Çankaya Üniversitesi Fen-Edebiyat Fakültesi,
Journal of Arts and Sciences Sayı: 12 / Aralık 2009

Comparison of the Microbiological Quality of Packed and Unpacked Ice Creams Sold in Bursa, Turkey*

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Abstract

In this study microbiological quality of packed (industrially produced, *n*=44) and unpacked (patisserie product, *n*=48) ice creams sold in Bursa, Turkey were compared. Total aerobic mesophilic bacteria (TAMB), yeast and mold counts in packed ice cream samples varied between $2.7x10^1$–$6.9x10^2$, <10–$1.0x10^1$, and <10–$2.0x10^1$ cfu/g, respectively. Coliforms and *Escherichia coli* counts of the same samples were <10 cfu/g. In unpacked samples TAMB, coliforms, *E. coli*, yeast and mold counts ranged between $8.1x10^1$–$1.0x10^6$, <10–$2.4x10^3$, <10–$8.4x10^2$, <10–$1.5x10^5$, and <10–$1.2x10^3$ cfu/g, respectively. *Listeria monocytogenes*, *Staphylococcus aureus,* and *Salmonella* spp. were not detected in any of the samples tested. All of packed samples conformed to Turkish Food Codex (TFC). In terms of TAMB, coliforms, *E. coli*, yeast and mold, percentage of the unpacked samples did not conform to TFC were 9.1%, 50.1%, 63.6%, 22.7% and 36.4%, respectively. Results showed that ice creams produced in patisseries in Bursa had poor microbiological quality. Application of the HACCP system for ice cream production should become legally mandatory to improve the product quality.

Keywords. Microbiological quality, Plain ice cream, Flavored ice cream.

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* Bu makale, birinci yazarın Yüksek Lisans tezinin bir bölümüdür

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Özet

Bu çalışmada, Bursa'da (Türkiye) satışa sunulan paketlenmiş (endüstriyel olarak üretilmiş, n=44) ve paketlenmemiş (pastanelerde üretilmiş, n=48) dondurma örneklerinin mikrobiyolojik kalitesi karşılaştırılmıştır. Paketlenmiş dondurma örneklerindeki toplam aerobik mezofilik bakteri (TAMB), maya ve küf sayısı, sırasıyla $2.7x10^1$–$6.9x10^2$, <10–$1.0x10^1$, <10–$2.0x10^1$ kob/g arasında değişmiştir. Aynı örneklerdeki koliform ve *Escherichia coli* sayısı ise <10 kob/g olmuştur. Paketlenmemiş örneklerdeki TAMB, koliform, *E. coli*, maya ve küf sayıları sırasıyla, $8.1x10^1$–$1.0x10^6$, <10–$2.4x10^3$, <10–$8.4x10^2$, <10–$1.5x10^5$, ve <10–$1.2x10^3$ kob/g arasında değişmiştir. Test edilen örneklerin hiçbirinde *Listeria monocytogenes*, *Staphylococcus aureus* ve *Salmonella* spp.ye rastlanmamıştır. Paketlenmiş örneklerin tamamı, Türk Gıda Kodeksi'nin (TGK) kriterlerine uygundur. Paketlenmemiş örneklerin sırasıyla %9.1, %50.1, %63.6, %22.7 ve %36.4'ü TAMB, koliform, *E. coli*, maya ve küf sayıları bakımından TGK kriterlerine uygun değildir. Sonuçlar, Bursa'daki pastanelerde üretilen dondurmaların mikrobiyolojik kalitesinin düşük olduğunu göstermiştir. Dondurma üretiminde HACCP sistemi uygulamasının yasal olarak zorunlu kılınması ürün kalitesini arttıracaktır.

Anahtar kelimeler: Mikrobiyolojik kalite, Sade dondurma, Çeşnili dondurma.

1. Introduction

Ice cream, a milk-based product, is a good media for microbial growth due to high nutrient value, almost neutral pH value (~pH 6–7), and long storage period of ice cream. It is produced by freezing pasteurized mixture of milk, cream, milk solids other than fat, sugars, emulsifiers, and stabilizers. Other ingredients include flavoring matters and water. Fruits, nuts, candies and syrups are optionally added into ice cream for flavor enrichment. It is sold in packages or in open containers at retail outlets/ice cream parlors, the open variety being distributed manually in scoops, cones, or sundaes across the counter (Champagne et al., 1994; Farrag and Marth, 1992; Marshall and Arbucle, 1996; Warke et al., 2000). During production, transportation and storage, it may become contaminated with several microorganisms. The microbiological quality of ice cream during retail marketing mainly depends upon the post production handling of the product, in addition to the efficiency and sanitary conditions during frozen storage. Contamination of ice creams by pathogenic microorganisms at some processing steps resulted in several disease outbreaks in numerous countries of Asia, Europe, and North America (Chug, 1996; Daniels et al., 2002; Djuretic et al., 1997). Ice cream is one of the major product of the dairy industry and continues to dominate the interest of large segments of the population. As most of the ice cream consumers are children of vulnerable age groups, it is required to be microbiologically safe (Warke et al., 2000).

In previous studies, high microbial loads in ice cream samples obtained from patisseries in Turkey have been reported (Coskun, 2005; Digrak and Özcelik, 1991; Digrak et al., 2000; Erol et al., 1998; Evrensel and Gunes, 1998; Kanbakan and Çon,

1999; Kivanc et al., 1994; Korel et al., 2005; Ozcan and Kurdal, 1997; Patir et al., 2006; Sezgin et al., 1997; Yaman et al., 2006). Also, it was observed that most of the ice cream samples obtained from patisseries did not conform to TFC (2001) in mentioned studies. Nevertheless, there are only a few studies about industrially produced ice cream sold in retail stores in Turkey (Bostan and Akin, 2002; Korel et al., 2005).

This study has been carried out in Bursa to determine the microbiological quality of ice cream offered for public consumption. Results of this study may help (a) to compare microbiological quality of industrially produced-packed ice cream with ice cream produced in patisseries and sold unpacked; (b) to understand the difference in the microbiological qualities between flavored and plain ice creams; and (c) to understand whether there is any improvement in the microbiological quality of ice cream offered for public consumption by making comparisons with previous researches.

2. Materials and Methods

2.1. Materials

Ice cream samples

A total of 44 industrially produced ice cream samples (packed, belonging to two different brands) were purchased from retail stores and 48 open ice cream samples were purchased from 3 different patisseries (from June to August 2003) in Bursa city. Samples were divided into two categories: plain and flavored (with nuts, fruits, cocoa, chocolate etc.). Producers and stores were chosen randomly. The samples were transported in a freezer bag and analyzed within 3 h after collection. Industrial ice cream producers and patisseries were represented by the letters A and B for the former and C-E for the latter groups.

2.1.1. Methods

Enumeration and isolation methods

Ten g of ice cream sample were diluted with 90 ml sterile peptone water in a conical flask containing glass beads. Three serial dilutions were plated in duplicates onto plate count skim milk agar (PCSMA), yeast extract-glucose-chloramphenicol agar (YEGCA), violet red bile agar (VRBA, using overlay method), chromocult coliform agar (CCA), and Baird-Parker agar (BPA), for enumeration of total aerobic mesophilic bacteria (TAMB), yeast and mold, coliform bacteria, *Escherichia coli,* and *Staphylococcus aureus,* respectively. PCSMA plates were incubated at 35 ºC for 48 h. VRBA and CCA plates were incubated at 37 ºC for 24 h, and BPA plates were incubated at 37 ºC for 48 h. YEGCA plates were incubated at 30 ºC for 48–72 h. To isolate *L. monocytogenes*, ice

cream samples (25 g) were added into Fraser *Listeria* selective enrichment broth (LEB). After 48 h incubation at 30 ºC, an inoculum from the LEB was plated onto Palcam *Listeria* selective agar and the plates were then incubated at 30 ºC for 24–48 h. Detection of *Salmonella* spp. was carried out by a two-step enrichment procedure in lactose broth and selenite cystine broth (37 ºC for 24 h), followed by streaking on *Salmonella-Shigella* agar, bismuth sulfite agar, and brilliant green agar. All plating and enrichment media were purchased from Merck, Germany. Suspected colonies were then identified using standard bacteriological procedures (Ice Cream Standard, 1992).

Statistical analysis

Plate-count data were transformed into logarithms before statistical treatment. Statistical analysis was carried out using the SPSS software. Differences between the $\log_{10}$ values were statistically compared by the Pearson's correlation coefficient analysis.

3. Results and Discussion

Findings obtained from the microbiological analysis of ice cream samples are categorized into three groups. Evaluations conducted according to manufacturing type (manufactured industrially-packed type/ manufactured in patisseries-unpacked type), type of product (plain ice cream/flavored ice cream), and different producers (factory/ patisserie) are provided in Tables 1 to 3, respectively. In Table 4, samples that do not comply with TFC (2001) are evaluated.

3.1. Microbiological quality of packed and unpacked ice creams

The microbiological qualities of packed and unpacked ice cream are shown in Table 1. In our study, the microbiological load of the industrially produced and packed ice cream samples were lower compared to the findings of Bostan and Akin (2002). In their study, TAMB, yeast-mold, and coliform counts of packed samples ranged between $1.0x10^2$ and $2.1x10^4$ cfu/g, <10 and $1.7x10^3$ cfu/g, and 0 and 9.3 MPN/g, respectively. They could not isolate *E. coli*, *S. aureus,* and *Salmonella* spp. Joshi et al. (2004) investigated the microbiological quality of 72 ice creams from 12 different factories in Kathmandu. TAMB, coliform bacteria, and *Staphylococcus* spp. varied between 10^3 and $1.52x10^8$ cfu/ml, 10^3 and $1.3x10^7$ cfu/ml, and 10^3 and $2x10^4$ cfu/ml, respectively. They isolated only one *Salmonella* spp. from the samples tested. M-E-Elahi et al. (2002) reported higher TAMB and coliform counts in packed ice cream than that found in this study. TAMB and coliform counts ranged between 2.8 x 10^3 and 5.6 x 10^4 cfu/ml, and between 4 and 42 cfu/ml, respectively, in their study.

Table 1. Microbiological loads of ice creams

Microorganism Count (cfu/g)	Unpacked ice cream (n = 48)	Packed ice cream (n = 44)	Total (n = 92)
TAMB	[a]$8.1x10^1$ – [b]$1.0x10^6$	$2.7x10^1$ – $6.9x10^2$	$2.7x10^1$ – $1.0x10^6$
Coliform count	<10 – $2.4x10^3$	<10	<10 – $2.4x10^3$
E. coli	<10 – $8.4x10^2$	<10	<10 – $8.4x10^2$
Yeast	<10 – $1.5x10^5$	<10 – $1.0x10^1$	<10 – $1.5x10^5$
Mold	<10 – $1.2x10^3$	<10 – $2.0x10^1$	<10 – $1.2x10^3$

[a] Minimum number of microorganisms

[b] Maximum number of microorganisms

The range of TAMB counts in the patisserie sample was $8.1x10^1$ to $1.0x10^6$ cfu/g. Several investigators reported higher TAMB counts in unpacked ice cream samples than that found in this study; $4.8x10^2$ to $5x10^8$ cfu/g (El-Sharef et al., 2006); 10^3 to $1.0x10^8$ cfu/g (Erol et al., 1998); $<10^3$ to $7.5x10^6$ cfu/g (Kivanc et al., 1994); $2.5x10^2$ to $3.0x10^7$ cfu/g (Korel et al., 2005); $>10^6$ cfu/g (Yaman et al.,2006).

Although coliform count in the unpacked samples of the current study ranged between <10 and $2.4x10^3$ cfu/g, those numbers varied between 11 and $8.5x10^6$ cfu/g in similar studies (Coskun, 2005; Erol et al., 1998; Evrensel and Gunes, 1998; Kivanc et al., 1994; Korel et al., 2005; Ozcan and Kurdal, 1997; Patir et al., 2006; Warke et al., 2000). El-Sharef et al., (2006) stated that coliform counts in 71% of ice cream samples were higher than the limits permitted (>10 cfu/g) by the Libyan Standards for Ice Cream. The current results on the coliform count of patisserie samples were similar to the findings of Kanbakan, et al. (2004): minimum of $2.5x10^2$; maximum of $2.4x10^3$ cfu/g).

In our study, *E. coli* was found in 29.16% of unpacked ice cream samples (14/48), and the counts varied between 10 and $8.4x10^2$ cfu/g. The ratio of samples containing *E. coli* was reported as 50% by Coskun (2005); 22.09% by Digrak et al. (2000); 6% by El-Sharef et al. (2006); and 29% by Patir et al. (2006). Erol et al. (1998) isolated *E. coli* from 2% of coliform-containing samples (0.36–2 MPN/g), and Kivanc et al. (1994) isolated *E. coli* (16 –$3.0x10^2$ cfu/g) from 22% of samples. *S. aureus*, *L. monocytogenes,* and *Salmonella* spp. were not detected in any of the 92 ice cream samples tested. Some researchers reported *S. aureus* count in the range of <10 to 10^5 (Erol et al., 1998; Kanbakan et al., 2004; Kivanc et al., 1994; Warke et al., 2000). Baek et al. (2000) isolated *L. monocytogenes* from 6.1% of samples; El-Sharef et al. (2006) from 6% of samples; and Warke et al. (2000) isolated *L. monocytogenes* from 100% of their samples. El-Sharef et al. (2006), Erol et al. (1998), and Korel et al. (2005) reported isolation rates of *Salmonella* spp. as 6%, 4.54%, and 14%, respectively.

Maximum yeast and mold counts in the unpacked ice cream samples tested in this study were $1.5x10^5$ and $1.2x10^3$ cfu/g, respectively. Yeast-mold counts in similar studies were close to or higher than the current findings: Erol et al. (1998) <2.0 x 10^2–10^6 cfu/g; Evrensel and Gunes (1998) $1.0x10^2$–$2.4 x10^4$ cfu/g; Kanbakan et al. (2004) $3.9x10^5$ cfu/g; Kivanc et al. (1994) $4.6x10^4$–$5.5x10^6$ cfu/g; Korel et al. (2005) <10–$3.0x10^4$ cfu/g; and Warke et al. (2000) $4.3x10^3$–$7.4x10^5$ cfu/g. In general, microorganism counts were higher in the unpacked ice cream samples than in the industrially produced ice creams with reference to all microbiological criteria tested (Table 1). The variation analysis on microbiological qualities of the packed and unpacked retail products showed a significant difference ($p<0.001$).

3.2.1. Microbiological quality of plain and flavored ice cream samples

The minimum and maximum microorganism counts of plain and flavored ice cream samples are shown in Table 2. In this study, occurrence of *E. coli* was more frequent in plain ice cream samples (50%) than in flavored ones (26.2%). The *E. coli* count of plain samples was significantly higher ($p<0.05$) than the flavored samples. The reason for this occurrence is considered the lower pH of flavored ice cream. Korel et al. (2005) stated that the average pH of plain ice cream was 6.08, whereas flavored ones had a pH of 3.36. Coşkun (2005) determined the pH range of plain and strawberry ice creams as 6.22–6.52 and 3.2–6.16, respectively. Researcher isolated *E. coli* from 68% of plain and 47% of strawberry ice creams. Patir et al. (2006) reported the range of coliform counts of plain and flavored ice creams from patisseries as $1.0x10^2$–$9.9x10^4$ cfu/g and <1–$5.5x10^5$ cfu/g, respectively. Their *E. coli* isolation rate was higher in coliform-containing plain ice cream, and *Enterobacter* spp. isolation was higher in flavored ones. Flavored ice cream samples from patisseries in the study of Ozcan and Kurdal (1997) had the following minimum and maximum TAMB and coliform counts, respectively: $3.9x10^3$–$5.7x10^5$ cfu/g; $5.0x10^1$–$1.53x10^4$ cfu/g. Although their specified TAMB count was closer to the findings of this study, the coliform count was higher. There was no significant difference in the TAMB, coliform, yeast counts between plain and flavored packed ice cream samples ($p>0.05$) in this study, similar to the results of Bostan and Akin (2002), except the TAMB and *S. aureus* counts. Joshi et al. (2004) reported higher TAMB and coliform counts in plain and flavored ice cream samples than that found in this study. They isolated *Salmonella* spp. from one plain sample. Generally, they stated that flavored packed samples had a higher microbial load. In the study of Korel et al. (2005), packed plain/flavored ice cream samples had the following minimum and maximum TAMB, yeast-mold, and coliform counts, respectively: $2.5x10^2$–$9.9x10^3$ / $2.5x10^2$–$9.9x10^6$ cfu/g, <10 / <10 cfu/g, <30 / <30 cfu/g, and they reported not being able to isolate *Salmonella* spp. Although they determined that the TAMB count in flavored ice cream was higher than that in plain ice cream, similar to the current study, their TAMB count was higher than the values obtained in this study for both types of samples. The findings of this

study showed similarity concerning yeast, mold, and coliform counts. According to our results, the microbiological load of plain ice cream was lower than that of flavored ones. However, a statistically significant difference ($p<0.05$) was only detected between the mold and *E. coli* counts.

Table 2. Minimum and maximum numbers of microorganisms in ice cream samples, grouped according to ice cream types.

Microorganism Count (cfu/g)	Plain ice cream (n = 18)	Flavored ice cream (n = 74)
TAMB	[a] $2.4x10^2 – 7.5x10^3$	$8.1x10^1 – 1.0x10^6$
	[b] $6.1x10^1 – 5.5x10^2$	$2.7x10^1 – 6.9x10^2$
Coliform	$2.0x10^1 – 1.9x10^3$	$<10 – 2.4x10^3$
	<10	<10 – 10
E. coli	$<10 – 8.4x10^2$	<10 – 35
	<10	<10
Yeast	$<10 – 4.7x10^3$	$<10 – 1.5x10^5$
	$<10 – 1.8x10^1$	$<10 – 1.8x10^1$
Mold	<10	$<10 – 1.2x10^3$
	$<10 – 2.0x10^1$	$<10 – 1.4x10^1$

[a] Minimum and maximum numbers of microorganisms in unpacked ice cream samples collected from patisseries.

[b] Minimum and maximum numbers of microorganisms in industrially produced, packed ice cream samples.

3.2.2. Evaluation based on producers

All the industrially produced packed samples tested in this study complied with the TFC (2001) standards. A similar result was observed by Korel et al. (2005). Minimum and maximum microorganism counts of samples from two different factories and three different patisseries are provided in Table 3. There was no significant difference in the microbiological qualities ($p>0.05$) between the samples collected from two factories (producers A and B). Ice cream samples that did not comply with the TFC (2001) norms (n = 22) were plain with 82 % and flavored with 18 %, prepared by the patisseries. Minimum and maximum microorganism counts in incompliant patisserie samples and the permissible limits laid down by the TFC are shown in Table 4. According to TFC (2001), ice creams must not contain *Salmonella* spp. and *L. monocytogenes* (in 25 g) and the acceptable limit of *S. aureus* count, according to the same standards, is <10 cfu/g. All the samples tested conform to the TFC criteria for *Salmonella* spp., *L. monocytogenes,* and *S. aureus*. The ratio of samples produced by C, D and E, which exceeded the TFC limits, were 13.64%, 54.55%, and 31.81%, respectively. Especially, 50% of samples from patisserie D did not

comply with the standards concerning 3–5 microbiological criteria. TAMB, yeast- mold, and coliform counts in the samples from producer D were higher than those in the samples from the other two patisseries ($p<0.05$). There was no significant difference among the three patisseries with reference to *E. coli* counts ($p>0.05$). The mold count in samples of producer E was higher than that in samples of producer C; however, no significant difference could be detected between the samples from producers C and E for other microorganism counts. Many studies on the microbiological quality of ice cream produced under unstandardized conditions by small-scale manufacturers have shown remarkable abundance of samples incompliant with standards in Turkey (Coskun, 2005; Digrak and Özcelik, 1991; Digrak et al., 2000; Erol et al., 1998; Evrensel and Gunes, 1998; Kanbakan and Çon, 1999; Kivanc et al., 1994; Ozcan and Kurdal, 1997; Patir et al., 2006; Sezgin et al., 1997; Yaman et al., 2006). This situation is replicated in the current study.

Table 3. Microbiological quality of ice cream samples according to producer.

Producer	Microorganism count (cfu/g)				
	TAMB	Coliform	*E. coli*	Yeast	Mold
*A (n = 22)	[a]$7.0x10^1$ – [b]$5.5x10^2$	<10	<10	<10 – $1.8x10^1$	<10 – $1.4x10^1$
B (n = 22)	$2.7x10^1$ – $6.9x10^2$	<10 – $1.0x10^1$	<10	<10 – $1.8x10^1$	<10 – $2.0x10^1$
C (n = 14)	$8.1x10^1$ – $2.6x10^3$	<10 – $1.3x10^3$	<10 – $8.4x10^2$	<10 – $9.5x10^1$	<10 – $2.7x10^1$
D (n = 14)	$4.4x10^2$ – $1.0x10^6$	<10 – $2.4x10^3$	<10 – $5.4x10^1$	<10 – $1.4x10^4$	<10 – $1.2x10^3$
E (n = 20)	$1.3x10^2$ – $1.3x10^4$	<10 – $5.5x10^2$	<10 – $3.3x10^1$	<10 – $5.9x10^2$	<10 – $3.2x10^2$

*A and B represent factories; C, D, and E represent patisseries.

[a] Minimum number of microorganisms

[b] Maximum number of microorganisms

Table 4. Unpacked ice cream samples that did not comply with TFC specifications.

Microorganism Count (cfu/g)	Minimum	Maximum	n	%	TFC*	
TAMB	$2.4x10^5$	$1.0x10^6$	2	9.1	[a]$1.0x10^5$	[b]$2.0x10^5$
Coliform	$5.5x10^2$	$2.4x10^3$	13	50.1	95	$4.6x10^2$
E. coli	$1.0x10^1$	$8.4x10^2$	14	63.6	9	9
Yeast	$1.9x10^2$	$1.5x10^5$	5	22.7	-	$1.0x10^3$
Mold	$1.2x10^3$	$1.2x10^3$	8	36.4	-	$1.0x10^2$

* Permissible limits of the Turkish Food Codex (20)

[a] Limits for plain ice cream

[b] Limits for flavored ice cream

4. Conclusion

It is concluded that, the microbiological quality of ice cream produced in patisseries has not shown any improvement from earlier periods to the present day. The poor microbiological quality of ice creams produced in patisseries when compared to industrially produced ice cream is caused by inadequate personnel hygiene, use of unstandardized raw ingredients, unhygienic production process, and retail-storage conditions. The presence of coliforms and *E. coli* in ice creams sold in patisseries may pose a potential risk for public health, especially for vulnerable groups such as children. As a solution, relevant public authorities should inspect these types of manufacturers more frequently and conduct appropriate training programs, such as courses and seminars toward taking necessary precautions. Also, the mandatory use of the HACCP system in ice cream production will increase the quality of the product significantly. On the other hand, consumers prefer ice cream produced in patisseries because of its low cost, and public opinion is in favor of the tastes of these ice creams. Therefore, consumers should be better informed on the subject and be directed to products that are industrially and hygienically produced.

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