

Heat Treated Turkish Style Sucuk: Evaluation Of Microbial Contaminations in Processing Steps

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Abstract: In this work, we determined the trends in contaminations for important microbial groups in heat treated Turkish style sucuk processing and verified the contamination routes for these groups. Samples were taken from deboned and cubed meat, post-blending and kneading, post-grinding, post-filling and from the final product after heat treatment. We also analysed samples of spices, casing, knife, meat cutting surface, batter vat, grinding machine, filling machine, workers' aprons, workers' hands, potable water used in the plant and production and cold room area air as possible origins for contamination and/or recontamination.

Statistical data revealed the following enlisted sources as primary agent(s) of contamination at indicated steps: knife (enterococci, $p<0.001$), meat cutting surface and cold room area (total aerobic mesophilic bacteria, $p<0.05$) in deboning; spices (enterococci, yeast and mold, $p<0.01$) in post-blending; batter vat (yeast and mold, $p<0.05$) and grinding machine (enterococci, $p<0.001$) in post-grinding; filling machine (total aerobic mesophilic bacteria, $p<0.05$; coliform, $p<0.01$) and casing (coliform, $p<0.01$; yeast and mold, $p<0.001$) in post-filling. Workers' hands were very important contamination/recontamination sources in blending (enterococci, staphylococci, yeast and mold, $p<0.05$), in grinding (enterococci, $p<0.001$; staphylococci, $p<0.05$) and in filling (coliform, $p<0.001$) steps.

Key Words: Sausage, sucuk, soudjouk, processing steps, microbiological contamination.

Isıl İşlem Uygulanmış Türk Tipi Sucuk: Üretim Aşamalarındaki Mikrobiyal Kontaminasyonların Değerlendirilmesi

Özet: Bu çalışmada, ısıl işlem uygulanmış Türk tipi sucuk üretiminde önemli mikrobiyal grupların kontaminasyon durumları ve oranları belirlendi. Kemiksiz et, kuşbaşı et, karıştırma ve yoğurma sonrası, çekim sonrası, dolum sonrası ve ısıl işlem sonrası son üründen örnekler alındı. Aynı zamanda, olası kontaminasyon ve/veya rekontaminasyonun belirlenmesi için baharatlar, barsak, bıçak, et parçalama tezgahı, hamur teknesi, kıyma makinesi, dolum makinesi, işçi önlük ve elleri, işletmede kullanılan su, soğuk depo havası da analiz edildi.

İstatistiksel verilere göre, kemiklerden ayırma aşamasında, bıçak (enterokoklar, $p<0.001$), et parçalama tezgahı ve soğuk depo ortamı (toplam aerobik mezofilik bakteri, $p<0.05$); çekim sonrasında, baharatlar (enterokoklar, maya ve küf, $p<0.01$); yoğurma sonrasında, hamur teknesi (maya ve küf, $p<0.05$) ve kıyma makinesi (enterokoklar, $p<0.001$); dolum sonrasındaki aşamada, dolum makinesi (toplam aerobik mezofilik bakteri, $p<0.05$; koliform bakteri, $p<0.01$) ve barsak (koliform, $p<0.01$; maya ve küf, $p<0.001$) kontaminasyonun başlıca etkenleri olarak belirlendi. İşçi ellerinin, yoğurma (enterokoklar, stafilocoklar, maya ve küf, $p<0.05$), çekim (enterokoklar, $p<0.001$; stafilocoklar, $p<0.05$) ve dolum aşamalarında (koliform bakteriler, $p<0.001$) çok önemli kontaminasyon ve/veya rekontaminasyon kaynağı olduğu saptandı.

Anahtar Kelimeler: Sausage, sucuk, soudjouk, üretim aşamaları, mikrobiyolojik kontaminasyon.

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Introduction

Turkish style sausage (sucuk) is the most popular dry fermented meat product in Turkey, and similar products are known in most of the Middle Eastern countries and Europe⁴. Sucuk is mostly produced by traditional methods in small scale plants by air-drying or in climatized rooms. This product, which is called “dry fermented sucuk” in Turkey is prepared by mixing ground lean or semi lean beef and/or sheep meat, tallow fat, garlic, salt, nitrate, ascorbic acid, sucrose, spices (such as red pepper, black pepper, cummin, allspice) and then filling this batter into natural or artificial casings. Then, they are hung for fermentation and dried at ambient temperatures for 20 or 25 days^{7,10}.

In recent years, some sucuk manufacturers started to prefer heat treatment of sucuks after a very short period of fermentation after the casing step²⁷. Primary aims of this type of production are: shortening of production time, elimination of pathogens during heat treatment, economical production. Due to the short fermentation period of this type of sucuks, nitrite is used instead of nitrate in the batter. Batter of this type of sucuk is incubated at 20 °C (ambient temperature) for 12 h in the casing, and then heat treated for 30 min after the core temperature reaches to 63 °C.

Economical losses related to texture and aroma deficiencies, and public health risks increase in manufacturing under improper and/or non-hygienic conditions^{14,24,26}. Several researchers have reported infections and intoxications as a result of consumption of raw or insufficiently heat treated or post-contaminated sucuks. To the best of our knowledge, despite the presence of many reports on dry fermented sucuk^{1,13} in Turkey, there is no study conducted related to heat-treated sucuks, which occupy a growing market share in our country.

In this study, we have evaluated the microbial status of heat treated Turkish style sucuk: we have determined the contamination sources and assessed the relationship of the routes of contamination by spesific microbial groups.

Materials and Methods

Sucuk processing

Turkish heat treated sucuks were prepared in a local meat processing plant in Bursa, Turkey.

Sucuk processing was performed between June and December 2004, and this was replicated 10 times (Figure 1).

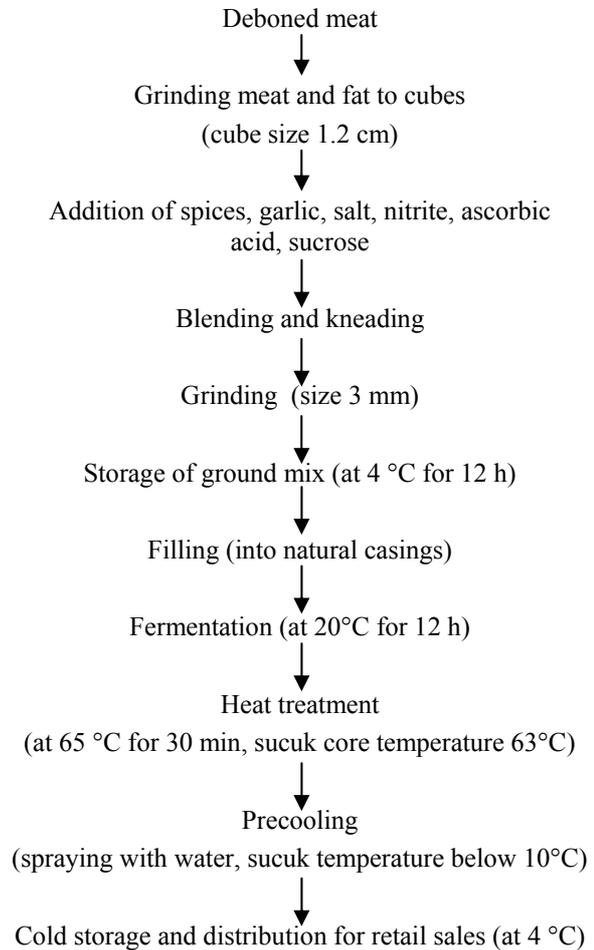


Figure 1.

Flow diagram for Turkish heat treated sucuk processing.

Şekil 1.

Isıl işlem uygulanmış Türk sucuklarının üretim akış şeması.

Deboned lean beef meat, which was chilled at 4 °C for 24 h, was cubed by a grinder (Çağdaş Makina Co., İstanbul, Turkey) with a hole size of 1.2 cm diameter, and overnight chilled fat (at -5 °C) cubed at the same size (rendered from the kidney and from the sheep tallow) was added at the ratio of 15 % to the cubed meat. Then the other ingredients (2 % sodium chloride, 0.5 % ascorbic acid, 1 % garlic, 0.5 % sucrose, 1 % red pepper, 0.5 % black pepper, 1 % cummin, 0.5 % allspice and 150 ppm sodium nitrite) were added. Meat-fat cubes and all other ingredients were

manually blended and kneaded thoroughly. This blended material was ground through a grinder with a hole size of 3 mm diameter and was kept at 4 °C for 12 h, in order to increase the penetration of ingredients into the meat. Then, sucuk batter was filled into natural casings (dried small intestine of cattle obtained from a private slaughterhouse in Bursa, Turkey) with a diameter of 36 mm, using a hydraulic filling machine (Yuneka Metal Co., Bursa, Turkey), and was kept at 20 °C for 12 h for fermentation. After fermentation, sucuks were heated in a steam chamber at 65 °C for 30 min (Yuneka Metal Co., Bursa, Turkey) with a core temperature of 63 °C. After heat treatment, sucuks were cooled below 10 °C. The temperature decline was carried out by spraying water on sucuks. Finished products were stored at 4 °C until retail sales.

Sampling procedure

Five hundred gram samples from the meat and/or production stages, namely deboned meat, cubed meat, post-blending and kneading, post-grinding, post-filling, after heat treatment, were taken aseptically by using sterile knives. Each sample was transferred into a sterile stomacher bag and transported to the laboratory at 4 °C in prechilled insulated containers with chiller packs and analysed within 1 h³². Samples taken from spices and casings were placed into sterile stomacher bags and transported without refrigeration²⁰.

Sterile cotton swabs were used to sample a 10 cm² area from the processing lines and from the surfaces of the following processing equipment: knife, meat cutting surface, batter vat, grinding machine, filling machine, and workers' aprons. If the surface was dry, swabs were pre-moistened with 0.1 % sterile peptone water (OXOID, CM 9). Swabs were transported to the laboratory in tubes containing 10 ml of 0.1 % sterile peptone water, were kept at 4 °C and analyzed on their arrival. All of the swab samples were taken during work hours⁹.

Samples from the workers' hands were taken as follows: Workers were let to wear sterile latex gloves and 20 ml 0.1 % sterile peptone water was carefully pipetted into the gloves. Hands in gloves were massaged completely and the gloves were carefully taken off, tied at the top and were transferred laboratory at 4 °C in prechilled insulated containers with chiller packs, and analyzed in the laboratory upon arrival⁸.

Approximately 200 milliliters of potable water samples were taken from the plant and transferred to the laboratory in pre-sterilized screw-capped bottles containing 5 % Na₂S₂O₃ at 4 °C, and analysed on their arrival to the laboratory³¹.

Air samples were taken from production and cold room areas, by keeping specific agar plates open in designated places for 15 minutes, where there was normal air circulation during production¹⁷.

Microbiological analyses

For microbiological analyses, 25 g samples were taken aseptically from each 500 g sample unit, added into 225 ml of sterile 0.1 % peptone water and homogenized in a stomacher (Seward Medical Head Office, BA 6020 Model, London, England) for 3 min at room temperature. Serial decimal dilutions were made using the same medium and then plated as duplicates for bacterial counts. The results were expressed as log cfu/g⁴.

Enumeration of total aerobic mesophilic bacteria: Aerobic mesophilic bacteria were enumerated by spread plate method on Plate Count Agar (PCA, OXOID CM 325). All colonies were counted after aerobic incubation at 30 °C for 48 h¹⁴.

Enumeration of coliforms: Coliform bacteria were enumerated by pour plate method in Violet Red Bile Agar (VRBA, OXOID CM 107), which was overlaid with 5 ml of the same medium, after incubation at 37 °C for 24 h in aerobic conditions¹⁶.

Enumeration of *Escherichia coli* (*E. coli*): Typical colonies observed in VRBA (indicated above in 2.3.2.) were transferred to Lactose Broth (OXOID CM 137) and incubated at 37 °C for 48 h. From the cultures with gas and turbidity, sub cultures were made on Eosin Methylene Blue Agar (EMBA, OXOID CM 69) and incubated at 37 °C 24 h. *E. coli* isolates were biochemically characterized by IMViC tests¹⁶.

Enumeration of enterococci: Enterococci were enumerated by spread plate method in Slanetz Bartley Medium (SB, OXOID CM 377), which was overlaid with 5 ml of the same medium after 24 h incubation at 37 °C³⁰.

Enumeration of staphylococci: Staphylococci were enumerated by spread plate method on Baird Parker Agar (BPA, OXOID CM 275) prepared by adding Sterile Egg Yolk Tellurite Emul-

sion (OXOID SR 54). All typical colonies were counted after aerobic incubation at 37 °C for 24 h²⁷.

Enumeration of coagulase positive staphylococci: Typical colonies which were observed in BPA (indicated above in 2.3.5.) were transferred to Brain Heart Infusion Broth (OXOID CM 225) and incubated at 37 °C for 24 h. Loopful of cultures were streaked on designated spots on the staphylase test kit card (OXOID DR 595) and coagulase test was performed. Counts were determined accordingly³⁰.

Enumeration of lactic acid bacteria: Lactic acid bacteria were enumerated by pour plate method in Mann Rogosa Sharpe Agar (MRS, OXOID CM 361) overlaid with 5 ml of the same medium, and incubated at 30 °C for 72 h in an anaerobic culture jar¹¹.

Enumeration of yeast and molds: Yeasts and molds were enumerated by spread plate method on Potatoe Dextrose Agar (PDA, OXOID CM 139), where plates were aerobically incubated at 20 °C for 4 to 5 d³⁰.

Analysis of water: For enumeration of total aerobic mesophilic bacteria, 0.1 ml from each water sample was pipetted and spread onto plate count agar (PCA, OXOID CM 325) and incubated at 30 °C 48 h. MPN method was used for the enumeration of coliforms and *E. coli*².

Statistical analysis

Statistical software SPSS and MINITAB for Windows were used for statistical analyses. Microbial levels at production stages were analysed by One Way Analysis of Variance (ANOVA), and Tukey-Kramer multiple comparisons tests were applied as post-test, when significant differences were determined²⁸. In addition, Regression Analyses were used to determine the effect of spices, casing, surfaces of various processing equipment, workers' hands and environmental factors on microbial load in production stages²¹.

Results and Discussion

Aerobic mesophilic bacteria counts in deboned meat and cubed meat in this study are similar to the counts reported in the previous studies^{6,20,25}, whereas other microbial counts from these meats were found to be higher than the counts indicated in other studies^{20,25} (Table I).

Reasons for these types differences could be due to variations in the hygienic quality of raw materials (related to pre and post mortem applications), and inadequate handling and storage practices. Statistics on deboned meat revealed that meat cutting surface was related to the increase in total aerobic mesophilic bacteria counts ($p < 0.05$) and knife was related to increases in enterococci counts ($p < 0.001$).

There were meaningful increases in the numbers of all microbial groups in post-blending and kneading samples ($p < 0.05$), which had been taken after the addition of all spices and additives to cubed meat (Table I). The total aerobic mesophilic bacteria counts (10^6 log cfu/g) for post-blending and kneading samples are within the range of aerobic mesophilic counts (10^5 - 10^8 log cfu/g) in related studies^{3,4,13,19}. Out of a total of 10 samples taken after blending and kneading, 4 of the samples (mean value = 3.92 log cfu/g) were found to harbor *E. coli*. Current literature indicate that increases in microbial counts in general had been related to the addition of spices and additives to the batter^{6,25}. Therefore, we performed statistical analysis to determine if spices, and workers' hands gave rise to microbial counts after blending and kneading in our processing. This analysis convinced us that spices had a significant effect on the increase of enterococci and yeast and mold counts ($p < 0.01$). Also, we determined that enterococci, staphylococci and yeast and mold counts inclined considerably due to possible contaminations from workers' hands ($p < 0.05$). Count for lactic acid bacteria, which was determined as approximately 10^6 log cfu/g in our study, was slightly higher than previously reported^{3,7,19}.

In post-grinding samples, counts were substantially higher than post blending and kneading of sucuk batter sample counts ($p < 0.05$). From statistical analyses of the possible suspect sources as batter vat, grinding machine and workers' hands, we have determined that all 3 of these sources caused increases in yeast and mold counts in post-grinding samples ($p < 0.05$). Workers' hands were found to be a significant contamination source for staphylococci ($p < 0.05$) and enterococci ($p < 0.001$) in this processing step.

Samples from post-filling, in general, yielded higher microbial counts than post-grinding (Table I). These counts obtained from sucuk batter samples filled into natural casings

Table I. Results of the microbiological analyses of the samples (n=10) collected during heat treated Turkish style sucuk processing steps.

Tablo I. Isıl işlem uygulanmış Türk sucuklarının üretim aşamaları sırasında alınan örneklerin (n=10) mikrobiyolojik analiz sonuçları.

Microorganism	Total aerobic mesophilic bacteria		Coliforms		Enterococci		Staphylococci		Yeast and molds		Lactic acid bacteria	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Samples												
Deboned meat ^a	5.47CD ^b	0.47	3.57D	0.13	3.80BC	0.44	4.40D	0.68	5.10D	0.71	3.71D	0.12
Cubed meat	5.81CD	0.41	3.98CD	0.40	3.94ABC	0.44	4.80CD	0.14	5.71C	0.70	4.65C	0.29
Post-blending and kneading	6.13BC	0.42	4.32BC	0.35	4.32AB	0.40	5.14BC	0.27	6.02ABC	0.43	5.48B	0.23
Post-grinding	6.60B	0.56	4.68AB	0.28	4.68AB	0.22	5.41AB	0.20	6.20AB	0.56	5.73AB	0.10
Post-filling	7.16A	0.75	5.02A	0.28	4.79A	0.15	5.67A	0.19	6.31A	0.57	5.92A	5.34
After heat treatment	4.60E	0.27	0.46E	0.76	1.32D	1.53	3.70E	0.43	2.85E	0.82	4.43C	0.31

^a, log cfu/g

^bA-E: Differences between the processing stages demonstrated with different capital letters in the same column are significant (p<0.05).

Table II. Results of the microbiological analyses of the samples (n=10) collected from spices, casing, equipment, workers' aprons, and hands during heat treated Turkish style sucuk processing steps.

Tablo II. Isıl işlem uygulanmış Türk sucuklarının üretim aşamaları sırasında baharatlar, barsak, ekipman, işçi önlükleri ve ellerinden alınan örneklerin (n=10) mikrobiyolojik analiz sonuçları.

Microorganism	Total aerobic mesophilic bacteria		Coliforms		Enterococci		Staphylococci		Yeast and molds		Lactic acid bacteria	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Samples												
Spices ^a	5.89	0.65	1.94	0.68	3.62	0.27	5.28	0.72	3.74	0.38	5.70	0.20
Casing	5.66	0.24	3.16	0.47	3.42	0.29	4.87	0.13	4.11	1.00	4.45	0.28
Knife ^b	5.42	0.79	3.52	0.64	3.22	0.66	4.51	0.77	4.22	0.49	2.59	0.29
Meat cutting surface	5.37	0.69	3.18	0.52	1.83	1.67	4.16	0.47	3.68	0.74	3.67	0.19
Batter vat	4.69	0.64	3.42	0.27	1.41	1.83	3.03	0.48	4.04	0.76	2.66	0.22
Grinding machine	4.90	0.53	4.01	0.52	3.71	0.42	3.85	0.64	3.87	0.59	3.60	0.28
Filling machine	5.58	0.11	3.78	1.06	3.25	0.90	4.19	0.66	3.96	0.58	4.30	0.31
Workers' aprons	4.33	0.64	1.78	1.63	1.31	1.15	3.27	0.93	3.30	0.77	4.19	0.36
Workers' hands ^c	5.23	0.51	1.11	0.97	0.57	1.20	4.49	0.24	4.42	0.90	3.13	0.38

^a, log cfu/g

^b, log cfu/cm²

^c, log cfu/ml

were also found to be higher than the counts reported in other studies^{6,11,24,25}. The filling machine's effect on the increase of the total aerobic mesophilic bacteria ($p < 0.05$) and coliform bacteria ($p < 0.01$) in samples from post-filling were found to be significant. From a total of 10 samples taken from the filling machine, 2 samples were found to harbor *E. coli*. Also we have recorded increases in coliform counts ($p < 0.01$), and yeast and mold ($p < 0.001$) counts in post-filling samples, which are related to contaminations from natural casing. Another source for increase in the coliform counts were related to workers' hands ($p < 0.001$).

In our study, we found that heat treatment reduced the numbers of all investigated microorganisms; particularly coliforms and yeast and molds. Calicioglu, Faith, Buege, and Luchnsky⁵ indicated that heat treatment of Turkish sucuk after fermentation and drying had decreased pathogen counts below detection levels by conventional methods. Parallel to this, *E. coli* and coagulase positive staphylococci could not be detected in the heat treated sucuks in this study. Also we have observed notable decline in microbial counts between post-filling and after heat treatment ($p < 0.05$) (Table I). Relatively low reductions in the enterococci and lactic acid bacteria counts after heat treatment indicate their ability to survive this process^{3,12,15}.

As indicated in various parts of our results, hands of the personnel working in sucuk processing were critical contamination sources for various types of microorganisms tested in the study. We have to note here that we have detected coliforms and staphylococci in workers' hands at 1.11 and 4.49 log cfu/ml levels, respectively (Table II), however no *E. coli* or coagulase positive staphylococci were identified. In addition, workers' aprons were important factors in the increase of lactic acid bacteria counts in the workers' hands ($p < 0.05$). These results indicate that hygienic practices were insufficient and the workers were possibly one of the sources for secondary contaminations^{22,29}.

Since the water used for precooling had been reported as a source of contamination for the product²⁰, we investigated microbiological quality of the water by examining 10 samples from the processing plant. We determined the total aerobic mesophilic bacteria counts as 1.02 log cfu/ml, and did not detect any coliforms or *E. coli*. These results show that the water used in the plant did not pose microbiological risk to sucuk processing.

Previous studies indicated that production area and cold room area as respective critical contamination and recontamination points accounting for shortening of shelf life^{18,23}. In our study, total aerobic mesophilic bacteria and yeast and mold counts were 1.56 and 1.7 log cfu/plate in the production area, and 1.31 and 1.19 log cfu/plate in the cold room. Analysis of the effect of air from cold room area to increase the total aerobic mesophilic bacteria counts on deboned meat was found significant ($p < 0.05$). Also, production area air was found to have no negative effect on the total aerobic mesophilic bacteria and yeast and mold counts throughout the processing.

This study showed that heat treatment resulted in reduction of microbial counts in the final product, which should not be the sole step to be depended on for production of safe sucuk. Data analysed in this work revealed that there were various sources, which contribute to contamination and recontamination to the process or to the final product. These can be summarized as follows: total aerobic mesophilic bacteria increase due to contaminations from meat cutting surface, filling machine and cold room area; coliforms increase after filling, casing and wherever the workers' hands were introduced; enterococci counts incline with the addition of spices and also with workers' hands and equipment surfaces; spices, casing, batter vat and workers' hands contributed to increases in yeast and mold counts. Workers' hands were found to harbor staphylococci and were the primary contamination sources for this bacterium mainly in post-blending and kneading, and post-grinding samples.

For a high quality and safe sucuk, certified suppliers should be used to ensure the microbial safety in all raw material. Additionally, plant personnel should continuously be trained and assure the followings before, during and after the processing, both for their safety and the product's wholesomeness: apply good hygiene practices for the plant, take actions in processing and storage areas for cross, post or recontamination.

All given recommendations are to overcome the problems encountered in the production of heat treated Turkish style sucuk.

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