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Determination of growth and toxin production potential of Staphylococcus *aureus* and *Clostridium perfringens* during döner production process

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Abstract: Döner is a traditional meat meal that is commonly consumed in Turkey. Although it receives heat treatment during processing, some microorganisms including pathogens may remain alive. Therefore, this study was carried out to determine the changes in the number of microorganisms (total aerobic mesophilic bacteria, Enterobacteriaceae, *Escherichia coli*, aerobic mesophilic spore-forming bacteria, *Staphylococcus-Micrococcus* spp.) and survival potential of some pathogenic bacteria (*Staphylococcus aureus* and *Clostridium perfringens)* during the cooking period. Experimentally prepared döner kebabs were cooked continuously or intermittently. This process resulted in a reduction of microorganisms depending on the species and the use of low quality raw materials, and a number of bacteria survived at significant levels after cooking. The remaining bacteria multiplied and reached unacceptable levels with interrupted heat treatment over a long period of time. However, staphylococcal enterotoxins were not detected in intentionally contaminated samples that were kept at room temperature for 18 h after cooking. On the other hand, no significant microbiological changes appeared in raw sections of döner during cooking when the heat source was turned off.

Key words: Döner, microbial quality, *Staphylococcus aureus*, *Clostridium perfringens*, heat treatment

1. Introduction

Döner is a traditional meat meal that is commonly consumed in Turkey. Its history dates back to the 1800s in Bursa and the Middle East and its consumption spread due to the rapid industrialization and urbanization of the Ottoman Empire. Today it is served in different formulations and types, such as a döner, iskender, and cağ kebab. In many countries (e.g., Germany, Greece, Lebanon, Arab countries, and the USA) döner has entered the culinary culture and has become a regional food with diverse names, such as gyro, doner kebab, shawarma, chawarma, and souvlaki (1,2).

Döner is made from beef, pork, or mutton meat and mixed with flavoring ingredients such as tail fat, onion, pepper, oil, milk, yogurt, tomato, egg, and vinegar or lemon juice based on consumer preferences. In Turkey, 2–3-year-old bovine meat is generally preferred for döner production and it must have been previously ripened (pH of <6). Meat is procured from the hind, back, legs, and waist of the carcass. A meat part known as the 'rump tail' is particularly used as a döner raw material (3–5). Before the cooking process, döner meat is marinated with salt, phosphates, organic acids, spices, and the previously listed flavoring ingredients. This method tenderizes, crisps, and increases the meat flavor (6,7).

The quality of the flora in raw döner kebab meat depends on numerous factors, such as the microbial load in the raw materials, processing, and plant hygiene. The use of low quality meat and other ingredients, poor hygiene at the plant, and staff inattention can result in increased microbial loads. According to Turkish standards, the presence of coagulase-positive *Staphylococcus aureus* and *Clostridium perfringens* is limited to 10^4 colony forming units (CFU)/g for döner kebab meat and a maximum of 10^6 CFU/g total aerobic bacteria. The pathogens *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 are not allowed in döner kebab meat. Legal limits were also established in the Turkish Food Codex (5) for cooked döner meat. The maximum limit for coagulase-positive *S. aureus* and *C. perfringens*, mold, and yeast is 10^3 CFU/g, while Salmonella spp., *L. monocytogenes*, and *E. coli* O157:H7 are not allowed.

As a fresh dish, döner kebab must be served immediately after cooking and there are some unique risks associated with the cooking process. The standard temperature used during the cooking application is generally enough

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to inactivate vegetative forms of pathogen bacteria. However, some mistakes may be made by staff at times when consumer demand is increased. The döner meat may be served under inadequate cooking conditions or cut into overly thick slices that may contain uncooked parts (8). While the surface temperature of a döner block is high, the central temperature of 23–25 °C facilitates the proliferation of microorganisms (2,9). The effect of heat treatment depends on total microorganism count; it may be inadequate to eliminate the pathogenic bacteria that proliferate in the central part of a döner block. Also, at some restaurants, the remaining döner block is kept and used the following day, which may result in an increase in the surviving bacteria. If cooked products are maintained under suitable conditions, an enhancement in the number of microorganisms that are present in the medium could be seen or the product may be subsequently contaminated (10). Several studies on the microbiological quality of döner kebab meat support this knowledge. It may contain various pathogenic microorganisms that could have an adverse effect on consumer health (1,11–19).

Meat and meat products are significantly risky for causing food poisoning when contaminated. They have optimum conditions for microbiological growth via parameters such as richness of nutritional items, pH, and water activity capacity (a_w). Microorganisms that may proliferate among wide pH and high a_w values use meat as an ideal medium. *Staphylococcus aureus* is one of the major microorganisms mentioned, playing an important role in food poisoning related to meat and meat products (20–22). Food contamination can be from animal origin as well as from personnel. To develop in the environment, *S. aureus* needs wide temperature, pH, and a_w values of 7–47.8 °C, 4–9.8, and 0.83–0.98, respectively. The meat composition also provides these conditions in this context. However, *S. aureus* can produce more than 20 types of extracellular toxins, which are also known as enterotoxins, highly robust and showing resistance to high temperatures and low pH values, when these conditions occur (23,24). Typical research supports that döner kebab may be contaminated with *S. aureus* at various levels (12,16,17,25). *Clostridium perfringens* is another important pathogen in food poisoning cases. *C. perfringens* is widespread in the environment and survives in soil, air, water, sewage water, and feces of humans and animals. Due to the wide range of its reservoir network, it is inevitable that it contaminates food. *C. perfringens* causes gangrenous emphysema and enterotoxemia in both humans and animals. It can grow at high temperatures and the optimum heat is 43–45 °C. The tolerable pH and a_w values of *C. perfringens* are 5.5 and 0.95, respectively (26). Meat and meat products are the main foods in which *C. perfringens* is mostly seen. Poisoning related to *C. perfringens* occurs generally between 8 and 22 h after ingestion of toxins in contaminated foods. The present spores germinate and the number of vegetative cells increases to 10^6 CFU/g in foods either cooked or not chilled quickly after cooking. This is the limit value that is enough to create food poisoning in humans (24,27). Several studies carried out at different points showed that *C. perfringens* is another pathogen that may occur in döner flora at various levels (9,12,28,29).

Based on this, we aimed to investigate the microbiological changes of döner kebabs both in raw (central) and cooked (surface) samples in the present study during the cooking process. Survival potentials of *S. aureus* and *C. perfringens* were examined in döner meat that was contaminated experimentally and also kept until the next day after cooking.

2. Materials and methods

The experimental manufacture of döner was carried out in the meat processing unit of our faculty. To begin, 10 kg of raw minced meat (beef) was mixed with a variety of ingredients (tomato, egg, yogurt, milk, olive oil, sugar, and some spices) in fixed proportions as listed in Table 1 by a fork-armed dough kneading machine (Boğaziçi, Turkey). To provide homogeneity in microbiological analyses, all döner was produced with minced meat and approximately 10 kg of meat was used to produce each döner sample.

Immediately after mixing, the raw döner mixture was kept at 4 °C overnight to marinade and it was then spitted. Before starting the cooking process, the döner was kept

Table 1. Materials used in döner manufacturing.

Materials	Quantity	%
Minced meat	10 kg	78.3
Salt	160 g	1.25
Black pepper	80 g	0.62
Onion (fine-cut)	500 g	3.91
Tomato paste	200 g	1.56
Tomatoes (fine-cut)	200 g	1.56
Yogurt (bag)	200 g	1.56
Milk	250 g	1.95
Olive oil	100 g	0.78
Egg	10 units (500 g)	3.91
Soybean flour – texturized	200 g	1.56
Soybean flour – isolated	300 g	2.35
Sugar	50 g	0.39
Thyme	20 g	0.15
Total	12.760 g	

in a freezer for 15 min to ensure a rigid structure. To ensure an equal effect of the heat treatment, döner samples were molded into a cylindrical shape. We would like to emphasize here that the radius values of our döner samples were not standardized. In the industry, there is no standard value for döner radius. After that, raw döner was placed on a special gas machine (Pimak, Turkey) and the distance between the döner surface and the heat source was set at 20 cm. The cooking process was carried out rotationally. Cooked parts of the döner were cut by an electric döner knife (Tekin, Turkey) at a standard thickness of 2 mm.

Four experimental types of döner production were evaluated.

In Group A, the döner samples were prepared based on the traditional method. Fresh and high quality minced meat was supplied by a meat plant as the raw material. To determine the raw meat's quality, microbiological parameters were evaluated based on the Communique on Microbiological Criteria (30). The cooking process was continuous.

In Group B, döner samples were prepared using low quality minced meat supplied by local markets. The döner was produced in the same way as in Group A and raw meat quality was again defined according to the Turkish Food Codex (30). The cooking process was intermittent (discontinuous), repeating 15 min of cooking followed by 15 min of break in which the heat was off, and this process was repeated for 6 h.

In Group C, high quality minced meat was used for döner production and the raw döner mixture was intentionally contaminated with *S. aureus*. Before contamination, raw minced meat and the other ingredients were analyzed for the presence of *S. aureus*. When the raw materials were found to be free of the mentioned microorganism, the döner mix was prepared. Later, the concentration of culture suspension was arranged as 10^8 CFU/g by using McFarland turbidity tubes for contamination. From this suspension, 10 mL was inoculated to the döner mixture, which was kneaded to reach a homogeneous distribution. Final concentration was regulated as 10^5 CFU/g in the final product. After that, inoculant density was measured by McFarland densitometer (DEN-1, Biosan, EU) Before contamination, the inoculation cocktail was checked for effectiveness of *S. aureus* via cultural methods. The inoculation process was done aseptically in a separate kneading trough using sterile hand gloves. The tubes with inoculum liquid were poured into the marinated döner mixture and it was kneaded in the trough by hand to reach a homogeneous distribution. The *S. aureus* strain used in this assay had the ability to produce enterotoxin A. It was previously isolated and identified by us in a project supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK, Project No. 107T266). The cooking process was discontinuous, lasting about 6 h. In this technique, the döner was cooked for 15 min and then a break of 15 min followed in which the heat was off, and these steps were repeated for 6 h. After this process ended, the döner was kept at room temperature for 18 h and then cooked again.

In Group D, the quality of the raw meat and the cooking process was the same as in Group C, but in this assay, the raw material was intentionally contaminated with *C. perfringens* (ATCC 13124, Wesel, Germany) supplied by a laboratory (Food and Environmental Analyses Laboratory, Turkey). Before contamination, raw minced meat and the other ingredients were analyzed for the presence of *C. perfringens*. When the raw materials were found to be free from the mentioned microorganism, the döner mix was prepared. Later, the concentration of culture suspension was arranged as 10^8 CFU/g by using McFarland turbidity tubes for contamination. From this suspension, 10 mL was inoculated to the döner mixture and it was kneaded to reach a homogeneous distribution. Final concentration was regulated as 10^5 CFU/g in the final product. After that, inoculant density was measured by McFarland densitometer (DEN-1, Biosan, EU). Before contamination, the inoculation cocktail was checked for effectiveness of *C. perfringens* via cultural methods. The inoculation process was done aseptically in a separate kneading trough using sterile hand gloves. The tubes with inoculum liquid were poured into the marinated döner dough and it was kneaded in the trough by hand to reach a homogeneous distribution. *C. perfringens* is one of the major foodborne pathogens that produces multiple enterotoxins (>15). It also has many strains, separated into five typical groups (A–E). Of these, mostly type A and rarely type C cause foodborne diseases in human. Therefore, in the assays, the *C. perfringens* type A strain was used. This strain is characterized by expressing the alpha-toxin, which has lethal, necrotic, and hemolytic effects on the human intestines.

All experiments were repeated three times. In Groups A and B, the total aerobic mesophilic bacteria (TAMB), Enterobacteriaceae, *E. coli*, spore-forming aerobic mesophilic bacteria, sulfide-reducing anaerobic bacteria, *Staphylococcus* spp. and *Micrococcus* spp., and *S. aureus* counts were analyzed. *S. aureus* and *C. perfringens* were evaluated in Groups C and D, respectively.

2.1. Microbiological analyses

A sample of 10 g of döner for each analysis was homogenized with 90 mL of sterile Peptone Salt Broth (PBS, Oxoid CM 733R) and serial decimal dilutions were prepared. Appropriate dilutions were spread on standard plate count agar (PCA, Oxoid CM 463) and incubated at 37 °C for 48 h to determine TAMB (31). For the determination of Enterobacteriaceae, violet red bile glucose

agar (VRBG, Oxoid CM 485) was used. This medium was poured into petri dishes to obtain a double layer and then incubated at 37 °C for 24 h (32). *E. coli* was enumerated on Tryptone bile X-glucuronide agar (TBX, Oxoid CM 945) and incubated at 44 °C for 24 h (33). Spore-forming aerobic mesophilic bacteria were determined based on the method of Harrigan (34). Next, 10 mL of homogenate samples were transferred into sterile tubes and kept at 80 °C for 1 min in a bain-marie and then cooled. Serial decimal dilutions were prepared from these tubes and PCA enumeration was used. Plates were incubated at 30 °C for 2 days. Sulfide-reducing bacteria were verified on sulfite polymyxin sulfadiazine agar (SPS, Merck 1.10235). One milliliter of the decimal dilutions was placed into the tubes and SPS was added. Paraffin was supplemented after incubation at 37 °C for 48 h (35).

For the isolation and enumeration of *S. aureus*, *Staphylococcus* spp., and *Micrococcus* spp., the appropriate International Organization for Standardization (ISO) method (36) was applied. Serial decimal dilutions were inoculated onto Baird-Parker agar (BPA, Oxoid CM 0275) and incubated at 37 °C for 48 h. Bright colonies colored with black-gray and opaque zones were defined as *S. aureus*, while nonzoned black colonies were defined as *Staphylococcus* spp. and *Micrococcus* spp. (37). In Groups A and B, typical colonies were passaged to mannitol salt phenol agar (MSA, Oxoid, CM 085) and incubated at 37 °C for 24–48 h. Yellow zoned colonies were confirmed with Hucker's Gram stain catalase (38) or the Thermonuclease and Latex Agglutination (Oxoid, Staphytect Plus, DR 850) test in Groups A and B, respectively. For the determination of *C. perfringens*, tryptose sulfide cycloserine agar (TSC, Oxoid CM 587) was used. One milliliter of the main homogenate was poured into petri dishes and a second layer (agar without egg yolk) was added. Plates were incubated at 35 °C for 24 h in an anaerobic jar by using an AnaeroGen kit (Oxoid AN0035) and black colonies of 2–4 mm in diameter with opaque zones were enumerated (39).

2.2. Detection of Staphylococcal Enterotoxins

The Staphylococcal Enterotoxin Reversed Passive Latex Agglutination Test Kit (SET RPLA, Oxoid TD900) was used to detect toxin production in döner contaminated with enterotoxigenic *S. aureus*. As per the instructions, samples of döner of 10 g were homogenized with 90 mL of NaCl (0.85%) by a lab blender. After a cooling process, samples were centrifuged at 3000 rpm for 30 min. Supernatants were leached from a 0.2-µm filter (Millipore). Filtrates were treated with staphylococcal enterotoxin (A, B, C, and D)-sensitive reactive latex in compliance with the double dilution system. Plates were kept at room temperature for 24 h, after which the presence of agglutination was evaluated; agglutination was accepted as positive for enterotoxin production.

2.3. Statistical analyses

The number of microorganisms in raw and cooked döner samples was expressed as $\log_{10}$ CFU/g. Differences between average numbers in döner samples taken at different times of production were evaluated by analysis of variance using SPSS (40). $P < 0.05$ was considered statistically significant.

3. Results

The results given in the tables comprise the average numbers of three trials per group. Table 2 shows the changes of TAMB count in döner samples in Group A (high quality) and Group B (low quality). As given in Table 2, a significant reduction of TAMB count occurred in Group A by the end of cooking both in raw (central) and cooked (surface) samples. In Group B, it was seen that the microbial load of raw samples was still maintained while a similar reduction occurred in cooked samples as in Group A, as anticipated.

In Table 3, the changes of Enterobacteriaceae counts in Group A and B are given. According to our results, no essential change was recorded in raw samples of Group A while a total reduction occurred and no bacteria were enumerated in cooked samples. In Group B, initial mean count of Enterobacteriaceae was maintained, as shown in Table 3.

Changes in *E. coli* populations are given in Table 4. In Group A, total count of *E. coli* decreased from 2.35 to 1.25 log CFU in raw samples while it was completely inactivated in cooked samples. On the other hand, a contrary change was seen in Group B. The microbial load showed an increase during the cooking period in raw samples while it kept its presence in cooked samples.

In this experimental study, spore-forming bacterial load showed no numerical change in either Group A or B, as expected. These findings are shown in Table 5. The number of sulfide-reducing bacteria was found to be low, with no significance in the samples of all groups in our study.

According to another outcome, given in Table 6, no significant change was detected in the load of *Staphylococcus* spp. and *Micrococcus* spp. in döner samples in the first hours of cooking. However, an important decrease occurred at the 5th and 6th hours. In Group A, a reduction of 2 log levels was observed both in raw and cooked samples, and after this, no bacteria could be enumerated in cooked samples. Similarly, a decrease was seen in the samples of Group B like in Group A, but there were still bacteria at about 2.66 log CFU level, by a narrow margin.

In Table 7, data about the döner experimentally contaminated with. *S. aureus* are given (Group C). The initial bacterial load showed a reduction of about 2 log levels in the last hours of cooking in raw and cooked

Table 2. Changes of total aerobic mesophilic count in döner samples during cooking process in Groups A (high quality) and B (low quality).

Sample	Radius (cm)		Central temperature (°C)		Log cfu/g (mean ± SE)			
					Raw		Cooked	
	A	B	A	B	A	B	A	B
Before marinating (initial load)	-	-	-	-	5.60 ± 0.17^{ab}	6.76 ± 0.19^{b}	-	-
After marinating	-	-	-	-	5.94 ± 0.40^{ab}	6.90 ± 0.05^{ab}	-	-
1st hour	9.93	9.86	6.33	5.66	6.03 ± 0.38^{b}	6.96 ± 0.14^{ab}	4.71 ± 0.44^{a}	5.40 ± 0.09^{a}
2nd hour	8.46	9.16	21.3	11.3	6.17 ± 0.36^{b}	7.03 ± 0.15^{ab}	4.57 ± 0.39^{a}	5.03 ± 0.11^{ab}
3rd hour	7.50	8.73	34.3	25.3	6.17 ± 0.35^{b}	7.13 ± 0.15^{ab}	4.26 ± 0.38^{a}	4.81 ± 0.05^{bc}
4th hour	6.53	7.80	48.3	36.3	5.56 ± 0.28^{ab}	7.42 ± 0.25^{a}	3.96 ± 0.51^{ab}	4.79 ± 0.12^{bc}
5th hour	5.56	6.96	61.0	42.0	5.39 ± 0.18^{ab}	7.48 ± 0.03^{a}	3.58 ± 0.40^{b}	4.69 ± 0.15^{bc}
6th hour	3.73	5.96	67.6	48.3	4.88 ± 0.42^{a}	6.62 ± 0.33^{b}	3.41 ± 0.44^{b}	4.36 ± 0.33^{c}

^{abc}: Differences between averages shown with different letters in the same column are statistically significant ($P < 0.05$).

Table 3. Changes of Enterobacteriaceae count in döner samples during cooking process in Group A (high quality) and B (low quality).

Sample	Radius (cm)		Central temperature (°C)		Log cfu/g (mean ± SE)			
					Raw		Cooked	
	A	B	A	B	A	B	A	B
Before marinating (initial load)	-	-	-	-	2.97 ± 0.24^{a}	4.69 ± 0.15^{a}	-	-
After marinating	-	-	-	-	3.12 ± 0.29^{a}	4.78 ± 0.09^{a}	-	-
1st hour	9.93	9.86	6.33	5.66	3.14 ± 0.37^{a}	4.87 ± 0.24^{a}	<10^{1}	2.13 ± 0.27^{a}
2nd hour	8.46	9.16	21.3	11.3	3.01 ± 0.28^{a}	4.94 ± 0.50^{a}	<10^{1}	2.25 ± 0.06^{a}
3rd hour	7.50	8.73	34.3	25.3	3.21 ± 0.22^{a}	5.06 ± 0.30^{a}	<10^{1}	2.06 ± 0.13^{a}
4th hour	6.53	7.80	48.3	36.3	3.08 ± 0.10^{a}	5.05 ± 0.18^{a}	<10^{1}	2.24 ± 0.15^{a}
5th hour	5.56	6.96	61.0	42.0	2.72 ± 0.29^{a}	4.77 ± 0.30^{a}	<10^{1}	1.89 ± 0.44^{a}
6th hour	3.73	5.96	67.6	48.3	2.51 ± 0.19^{a}	4.40 ± 0.42^{a}	<10^{1}	1.81 ± 0.17^{a}

^{abc}: Differences between averages shown with different letters in the same column are statistically significant ($P < 0.05$).

samples. However, in samples taken from döner retained after cooking (for 18 h) until the next day, the number of present microorganisms increased by about 3 log levels and reached 6.08 and 4.13 log CFU in raw and cooked samples, respectively. In the samples taken from raw and cooked sections of döner experimentally contaminated with *C. perfringens* (Group D), no significant change in microorganism load was seen during cooking (Table 8). It was also found that the number of microorganisms increased by about 2.5 log levels in raw and cooked döner samples stored until the next day after cooking (for 18 h).

4. Discussion

Today, consumer eating habits have changed to accommodate our busy lives. The increases in urbanization and industrialization have resulted in an increase in the popularity of ready-to-eat foods. Döner kebab is one preferable fast food in this context. It has wide sales potential in fast food markets.

In the present study, different heat treatments and cooking principles were applied to experimentally manufactured döner (Groups A–D). The microbial loads of samples taken from the raw (central) and cooked

Table 4. Changes of *E. coli* count in döner samples during cooking process in Group A (high quality) and B (low quality).

Sample	Radius (cm)		Central temperature (°C)		Log cfu/g (mean ± SE)			
					Raw		Cooked	
	A	B	A	B	A	B	A	B
Before marinating (initial load)	-	-	-	-	1.99 ± 0.[33abc]	2.53 ± 0.74 [a]	-	-
After marinating	-	-	-	-	2.16 ± 0.29[bc]	2.55 ± 0.73 [a]	-	-
1st hour	9.93	9.86	6.33	5.66	2.35 ± 0.23 [c]	2.58 ± 0.78 [a]	<10[1]	0.53 ± 0.53 [a]
2nd hour	8.46	9.16	21.3	11.3	2.41 ± 0.24 [c]	2.60 ± 0.76 [a]	<10[1]	0.90 ± 0.45 [a]
3rd hour	7.50	8.73	34.3	25.3	2.51 ± 0.29 [c]	2.80 ± 0.77 [a]	<10[1]	0.60 ± 0.60 [a]
4th hour	6.53	7.80	48.3	36.3	2.05 ± 0.20[bc]	3.10 ± 0.74 [a]	<10[1]	1.12 ± 0.60 [a]
5th hour	5.56	6.96	61.0	42.0	1.44 ± 0.20[ab]	3.35 ± 0.33 [a]	<10[1]	0.81 ± 0.42 [a]
6th hour	3.73	5.96	67.6	48.3	1.25 ± 0.10 [a]	3.21 ± 0.28 [a]	<10[1]	0.40 ± 0.40 [a]

[abc]: Differences between averages shown with different letters in the same column are statistically significant ($P < 0.05$).

Table 5. Changes of spore-forming aerobic mesophilic bacteria count in döner samples during cooking process in Group A (high quality) and B (low quality).

Sample	Radius (cm)		Central temperature (°C)		Log cfu/g (mean ± SE)			
					Raw		Cooked	
	A	B	A	B	A	B	A	B
Before marinating (initial load)	-	-	-	-	3.45 ± 0.24 [a]	4.16 ± 0.28 [a]	-	-
After marinating	-	-	-	-	3.36 ± 0.30 [a]	4.25 ± 0.30 [a]	-	-
1st hour	9.93	9.86	6.33	5.66	3.53 ± 0.22 [a]	4.20 ± 0.32 [a]	3.11 ± 0.37 [a]	3.66 ± 0.17 [a]
2nd hour	8.46	9.16	21.3	11.3	3.81 ± 0.22 [a]	4.27 ± 0.29 [a]	3.17 ± 0.43 [a]	3.73 ± 0.03 [a]
3rd hour	7.50	8.73	34.3	25.3	3.70 ± 0.04 [a]	4.21 ± 0.34 [a]	3.03 ± 0.25 [a]	3.61 ± 0.19 [a]
4th hour	6.53	7.80	48.3	36.3	3.46 ± 0.14 [a]	4.26 ± 0.32 [a]	2.85 ± 0.12 [a]	3.44 ± 0.23 [a]
5th hour	5.56	6.96	61.0	42.0	3.35 ± 0.19 [a]	4.22 ± 0.23 [a]	2.82 ± 0.03 [a]	3.53 ± 0.18 [a]
6th hour	3.73	5.96	67.6	48.3	3.82 ± 0.04 [a]	4.05 ± 0.38 [a]	2.58 ± 0.14 [a]	3.16 ± 0.24 [a]

[abc]: Differences between averages shown with different letters in the same column are statistically significant ($P < 0.05$).

(surface) sections of döner and their interaction with heat throughout the cooking period were examined periodically. We also investigated the probable risks associated with the consumption of döner for consumer health by evaluating the microbial quality of döner and the toxin production potential of *S. aureus* and *C. perfringens*, which may be present.

TAMB is a general parameter used to evaluate food hygiene by providing the microorganism load in food. According to our results, the mean TAMB loads were 5.94 log CFU/g and 6.90 log CFU/g in Groups A and B, respectively, after the döner had been marinated. In raw samples taken from the center of döner during 5 h of cooking, no serious change was detected in TAMB counts. However, in the last hour of cooking (6 h), the TAMB count decreased to 4.55 log CFU/g and 6.62 log CFU/g in Groups A and B, respectively (Table 2). This significant reduction, especially in Group A ($P < 0.05$), was thought to be associated with a diminishing döner radius ratio (3.73 cm) and increase in central temperature (67.6 °C) (41). On the other hand, no significant change in TAMB was detected in Group B. The higher bacterial load in Group B was a result of using low quality raw materials, as anticipated. This may also have been due to the discontinuous (intermittent) cooking technique and the döner radius at 6 h; it was thicker and the

Table 6. Changes of *Staphylococcus-Micrococcus* spp. count in döner samples during cooking process in Group A (high quality) and B (low quality).

Sample	Radius (cm)		Central temperature (°C)		Log cfu/g (mean ± SE)			
					Raw		Cooked	
	A	B	A	B	A	B	A	B
Before marinating (initial load)	-	-	-	-	4.34 ± 0.22 [a]	4.84 ± 0.30 [a]	-	-
After marinating	-	-	-	-	4.31 ± 0.22 [a]	4.89 ± 0.26 [a]	-	-
1st hour	9.93	9.86	6.33	5.66	4.25 ± 0.31 [a]	5.00 ± 0.31 [a]	3.00 ± 0.25 [a]	3.39 ± 0.15 [a]
2nd hour	8.46	9.16	21.3	11.3	4.08 ± 0.28 [a]	5.08 ± 0.22 [a]	2.74 ± 0.27[ab]	3.44 ± 0.13[ab]
3rd hour	7.50	8.73	34.3	25.3	3.83 ± 0.37 [a]	5.06 ± 0.25 [a]	2.60 ± 0.24[ab]	3.32 ± 0.25[ab]
4th hour	6.53	7.80	48.3	36.3	3.54 ± 0.33[ab]	4.97 ± 0.90 [a]	2.17 ± 0.29 [b]	2.82 ± 0.05[ab]
5th hour	5.56	6.96	61.0	42.0	2.83 ± 0.30 [b]	4.98 ± 0.10 [a]	<10[1] [c]	2.86 ± 0.21[ab]
6th hour	3.73	5.96	67.6	48.3	2.70 ± 0.32 [b]	4.91 ± 0.20 [a]	<10[1] [c]	2.66 ± 0.39[b]

[abc]: Differences between averages shown with different letters in the same column are statistically significant ($P < 0.05$).

Table 7. Changes of *S. aureus* count in döner samples of Group C contaminated intentionally with *S. aureus*.

Sample	Radius (cm)	Central temperature (°C)	*S. aureus* (mean ± SE; log cfu/g)	
			Raw	Cooked
Before marination (initial load)	-	-	5.39 ± 0.05[ab]	-
After marination	-	-	5.28 ± 0.11[ab]	-
1st hour	9.56	11.0	5.47 ± 0.27[ab]	3.11 ± 0.18 [b]
2nd hour	9.00	21.6	5.02 ± 0.11 [b]	2.82 ± 0.24[bc]
3rd hour	8.40	35.7	4.69 ± 0.09[bc]	2.67 ± 0.20[bc]
4th hour	7.66	45.6	4.14 ± 0.30 [cd]	2.46 ± 0.23[bcd]
5th hour	6.56	50.0	3.72 ± 0.47 [de]	2.19 ± 0.14 [cd]
6th hour	5.76	56.3	3.10 ± 0.39 [e]	1.82 ± 0.10 [d]
24th hour*	-	-	6.08 ± 0.24 [a]	4.13 ± 0.38 [a]

[abcde]: Differences between averages shown with different letters in the same column are statistically significant ($P < 0.05$).

*: Between the 6th and 24th hours, the cooking process was suspended.

central temperature was higher than that in Group A. The discontinuous cooking technique caused the central temperature to rise slowly and stay at proper levels (25–40 °C) for a long time for microbial growth. In addition, this may have been associated with the hygienic quality of the raw materials. If the initial microorganism load is higher in raw materials, most of them may still remain alive after the heating process (42). Çoksöyler and Avşaroğlu (41) also suggested that if the number of microorganisms is higher in the environment, thermal resistance becomes greater. The authors correlated this with the protective components that inactive microorganisms excrete out of the cell and they noted that these components, which are of carbohydrate, protein, and fat structure, show a protective effect on living microorganisms.

From the first hour of cooking, TAMB counts from cooked (surface) samples showed about 1.32 log (Group A) and 1.6 log (Group B) reductions when compared with raw samples taken at the same time from the center of the döner. This may be explained by the fact that the general microflora in our döner samples mostly consisted of heat-resistant microorganisms. In cooked samples, total TAMB counts decreased over time. As seen in Table 2, this reduction was detected after 5 and 6 h of cooking in Group A, while it

Table 8. Changes of *C. perfringens* count in döner samples of Group D contaminated intentionally with *C. perfringens*.

Sample	Radius (cm)	Central temperature (°C)	*C. perfringens* (mean ± SE; log cfu/g)	
			Raw	Cooked
Before marination (initial load)	-	-	5.50 ± 0.12[b]	-
After marination	-	-	5.68 ± 0.11[b]	-
1st hour	9.80	8.3	5.77 ± 0.12[b]	2.65 ± 0.13[b]
2nd hour	9.26	21.7	5.59 ± 0.23[b]	2.80 ± 0.11[b]
3rd hour	8.46	36.7	5.84 ± 0.19[b]	2.83 ± 0.14[b]
4th hour	7.86	46.3	5.62 ± 0.22[b]	2.75 ± 0.21[b]
5th hour	7.20	53.3	5.80 ± 0.30[b]	2.90 ± 0.24[b]
6th hour	6.46	59.7	5.49 ± 0.37[b]	2.55 ± 0.24[b]
24th hour*	-	-	7.89 ± 0.30[a]	5.05 ± 0.22[a]

[ab]: Differences between averages shown with different letters in the same column are statistically significant ($P < 0.05$).

*: Between the 6th and 24th hours, the cooking process was suspended.

was at 3 h in Group B. These differences might be related to the weakening of surface bacteria before cooking. Döner is fixed to a skewer and frozen, so the temperature below the surface is lower. However, as the central temperature increases over time, microorganism resistance decreases partially during cooking. This may be associated with differences in heat resistance during the microbiological growth phase. Cells are more resistant in the stationary phase than in the logarithmic phase (43). In this study, the microorganisms were in stationary phase because the döner had been kept in cold storage. Thus, during cooking, the microorganisms proceeded to a logarithmic phase and were more affected by the heating process.

The reduction effect of the thermal process on the present microorganism count depends on factors such as the bacterial count and strain, heating degree, and time. Kayisoglu et al. (15) reported that TAMB counts of raw döner samples were 5.68 log CFU/g (chicken) and 5.59 log CFU/g (meat) and that cooked counts dropped to 4.92 log CFU/g and 4.99 log CFU/g, respectively. Gonulalan et al. (44) also noted that TAMB counts of raw döner samples decreased from 5.03 log CFU/g to 4.62 log CFU/g after a heat treatment. These results are similar to ours. However, some other studies showed a greater decreasing ratio in microbial load by cooking (15,45–48).

Enterobacteriaceae is a widespread bacterial group in nature. It includes many pathogenic species that form part of the natural flora of intestinal systems (49). In our study of Group A, no numeric change in the Enterobacteriaceae load from the center of the döner was recorded after 4 h of cooking, and little reduction was detected after 5 and 6 h. Similarly, Group B had a higher microbial load and no important change was detected (Table 3). We believe that this may be due to the adaptation of microorganisms to changeable conditions (especially Group A). However, in Group A, a significant reduction of Enterobacteriaceae occurred and it reached negligible levels in cooked samples with the effect of the heating process. This may be associated with Group B, having a higher initial microbial level and different cooking technique. This is in accordance with the results of Cebirbay (9), Jockel and Stengel (14), and Acar and Çiftçioğlu (45). On the other hand, different Enterobacteriaceae levels have also been reported (8,9,15,16,50,51).

E. coli is one of the important members of Enterobacteriaceae. It is a fecal contamination microorganism (indicator) that is normally found in human and animal intestinal tracts. Therefore, if it is detected in cooked products, this shows that the product had been recontaminated after cooking via a fecal source (52,53). *E. coli* is a sensitive microorganism that is inactivated at 61–73 °C (24,54), and the standard temperature of döner reaches this level during cooking. Changes in *E. coli* populations are given in Table 4. In our study, *E. coli* was completely inactivated by heating in cooked samples. This was also reported by Yilmaz et al. (55); however, many researchers found different *E. coli* levels in cooked döner samples (8,12,16,48,56). In Group B, another improvement was observed. The microbial population increased in raw samples while it maintained its stability in cooked samples. Although *E. coli* is a heat-sensitive microorganism, it may have been detected in those samples after the cooking process because of the hygienic quality of the raw material and cooking conditions.

Spore-forming aerobic mesophilic bacteria are resistant to high temperatures, so the cooking process has no effect on this group (52,54). In this experimental study, changes in the bacterial population gave us inconsistent results (Table 5). These values were significantly lower than the number of other microorganisms examined in our study. Thus, the mean values of these microorganisms were not taken into consideration. This may be due to the fact that optimum conditions for the growth of spore-forming bacteria were not provided in the central parts of döner. Also, sulfide-reducing anaerobe bacteria were evaluated in our study. Sulfide-reducing anaerobes were found occasionally in samples, but the numbers were not more than 10^2 CFU/g at any stage. This situation might be associated with the microbiological quality of raw meat and the other ingredients from which these microorganisms originate. Bostan et al. (8) and Hampikyan et al. (56) reported that they did not find countable levels of bacteria in their studies. On the other hand, in some other survey studies, many different levels of sulfide-reducing anaerobe bacteria were found (9,57).

Staphylococcus spp. and *Micrococcus* spp. are a large bacterial group belonging to the Micrococcaceae. Some strains have pathogenic effects on human health (58). These microorganisms are not competitive in the environment so they cannot grow in raw food (59–61). In this context, we obtained good results from our study. No numeric changes in bacterial load in the center part of the döner were observed in either Group A or B during the cooking period. However, serious reductions were detected after 5 and 6 h in Group A due to the high temperature, whereas there was no reduction in Group B. Also, the microorganism load in the cooked samples decreased significantly after 5–6 h of cooking ($P < 0.05$; Table 6). We also examined *S. aureus* in this assay. *S. aureus* is one of the most important microorganisms related to food poisoning. It is a noncompetitive and recessive bacterium. As was anticipated, no typical colony of *S. aureus* was enumerated in our study. However, this does not mean that it was not there previously. There are many different organisms in döner flora that could affect the growth of *S. aureus*. In many studies the presence of *S. aureus* in döner was reported to some degree and these findings do not support ours (1,9,11,12,16,17,25,57,62). It may have been due to recontamination, or *S. aureus* in the raw material may have survived despite heat treatment.

In light of all these findings, many kind of risks about döner can be mentioned. One of the probable risks when döner is cooked is that the central temperature promotes the present microorganisms to multiply at optimum levels (2,9). This risk grows when the döner is skewered with big diameters and the sales period is longer (14,62). Although the temperature is higher at the surface of döner, the central temperature is lower. In Acar and Çiftçioğlu's study (45), it was stated that the central temperature did not have the anticipated effect on microflora. However, this temperature, which is insufficient to inactivate the microorganisms, could remain levels supporting the growth of microorganisms and toxin production. In our study, the central heat degrees showed a regular increase in all groups as a result of the decrease in döner diameter. Particularly, the central temperature reached 48 °C at the 6th hour of cooking in Group B. When the central temperatures were evaluated in this study, it seemed that heat generally stayed at growth-promoting levels of 20–45 °C for at least 2 h. Another important point is the raw material. For instance, the minced meat that is used as a raw material in döner mix has great microorganism activity, but when it is marinated with the other ingredients, it does not show the same influence due to the change of environmental conditions. The first stage of microorganisms' growth is the latent phase and no numerical production occurs in this phase. The length or shortness of it depends on various factors such as environmental conditions and composition of foods (42). Instead of meat, other ingredients could be discussed in this study. Even if spices have an antimicrobial effect, the rate we used in the döner was lower. On the other hand, onion has the same feature as spices, due to having a component like allicin (63,64). It could be said that the usage ratio of onion in our study was rather high (3.91%) and that could inhibit the microbial growth. Another ingredient, egg, also shows an important effect on microbiota with lysozyme. It is effective on both gram-positive and gram-negative bacteria (65–69). In this context, the quantity of eggs we used for each experiment (10 eggs for each döner) could be associated with the reduction of flora. Also, the yogurt could be effective on the microbial population in raw döner. As is known, yogurt is highly rich in lactic acid bacteria (9).

One of the many risks for döner stems from interrupting the cooking process for a long time and multiplying the present vegetative microorganisms at that time (10). It is known that at some sales points the döner that is not sold that day is kept until the next day. When this is the case, both the inside and the outside of the döner becomes a proper medium for growing bacteria (70). In this study, we evaluated the changes of *S. aureus* and *C. perfringens* counts in döner samples of Groups C and D, which were kept at room temperature for 18 h after cooking.

In this study, Group C döner was experimentally contaminated with *S. aureus* at 10^5 CFU/g level. Although the initial mean of *S. aureus* was higher, a significant decrease was seen in the last hours of cooking, such as 2 log CFU both in raw and cooked samples. In this assay, group C döner was kept at room temperature for 18 h

after the cooking process and the changes of *S. aureus* count were examined. After 18 h, a significant increase was detected in the *S. aureus* load compared with 6 h of cooking, reaching 6.08 log CFU/g ($P < 0.05$) (Table 7). Also, in reheated döner samples, the *S. aureus* load was about 4.13 log CFU/g and it was found higher compared with the initial load. This increase of *S. aureus* population could be a sign of recontamination after cooking. It may also be associated with a numeric reduction of competitive microflora due to the change of environmental conditions or insufficient storage (71). All these factors make döner hazardous for consumers.

C. perfringens is another important thermophilic, anaerobic, and spore-forming microorganism associated with food poisoning. It has many strains, separated into five typical groups (A–E). Of these, types A and C are known as primarily human pathogens causing foodborne diseases. *C. perfringens* also produces multiple enterotoxins. These toxins are alpha, beta, gamma, epsilon, and iota. The alpha toxin is produced by all of these types of *C. perfringens.* Alpha toxin is also lethal, necrotic, and hemolytic. Enterotoxins occur in spore cells and they are released after lysis of the cells.

The vegetative form of *C. perfringens* is sensitive to heat treatment and unable to survive over 50 °C while germinated spores are not affected by standard thermal processes. The surviving spores transform into active cell forms if they are maintained under suitable temperatures for a long time. In this condition, the environment becomes anaerobic by removing the available air and a decrease is seen in the presence of other microflora. Via this support, *C. perfringens* can show its effects in poisoning (72,73). *C. perfringens* especially needs amino acids, so it is generally found in meat and meat products. Regarding this issue, meat dishes that are ready to eat are risky. Particularly, foods that are cooked but not cooled quickly or cooked insufficiently are more likely to contain *C. perfringens*. Many studies also reported that *C. perfringens* was found at variable levels in döner samples collected from markets (2,9,11,12,14–16,57).

In the present study, Group D döner was experimentally contaminated with *C. perfringens* (vegetative form) at 10^5 CFU level. Group D döner was also kept at room temperature for 18 h after the cooking process and changes in *C. perfringens* load were evaluated. The initial mean of *C. perfringens* was about 5.68 log CFU/g before cooking and no change was detected after 6 h of cooking. However, approximately 3 log of decrease in *C. perfringens* load was observed in cooked samples when compared with raw samples, a significant result. Surviving bacterial counts showed no change during cooking (Table 8). The initial *C. perfringens* load was high in this group; thus, the surviving bacterial load remained at the same level after the heating process. One of the possible risks during cooking is that the central temperature in döner provides conditions suitable for microorganisms to reproduce, especially in döner with a large diameter and long sales period (2,9,45). It is also known that at some sales points like restaurants, döner meat that is not consumed is kept for the next day and served again. The resulting temperature of unconsumed döner provides an ideal environment for microorganisms to reproduce (70). The *C. perfringens* load was higher by about 2.5 log in reheated samples compared with 6 h of cooking. In raw döner samples, nearly the same increasing ratio (2.4 log) was observed (Table 8). This increase can be related to germinated spores along the waiting period. Also, it is known that the germination time of *C. perfringens* is short; hence, the number of bacteria counted in our study was high (74,75). These findings show that döner meat, especially when cooked and then left to wait, could be hazardous for human health due to an increase in *C. perfringens* load.

In our study, staphylococcal enterotoxin production was evaluated in Group C. Although the *S. aureus* strain used for contamination was enterotoxigenic, it was not detected in either the cooked döner or the döner that was left for 18 h. The enterotoxin value for staphylococcal food poisoning is 100 ng, and because of this, *S. aureus* loads must be approximately 10^5 CFU/g for toxin production (71,76). Despite the fact that there were enough *S. aureus* colonies and that the analysis kit used was highly sensitive, no enterotoxin production was detected in this study. For toxin production, optimal conditions must be 40–45 °C, pH 6–7, and a_w of 0.98, and in our study, all of these conditions were within tolerable limits. However, *S. aureus* cannot produce enterotoxin under anaerobic conditions (77). During the cooking process, the central part of döner becomes anaerobic as a result of distancing of the present oxygen by heat effect. The lack of toxin in our study may be associated with this. It is claimed that *S. aureus* bacteria that survive heat treatment lose some of their pathogenic features due to heat stress. In a study by Batish et al. (78), although no change was seen in species exposed to heat treatment at 50 °C, it was detected that the species lost their coagulase activity at 55 and 62.5 °C. Also, the DNAse activity decreased and no enterotoxin production was seen in some of them after exposure to heat treatment.

On that note, attention must be given to some critical points during döner production. The quality of raw materials affects the quality of the final product, so the selection of these materials should be considered carefully. Furthermore, the heat treatment should never be interrupted or diminished during the cooking process. If the döner will be kept and served the next day, it should be kept at 4 °C.

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