

Araştırma Makalesi

THE PRESENCE OF *STAPHYLOCOCCUS AUREUS* AND STAPHYLOCOCCAL ENTEROTOXINS IN READY-TO-COOK MEATBALLS AND WHITE PICKLED CHEESE

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Hazır Köfte ve Beyaz Peynirlerde *Staphylococcus aureus* ve Stafilokokkal Enterotoksinlerin Varlığı

Özet : Bu çalışmada *Staphylococcus aureus* yönünden riskli gıdalar arasında yer alan hazır köftelerde (30 adet) ve beyaz peynirlerde (30 adet) ELISA tekniği ile stafilokokkal enterotoksinlerin (SE) varlığı araştırılmıştır. Çalışma materyali İstanbul'daki çeşitli satış noktalarından sağlanmıştır. Örneklerde aynı zamanda toplam *Staphylococcus aureus* sayısı, DNase pozitif *Staphylococcus aureus* sayısı ve termonükleaz aktivitesi de belirlenmiştir. Yapılan analizler sonucunda hazır köfte örneklerinde $3.0 \times 10^1 - 5.2 \times 10^4$ kob/g; beyaz peynir örneklerinde $<10 - 9.2 \times 10^3$ kob/g arasında *Staphylococcus aureus* saptanmıştır. Hazır köftelerin 9'unda, beyaz peynirlerin 11'inde DNase pozitif bakteri üremesi kaydedilmemiştir. Buna rağmen, her iki gıda grubuna ait örneklerin hiçbirinde SE saptanmamış ve tamamında termonükleaz testi negatif sonuç vermiştir. *Staphylococcus aureus* sayısı bakımından peynir örneklerinin % 63'ü, hazır köfte örneklerinin % 43'ü Türk Gıda Kodeksi Yönetmeliği'ne uygunluk göstermemektedir. Bu çalışmada elde edilen bulgular; incelenen köfte ve peynir örneklerinin mikrobiyolojik kalitesi düşük olmakla birlikte stafilokokkal enterotoksinlerin bulundurmadığını göstermektedir.

Anahtar Kelimeler: *Staphylococcus aureus*, enterotoksin, peynir, köfte

Summary: In this study 30 ready-to-cook meatball and 30 white pickled cheese samples, obtained from different sales outlets in Istanbul, were analysed for the presence of staphylococcal enterotoxins (SE) with the aid of the ELISA technique. Additionally, total *Staphylococcus aureus* count, DNase positive *Staphylococcus aureus* count and thermonuclease activity were determined. The meatball samples were found to contain between 3.0×10^1 and 5.2×10^4 cfu/g of *Staphylococcus aureus* while in the white cheese samples, the count varied between $<10 - 9.2 \times 10^3$ cfu/g. In 9 meatball samples and 11 white cheese samples, no DNase positive bacteria growth could be detected. Despite these results, SE could not be identified in any of the samples of both food groups, and all samples gave negative results in the thermonuclease test. With respect to *Staphylococcus aureus* count, 63% of the white cheese samples and 43% of the meatball samples did not comply with Turkish Food Codex. The findings of

this study indicate a low microbiological quality of the analysed meatballs and white cheese samples. However, none of the samples contained staphylococcal enterotoxins.

Keywords: *Staphylococcus aureus*, enterotoxin, cheese, meatball

Introduction

Staphylococcus aureus is one of the bacteria that produce toxins in foods. Staphylococcal enterotoxins are serologically classified are seven groups (SEA, SEB, SEC1, SEC2, SEC3, SED, SEE). Type A and type D are especially responsible for food (1, 8). This organism is salt-tolerant and able to grow under a wide range of conditions; low acid production may allow staphylococci grow and produce enterotoxin (1). The presence of 100 ng toxin in food results in clinical symptoms (4). Bacteria counts of $10^5 - 10^6$ /g are required before their enterotoxins can cause food-related poisoning. The toxin in food is not destroyed by pasteurisation, cooking or any other simple heat treatment, and is not affected by digestive enzymes. Their stability is an important reason for frequent staphylococcal food poisoning. Not all *Staphylococcus aureus* strains are enterotoxigenic. However, all food products with a pH higher than 5.0 and an a_w higher than 0.86 support growth of *Staphylococcus aureus* and the production of toxins. For raw meat products and cheese, rather high levels of *Staphylococcus aureus* and staphylococcal enterotoxins have been detected in microbiological analyses (15, 19, 21, 22, 23). Wieneke et al. (28) reported that the most frequently implicated foods in staphylococcal food poisoning cases were meat and meat products (75%), fish and shellfish (7%) and milk and milk product (8%). Detection of *Staphylococcus aureus* in food by culture methods are not sufficient to prove of the safety of the food in question with respect to enterotoxins. Technological procedures applied to foods can eliminate the present bacteria, but the heat resistant toxins produced by these bacteria may still be present in the food. In addition to that, some staphylococci strains, which have no activities considered as a criteria for toxigenicity such as coagulase and thermonuclease activities are also known to produce enterotoxins (26). For these reasons, suspected foods should be analysed for the presence of enterotoxins. Various methods are employed for staphylococcal enterotoxins determination in foods. One of the most simple and sensitive is the ELISA technique which allows the detection of microbial toxins in concentrations in the ng/kg range (12, 14, 16, 17). This research was undertaken determining the presence of staphylococcal enterotoxins in ground meat products (ready-to-cook meatballs) and white pickled cheese with the aid of the ELISA technique, as well as carrying out a *Staphylococcus aureus* count and determining the thermonuclease activity in order to establish the connection with the presence of toxins.

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Material and Methods

Materials

In this study a total of 30 ready-to-cook meatballs and 30 samples of white pickled cheese marketed without packaging were analysed. The samples were collected from various markets and other sales outlets in Istanbul periodically from May through August 2001. All the samples were placed in sterile plastic bags and transported to the laboratory at low temperature (<7 °C) and stored at 4 °C, until testing.

Microbiological analyses

A 10 g portion of each sample was homogenised 1:10 (w/v) with sterile 0.1 % peptone water for 2 min in Stomacher Lab-Blender (Seward). Serial 10-fold dilutions were made as required and inoculated in appropriate growth media (9).

Staphylococcus aureus count was determined on Baird Parker Agar (BP; Oxoid, CM 275) supplemented egg yolk and tellurite. The plates were incubated at 37 °C for 24 h. Colonies with typical black appearance and surrounded by clear zone were enumerated as *Staphylococcus aureus*. For DNase positive *Staphylococcus aureus* counts, 10 representative colonies in BP were transferred with a loop onto DNase Agar (MERCK, 1.10449). After incubation for 18-24 hrs at 37°C, 1 N HCl solution was poured over the colonies. Formation of a clear zones around colonies when hydrolysis occurred, was considered an evidence of DNase activity (3, 9).

Thermonuclease test

For the identification of thermonuclease positive *Staphylococcus aureus* in the samples, the Ridascreen *Staphylococcus aureus* test kit (Biopharm, R4001) was used. To prepare the extract, 20 g of the sample was homogenized together with 5 g of fat-free and thermonuclease-free milk powder in 50 ml of distilled water. The pH was adjusted to 3.8 with 1N HCl and the mixture was then centrifuged for 35 min. After centrifugation, the supernatant was separated and 2 ml of cold 3 M trichloro acetic acid were added. It was centrifuged again and the supernatant was removed. To the pellet 1ml of tris buffer was added and the pH adjusted to 8.5 with 2 N NaOH. Then tris buffer was added to achieve a total volume of 2 ml. After 15 min holding in a water bath at 100°C the sample was ready for analysis. An agar plate with pairs of wells was used for the test. Into those wells – opposite of each other – 10 µl each of sample extract and antibody solution were added. After covering the agar plate it was incubated for 4 hrs at 37°C. Formation of a pink plaque around the wells was considered that sample was thermonuclease positive.

Detection of staphylococcal enterotoxins

Presence of staphylococcal enterotoxins (A, B, C, D, E) were determined qualitatively by ELISA-based Transia Plate Staphylococcal Enterotoxin Kit (Diffchamb, ST0796). Detection limit of this test kit was 0.25 ng/g food. To prepare the extract, 20 ml of distilled water were added to 20 g of the sample. The mixture was homogenized and settled the toxin to diffuse. After 20 min, it was filtered to eliminate food particle. The pH of the aqueous phase was then adjusted to 7.0-7.5. In the case of the meat samples, raw meat extract solution 1 (Diffchamb, AK0220) and 2 (Diffchamb, AK0220) were added to the extract following the manufacturer's instructions. The test was performed with 100 µl of extract. For the test, a sufficient amount of negative control culture supernatant, positive control supernatant and sample extract were added into the plate wells. The plate was left at room temperature for 30 min and then was washed 5 times in a washer (ELX50, Auto Strip Washer, BIO-TEK). A one hundred µl of conjugate were added to the wells and the plate left at room temperature for 30 min. It was washed again 5 times. In the subsequent step, 100 µl of the substrate/chromogen mixture were added and the plate again left at room temperature for 30 min. Finally 50 µl of stop solution were added into each well. The optical densities were measured at 450 nm with a plate reader (ELX800, Universal Microplate Reader, BIO-TEK).

Results

Staphylococcus aureus was present in the all ready-to-cook meatball samples analyzed with an average of 9.6×10^3 cfu/g (Table 1). The count of DNase positive bacteria among them was found to be 5.2×10^3 cfu/g on average (Table 2). In thirteen of the samples (43%), the *Staphylococcus aureus* count exceeded 5.0×10^3 cfu/g. Although *Staphylococcus aureus* was detected in all samples, DNase positive *Staphylococcus aureus* was not detected in 9 samples (30%).

Table 1: *Staphylococcus aureus* counts in meatballs and white pickled cheese (cfu/g)

Table 1: Köfte ve beyaz peynirlerde *Staphylococcus aureus* sayısı (kob/g)

Food Group	n	Lowest (n)	Highest	Average
Ready-to-cook meatballs	30	3.0×10^1	5.2×10^4	9.6×10^3
White cheese	30	<10 (9)	9.2×10^3	1.6×10^3

Table 2: DNase positive *Staphylococcus aureus* counts in meatballs and white cheese (cfu/g)

Table 2: Köfte ve beyaz peynirlerde DNase positive *Staphylococcus aureus* sayısı (kob/g)

Food Group	n	Lowest (n)	Highest	Average
Ready-to-cook meatballs	30	<10 (9)	3.6×10^4	5.2×10^3
White cheese	30	<10 (11)	2.5×10^3	3.0×10^2

In the 30 samples of analysed white pickled cheese, the average *Staphylococcus aureus* count was determined as 1.6×10^3 cfu/g (Table 1). The DNase positive *Staphylococcus aureus* were found in average numbers of 3.0×10^2 cfu/g (Tables 2). In nine of the samples (30%), no *Staphylococcus aureus*, and in 11 samples (36.6%) no DNase positive *Staphylococcus aureus* was detected.

The thermonuclease test did not yield a single positive result for white cheese and ready-to-cook meatballs samples. Also staphylococcal enterotoxins were not detected by the ELISA technique in any of the samples tested.

Discussion and Conclusion

In 43% of the samples *Staphylococcus aureus* counts were higher than 5.0×10^3 cfu/g, which is permitted by the Turkish Food Codex Directive on Meat Mixtures (25). The average *Staphylococcus aureus* count in the white cheese samples was found considerably lower than in the meatballs. And while *Staphylococcus aureus* was detected in all meatball samples, 30% of the white cheese samples were below the detectable level. In 63% of samples, however, *Staphylococcus aureus* counts were above the maximum tolerable microbiological limit (10^2 cfu/g) for cheese according to the Turkish Food Codex (24).

In other studies, various *Staphylococcus aureus* counts have been reported in analysed food and cheese samples. Sokari (21) found that 552 (62%) of 880 meat, fish and vegetable ready-to-cook foods samples widely consumed in Nigeria contained coagulase-positive *Staphylococcus aureus*, with 269 (48%) of the strains being enterotoxigenic. Tawfek et al. (22) reported that all of 100 Kerish cheese samples contained staphylococci in an average concentration of 92×10^3 cfu/g. In a study conducted in Brasil, 95% of all cheese samples analysed contained staphylococcus above the legally permitted level (19). Olarte et al. (15) also reported that *Staphylococcus aureus* counts above 2 log were found 55% of the Cameros cheese studied. In a study carried out in Turkey (23), mean *Staphylococcus aureus* count in white pickled cheese was lower than those reported in this present study.

Despite the high prevalence, the *Staphylococcus aureus* counts found were not high enough in any sample food poisoning. *Staphylococci* usually need to grow to

approximately 10^6 cfu/g to produce enterotoxin (1, 10, 27). In 359 cases of *Staphylococcus aureus*-related food poisoning reported in United Kingdom between 1969 and 1990, the highest concentration was 1.5×10^{10} cfu/g and the average concentration was 3.0×10^7 cfu/g. However, even though viable *Staphylococcus aureus* was not detected, enterotoxins were detected in implicated food (cheese) samples in two cases of staphylococcal food poisoning (28). In eggs, the enterotoxins A and C could only be detected with *Staphylococcus aureus* counts of 4.0×10^7 cfu/g and higher (7). In this study, the highest count of *Staphylococcus aureus* was found to be 5.2×10^4 cfu/g for meatballs and 9.2×10^3 for white cheese. DNase positive *Staphylococcus aureus* counts, which are an indicator of toxigenicity were even lower.

With the ELISA technique, staphylococcal enterotoxins were not detected neither in the meatball samples nor in the white cheese. Tawfek et al. (22) reported much higher counts of staphylococci in the 100 cheese samples they analysed, however, they also could not detect any enterotoxins (A, B, C or D) in any of the samples. According to the Turkish Food Codex, staphylococcal enterotoxins should not be detected in foods. In the present study, all of the meatball and white cheese samples fulfilled the requirements. It is known that not all *Staphylococcus aureus* strains are enterotoxigenic. Peeva and Gogov (18) reported that only 3.3% of the 243 strains of *Staphylococcus aureus*, which is isolated from foods, produced the enterotoxin D. Similarly, Isigidi et al (11) who identified 185 *Staphylococcus aureus* strains in different foods and other sources reported that only 30 of those strains produced enterotoxins. The results of the samples analysed in this study show that coagulase positive *Staphylococcus aureus*, which is accounted responsible for the production of enterotoxins was either not present or at such a low concentration as not to produce any detectable toxins. This was confirmed by the fact that the thermonuclease test, which is used to identify the presence of toxigenic *Staphylococcus aureus* in food, gave a negative result. However, this test only gives positive results if the concentration of microorganisms is at least 10^5 - 10^6 /g. Göckler et al. (7), who studied on egg-noodles, reported that thermonuclease usually is produced at all conditions which allow growth of *Staphylococcus aureus*. They, therefore, proposed the evidence of thermonuclease for screening for staphylococcal enterotoxins. Various studies based on the experimental production of cheese from milk contaminated with enterotoxigenic *Staphylococcus aureus* showed an initial phase of *Staphylococcus aureus* count increase followed by subsequent reduction in its number. In the late phase of ripening the *Staphylococcus aureus* count may even drop to such level as to become undetectable. However, enterotoxins could be detected in those samples with their amounts varying in depending on the inoculation level and inoculated strain of *Staphylococcus aureus* (5, 6, 13). In addition to microbial count, production of enterotoxins by *Staphylococcus aureus* in foods also depend on factors such as the presence of other microflora, the a_w and pH of medium, storage temperature. The conditions for enterotoxin production is more fastidious than bacterial growth (1, 2, 8, 20). In this study, another possible reason why no

staphylococcal enterotoxins could be detected in the analysed samples may be that the enterotoxigenic *Staphylococcus aureus* strains did not find the conditions necessary for toxin production

In conclusion, the results of this study indicate that the microbiological quality of the analysed meatballs and white pickled cheese samples were low but none of samples had a potential health risk with regard to staphylococcal enterotoxins.

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