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Microbiological Examination of Meatball, Cream Cake and Turkish Delight (Lokum)

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Abstract: The aim of the present study was to evaluate, the incidence of some pathogen and hygienic microorganism in meatball, cream cake and Turkish delight samples. A total 63 sample (25 raw meatball, 17 cream cake and 21 Turkish delight samples) were analyzed. For this aim, drop and spread method were applied on to specific media. As a result, *Salmonella* sp. were present in meatball and *B. cereus* was found in cream cake samples. Coagulase positive staphylococci were present meatball samples up to 10^4 cfu g^{-1} levels. Sulphite reducing bacteria were not detected in any samples analyzed. Meatball samples may be potential risk for staphylococcal intoxication to consumer during shelf-life. Cream cake samples could be potential risk for *B. cereus*. Additionally, analyzed samples were also contaminated with other enteric and spoilage bacteria. Majority of Turkish delight samples were of satisfactory/acceptable microbiological quality. Contrast to Turkish delight, microbiological qualities of the meatball and cream cake samples analyzed were unsatisfactory and the product could be cause of food poisoning. To minimize contamination, GHP or GMP and HACCP systems could be applied to the control of the pathogenic and spoilage bacteria at all stages of manufacture, storage, transport and retail step.

Key words: Cream-cake, meatball, Turkish delight, *Salmonella*, *B. cereus*, Coagulase positive staphylococci, *micrococci/staphylococci*, sulphite reducing bacteria

INTRODUCTION

In the past, people used to preparing own meal at home and consumed freshly. Today, however, especially in developing and developed country, eating habitants of the people have changed according to technological developing in food sectors. There have been many kind of Ready to Eat Food (RTE) or semi-ready meat products at the supermarkets. Meatball a raw food products of animal origin forms a significant portion of the diet of the Turkish people. In Turkey, people are consumed meat products at the rate of around 11% of produced meat and increasing consumer demands for RTE and semi-ready meat products prepared from fresh ground beef such as meatball. Increase in demand for meat and semi-ready meat products without the infrastructure for proper sanitary handling may lead to transfer of pathogenic organisms from the animals to consumer.

Like meatball, cream cake is an excellent growth medium for many kinds of microorganisms, as it provides rich nutrient for microorganism, is high in moisture and has neutral pH. The water activity (a_w) of cream cake is high (a_w>85). The ingredients of cream cake such as chocolate, fruit, milk and butter cream has also perishable food (Pyler, 1988). So, these kinds of ready and semi-ready to food products and cream cake are not shelf stable and pose a potential public health risk if subjected to temperature abuse at any stage of the product's production, storage, distribution and marketing as well as production under unhygienic condition.

Staphylococcal food poising is caused by the ingestion of food that contains one or more preformed toxins produced by *S. aureus* (Stewart *et al*., 2003). In outbreaks associated with cream-filled baked goods such as custard-filled puffs and Boston cream pies, it is almost always the cream or custard filling that is the contaminated portion of the food, since these fillings are excellent microbial growth monstrous. Foodborne outbreaks associated with cream-filled baked goods are attributed primarily to contamination by food handlers followed by inadequate refrigeration during manufacture and/or storage (Bryan, 1976).

Turkish delight (lokum) is a confection made from starch and sugar. In briefly, Turkish delight is produced

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by mixing sugar, corn starch and water in certain fractions and by adding fruits or nuts to the mixture. This mixture is heated for a certain time at a certain temperature in an open vessel or steam jacketed tank with agitator. Heating time is changed from 1-2 h according to the vessel's type. Hot lokum fluid is dripped onto a wooden table or steel tray, which has some starch on it, with lower edges and after sprinkling some starch lokum is cut into small pieces. Powdered sugar is added on these lokum pieces and they are stored and sold (Batu and Kirmaci, 2009).

Turkish delight, cream pastry and meatball are generally eaten from child, young and old peoples in the Turkey and other part of the world. There has not been any data for microbiological quality of Turkish delight (lokum). To ensure of microbiological quality of them is very important to public health. Therefore, this study investigated the microbiological qualities of meatball, cream cake and Turkish delight from catering establishments and supermarkets.

MATERIALS AND METHODS

A total of 63 samples (25 raw meatball, 17 cream cake and 21 Turkish delight samples) were purchased from different catering establishments and supermarkets in Samsun and were immediately analyzed for the presence of microorganisms.

To determine the microbial counts of samples, the Aerobe Plate Count (APC), lactobacilli, micrococci/ staphylococci, Coagulase Positive Staphylococci (CPS), Enterobacteriaceae, coliforms, enterococci and mould/ yeast counts were obtained by the drop plating technique, *Pseudomonas* sp., *B. cereus* and sulphite-reducing anaerobic bacterial counts were carried out using the spread plating technique.

For the enumeration methods, 10 g of each of the samples was weight aseptically into a sterile stomacher bags, diluted with 90 mL Peptone Water (PW) (Oxoid CM 9, UK) except for *Salmonella*. The sample was then homogenized in a Stomacher (Interscience-Bag Mixer 400) for up to 2 min. Further, ten-fold serial dilutions were made as required using 1 mL of the homogenate and 9 mL of 0.1% PW and inoculated onto specific culture media for total Aerobic Plate Count (APC), Lactobacilli (LAB), *micrococci/staphylococci* (MS), CPS (Baird Parker Medium), Enterobacteriaceae (EB), Coliforms (CB), Enterococci (ENB), Sulphite Reducing Anaerobic Bacterial counts (SRAB) and Moulds/Yeasts (MY). For the isolation of *Pseudomonas* sp, oxidase tests were done and oxidase positive colonies grown on CFC (*Pseudomonas* agar base) media plates were presumed positive for *Pseudomonas* sp. Enumeration of *B. cereus* (BS); the colonies suspected of being *B. cereus* onto CSM (Cereus selective agar) were selected and identified by gram stained. The culture media and incubation conditions are shown in Table 1.

For the isolation of CPS, up to 5 typical colonies (black or grey colonies) grown on BP agar were selected and transferred to tubes contained Brain Heart Infusion Broth (BHI-Oxoid CM 225, UK). The tubes were incubated at 37°C for 24 h. After the incubation, coagulase tests were done (Thatcher and Clark, 1978). For the isolation of *E. coli*, one presumptive colony on Violet red bile lactose agar (VL) was selected and directly streaked onto Endo Agar Base and incubated for up to 48 h at 37°C. One suspected of being *E. coli* on the EA was selected and identified by Indole, Methyl red, Voges Proskauer and Simmons citrate tests (IMViC tests).

Isolation of *Salmonella* sp.: The isolation of *Salmonella* sp. was carried out in two enrichment steps; 25 g of each sample was aseptically transferred into sterile plastic bags containing 225 mL Buffered Peptone Water (BPW), homogenized for 1-2 min and then incubated at 37°C for 24 h. Following incubation, 0.1 mL of each BPW incubated was transferred into culture tubes containing 10 mL of Rappaport Vassiliadis *et al.* (1978) enrichment broth and incubated again at 43°C for 24. The culture was then streaked onto brilliant green (modified) agar plates and incubated for 24-48 h at 37°C. The colonies suspected of being *Salmonella* sp. were selected and identified by gram staining and standard biochemical tests (triple sugar iron agar, lysine iron agar, urease test and Simmons citrate) and was confirmed with *Salmonella antiserum* (O and H-Vi *Polyvalan antiserum*, Difco 2264-47-2).

RESULTS AND DISCUSSION

The microbiological analyses of all the samples are shown in Table 2. According to analysis, 88% of the meatball and 35.4% of the cream cake samples were found have APC at level of $\geq 10^6$ cfu g^{-1}, respectively. In one Turkish delight sample contained APC at level of 10^5 cfu g^{-1}. Two (8%) meatball and one (5.9%) cream cake sample contained 10^6 cfu g^{-1} lactobacilli. *Pseudomonas* sp. were detected in 2 (8%) the meatball samples at level of 10^3 cfu g^{-1}. Enterobacteriaceae and coliforms were present in 8 and 11.8% of the meatball and cream cake samples, respectively at level of $>10^4$ cfu g^{-1}. In the Turkish delight samples, while Enterobacteriaceae was detected in 2 (9.5%) samples at 10^5 cfu g^{-1} level, coliforms were not detected. Enterococci were detected in 1 (4.7%) of Turkish delight, 2 (8%) of meatball and 1(5.9%) of cream

Table 1: The mediums used for the microbiological analysis and incubation conditions

Microorganisms	Medium	Incubation conditions		Conditions anaerobic/ aerobic
		Temperature (°C)	Time (h)	
Total aerobic plate count	Plate Count Agar (CM509)	30	24-72	Aerobic
micrococci/staphylococci	Baird-Parker Agar (CM275)	37	24-48	Aerobic
Lactobacilli	MRS Agar (CM361)	30	24-72	Anaerobic
Enterobacteriacea	Violet Red Bile Glucose Agar (CM485)	37	24-48	Anaerobic
Coliform	Violet Red Bile Agar (CM107)	37	24-48	Anaerobic
E. coli	Endo Agar Base (Food Hygiene and TechnologyCM479)	-	-	-
	MR-VP Agar (CM0043)	-	-	-
	Cimmon citrate Agar	-	-	-
	Tryptone Water	37	24-48	Aerobic
Enterococci	Slanetz and Bartley Medium (CM377)	37	24-48	Aerobic
Pseudomonas sp.	*Pseudomonas* agar Base (CM559, SR103, BR0064A)	30	24-48	Aerobic
B. cereus	*Bacillus cereus* agar base (CM617)	30	24-48	Aerobic
Mould/Yeast	Rose Bengal Chloramphenicol Agar (CM549)	25	2-5 day	Aerobic
Sulphite-reducing anaerobic bacteria	Perfringens agar base (CM 543, A-SR 76, B SR 77, BR38)	37	24-48	Anaerobic
Salmonella sp.	Buffered Pepetone Water (BPW) (CM509),	37	24	Aerobic
	Rappaport-Vassiliadis (RV) Enrichment Broth (CM669)	43	24	-
	Brilliant Green Agar (Modified) (CM 329, Sulphamandelate supll., SR87),	37	24-48	-
	Triple Sugar Iron Agar (CM277),	37	24-48	-
	Lysine Iron Agar (CM381)	37	24-48	-

*All media were used Oxoid, Basingstoke, UK)

Table 2: The results of the microbiological examination of food samples (Log_{10} cfu g^{-1})

No. of Samples	Level of microorganisms (log)	APC		LAB		MS		CPS		EB		CB		EnC		CFC		M/Y		BC		SRB		*E. coli*	*Salmonella* sp.
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Meatball	<2	0	0.0	0	0.0	0	0.0	19	76.0	0	0.0	0	0.0	0	0.0	21	84.0	8	32.0	25	100.0)	25	100.0	Negative	Positive
(n = 25)	2	0	0.0	13	52.0	3	12.0	1	4.0	11	44.0	11	44.0	4	16.0	2	8.0	0	0.0	0	0.0	0	0.0	(n = 1)	(4%)
	3	0	0.0	2	8.0	2	8.0	0	0.0	10	40.0	10	40.0	13	52.0	2	8.0	7	28.0	0	0.0	0	0.0		
	4	0	0.0	4	16.0	17	68.0	5	20.0	2	8.0	2	8.0	6	24.0	0	0.0	6	24.0	0	0.0	0	0.0		
	5	3	12.0	4	16.0	3	12.0	0	0.0	2	8.0	2	8.0	2	8.0	0	0.0	2	8.0	0	0.0	0	0.0		
	6	10	40.0	2	8.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	8.0	0	0.0	0	0.0		
	7	6	24.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		
	8	5	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		
	9	1	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		
Cream	<2	0	0.0	0	0.0	0	0.0	16	94.1	0	0.0	0	0.0	0	0.0	100	0.0	4	23.5	6	35.2	17	100.0	Negative	Negative
cake	2	0	0.0	12	70.6	5	29.5	1	5.9	13	76.4	12	70.6	5	29.5	0	0.0	8	47.1	10	59.0	0	0.0		
(n = 17)	3	0	0.0	2	11.8	7	41.3	0	0.0	2	11.8	3	17.6	8	47.1	0	0.0	4	23.5	1	5.9	0	0.0		
	4	5	29.5	2	11.8	1	5.9	0	0.0	0	0.0	0	0.0	3	17.6	0	0.0	1	5.9	0	0.0	0	0.0		
	5	6	35.2	0	0.0	3	17.6	0	0.0	2	11.8	2	11.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		
	6	5	29.5	1	5.9	1	5.9	0	0.0	0	0.0	0	0.0	1	5.9	0	0.0	0	0.0	0	0.0	0	0.0		
	7	1	5.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		
Turkish	<2	0	0.0	7	33.4	0	0.0	21	100.0	0	0.0	21	100.0	0	0.0	21	100.0	20	95.3	21	100.0	21	100.0	Negative	Negative
delight	2	8	37.3	14	66.6	20	95.3	0	0.0	16	76.3	0	0.0	19	90.6	0	0.0	1	4.7	0	0.0	0	0.0		
(n = 21)	3	9	42.8	0	0.0	1	4.7	0	0.0	1	4.7	0	0.0	1	4.7	0	0.0	0	0.0	0	0.0	0	0.0		
	4	3	14.2	0	0.0	0	0.0	0	0.0	2	9.5	0	0.0	1	4.7	0	0.0	0	0.0	0	0.0	0	0.0		
	5	1	4.7	0	0.0	0	0.0	0	0.0	2	9.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		

APC: Aerobe Plate Count, LAB: Lactobacilli, MS: *Micrococci\Staphylococci*, EB: Enterobacteriaceae, CB: Coliform Bacteria, EnC: Enterococ, PS: *Pseudomonas* sp., M/Y: Mould and Yeast, BC: *B. cereus*, SRB: Sulphite Reducing Bacteria

cake samples at 10^4, 10^5, 10^6 cfu g^{-1} levels, respectively. *micrococci/staphylococci* were found in one (4.7%) Turkish delight samples at level of 10^3 cfu g^{-1}, while they were detected in 3 (12.0%) meatball and 4 (23.5%) cream cake samples at levels of $>10^4$ cfu g^{-1}, respectively. CPS were detected in 5 (20%) of meatball and one (5.9%) of cream cake samples at levels of 10^4 and 10^2 cfu g^{-} ,^{1} respectively. Although, *B. cereus* was detected in 10 (59.0%) and one (5.9%) cream cake samples at level of 10^2 and 10^3 cfu g^{-1}, respectively, it was not detected in meatball and Turkish delight samples. *Salmonella* sp. was found only one (4%) meatball sample. However, *E. coli* and sulphite-reducing anaerobic bacteria were not detected in any of the samples.

In the Turkish delight samples, one (4.7%) sample contained APC at level of 10^5 cfu g^{-} .^{1} While, Enterobacteriaceae was detected in 4 (19%) of the samples between at 10^4 and 10^5 cfu g^{-1} levels, coliforms and *E. coli* were not detected in any of the samples. In this respect, Turkish delight samples analyzed were found to be above permitted limit in Turkish Food Codex Regulations for APC, coliform, yeast/mould counts; they were suitable for *E. coli.* This study results has shown that majority of Turkish delight samples were of satisfactory/acceptable microbiological quality according to Turkish regulation. However, it is not same for the meatball. Hence, according to Turkish Regulation for meatball, APC, CPS, yeast/ moulds and *Salmonella* sp. have to be 10^5-10^6, 10^2-10^3, 10^3-10^4 cfu g^{-1} levels and absent, respectively. For his respect, contrast to Turkish delight samples, 12 (48%) of the samples for APC, 5 (20%) of the samples for CPS, 4 (16%) of the samples for yeast/moulds and one (4%) of the samples for *Salmonella* sp. were above permitted level of Turkish Food Codex Regulation.

For raw meat products such as meatball, potential safety and quality can be estimated with the use of indicator microorganisms. Generally, higher APC usually relates to poorer quality and a reduced shelf-life. It is also, known that coliform and *E. coli* counts provide an estimate of fecal contamination and poor sanitation during processing. In this respect, the present study, APC was found to be 10^5 cfu g^{-1} in 3 (12%) meatball and $>10^6$ cfu g^{-1} in 12 (48%) meatball samples. Both coliform and Enterobacteriaceae were detected between 10^4 and 10^5 cfu g^{-1} in 4 (16%) of the meatball samples. In the cream cake samples, APC was detected in 10^5-10^7 cfu g^{-1} levels in 12 (70.6%) of the samples; both total coliform and Enterobacteriaceae were found to be 10^5 cfu g^{-1} in 2 (11.8%) of the samples. For Turkish delight, APC and Enterobacteriaceae were detected in 10^5 cfu g^{-1} level in 1 (4.7%) and 2 (9.5%) of the samples, respectively. However, *E. coli* was not detected in all the samples. According to these results, hygienic quality of the most of meatball and cream cake samples is low. In this study, *Pseudomonas* was found at 10^3 level in 2 (8%) of the meatball samples. It was not detected in cream cake and Turkish delight samples. It was not high level and not affect on shorting of shelf life of meatball samples.

Raw ingredients used to prepare meatball, cream cake and other products may introduce some foodborne pathogen bacteria. For meatball, one of the main contamination sources may be ground beef. Several studies have indicated that *Salmonella* sp. are present in beef carcasses (median of 3.3%) (Roberts *et al*., 1980; Sofos *et al*., 1999). The prevalence of *Salmonella* sp. in ground beef is also considerably higher (median of 9.7%) (Erol, 1999; Heredia *et al*., 2001; Zhao *et al*., 2002; Siriken, 2004). In the present study, *Salmonella* sp. was not detected in Turkish delight and cream cake samples. However, it was detected in 4% of the meatball samples. Similar result was obtained from Yilmaz *et al*. (2002). They reported that it was detected in only one meatball (raw) samples. They also, reported that no *C. perfringens* was found.

There have been several studies for *B. cereus* in foods (Van Netten *et al*., 1960; Wong *et al*., 1988) and researchers reported that milk products and other foods are potential risk for *B. cereus*. For instance, Wong *et al*. (1988), reported that *B. cereus* was isolated from fermented milk, ice cream, pasteurized milk and milk powder. Van Netten *et al*. (1960) also, reported that *B. cereus* was isolated from 19%, 11% cream cake and 8% of pasteurized milk at levels of 2 and >5 log/g mL. In the present study, *B. cereus* was detected in 11 cream cake samples. According to above study results, milk and meat products could be potential risk for *B. cereus*. According to the present study result for *B. cereus*, cream cake samples could be potential risk, too.

Another spore-forming bacterium is *C. perfringens*. In the present study, we could not detected it any of analyzed samples. So, we can say that the samples analysed in this study were safety for *C. perfringens*. *C. perfringens* food poising that the contaminated food is almost always heat-treated, which kills competing flora while, the *C. perfringens* spores survive (Andersson *et al*., 1995). *C. perfringens* is then frequently the dominating flora, sometimes accompanied by other spore formers such as *B. cereus*. This theory may be explained, why it could not isolated from meatball in the present study.

Several investigators have reported on the prevalence of *S. aureus* in cream-filled baked goods from different country (Escartin *et al*., 1998). From Nebraska, Sumner *et al*. (1993) reported isolating *S. aureus* from 30%

cream puffs and 11.1% of long johns obtained from local bakeries. It is reported that *S. aureus* in 0.5% of desserts and cakes examined in the United Kingdom. From Turkey, Erol *et al.* (1996) reported that CPS were found at the mean of 10^2-10^3 cfu g^{-1} in cream cake samples. This result is similar the present study and CPS was detected in only one (5.9%) cream cake samples at level of 10^2 cfu g^{-1}. In meatball, it was detected in 5 (20%) samples at level of 10^4 cfu g^{-1}. However, Food and Drug Administration has established that staphylococcal toxin dose of <1.0 μg in contaminated food will produce symptoms of staphylococcal intoxication and that this toxin level is reached when *S. aureus* populations exceed 10^5 cfu g^{-1}. This limit was not exceeded in any of the analyzed samples. Another study from Turkey, Aycicek *et al.* (2005) reported that *S. aureus* was detected in 17 out of 144 samples at levels of 3.7-4.1 log. Contrast to Turkish delight and cream cake, meatball samples may be potential risk for staphylococcal intoxication to consumer during shell-life. Therefore, the maximum level of CPS limited by Turkish Food Codex Regulation is 10^2-10^3 cfu g^{-1} in meatball samples. So, according to this regulation, although 20% of meatball samples are not suitable and they are considered as non-consumable products.

Foodborne outbreaks due to *S. aureus* contamination of bakery product from United States (1962), United Kingdom/Wales 1969-1972, France 1969, Sheffield, UK, Caribbean Cruise Ship 1983, Spain (NK), Brazil 1994, Thailand 1990 and Mexico 1984 that 2-1800 cases poised 10^6-10^9 cfu g^{-1} levels of *S. aureus*. It was also, reported *S. aureus* toxin type (Stewart *et al*., 2003). From Turkey, Kisa *et al*. (1996) reported that among 96 coagulase-positive staphylococci isolates, isolated from 25 (26.0%) cream cake, contained enterotoxigenic CPS. Now-a-days, in Turkey, butter cream have to be pasteurized before selling and the use of raw butter cream for production of cream cake is forbidden. So, the study results of cream cake may be confirmed to pasteurized butter cream was used for cream cake production.

The food categories related to the system used by FSAI to relate different food types to the expected aerobic colony counts, *S. aureus*, *E. coli*, *Salmonella* sp. and *B. cereus* levels (Gilbert *et al*., 2000).

For example, cakes, pastries, slices and deserts-with dairy cream were placed into food category 3 where there were ACC and *B. cereus* colony counts limits were ≥10^6 for APC and ≥10^5 cfu g^{-1} and these limits were unacceptable. According to same researchers, 10^4 and <10^5cfu g^{-1} for *B. cereus* limits were unsatisfactory. A 10^5 and <10^6 cfu g^{-1} limits for APC and 10^3 and <10^4 cfu g^{-1} limits for *B. cereus* were acceptable and <10^5 cfu g^{-1} limits for APC and <10^3 cfu g^{-1} limits for *B. cereus* were satisfactory. In this respect, 6 (35.4%) cream cakes samples analyzed were unsatisfactory, 6 (35.2) samples were acceptable, 5 (29.5%) samples were satisfactory for APC. While, 16 (94.2%) cream cake samples were satisfactory and only one (5.9%) sample was acceptable for *B. cereus*, there were no unacceptable samples for *B. cereus*.

CONCLUSION

The present study demonstrated that *Salmonella* sp. were present in meatball and *B. cereus* was found in cream cake samples. Coagulase positive staphylococci were present meatball samples up to 10^4 cfu g^{-1} levels. So, meatball samples may be potential risk for staphylococcal intoxication to consumer during shell-life. Additionally, analyzed samples were also, contaminated with other enteric and spoilage bacteria. These results indicate that, the microbiological qualities of the meatball and cream cake samples analyzed were unsatisfactory and in contrast to Turkish delight, the product could be cause of food poisoning. To minimize contamination, GHP or GMP and HACCP systems that are specific to the control of the pathogenic and spoilage bacteria at all stages of manufacture, storage, transport and retail could be applied.

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