Examining The Ice Creams Offered for Sale in Istanbul in Terms of Listeria Monocytogenes and Enterobacteriaceae SPP. Existence

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Abstract

Today, food requirements of the people have increased in parallel with the rapid population growth of the world. Consumption of convenience food is in high demand along with the development of industrial production. Nonetheless, foodborne infections in convenience food consumed in ample amounts pose a threat to the public health. One of these foods is ice cream notably attracting consumers in summer months. In Turkey, pastry shops and small-scale enterprises produce and sell ice-cream in addition to packaged and branded ice cream. Categorized in pathogen group and can be present in ice cream, *Listeria monocytogenes* is a species of bacteria that threats human health. Though *Listeria monocytogenes* is ubiquitous in the environment, it can be inhibited thanks to pasteurization process of milk during production. If present in foodstuff, this bacteria causes listeriosis in humans and some animals. Listeriosis causes meningitis, cepticemia and spontaneous abortion in advanced cases. *Enterobacteriaceae Spp.* is a large family widely present in quite many ecosystems in the nature and at the same time includes numerous microorganisms that are components of human and animal gastrointestinal system flora, some of which may be pathogenic. Existence of *Enterobacteriaceae* Spp. in food in excess of limit values specified is an indication of insufficient conformity to hygiene and sanitation rules. In this study, the presence of *Listeria monocytogenes* and *Enterobacteriaceae* Spp. in total 70 pieces of plain, chocolate and mixed ice cream, offered for sales in Istanbul market, 33 of which are branded from different companies and 37 from open sales shops [in individual servings], was examined. The findings revealed that 5 of the packaged ice creams and 15 of the open sales ice creams are above the limit values specified in the Turkish Food Codex in terms of *Enterobacteriaceae* Spp. The count of *Enterobacteriaceae* Spp in packaged ice creams was found as $3x10^{2-}$ $23x10^{2}$ and as $22x10^{2-}$ $66x10^{3}$ in open sales ice creams. Both *Enterobacter cloacae* and *Enterobacter asburiae* species were found in 7 samples and *Klebsiella pneumoniae* in 1 sample out of 20 suspicious samples in terms of *Enterobactericeae* Spp. by means of identification analyses conducted with VITEK-MS working on MALDI TOF MS principle. Total 10 L. *monocytogenes* suspicious isolates obtained from 70 samples examined were typified with MALDI TOF MS based VITEK-MS and then validated with Real-time PCR. However, existence of *L. monocytogenes* was not detected. 1 *E. faecalis* and 1 *L. innocua* were identified

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by means of VITEK-MS. This study determined that some ice cream samples consumed by the public in their daily lives in Istanbul contain *Enterobacteriaceae* Spp. above the limits of the Turkish Food Codex, and that specifically the open sale ice creams are exposed to more contamination and may pose more risk to the public health.

Keywords: *Ice Cream, Listeria monocytogenes, Enterobacteriaceae Spp., Food Safety, MALDI TOF MS.*

1. Introduction

As per the definition in the Regulation on Turkish Food Codex, ice cream is a food product which contains milk and/or milk products, potable water, sugar and other allowed additives according to taste and type as well as other ingredients such as salep, egg and/or egg products, aromatic substances and flavoring substances when desired, and offered for consumption in soft form or after hardening, obtained by processing and freezing according to the applicable technique following the pasteurization of the mixture [21]. The basis of production stages of both industrial ice cream and open-sale ice creams include mix preparation, conditioning and freezing processes. During freezing stage, air is applied onto mix [19]. Today, all substances and materials included in the composition of developing modern ice cream technology are mixed well; then left aside for 15 - 20 minutes for the stabilizer to achieve the desired viscosity by intake of water and later on, the mixture is pasteurized for 30 minutes at 68ºC or 25 seconds at 80ºC or UHT method is applied at 138ºC [20].

Many people suffer from food poisoning due to certain bacteria they take in together with the food consumed. Most of the bacteria that cause food poisoning live in the natural environment. Risks of presence of microorganisms in final products offered to consumers can be grouped in 3 main areas as microorganisms naturally present in the raw material, microorganisms that might transmit during production and microorganisms that might transmit during packaging, storage or transportation and handling of final product.

Today, *Listeria monocytogenes* is one of the major foodborne pathogens that might cause numerous infectious diseases in humans. *Listeria* genus includes 6 species, which are *L. monocytogenes*, *L. innocua, L. seeligeri, L. ivanovii, L. welshimeri* and *L. grayi*. Among the above, only *Listeria monocytogenes* is pathogenic for humans [17,7,14]. *L. monocytogenes* is ubiquitous in nature, and detected in many places such as water, silage, sewage, slaughterhouse wastes, milk of healthy and mastitis cows, human and animal feces [19]. Although not posing a risk for most of the health people, these bacteria can be seen in every segment of the society and generally seen more in fetus, newborns, babies, pregnant women, the old and people with poor immune system [16].

In the studies made, *Listeria monocytogenes* was isolated from food products like sea food, ice cream, milk, fish, vegetables and fruits[19]. *Listeria monocytogenes* is a zoonotic, Gram-positive, facultatively anaerobic, non-capsular and non-spore forming bacteria widely present in the environment. In humans, it can cause meningitis, cepticemia, conjunctivitis, skin and mucosa localizations, in especially pregnant women, abort, stillborn, premature death of newborns or congenital anomalies since it can be transmitted to the fetus through placenta. Given the psychrophilic nature of *Listeria monocytogenes* that can continue reproduction even at 4°C, it is detected in higher rates in convenience food stored at lower temperatures [1].

Enterobacteriaceae Spp. are microorganisms frequently found in soil, water, plants, humans and natural intestinal flora of most animals all around the world. They include more than 40 genus such as *Escherichia, Salmonella, Shigella, Yersinia, Klebsiella*, *Proteus, Enterobacter, Serratia* and *Citrobacter* [9]. In this large family, the coliform bacteria, faecal coliforms, *E. coli* (type 1), *E. coli* O157:H7 serotype, *Salmonella, Shigella* and *Yersinia enterocolitica* are looked for and/or counted in routine analyses in many food microbiology laboratories.

Enterobacteriaceae species are structurally Gram (-), facultatively anaerobic bacilli that ferment sugar. They produce catalase; rarely in oxidase positive form, reducing nitrate to nitrite. They are present in natural intestinal flora of humans and animals. This is the group demanding the most fight for infections in the medical field. *Enterobacteriaceae* Spp is the primary cause of numerous diseases such as urinary system infections, wound infections, digestive system infections, meningitis and pneumonia [32]. Existence of high number of *Enterobacteriaceae* in food is considered an indication that hygiene and sanitation rules are not observed.

This study aims at detection of existence of *Enterobactericeae Spp.* and *Listeria monocytogenes* bacteria in ice cream sold to consumers in Istanbul.

2. Material and Methods

Samples

In this study, ice cream sold as individual servings and as packaged in the Istanbul market constituted the research material. Total 70 ice creams were analyzed for determination of existence of *Enterobactericeae spp.* ve *Listeria monocytogenes.* Packaged ice creams were taken from 15 different shops as chocolate ice cream, plain and mixed ice creams of 4 different brands. Open sales ice creams, on the other hand, were taken from 15 different shops in 100 grams of servings as chocolate, plain and mixed ice creams. Samples were put into sterile containers under aseptic conditions and analyzed right after being taken, in cold chain, to the laboratory.

Microbiological analysis

The count and isolation of *Enterobactericeae s*pp. were done according to the ISO 21528-2 method and the count and isolation of *Listeria monocytogenes* according to the ISO 11290-1 method. *Enterobactericeae* Spp. suspicious colonies detected via the classic method were typified by using the MALDI TOF MS technical based VITEK MS, (BioMerièux, France) device. *Listeria monocytogenes* suspicious colonies were also typified by means of VITEK MS and at the same time by Real-Time PCR device (SLAN-96S).

Enterobactericeae Spp. Count

For *Enterobactericeae spp.*; 10 grams of each sample were weighed in precision balance under aseptic conditions; put inside sterile bags and homogenized in Stomacher with 90 ml sterile Maximum Recovery Diluent (MRD) (Merck, 112535). Sub-dilutions were prepared from the homogenized main dilution at a rate of 1/10, and 1 ml of each dilution was put into two separate Petri dishes. 10 ml Violet Red Bile Glucose (VRBG) Agar (Merck, 146127) was poured over the dilutions and it was ensured that it mixed with the inoculum by moving the Petri dish in horizontal and circular movements. After solidification of the mixture, another 15 ml VRBGA was added and a suitable environment was created for the facultative anaerobic bacteria. Following solidification, the reversed Petris were incubated for 24 hours at 37ºC. The colonies in pink, red and purple colors that formed or not formed zone in the VRBG Agar were considered as characteristic colonies. For the process, characteristic colonies in the VRBG Agar were transferred to Nutrient Agar (Merck, 105450) and left for incubation of 24 hours at 37 ˚C. After incubation, oxidase test (Merck 1.13300) was conducted on the colonies purified in the Nutrient Agar. Oxidase negative colonies were cultivated into Glucose Agar for fermentation test purposes and incubated at 37°C. The yellow color was considered as positive. At the end of all these processes, oxidase negative, glucose positive colonies were considered as *Enterobactericeae*. Positive isolates were further identified by means of MALDI TOF MS based VITEK®MS device.

Listeria monocytogenes Count

For the analysis of *L.monocytogenes*; 25g sample was put into sterile Stomacher bag for pre-enrichment; made up with 225 ml Half-Fraser Broth (Oxoid, BO0793) addition; homogenized in the Stomacher device and left for incubation of 24-36 hours at 30°C. 0.1 ml was taken from the pre-enrichment culture for selective enrichment; transferred to 10 ml Fraser Broth (Merck, 110398) flasks again incubated for 48 hours at 37°C. Following the incubation, cultivation was done into the selective solid medium (Palcam Agar, Merck, 111755) and left for incubation for 24 hours at 37°C. Colonies of 1.5-2 mm in diameter, with olive green-gray color and black zones in the Palcam Agar were accepted as suspicious and validated. For this purpose, gram staining, catalase test and beta-haemolysis test in blood agar were performed onto the isolated *L.*

monocytogenes suspicious colonies. Catalase positive, Gram positive and beta haemoysis positive colonies were comparatively validated in by means of MALDI TOF MS (VITEK®MS) and Real-time PCR.

Matrix-Assisted Laser Ionization Mass Spectrometry (MALDI TOF MS) Analysis

MALDI TOF (MALDI TOF MS; 'Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry') is a system defining microorganisms on basis of mass spectrometry and an identification method based on plotting protein profiles of bacteria cells and comparing them with a reference spectrum available in the database [22].

VITEK-MS device was developed for rapidly making only microbiological analyses by sticking with these principles.

Using VITEK MS (bioMérieux, France), first of all, bacteria isolation of the samples was done according to the classical microbiologic methods described above. A small amount taken from the suspicious colony with single-use sterile extract was smoothly and homogenously placed on specified areas on the metal plates of the device called "target" and left for drying. As the positive control, *E. coli* ATCC 8739 reference control strain was spread on the relevant area of the plate as mentioned, and then 1 µL matrix (α-cyano-4-hydroxycinnamic acid saturated inside 50% "acetonitrile" and 2.5% "trifluoroacetic acid") was added onto the positive control strain and the plate was left to dry in air again. After the samples were prepared, the plate was loaded on VITEK MS, (bioMérieux, France) device and the process was initiated. Entering into the main menu of the device software, the slide prepared was bar-coded and the areas with the samples were marked. The device does laser shots on each area where the samples are spread and, for example, the molecules become ionized and start flying inside the device tube according to the molecular weight. Data converted into digital format are accumulated to create TOF (time of flight) and detected by means of a detector available inside the device. The analysis of the data obtained is done with the database and the microorganisms on which the work is done are named.

DNA isolation

DNA material from the suspicious *L. monocytogenes* strains was obtained by applying the Eurofins GENESpin DNA isolation kit (Eurofins GeneScan, Germany) procedure. Genetic materials obtained in completion of the isolation were kept at -20ºC for the purpose of using in Real-time PCR stage.

Real-time PCR

For validation of *L. monocytogenes* suspicious isolates by means of Real-time PCR method, Qiagen QIAsymphony Mericon *L. monocytogenes* Kit was used and the kit procedure was followed. SLAN-96S model Real-time PCR device was used for

this purpose. 10 µl of master mix was prepared and 20 µl of final volume was obtained by making up with 10 µl DNA. 40 cycles were applied on PCR tubes as 5 minutes at 95ºC, 15 seconds at 95ºC, 15 seconds at 60ºC and 10 seconds at 72ºC. FAM wavelength light (495/520 nm) was chosen for the target DNA and MAX wavelength light (524/557 nm) for internal control purposes. The results were evaluated according to the standard curve seen in the device.

3. Results

70 samples of ice cream, as industrially packaged product or traditionally produced and sold in open stands with minimum 10 grams were collected according to proper methods from pastry shops, markets, delicatessen, etc. in various districts of Istanbul from places where the analyzed samples sold, and examined by studying on parallel samples for the purpose of determination of existence of *Enterobactericeae* spp. and *Listeria monocytogenes*. The samples taken consisted of 33 packaged and 37 open-sales ice creams. 11 of the packaged ice creams were plain, 11 chocolate and 11 mixed; on the other hand, 12 of the open-sales ice creams were plain, 12 chocolate and 13 mixed.

Enterobactericeae Spp. Analysis Results

Following classical microbiological processes of the samples, *Enterobactericeae spp.* suspicious isolate was detected in 35 of the 70 samples. 20 of these samples do not conform to the limited set out in the Turkish Food Codex. The findings revealed that 5 of the packaged ice creams and 15 of the open sales ice creams are above the limit values specified in the Turkish Food Codex (Table 1).

Enterobacteriaceae Spp count was found as $3x10^2$ - $23x10^2$ in the above-limit packaged ice creams, and $22x10^2$ - $66x10^3$ in open sales ice creams (Figure 3). The remaining 50 ice creams were in the range of 0-$6x10^1$ kob/g, that is, conforms to the 10^2 kob/g limit specified in the Codex.

Both *Enterobacter cloacae* and *Enterobacter asburiae* species were found in 7 samples out of 20 in the identification study of the non-conforming isolates made by VITEK-MS. *Klebsiella pneumoniae* species was found in 1 sample. 12 isolates out of 20 were not typified according to the VITEK-MS results.

The Figure 1 below shows the *Enterobactericeae* spp. count and breakdown of packaged ice creams, and Figure 2 shows the open sales ice creams in graphs.

Table 1: *Enterobactericeae* Spp. Microbiologic Count Results

Type of Sample		Number of Sample (n)	Limit in the Turkish Food Codex	Values Detected	Number of Non-Conforming Sample (Above-Limit)
Type of Package	**Type of Ice Cream**				
Packaged	Plain ice cream	11	Max 10^2 cfu/g	$17x10^2$ cfu /g	1
	Chocolate ice cream	11		$19x10^2$ -$23x10^2$ cfu /g	2
	Mixed ice cream	11		$3x10^2$ -$5,6x10^2$ cfu /g	2
Open Sales	Plain ice cream	12		$22x10^2$ -$66x10^3$ cfu /g	8
	Chocolate ice cream	12		$3,1x10^2$ -$16,4x10^2$ cfu /g	5
	Mixed ice cream	13		$4,7x10^2$ -$8,1x10^2$ cfu /g	2

Figure 1. Breakdown of Packaged Ice Creams Above Limits in terms of *Enterobactericeae spp*.

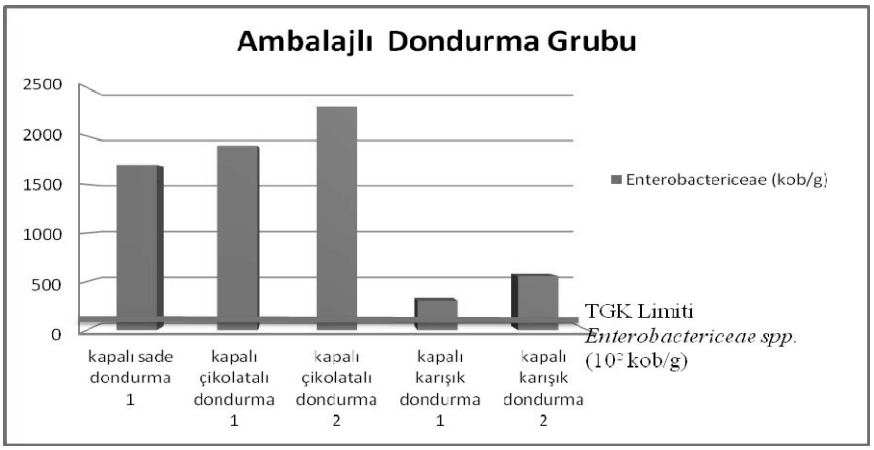


Figure 2: Breakdown of Open Sales Above-Limit Ice Creams in terms of *Enterobactericeae* Spp.

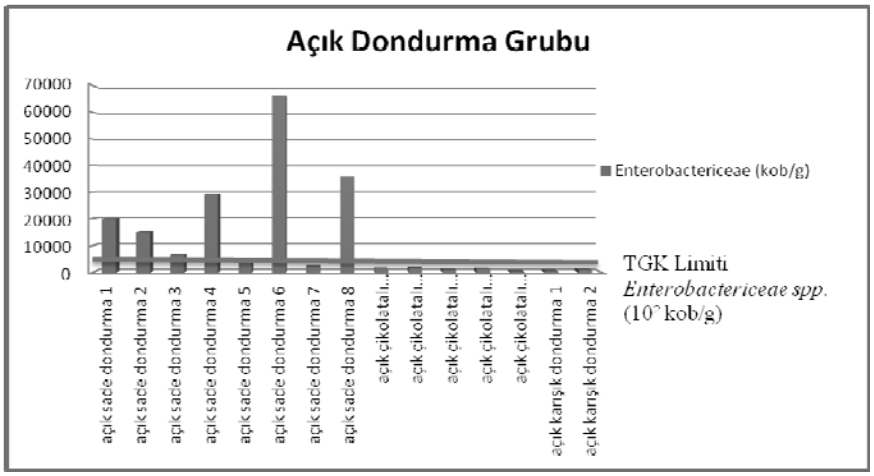
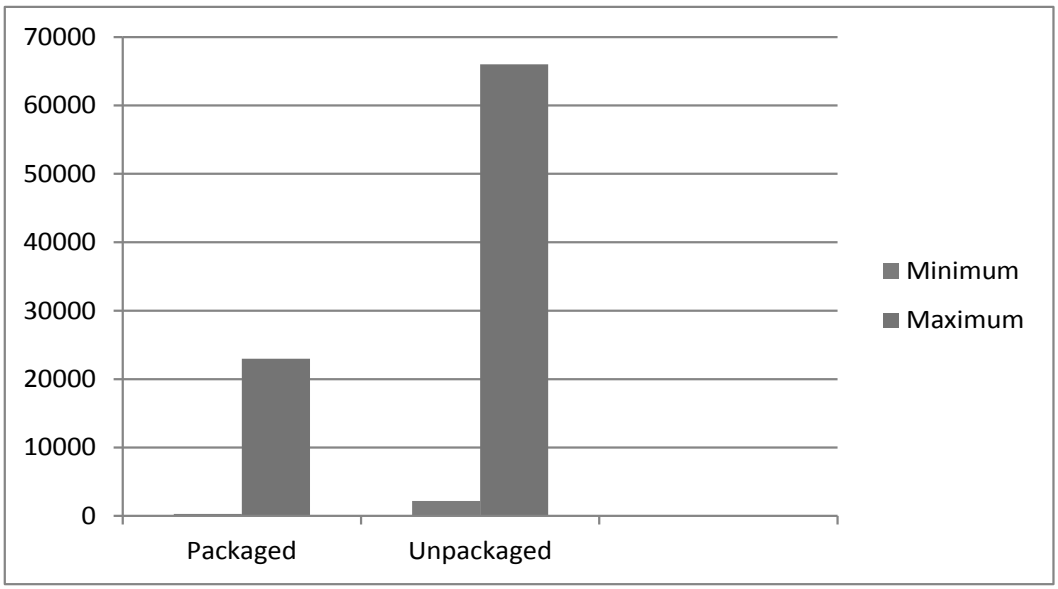


Figure 3. Comparison of Packaged and Open Sales Ice Creams in terms of *Enterobacteriaceae* Spp. Counts



***Listeria monocytogenes* Analysis Results**

Total 10 *L. monocytogenes* suspicious isolates in 70 samples were detected in the microbiological analysis.

Table 3: Results of *L. monocytogenes* Count of Samples

Type of Sample		**Number of Sample (n)**	**Limit in the Turkish Food Codex**	**Values Detected**	**Number of Non-Conforming Sample (Above-Limit)**
Type of Package	**Type of Ice Cream**				
Packaged	Plain ice cream	11	0 cfu/ 25g	-	Conforms
	Chocolate ice cream	11		-	Conforms
	Mixed ice cream	11		-	Conforms
Open Sales	Plain ice cream	12		-	Conforms
	Chocolate ice cream	12		-	Conforms
	Mixed ice cream	13		-	Conforms

The isolates prepared were typified using VITEK-MS and *L. monocytogenes* species was not found. However, 1 *Enterococcus faecalis* and 1 *Listeria innocua* were identified from the isolates in the VITEK-MS. Suspicious isolates were likewise analyzed by means of Real-time PCR kit procedure and it was verified that none had *Listeria monocytogenes*.

4. Discussion and Conclusions

Ice cream is important in nutrition thanks to its nutritious values and liked and consumed by a large segment of the society; however, it is also a rather conducive medium for growth of many pathogenic microorganisms. Ice creams that contain pathogenic microorganisms or toxins of such microorganisms due to use of non-hygienic raw material, primitive production technology and insufficient personnel hygiene lead to food infections and intoxications and constitute great problems in terms of food safety and public health. Notably, ice creams produced in small-size shops and sold openly can be infected with numerous contaminants during production, storage and sales stages [26].

Species of microorganisms forming the ice cream microflora also affect the ice cream quality. *Basillus* spp. spores, micrococcus, enterococcus, corynebacteria play important roles in formation of undesired and disliked tastes in ice creams. A bitter and oxidized taste occurs based on reproduction in very large numbers of the foregoing. These microorganisms are mostly transmitted through milk and milk powder used in ice cream production [28].

Most of the infections caused by *Listeria spp.* are conveyed through raw milk. Since ice cream is made with milk, it contains risks. Milk protects *Listeria* bacteria against the effects of stomach acid. Therefore, it has a significant effect in infections and dissemination of the disease. *L. monocytogenes* either exists freely inside milk or commonly inside leucocytes. Intracellular nature of the *Listeria* bacteria can protect it against quite many substances such as immunoglobulin, lysozyme, peroxidase and lactoferrin present in milk. Thus, these bacteria can still be alive even after pasteurization of milk, reproduce in fridge temperature and be taken in to the body together with the food [5].

Due to the fact that *Listeria spp.* can develop even in fridge temperature, survive drying, heating, freezing and cooling processes, there is a high risk that it can exist in ice cream and it can pose problems in ice cream production; *Listeria spp.* is one of the bacteria species that is difficult to isolate for reason of less number in the environment [26].

Detection of *Enterobacteriaceae* family, which is the most important family in terms of food microbiology and the coliform bacteria within this family and bacteria of faecal origin like *E.coli* within the coliform bacteria is an indication of the microbiological quality of food and therefore is meaningful as a quality criterion of hygiene and sanitation. Bacteria from

Enterobacteriaceae family may have origins in the environment or water or their existence in raw milk shows that milk contacts with any source of intestine origin and the same milk can host the microorganisms that might cause numerous diseases [3]. Some ice cream producers use raw milk while making ice cream instead of pasteurized milk. Many producers stated at the observations done during sampling that they skip pasteurization stage for reason of its adverse effect on the taste of the ice cream. In this case, direct intake of *Enterobactericeae* spp. and other pathogens into the body together with the food pose a threat to the public health.

Many studies conducted in Turkey draw attention to the fact that ice creams available in the market are contaminated with microorganisms to a significant level and might be a threat to the public health.
Erol *et al.* (1998) examined total 100 ice cream samples, 30 of which vanilla ice cream, 26 chocolate and 44 fruit ice creams, from various pastry shops in Ankara in microbiological terms and found out that considering the total aerobic mesophilic bacteria and coliform counts, respectively, 63.2 % and 73.1 % of vanilla ice cream samples, 73.0 % and 57.4 % of chocolate ice creams and 61.3 % and 52.1 % of the fruit ice cream samples did not conform to the ice cream standard of the Turkish Standards Institute. They concluded that the samples they examined in their study might pose a potential risk in terms of public health.

In another study carried out by Mukan and Evliya (2002) on 24 ice cream samples taken from various pastry shops, production and sales points for the purpose of evaluation of microbiological quality of clotted cream ice creams sold in Adana, they detected coliform bacteria in 87.5% of the samples and found the microbiological quality of ice creams sold in Adana was low.

Patir *et al.* (2004) analyzed the species and breakdown of coliform in clotted cream (plain) and fruit aroma ice creams in open sales in Elazig in total 100 samples as 50 clotted cream ice creams and 50 fruit aroma ice creams (10 from each lemon, cacao, pistachio, sour cherry and strawberry aromas). The coliform counts in their samples were found as <1,00 -5,74 log10 kob/g. The researchers who isolated total 632 strains from the ice cream samples found out that 41 (22.04%) of 186 strains isolated were *E. coli*, 89 were *Escherichia* spp. (47.85 %), 45 *Citrobacter* spp. (24.19%), 32 *Enterobacter* spp (17.20%) and 20 *Klebsiella oxytoca* (10.75%) in the clotted cream ice creams. In the fruit aroma ice creams, 26 (5.83 %) were *E. coli*, 190 (42.60 %) were *Enterobacter* spp., 103 (23.09 %) *Escherichia* spp, 96 (21.52 %) *Citrobacter* spp. and 57 (12.78 %) were *Klebsiella oxytoca* of 446 strains isolated. They determined that *Escherichia* species was more common in the clotted cream ice creams and *Enterobacter* species was more common in the fruit aroma ice cream samples. This study revealed that because the *E. coli* and *Enterobacter* spp. existed in ice creams sold for consumption in Elazig,

they were not produced under hygienic conditions and therefore not safe in terms of public health.

Agaoglu and Alemdar (2004) detected in their study conducted for the purpose of investigation of existence of certain pathogenic bacteria, significant in terms of public health, in ice creams sold in the city of Van that out of total 75 samples of plain, chocolate and fruit ice creams taken from various pastry shops, 8 % had *L. monocytogenes*, 25.3 % *K. pneumoniae*, 17.3 *Salmonella* spp., 13.3 *E. coli* and 13.3 % had *S. aureus*.

Akarca and Kuyucuoglu (2008) determined that out of 50 samples of plain ice cream taken from ice cream sales places in the city center of Afyonkarahisar, 22 %, 44 % and 22 % were above the limit values specified in the 4265 Ice Cream Standard of the Turkish Standards (TS) and the Communiqué on Ice Cream of the Bylaw of Turkish Foodstuff, respectively, in terms of total aerobic mesophilic bacteria, coliform bacteria and *S. aureus* counts.

Keskin *et al.* (2007) revealed that 12.7% of the ice cream offered to consumers within the provincial borders of Istanbul did not conform to the Communiqué on Food Codex Microbiology Criteria. Agaoglu and Alemdar (2004) found that 13.3 % of the ice cream sold in the city of Van was contaminated with *E. coli.* Their study showed this rate as 8 % in plain ice cream and 5.3 % in fruit ice cream.

Cinar (2010) found that the *Enterobacteriaceae* spp. count as <1 – $7{,}0 \times 10^5$ kob/g in the study that investigated the microbiological characteristics of 30 plain and 30 strawberry ice creams sold in the province of Tekirdag. None of the samples had *L. monocytogenes*.

Caglayanlar *et al.* (2009) showed that the microbiological quality of the ice cream sold in packages (industrially produced, n=44) and unpackaged ice cream and produced in pastry shops in the city of Bursa was lower. *Listeria monocytogenes, Staphylococcus aureus* and *Salmonella* spp. were not detected in any of the samples tested. All of the packaged samples conformed to the criteria stipulated by the Turkish Food Codex (TFC). The 9.1 %, 50.1 %, 63.6 %, 22.7 % and 36.4 % of the unpackaged samples did not conform tot the TFC criteria respectively in terms of TAMB, coliform, *E. coli,* yeast and mold counts.

Akman *et al.* (2000) examined total 58 ice cream samples, 28 from the city of Kahramanmaras and 30 from Adana, for the existence of *Listeria* spp. They detected the existence of *Listeria* species in 14 of the samples from Kahramanmaras and 10 of the samples taken from Adana. The biochemical species determination analysis conducted revealed that 22 samples out of 58 were contaminated with *L.grayi* and one each with *L.innocua, L.welshimeri;* in parallel with this study, they also stated that none of the samples had *L.monocytogenes.* Jaleli and Abedi (2008) revealed that 1.3% of the ice cream they examined had *L. monocytogenes*.

Similarly, Gonulalan (2010) detected that 24 % of the ice cream samples examined in a study carried out in Kayseri were positive in terms of *Listeria* species. Nawal *et al.* (1997) found out the frequency of existence of *Listeria* species in the ice creams sold in the United States of America as 8 % in their microbiological analyses. Cordano and Rocourt (2001) found *Listeria monocytogenes* at the rates of 3.5 - 7.4 % in the ice cream sold in Chile. Dhanashree and Otta (2003) determined the rate of existence of Listeria species in ice creams consumed in India as 17.5 %. It was seen that the most dominant phenotypes among the Listeria species were *L. innocua* and *L.monocytogenes*. Molla *et al.* (2004) found out the most common strain was *L. monocytogenes* in the ice cream samples they examined. It is of importance that none of the samples we analyzed showed the existence of *L. monocytogenes*. However, the fact that *Listeria monocytogenes* isolation could not be made from the ice cream samples does not mean that the samples taken were not risky. Listeria is still one of the bacteria that is most difficult to isolate in the food microbiology. Existence of Listeria in small numbers together with accompanying flora bacteria in the food reduces the rate of making an isolation.

In this study, the presence of *Listeria monocytogenes* and *Enterobacteriaceae* Spp. in total 70 pieces of plain, chocolate and mixed ice cream, offered for sales in Istanbul market, 33 of which are branded from different companies and 37 from open sales shops [in individual servings], was examined. The findings revealed that 5 of the packaged ice creams and 15 of the open sales ice creams are above the limit values specified in the Turkish Food Codex in terms of *Enterobacteriaceae* Spp. The count of *Enterobacteriaceae* Spp in packaged ice creams was found as $3x10^{2-}$ $23x10^{2}$ and as $22x10^{2-}$ $66x10^{3}$ in open sales ice creams. Both *Enterobacter cloacae* and *Enterobacter asburiae* species were detected in 7 out of 20 suspicious *Enterobactericeae Spp*. samples through the identification studies made by means of VITEK-MS running with the MALDI TOF MS principle. Ice cream samples that contained *Listeria monocytogenes* were not detected.

Detection of *Enterobacteriaceae* spp. and Coliform in numerous studies, previously conducted in various provinces of Turkey at various times, above the limits of the Turkish Food Codex, as was the case in our study, shows that these species can contaminate ice creams through many different sources and ways such as lack of personal hygiene, raw materials used in production (use of raw milk, use of well water in production and cleaning, use of contaminated flavor substances, etc.) and insufficient facility hygiene. Our study conducted in Istanbul and similarly the study by Caglayanlar *et al.* (2009) in Bursa found the *Enterobacteriaceae* spp. count in the open sales ice cream samples higher than the packaged ice creams. Even though the open sales ice creams are produced with the necessary care and attention, they have additional contamination risk during storage and even sales. Furthermore, it is clear that

ice creams produced at small-scale shops and facilities by people who do not have sufficient hygiene and sanitation knowledge using raw materials of unknown quality and generally by means of non-standardized production methods are more risky in hygienic terms.

Today, ice creams of many kinds and enjoyed by most people can be contaminated with various microorganisms for many reasons in many stages as production, transportation, storage and preparation for consumption. The study we made also detected *Enterobacteriaceae* spp. in counts that can negatively affect the public health and shows that ice cream sold in Istanbul is one of the important foodstuff that might threat the public health.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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