest contamination occurs in plain ice

cream and this may be due to the acidity level of strawberry flavored ice cream (28). Similarly, in our study, as given in Figure 1, the highest rate of *Enterobacteriaceae* occurred in plain flavored ice creams. However, on the contrary, the lowest rate was seen in nuts, not fruits.



**Figure 1.** Distribution of bacterial numbers (cfu/g) found in ice cream samples (n=75)

*S. aureus*, which is one of the major threats to human health with its many toxic substances, can occur as a result of cross-contamination in food and beverages, especially through hair, skin, hands and fingers (29). It manifests itself with sudden symptoms such as abdominal pain, vomiting and stomach cramps (30). *S. aureus*, which easily contaminates food, was detected in 7 enterprises among our samples. Antibioqram test was performed on the detected ice creams. Multiple antibiotic resistance was found in only one company's nut ice cream, while others were found to be sensitive to antibiotics. According to

the Turkish Food Codex Microbiological Criteria Regulation and Food Safety Criteria, more than  $10^3$  cfu/g puts the food at risk (15). It was observed that 4 (5.3%) of the samples we collected were in the risky group. *S. aureus* was not detected in any of the fruit ice creams in our study. It is thought that this situation may arise due to the acidity present in the fruits.

In the present study, *Salmonella* was not found in any of the 75 ice cream samples. Positive results were obtained for *Salmonella* in different studies conducted in various regions of Türkiye (31). For example, *Salmonella* positivity level

was found to be 2% in a study conducted in Ankara and 5% in a study conducted in Manisa (32). In the results of our study, it has been revealed that the samples we collected from Istanbul are more consumable than other regions of Türkiye, since there should be no Salmonella according to the Turkish Food Codex (14). Similar to our results, 150 ice cream samples were found negative for *Salmonella* in another study conducted in Istanbul (28).

Considering the flavors of the samples, it was found that 52% (n=13) of plain ice creams, 88% (n=22) of fruit ice creams and 60% (n=15) of nuts ice creams were suitable for consumption.

As a result of the examination of 75 ice cream samples taken from 25 enterprises, 32% (n=8) of the enterprises were found hygienically suitable for consumption on the grounds that they meet the conditions in the Turkish Food Codex on Microbiological Criteria Regulation (14,15).

## CONCLUSION

According to the samples taken from the enterprises, it was revealed that most of them did not meet the criteria determined by the Turkish Food Codex on Microbiological Criteria . Despite the mask and glove rules that emerged after the COVID-19 epidemic in the world, it is thought provoking that there is such a high contamination.

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