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Determination of microbiological properties of milk-based desserts presented for consumption in Ankara, Turkey

Bestimmung der mikrobiologischen Eigenschaften von zum Verzehr angebotenen Desserts auf Milchbasis in Ankara, Türkei

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Summary

Milk-based desserts are a popular consumer product. They are generally preferred over other sugar-based products, especially in summer. However, these popular desserts also constitute a microbiological risk and may result in gastrointestinal disease or food poisoning. The purpose of this study was to determine the microbiological properties of milk-based desserts available for public consumption in Ankara, the capital of Turkey, and to determine their compatibility with the relevant legislation. Fifty-two milk-based desserts sold in Ankara were subjected to microbiological analysis in terms of *Salmonella* spp. and staphylococcal enterotoxin, as required by law – rice pudding (sutlac) (36.54 %), white pudding with a caramelized surface (kazandibi) (32.69 %) and almond pudding (keskul) (30.77 %). The samples were also analyzed in terms of *Escherichia coli* O157, *Escherichia coli, Listeria monocytogenes,* and coagulase positive staphylococci. Although none of the 52 samples contained *Salmonella* spp., *L. monocytogenes, E. coli* O157, coagulase positive staphylococci, or staphylococcal enterotoxin, *E. coli* ($2.1x10^1$ – $1.1x10^4$ MPN/g) was found in 12 samples.

Keywords: Consumption, food, milk-based dessert, health, microbiological, public

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Introduction

Milk-based desserts enjoy a significant place in Turkish cuisine, are easier to prepare and digest than desserts made from dough and syrup, and have a higher nutritional value. The microbiological quality of the major components of milk-based desserts, which are particularly suitable for older people and children, is of considerable importance (Swai & Schoonman, 2011; De Silvaa et al., 2016). Various studies have therefore investigated the microbiological quality of milk-based desserts produced in Turkey (Öksüztepe et al., 2013; Seçim & Uçar, 2014; Gönül, 2017). Numerous local milk-based desserts are consumed, such as sutlac (rice pudding), kazandibi (white pudding with a caramelized surface), keskul (almond pudding) and gum pudding (Sevimli & Sönmezdağ, 2017). The microbiological quality of these desserts is important. Various studies performed concerning the microbiological quality of milk-based desserts produced in different parts of the world (El-Malt et al., 2013; Karthikeyan & Pandiyan, 2013; Łobacz et al., 2016). Poor milk-based dessert quality will impact on public health. The consumption of desserts of unreliable microbiological quality can result in food poisoning and may even be fatal (Öz et al., 2014). The microbiological criteria for milk-based desserts have been set out under national legislation (Turkish Food Codex Regulation on Microbiological Criteria, 2011). The purpose of this study was to determine the microbiological properties of milk-based desserts available for public consumption in the city of Ankara and to measure their compatibility with the relevant legislation.

Material and methods

Material

Fifty-two milk-based dessert samples, 19 sutlac, 17 kazandibi, and 16 keskul, were obtained from 19 patisseries in the Çankaya district of Ankara. One sutlac sample (19 in total) was obtained from each of the 19 patisseries in the Çankaya district. Kazandibi samples (17) were obtained from 17 of the 19 patisseries and keskul samples (16) from 16. The total number of samples subjected to analysis was thus 52. Samples were numbered and coded based on the order in which they were obtained. These samples kept in plastic containers at +4 °C and displayed in glass cases in patisseries were placed into sterile sample containers. They were then transported to the laboratory under cold chain and aseptic conditions for microbiological analysis.

Method

Milk-based dessert samples were analyzed in terms of *Salmonella* spp. and staphylococcal enterotoxin specified in the relevant legislation (Turkish Food Codex Regulation on Microbiological Criteria, 2011) (Table 1). They were also subjected to analysis for coagulase-positive staphylococci, *E. coli* O157, *L. monocytogenes* and *E. coli,* agents of gastrointestinal disease and food-based intoxication.

Weighing of the samples and preparation of dilutions

For *E. coli* and coagulase-positive staphylococci, 1 g and 10 g, respectively, of each sample were initially totally homogenized by addition of 9 mL and 90 mL of sterile peptone saline (EN ISO 6887-1, 2017). For *E. coli,* enough dilution was prepared to ensure that all tubes for the final dilution would yield a negative result. Additionally, for pre-enrichment of *Salmonella* spp. and *L. monocytogenes,* and for enrichment of *E. coli* O157, 25 g of each sample was placed into sterile stomacher bags and homogenized with 225 mL sterile buffered peptone water (Merck) for *Salmonella* spp., 225 mL Half Fraser Broth (LabM) for *L. monocytogenes,* and 225 mL modified Tryptone Soya Broth (mTSB+N, Merck), and with novobiocin for *E. coli* O157. After homogenization, for incubation-pre-enrichment-enrichment processes, they were incubated for *Salmonella* spp., *E. coli* O157, and *L. monocytogenes,* at 34–38 °C for 18±2 h, at 41.5 °C for 6–18 h, and at 30±1 °C for 24–26 h, respectively.

Determination of Salmonella spp. in the samples

The EN ISO 6579-1 method (2017) was used to identify *Salmonella* spp. in the samples. Following pre-enrichment, the samples were incubated at 41.5±1 °C for 24±3 h in Rappaport Vassilliadis Broth (Biokar) and at 37±1 °C for 24±3 h in Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn broth) (Biokar) media. At the end of incubation, they were incubated in Xylose Lysine Deoxycholate (XLD) Agar (Merck) medium at 37±1 °C for 24±3 h. In order to confirm the colonies identified following incubation, black-centered and colorless suspicious colonies growing in the medium were examined for usage of glucose, non-usage of lactose, non-usage of sucrose, gas formation from glucose, formation of H_2S, urea hydrolysis, L-lysine decarboxylase activity, and the indole test. Agglutination control, O antigens, Vi antigens, and H antigens were applied for serological confirmation analysis.

Determination of coagulase-positive staphylococci in the samples

EN ISO 6888-1 (1999) and EN ISO 6888-1:1999/A1 (2003) were used. First, 1 mL of the dilutions prepared was cultured into Baird-Parker Agar medium (Merck) and allowed to incubate at 35±1 °C for 48 ± 2 h. After incubation, typical colonies that were black or grey, shining, convex and surrounded by a clear zone were tested with coagulase for confirmation.

Determination of the presence of staphylococcal enterotoxin in samples

The 3M Tecra Staph Enterotoxin Visual Immunoassay test kit was used for this purpose. Briefly, 10 g of sample was placed into a sterilized stomacher bag, and 20 mL 0.25 M Tris Buffer (Sigma) was then added. The manufacturer's

TABLE 1: *Food safety criteria for all types of ready to eat (cooked) dessert (pudding, cream, ashura, water pudding etc.) in Annex-1 of the Turkish Food Codex Regulation on Microbiological Criteria (2011).*

Food	Microorganisms / toxins / metabolites	Sampling plan[1])		Limits[2])		Reference method[3])
		n	c	m	M	
All types of ready to eat (cooked) dessert (pudding, cream, ashura, water pudding etc.	Staphylococcal enterotoxins	5	0	Should not be present in 25 g		
	Salmonella	5	0	0/25 g -mL		EN ISO 6579

[1]): n: Number of samples; c; number of samples with a value between the m and M limit. [2]): Unless otherwise specified, the limit evaluated is cfu/g-mL. cfu: Colony forming unit (in solid medium). [3]): Recent versions of the standards set out in these regulations were used.

instructions were followed, and the color changes were compared with the colors on the control card to determine the presence of the toxin.

Determination of E. coli in the samples

The most probable number (MPN) technique was used to determine *E. coli* in the samples (ISO 11866-1, 2005). One milliliter from each dilution prepared was placed into three tubes containing single strength Modified Lauryl Sulfate Tryptose Broth (Merck) medium and incubated at 30 °C for 24–48 h ±2 h. After incubation, those tubes showing fluorescence and formation of indole were identified as positive for *E. coli*. The number of *E. coli* per gram of the milk-based dessert sample was determined by the MPN index. Also, the category 1 in the MPN index was selected.

Determination of E. coli O157 in the samples

ISO 16654 (2001) and EN ISO 16654:2001/A1 (2017) were used to determine E. coli O157. Immune-magnetic separation (IMS, LabM) was applied after enrichment. The sample was then allowed to incubate at 37 °C for 18-24 h in MacConkey (CT-SMAC) (Oxoid) agar medium. Indole and serological confirmation analyses were applied to confirm the suspicious, transparent, and light-yellowish brown colonies developing after incubation.

Determination of L. monocytogenes in the samples

The EN ISO 11290-1 method (2017) was used for this purpose. Following pre-enrichment, the sample was placed into Fraser Broth (Biokar) medium, and enrichment was performed at 37±1 °C for 24±2 h. After the enrichment process, the samples were inoculated on Agar Listeria according to Ottaviani and Agosti (Merck) medium and Oxford Listeria Selective Agar medium (Merck), and then incubated at 37±1 °C for 48±2 h. Presumptive *L. monocytogenes* colonies were defined as blue-green colonies surrounded by an opaque halo, on Agar Listeria according to Ottaviani and Agosti medium, and grayish green colonies with collapsed centers, surrounded by a black halo, on Oxford Listeria Selective Agar medium. Biochemical analyses such as "beta-hemolysis, L-rhamnose, D-xylose, the catalase test, Gram staining" and Microbact *L. monocytogenes* identification kit (Oxoid) were used for confirmation of presumptive *L. monocytogenes* colonies.

Results and discussion

Fifty-two milk-based dessert samples (36.54% sutlac, 32.69% kazandibi, and 30.77% keskul) sold in the Çankaya district of Ankara were subjected to microbiological analysis for *Salmonella* spp., coagulase positive staphylococci, staphylococcal enterotoxin, *E. coli* O157, *E. coli* and *L. monocytogenes*. No *E. coli* O157 colonies were determined in any of the samples. No growth of atypical or typical *L. monocytogenes* or coagulase-positive staphylococci colonies was observed on medium. No staphylococcal enterotoxin was also detected. Two black-centered and colorless suspicious *Salmonella* spp. colonies growing on the XLD Agar medium of the sutlac sample from patisserie no. 6 were subjected to biochemical and serological tests following EN ISO 6579-1 (2017). No *Salmonella* spp. were detected in 25 g of this sample.

Cream cake samples sold in different countries have been analyzed in previous studies and shown to be of insufficient microbiological quality (Can & Yalçın, 2011; El-Fadaly et al., 2016; Chaudhari et al., 2017). The microbiological quality of milk-based desserts from different countries has also been evaluated in various studies, which have concluded that insufficient food hygiene may cause diseases in humans (Abdelsamei et al., 2014; Asadia et al., 2015; Hervert et al., 2016; Barros et al., 2017). El-Gendi & El-Shreef (2013) evaluated the microbiological quality of dried mixes of dairy products consumed in large quantities in Egypt. Those researchers reported that *E. coli* O157:H7 and coagulase positive *S. aureus* could not be isolated from any of the samples examined, while *L. monocytogenes* was isolated from chocolate pudding at a rate of 26.7%. Ertaş et al. (2010) determined the presence of *S. aureus* and staphylococcal enterotoxin (SE) genes in milk-based dessert samples using the multiplex PCR (mPCR) technique. The researchers also noted that the presence of *S. aureus* and their SE genes in these desserts may constitute a potential risk to human health. Angelovski et al. (2010) investigated the microbiological quality of cakes and pastries sold directly to consumers in Skopje, Macedonia. All the samples were tested for *S. aureus, Enterobacteriaceae,* aerobic colony count (ACC), *E. coli, Salmonella* spp. and *L. monocytogenes.* High levels of ACC, *Enterobacteriaceae* and *S. aureus* were reported to reflect unsatisfactory hygienic practice during food processing from source to table.

In the present study, *E. coli* was found in 12 samples (23.08%) in quantities ranging between 2.1 x 10^1 and 1.1 x 10^4 MPN/g (Table 2). However, *E. coli* detection in milk-based desserts is not specified in the Turkish Food Codex Regulation on Microbiological Criteria (2011). *E. coli* was detected at 1.1 x 10^4 MPN/g, 1.1 x 10^3 MPN/g, and 1.1 x 10^3 MPN/g in sutlac samples from patisseries no. 1, 6, and 13, respectively, at 1.1 x 10^4 MPN/g, 2.1 x 10^1 MPN/g, and 2.3 x 10^1 MPN/g in kazandibi samples, and at 2.4 x 10^2 MPN/g, 4.3 x 10^1 MPN/g, and 9.3 x 10^1 MPN/g in keskul samples. *E. coli* was also detected in sutlac samples from patisseries no. 18 and 19, respectively, at 1.1 x 10^4 MPN/g and 2.4 x 10^2 MPN/g. Since kazandibi and keskul were not available in these patisseries, samples could not be obtained. *E. coli* was also found at 2.4 x 10^2 MPN/g in kazandibi from patisserie no. 17 *E. coli.* However, *E. coli* was not detected in sutlac from that patisserie. Keskul was also not available in that patisserie, and no sample could therefore be obtained.

TABLE 2: *E. coli counts in milk-based dessert samples (MPN/g).*

Number	Samples of Milk-Based Dessert	*E. coli*
1.	S1	1.1x10^4
2.	S6	1.1x10^3
3.	S13	1.1x10^3
4.	S18	1.1x10^4
5.	S19	2.4x10^2
6.	K1	1.1x10^4
7.	K6	2.1x10^1
8.	K13	2.3x10^1
9.	K17	2.4x10^2
10.	E1	2.4x10^2
11.	E6	4.3x10^1
12.	E13	9.3x10^1

S: Sutlac, K: Kazandibi, E: Keskul; MPN: Most Probable Number

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The findings of the present study suggest the presence of inadequate personnel and/or equipment hygiene, and even cross-contamination during the preparation and particularly the sale of milk-dessert products prepared in patisseries 1, 6, 13, 18, and 19. It also concludes that insufficient personnel and equipment hygiene conditions were established in the preparation and readiness for consumption of kazandibi in patisserie no. 17. Otherwise, the milk-based dessert samples in this study complied with the Turkish Food Codex Regulation on Microbiological Criteria (2011).

The presence of *E. coli* in 12 samples showed that hygiene conditions were inadequate, or that enough attention was not paid to hygiene during the production and sales of these products. Alişarli et al. (2003) subjected pudding samples from five different patisseries in the city of Van, Turkey to microbiological analysis. Their findings were consistent with those of the present study and indicated inadequate hygiene conditions or lack of proper attention to hygiene. Another study evaluated the bacteriological quality of ice cream sold in different areas of the town of Gilgit, with *E. coli* (highest) and *Salmonella* spp. (lowest) being isolated (Khalil et al., 2009).

Conclusions

In conclusion, hygiene rules during production and sale in Turkey are either insufficient, or else the requisite attention is not paid to hygiene. Continuous and effective control must therefore be established at production sites and during the sales of these desserts. Care is also required in terms of staff hygiene, and training on the subject should be provided. Additionally, in order to ensure safety, it is essential that the public and the industry be advised to pay close attention to several key points from preparation to consumption of milk-based desserts. Emphasis must also be placed on the introduction of HACCP in milk-processing establishments in terms of food safety.

Conflict of interest

Pınar Kaynar declares that she has no conflict of interest.

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