PAPER

DETERMINATION OF CONTAMINATION STAGES OF FROZEN CHICKEN DÖNER

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ABSTRACT

"Döner" is a traditional food product generally produced with beef, lamb, and/or chicken meat, and is a very popular product in Turkey. The aim of this study was to investigate the possible contamination sources during the processing stages of Chicken Döner. Total viable psychrotrophs, total lactic acid bacteria, *Enterobacteriaceae* spp., *Escherichia coli*, total yeast and mold, *Salmonella* spp., and *Staphylococcus aureus* counts were determined at 13 processing points. At the freezing stage, lactic acid bacteria and *Enterobacteriaceae* counts can decrease, but at this point the elimination of *E. coli* and *S. aureus* contaminations is also very important.

- Keywords: chicken döner, contamination, meat processing, microbiological quality, ready-to-eat -

INTRODUCTION

"Döner" (sometimes known by other names, such as gyro, donair, dona kebab, and shawarma) is generally made of beef, lamb, veal, and/or poultry meat and is a traditional meat product in the Middle East (TODD *et al.*, 1986; KILIÇ, 2003; KAYAARDI *et al.*, 2005). The traditional "Döner" is made from intact muscle, ground muscle, or slices of meat interleaved with layers of raw meat resembling minced meat (TODD *et al.*, 1986; KILIÇ, 2003; VAZGEÇER *et al.*, 2004). Meat pieces (thickness ranging from 1-6 mm) or ground meat is marinated (for 3-6 h) according to the producers' preferences with red or black pepper, salt, diced onion, onion juice, or onion powder; diced tomatoes or tomato sauce; olive oil; lemon juice or vinegar; white sugar; cumin; allspice; thyme; grape juice; milk or milk powder; yoghurt, and egg at 4°C for 12 h (KAYAHAN and WELZ, 1992; ANONYMOUS, 1995; VAZGEÇER *et al.*, 2004; KAYAARDI *et al.*, 2005). Then, döner dough is impaled on a vertical stainless steel döner stick. The döner is grilled in a vertical position in front of heating equipment (open gas or electric oven to cook the surface). When the meat surface is cooked, it is shaved off, and the cooked döner is thinly sliced. "Döner" slices are served either on a plate or on bread with sliced tomato, onions, and lettuce (ANONYMOUS, 1995; KAYIŞOĞLU *et al.*, 2003).

Recently, the use of chicken and turkey breast meat in "Döner" production has become very popular because of the low cost of production, the high nutritional value, and the ease of digesting the product (CHOULIARA *et al.*, 2007). When chicken breast meat is used to manufacture döner as described above, it is called "Chicken Döner" (KILIÇ, 2003). In recent years, Döner has become a ready-to-eat food product that is prepared in meat processing plants. After preparation, it is packaged in a plastic tray and held in refrigerated (+4°C, 24 h) or frozen conditions (-18°C, 6 months) until it is ready to be cooked. The aim of freezing is to control microbiological activity (KILIÇ, 2003; ERGÖNÜL and KUNDAKÇI, 2007).

The purpose of this study was to investigate the hygienic conditions and possible contamination sources in the processing stages of "Chicken Döner" that limit its shelf-life.

MATERIALS AND METHODS

Material

Sampling procedures

"Chicken Döners" were prepared four times in a local meat factory in Balıkesir, Turkey, at different times, and each sample from collected processing stages was analyzed twice (n = 4 x 2). Fresh, deboned chicken breast meat was obtained from another company, and all bone, skin, and subcutaneous fat were removed before use. Chicken breast fillets were marinated in sauce (tomato paste, yoghurt, vegetable oil, water, garlic, onion) for 12 h at 4°C. Immediately after, the fillets were massaged for 25 min at 10°C, after which they were given a cone shape and placed on a stainless steel döner spit. Plastic stretch film was used as the packaging material. The döner samples were frozen at -40°C, and subsequently stored at -18°C.

The microbiological analyses were conducted for the various stages of chicken döner production on the day of production. A sample of 100 g was collected from each stage of the production process, placed in a sterile Stomacher bag, and stored in a cooler for transportation to the laboratory, where the analyses were conducted within 2 h. The stages of sampling were as follows: 1) raw chicken breast meat (with bone), 2) the hands of the employees who were chopping the meat, 3) the meat cutting board and knife, 4) the meat collecting tray, 5) the fillets, 6) sauce, 7) marinated fillets, 8) fillets in sauce before massaging, 9) fillet in sauce after massaging, 10) the board used to skewer the fillets, 11) the hands of the employees who skewered the fillets, 12) the pre-frozen ready-to-eat chicken döner, and, 13) the post-frozen product.

Methods

Microbiological analysis

For the microbiological analyses, 25-g samples of chicken döner were weighed out aseptically, 225 mL of a sterile buffered peptone water (Oxoid CM0509) were added, and the mixture was homogenized in a Stomacher blender (Masticator, IUL Instruments, Spain) for 60 s at room temperature (20° ± 1°C). Decimal dilutions of the buffered peptone water solution were prepared and duplicated, and 1 or 0.1 mL of at least three appropriate dilutions were mixed or spread on the following agar media: Plate Count Agar (PCA; Oxoid CMO325) for total viable (TV) and psychrotroph counts, incubated at 35°C for two days and at 7°C for seven days, respectively (BERRUGA *et al.*, 2005); de Man-Rogosa-Sharp medium (MRS; Oxoid CM0361) for lactic acid bacteria, overlaid with the same medium and incubated at 37°C for 48 h and Rose Bengal Chloramphenicol Agar (RBC; Oxoid CM 549 supplemented with SR 78) for yeasts and molds, incubated at 25°C for five days (COULIARA *et al.*, 2007); Violet Red Bile Glucose Agar (VRBGA; Merck 1.10275) for *Enterobacteriacea* counts, incubated at 37°C for 24 h (GOVARIS *et al.*, 2007); Tryptone Bile X-glucuronide Agar (TBX - Oxoid, CM0945) medium for *E. coli* counts (ÇOLAK *et al.*, 2008), incubated at 37°C for 48 h; Streptomycin Thallous Acetate Actidione Agar (STAA, Oxoid CM0881 supplemented with SR0151) for *Brochothrix thermosphacta,* incubated at 22°-25°C for 48-72 h (LIN and LIN, 2002). Presumptive *Staphylococcus aureus* were determined on Baird-Parker Agar base

(Oxoid CM0275) enriched with Egg Yolk Tellurite (SR0054) and plates were incubated aerobically at 35°C for 2 d. For the detection of *Salmonella spp.*, 25 g of sample was inoculated for pre-enrichment in 225 mL of buffered peptone water, and homogenized in a stomacher blender and incubated at 35°-37°C. After 16-20 h, the pre-enrichment culture was transferred into Rappaport Vassiliadis Enrichment Broth (Oxoid CM0669), and Tetrathionate Broth (Oxoid CM0029) base media with a ratio of 0.1/10 and 1/10, incubated at 42°-43°C for 24 h, and 37°C for 24 h, respectively. A loopful of these two enrichments were streaked onto Xylose Lysine Desoxycholate Agar, (Oxoid CM0469) and MacConkey Agar (Oxoid CM0007) for selective growth, and were incubated at 35°-37°C for 18-24 h. The plates were examined for the presence of typical suspect colonies of *Salmonella*, i.e. pink colonies with or without black centers on XLD Agar, and colorless colonies on MacConkey Agar. Presumptive *Salmonella* colonies were then subjected to initial screening tests using Triple Sugar Iron Agar (Oxoid CM0277), Lysine Iron Agar (Merck, 1.11640.0500), Urea Broth (Merck, 1.08483.0500), and Lysine Decarboxylase Broth (Oxoid, CM308). All biochemical tests were performed at 37°C for 18-24 h. Positives were confirmed serologically utilizing *Salmonella* O grouping antisera (TEMELLI *et al.*, 2006; ÇOLAK *et al.*, 2008). Swab methods were used for the microbiological analyses of the meat collection tray, the cutting board and knife (EISEL *et al.*, 1998). Glove methods were used for the employees' hands (DE WIT and KAMPELMACHER, 1988). All samples were analyzed in duplicate, and the results were averaged for statistical analysis. Analyses were conducted separately on the materials from each package. All microbial counts were expressed as base-10 logarithms of colony forming (log CFU, g^{-1} or mL or swabbed sample), except *Salmonella* spp. In this study, the presence of *Salmonella* spp. was assessed using 25-g samples and the minimum detectable level from presence/absence test is typically one organism in a 25-g sample.

Statistical analysis

The SPSS 16.0 Professional Statistics package (SPSS Inc., Chicago, IL, USA) was used to determine mean ± standard deviation values of the microbiological counts.

RESULTS AND DISCUSSION

The results of the microbiological analysis showed: 4.45, 5.04, and 3.89 log CFU/g total viable count (TVC); 3.35, 3.61, and 3.88 log CFU/g psychrotrophs; 5.33, 4.12, and 3.50 log CFU/g total yeast and mold; 2.79, 3.67, and 2.68 log CFU/g *Enterobacteriaceae* for the raw chicken breast meat, the fillets, and the ready-to-eat frozen "Chicken Döner," respectively (Table 1).

Table 1 - Results of the microbiological analyses of the Chicken Döner Kebab samples and sauce (n = 4 x 2) collected from production stages.

Samples	Total viable count [a,b]	Psychrotrophs	Lactic acid bacteria	*B. thermosphacta*	*Enterobacteriaceae*	*E. coli*	Total yeast-mold	*S.aureus*
Raw chicken breast meat-bone	4.45±0.07	3.35±0.02	2.96±0.07	2.26±0.09	2.79±0.17	2.11±0.07	5.33±0.02	3.79±0.17
Fillet	5.04±0.02	3.61±0.06	2.66±0.06	2.13±0.15	3.67±0.12	2.34±0.06	4.12±0.41	3.75±0.24
Sauce	6.35±0.09	2.80±0.09	6.04±0.13	1.00±0.00	2.79±0.17	2.41±0.15	4.44±0.05	1.00±0.00
Marinated fillet	4.83±0.06	3.07±0.12	4.23±0.21	3.12±0.07	2.70±0.25	2.47±0.10	3.44±0.19	3.69±0.32
Fillet in sauce pre-massaging	4.89±0.06	3.24±0.05	4.23±0.18	3.24±0.32	3.83±0.21	2.73±0.02	3.26±0.32	3.29±0.05
Fillet in sauce post-massaging	4.97±0.04	3.49±0.09	4.26±0.04	3.37±0.16	3.92±0.05	2.90±0.09	3.29±0.45	3.32±0.09
Ready-to-eat döner pre-frozen	3.95±0.05	2.86±0.09	5.12±0.05	3.34±0.09	2.77±0.08	2.65±0.21	2.96±0.16	2.98±0.03
Ready-to-eat döner post-frozen	3.89±0.07	3.88±0.16	3.46±0.02	3.29±0.05	2.68±0.04	2.46±0.26	3.50±0.02	2.80±0.23

[a] Mean (± standard deviation) counts determined samples; [b] log CFU/g for chicken döner kebab samples and sauce.

Also, the TVC in fillets with sauce after marination (4.83 log CFU/g) were similar to raw chicken breast meat, although the sauce had a TVC that was considered high (6.35 log CFU/g). It was thought that because the marination was conducted in cold conditions (+4°C), this restricted the increase of TVC.

According to OKONKWO *et al.* (1994), unhygienic processing conditions and/or recontamination due to poor post-processing handling are usually responsible for the high viable counts of organisms in meat products. The influence of environmental sanitation on the microbial population is a highly significant factor that affects the quality of meat products. "Chicken Döner" have higher aerobic plate counts and psychrotrophic bacteria counts than beef döners, therefore, these could be a potential hazard for public health because of low hygienic quality (KAYIŞOGLU *et al.*, 2003). Bacterial counts (aerobes, *Salmonella* spp., *E. coli*) are higher on the breast area of broiler carcasses than on the thigh and drum areas. In addition, some microorganisms, particularly *Salmonella* spp., attach to the skin of the poultry and they are difficult to remove (KOTULA and DAVIS, 1999). A great risk may be incurred from these pathogens if chicken skin is added to the chicken döners (VAZGEÇER *et al.*, 2004). Also, hygienic quality is expected because of the normal prevailing conditions in the processing sites and the personal hygiene of the processors. This situation was explained by ELMALI *et al.* (2005), and ABDULLAHI *et al.* (2006), indicating that contamination of meat products can occur due to inadequate cleaning of processing equipment, such as the meat chopping board, knife, and the meat collecting tray, infected or unhygienic status of the handlers, and contamination from raw beef, among others.

Lactic acid bacteria (LAB) and *Brochothrix thermosphacta* are found to be significant components of the microflora of products stored at refrigeration temperatures, and both have been associated with the spoilage of meat and meat products (STOLLE *et al.*, 1993; SAMELIS *et al.*, 2000; CAYRE *et al.*, 2005). STOLLE *et al.* (1993) reported that LAB counts ranged from less than 2.30 log CFU/g to 7.32 log CFU/g in döner. In this study herein, the LAB count in raw meat (2.96±0.07 log CFU/g) was approximately two log units lower than marinated fillet (4.23±0.21 log CFU/g). It was thought that the use of sauce that contained yoghurt was responsible for increasing the count of LAB in the fillets with sauce after marination. Also, LAB counts were increased in the ready-to-eat, pre-frozen chicken döner due to cross-contamination caused by the staff, and the board that was used to skewer the fillets. However, there was no change in the counts of *B. thermosphacta* in the stages of the process. Nevertheless, it was identified that LAB counts were decreased after freezing. It is thought that LAB were subjected to adverse conditions, such as water crystallization and low temperatures, producing a degree of protein denaturation and bacterial membrane injury, with consequent decrease in viability and loss of reproductive capability, similar to that reported by DE GIULIO *et al.* (2005)

Species of *Enterobacteriacea*e, such as *Salmonella* spp. and *E. coli*, as well as gram-positives such as *Staphylococcus aureus*, may be found in considerable numbers in "Döners" and may create a high potential risk of foodborne diseases for consumers (KAYISOGLU *et al.*, 2003; VAZGECER *et al.*, 2004; ELMALI *et al.*, 2005; ARUN *et al.*, 2007). Among these, the presence of *E. coli* in both raw and cooked döners is a matter of particular concern (JOCKEL and STENGEL, 1984; ELMALI *et al.*, 2005). Ensuring the microbial safety of meat and meat products is based on application of proper process hygiene, managed through a HACCP-based system.

The results of the microbiological analyses showed: 2.11, 2.34, and 2.46 log CFU/g *E. coli* counts, and 3.79, 3.75, and 2.80 log CFU/g *S. aureus* for the raw chicken breast meat, fillet, and ready-to-eat frozen chicken döners, respectively (Table 1). Among the investigated bacteria, the maximum counts calculated from [log CFU/g], i.e., 3.70 for *E. coli*, and 3.70 for *S. aureus* for the raw meat used for various types of döners were in compliance with the Turkish Food Codex (ANONYMOUS, 2006) and, therefore, the meat samples tested in this study could be considered to have good hygienic quality. Nevertheless, it is widely known that humans are the primary sources of contamination in most of the poisonings caused by *S. aureus*, where its growth is inhibited at temperatures under 5°C. In the EU, verification of the hygienic functioning of the manufacturing process for meat/meat products is done through microbiological testing to determine whether process hygiene indicator organisms ("aerobic colony count" and/or *E. coli* count) are within given acceptable ranges (ANONYMOUS, 2005). The detection of these bacteria in raw chicken meat is a very important indicator of the cross-contamination caused by staff during the production process.

Results of the microbiological analysis of samples collected from equipment and the hands of personnel revealed that counts were notably high from the aspect of total viable count, *E. coli*, and *S. aureus* (Table 2). This may be an indication of insufficient sanitary applications in the plant.

Salmonella spp., which can cause infections even with low counts (1-10 cell/g), have been reported to form the natural microflora in poultry farms, where they are raised, and therefore, can be a risk factor for humans and animals. In our study, all samples taken from the various stages of production were assumed to have *Salmonella* colonies, but these were not detected in the samples by serological tests. Similar

Table 2 - Results of the microbiological analyses of possible contamination stages (n = 4 x 2) collected from Chicken Döner Kebab production stages.

Samples	Total viable count [a,b]	Psychrotrophs	Lactic acid bacteria	*B. thermosphacta*	*Enterobacteriaceae*	*E. coli*	Total yeast-mold	*S. aureus*
Hands of staff-meat chopping	4.44±0.03	2.96±0.08	1.00±0.00	3.28±0.03	1.00±0.00	1.00±0.00	4.95±0.03	3.39±0.05
Meat cutting board and knife	4.49±0.04	3.34±0.12	1.00±0.00	3.30±0.14	2.82±0.05	2.31±0.03	4.23±0.12	1.00±0.00
Meat carriage-collecting tray	4.04±0.05	4.65±0.04	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	5.20±0.08	1.00±0.00
Board of skewering fillets	3.59±0.09	3.04±0.03	3.02±0.09	3.46±0.06	1.74±0.35	1.00±0.00	1.00±0.00	1.00±0.00
Hands of staff-skewering fillets	5.42±0.03	3.72±0.05	4.09±0.16	3.34±0.02	2.16±0.12	1.00±0.00	2.96±0.04	3.24±0.12

[a] Mean (± standard deviation) counts determined processing stages; [b] CFU/swabbed sample for collection tray, cutting board and knife; CFU/mL for staff hands.

results were reported for "Döners" by VAZGECER *et al.* (2004).

In this study, yeast and mold count, which were at high levels, had decreased from 5.33 to 3.44 log cfu/g after marination. After this processing stage, the population of yeast-mold occurred steadily throughout the frozen periods (Tables 1 and 2). The high levels of yeast and mold counts before marination, can be related to contamination from the raw material, and poor hygiene applied in the plant. It was also thought that yeast and mold contamination is a problem in meat production and to avoid problems with these, it is important to monitor the hygienic quality of the environmental air and equipment, and follow up with corrective actions to prevent further contamination.

CONCLUSIONS

Based on the critical appraisal of the chicken döner production process, some of the potential safety and quality hazards should be appropriately controlled. Potential hazards associated with the production line and considerations include: i) the raw material could contribute to the possible growth of pathogenic bacteria, so it should be purchased only from reliable suppliers; and ii) the production process is subjected to significant contamination from both human and environmental sources, such as the cutting board and knife, the meat collecting tray, and the hands of the staff; therefore strict compliance with hygienic regulations, such as those required by the HACCP (Hazard Analytical Critical Control Points) and GMP (Good Manufacturing Practice) programs is very important at all stages, from the production to the consumption of chicken döner.

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