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Determination of microbial contamination sources at a Frankfurter sausage processing line

Ebru GÜNGÖR[1], Nalan GÖKOĞLU[2,*]

[1]Department of Food Engineering, Faculty of Agriculture, Akdeniz University, Antalya - TURKEY

[2]Department of Fisheries and Fish Processing Technology, Faculty of Fisheries,
Akdeniz University, Antalya - TURKEY

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Abstract: This study was conducted to determine microbial contamination sources during sausage processing. The samples were taken at 6 different stages of the sausage processing line in a local plant. Minced meat, sausage batter, stuffed sausage, cooked sausage, peeled sausage, and pasteurized sausage samples were examined microbiologically. Moreover, spices and ice water used in production, personnel hands, and equipment were examined. Counts of total aerobic mesophilic bacteria, *Staphylococcus aureus*, *Escherichia coli*, yeasts, and molds in minced meat were found to be 7.02, 3.83, 4.42, and 1.62 log cfu/g, respectively. *E. coli* and yeast-mold counts in sausage batter reached 3.99 and 1.72 log cfu/g, respectively. Heating for cooking was effective in reducing microbial counts. Total plate (3.93 log cfu/g) and *S. aureus* (1.08 log cfu/g) counts in cooked sausages decreased; *E. coli* and yeast-mold were not detected. According to the results, raw material and spices were found as primary contamination sources. Personnel hands and equipments were found as secondary contamination sources. Microbial counts in personnel hands showed significant correlations with the counts of the samples taken from all processing stages. Microorganism counts determined in overall processing were not at harmful levels for human health and the microbial load of the final product was within critical limits.

Key words: Microbial contamination, sausage processing, hygiene

Sosis işleme hattında mikrobiyal kontaminasyon kaynaklarının belirlenmesi

Özet: Bu çalışma sosislerin işlenmesi esnasında mikrobiyal kontaminasyon kaynaklarının belirlenmesi için yürütülmüştür. Örnekler yerel bir işletmenin sosis işleme hattında altı farklı aşamada alınmıştır. Kıyma, hamur, doldurulmuş çiğ sosis, pişmiş sosis, soyulmuş sosis ve pastörize sosis örnekleri mikrobiyolojik açıdan incelenmiştir. Ayrıca üretimde kullanılan baharat ve buz örnekleri ile alet ekipman ve personelden alınan örnekler de incelenmiştir. Kıymada toplam mezofilik aerobik bakteri, *Staphylococcus aureus*, *Escherichia coli*, maya ve küf sayıları sırasıyla 7,02, 3,83, 4,42 ve 1,62 log kob/g olarak bulunmuştur. Pişirme aşamasında uygulanan ısıl işlemin mikroroganizma sayısında önemli azalmaya neden olduğu belirlenmiştir. Pişmiş sosislerin toplam mezofilik aerobik bakteri (3,93 log kob/g) ve *S. aureus* (1,08 log kob/g) sayıları azalmış *E. coli* ve maya-küf ise tespit edilememiştir. Elde edilen sonuçlara göre birinci kontaminasyon kaynağı olarak hammadde ve baharatlar belirlenirken, ikinci kaynak personel ve alet-ekipman olarak belirlenmiştir. Sosis üretiminin bütün aşamalarında mikroorganizma sayılarının insan sağlığı için tehlike oluşturabilecek düzeyde olmadığı ve son ürünün mikrobiyolojik olarak kritik limitlere uyduğu saptanmıştır. Tüketici sağlığı açısından güvenli sosis üretimi için sosislerin uygun sıcaklık ve sürede pişirilmesi, son ürünün pastörize edilmesi ve üretimde kullanılan alet-ekipman temizliğine ve hammadde kalitesine dikkat edilmelidir.

Anahtar sözcükler: Mikrobiyal kontaminasyon, sosis işleme, hijyen

* E-mail: ngokoglu@akdeniz.edu.tr

Introduction

Frankfurters are cooked and smoked sausages. They are produced from fresh meat that is cured during processing, fully cooked, and smoked. A number of product characteristics influence the growth of microorganisms. Microbial hazards are a major concern in the production of food of animal origin. Studies related to microbial contamination have concentrated on carcass. However, meat and meat products can be contaminated with bacteria during manufacturing and packaging. Microorganisms gaining access into sausage from meat, spices, and other ingredients, from environment, equipment, and handlers during processing affect the microbiological status of the products (1). The safety of food of animal origin for human consumption has become an essential part of the public health debate. Several meat-processing plants have begun to utilize a program called the Hazard Analysis and Critical Control Point (HACCP) system to reduce pathogenic contamination. This program identifies the steps in the conversion of livestock to human food where the product is at risk of contamination by microorganisms (2). It is essential to identify potential risks related to product and microbial contamination sources for implementation of HACCP program in a sausage processing plant. The aim of this research was to determine microbial contamination sources at sausage processing line and to establish critical control points.

Material and methods

Preparation of sausages

Frankfurters were manufactured in a private meat processing plant in Antalya, Turkey. Sausages were produced by fresh boneless beef cuts from shoulder. Beef meat was trimmed of visible fat, minced through a 3 mm plate using a Mado mincer MEW 5102 and then placed in a cutter (Mado, MTK 661, Dornhan, Germany) along with the other ingredients. The following common ingredients were used per kg of batter: starch 382 g; sodium lactate 19 g; monosodium glutamate 24 g; nitrite salt 15 g; sodium tripolyphosphate 25 g; and spice mix 146 g.

Total mixing time was 10-15 min and the final temperature of the batter was 10.0-12.3 °C. Prepared frankfurter mixture was stuffed using a stuffer (Handtmann, VF100/240, Biberach, Germany) into 14 mm diameter casings. The frankfurters were hanged, smoked, and cooked for 25 min at 76 °C. The cooked frankfurters were showered in cold water and kept overnight at 4 °C. After the casings were removed, products were packed under vacuum with MULTIVAC vacuum packaging machine (R 230/719, Wolfertschwenden, Germany) and then pasteurized at 85 °C for 10 min.

Sampling procedure

Samples were collected at 6 different stages of sausage production, namely minced meat, batter, stuffed sausage, cooked sausage, peeled sausage, and pasteurized sausage. In addition, samples were collected from equipment surfaces, personnel hands, spice mix, and ice water. The samples of minced meat and spice mix were taken just prior to use for production. Samples of batters were taken from the cutter before stuffing. Sausage samples were taken just after each process (stuffing, cooking, peeling, and pasteurizing). All meat, batter, and sausage samples were taken from the same runs. Samples from the surfaces of equipment and tables were taken at the end of the work day after cleaning and sanitizing. The samples from the personnel hands were taken during working hours. Sampling procedure was repeated 6 times at different production days with 3-week intervals. Sampling points are shown in flow diagram of sausage production (Figure).

Ten grams of minced meat, batter, and sausage were sampled aseptically using sterile knives for microbiological analyses. All samples were diluted up to 10^{-7} with sterile ringer solutions (3). Water samples of 10 mL were taken using sterile pipettes and diluted up to 10^{-2} with sterile 0.1% (w/v) saline peptone water (0.1% peptone and 0.85% NaCl). Ten grams of homogenous samples from the spice mix were taken and serial dilutions were carried out.

The samples from personnel hands and equipment surfaces were collected by the swab method. A sterile paper template was used to outline a 10 cm^2 area, inside which a pre-moistened swab in 10 mL ¼ sterile ringer solution performed the swabbing. The swab

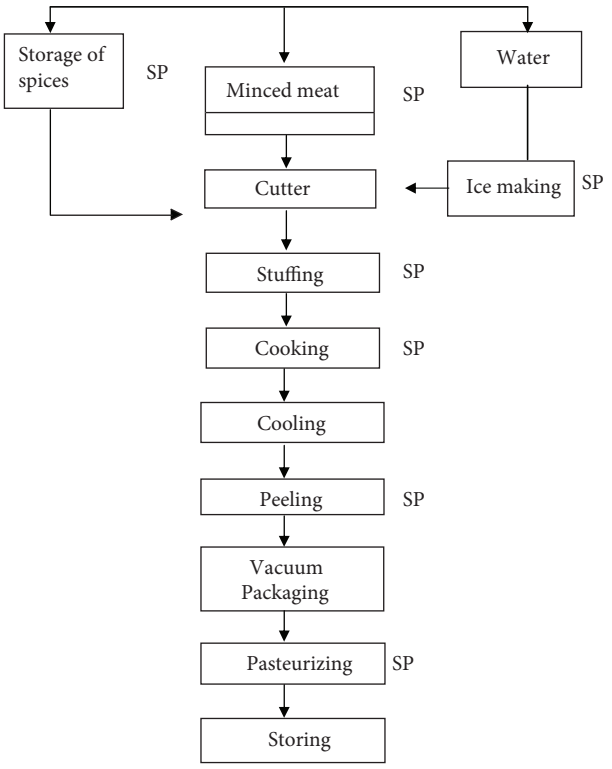

Figure. Flow diagram of sausage production and sampling points (SP)

was then placed in a tube containing 10 mL ringer solution, shaken, and squeezed in the diluents, and the rinse fluid plated in appropriate culture media.

All samples were immediately transferred to the laboratory in the same plant and analyzed. All analyses were performed in duplicates and the experiment was repeated for 6 times. The results were expressed as means of replicates.

Microbiological analyses

Total viable counts were determined by the spread plate method on Plate Count Agar (PCA Oxoid CM0463B, Hampshire, England). Plates were incubated at 35 °C for 48 h (4).

Enumeration of *Staphylococcus aureus* was performed by the spread plate method on Baird Paker Agar. Plates were incubated at 35 °C for 48 h. Typical *S. aureus* colonies were examined and coagulase test was performed to these colonies (4).

The presence of *Escherichia coli* was examined by transferring 1 mL of each sample dilution to sterile petri dishes followed by pouring 10 mL of Violet Red Bile Agar (VRBA Oxoid, Hampshire, England) tempered to 45 °C into plates. The plates were swirled, allowed to solidify, overlaid with 5-7 mL of VRB-MUG and then incubated at 35 °C for 18-24 h. These plates were examined under long wavelength UV lamb for the presence of fluorescent colonies. Fluorescent colonies were enumerated as *E. coli* (4).

Yeast-molds were enumerated with Potato Dextrose Agar (Merck, Darmstadt, Germany). Petri dishes were incubated at 25 °C for 5 days for yeast-mold. Petri dishes containing 30-300 colonies were enumerated (4).

All microbial counts were expressed as base-10 logarithms of colony forming units per gram (log CFU/g).

Statistical analyses

The samples taken from 6 different stages of sausage production were investigated. The experiments were performed in 6 replications. Means and standard errors were calculated. The significant differences between the means of microbial counts at different stages of sausage processing were analyzed by the t-test (paired comparison) using MINITAB program (version 13.0).

Results

The results found in minced meat, sausage batter, and stuffed sausage are shown in Table 1. There were no significant ($P > 0.01$) changes in total plate and *S. aureus* counts between minced meat and sausage batter but significant ($P < 0.01$) increases were observed in *E. coli* and yeast-mold counts.

Total plate count in stuffed sausage was higher ($P < 0.01$) than those of minced meat and batter. There were no significant ($P > 0.01$) differences in *S. aureus* counts of stuffed sausage compared to minced meat and batter. *E. coli* and yeast-mold counts of stuffed sausages were significantly ($P < 0.01$) different than those of minced meat but not significantly ($P > 0.01$) different than those of batter.

Microorganism counts of cooked sausage, which was cooked after stuffing at 76 °C for 25 min, are shown in Table 1. Total plate and *S. aureus* counts of cooked sausages were significantly ($P < 0.01$) lower

Table 1. Microbial counts at different stages of sausage processing in a meat processing plant.[1] (log cfu/g).

Processing stages	Total plate count	*E. coli*	*S. aureus*	Yeast-mold
Minced meat	7.02 ± 0.11a	3.83 ± 0.05a	4.42 ± 0.09a	1.62 ± 0.02a
Batter	7.04 ± 0.1a	3.99 ± 0.04b	4.51 ± 0.09a	1.72 ± 0.02b
Stuffed sausage	7.06 ± 0.1b	3.99 ± 0.02b	4.52 ± 0.09a	1.72 ± 0.02b
Cooked sausage	3.93 ± 0.1c	$<1.0 \times 10^1$c	1.08 ± 0.16c	$<1.0 \times 10^1$c
Peeled sausage	3.93 ± 0.09c	$<1.0 \times 10^1$c	1.06 ± 0.15c	$<1.0 \times 10^1$c
Pasteurized sausage	3.66 ± 0.18c	$<1.0 \times 10^1$c	0.73 ± 0.18d	$<1.0 \times 10^1$c

[1] All values reflect the mean values of 6 replicates and standard errors. Values in the same column bearing different letters are significantly different ($P < 0.01$)

than those of minced meat, batter, and stuffed sausage. *E. coli* and yeast-molds were not detected in cooked sausages.

There were no differences in microbial counts of sausages after the peeling process (Table 1). Total plate and *S. aureus* counts did not change after peeling. *E. coli* and yeast-molds were not detected in peeled sausages.

After the peeling process the sausages were packed in polyethylene bags under vacuum and pasteurized at 85 °C for 10 min. Total plate and *S. aureus* counts in pasteurized sausages were significantly ($P < 0.01$) lower than those of cooked and peeled sausages.

In our study the highest microbial count was found on the 3rd production day (one of replications) and the lowest count was found on day 4. The highest and lowest microbial counts at all processing stages were also found on days 3 and 4. Microbial counts of equipment surfaces showed significant ($P < 0.05$) correlations with minced meat ($r = 0.81$), batter ($r = 0.87$), stuffed sausage ($r = 0.87$), cooked sausage ($r = 0.93$), peeled sausage ($r = 0.94$), and pasteurized sausage ($r = 0.83$).

The mean counts of the total plate, *S. aureus* and *E. coli* in the samples taken from personnel hands at 6 different sampling days were found below the standard values (Table 2). Total plate count found in personnel hands affected the microbial load of sausages at all production stages. While the highest total plate count in personnel hands was found on the 3rd sampling day, the highest counts at all production stages were also found at the 3rd sampling day. Microbial counts in personnel hands showed significant ($P < 0.05$) correlations with the counts of minced meat ($r = 0.80$), batter ($r = 0.86$), stuffed sausage ($r = 0.85$), cooked sausage ($r = 0.92$), peeled sausage ($r = 0.93$), and pasteurized sausage ($r = 0.82$).

The counts of total plate, *S. aureus,* and yeast-mold in spice mix were found higher than that reported in the Turkish Food Codex. *E. coli* was not detected in spice mix (Table 2).

Table 2. Microbial counts of equipments surfaces, personnel hands, ice water, and spice mix in a meat processing plant.[1] (log cfu/g).

	Total Viable Count	*E. coli*	*S. aureus*	Yeast-mold
Equipment surfaces	4.46 ± 0.22	-	-	-
Personnel hands	4.46 ± 0.22	3.97 ± 0.08	2.67 ± 0.12	-
Ice water	1.71 ± 0.1	-	-	-
Spice mix	7.61 ± 0.17	nd[2]	3.41 ± 0.16	3.17 ± 0.17

[1] All values reflects the mean values of 6 replicates and standard errors.
[2] nd= non detected

Discussion

The counts of total plate, *E. coli, S. aureus* and yeast-mold in raw minced meat were found above the microbiological standards of the EU Council (94/65/EC) (5) and Turkish Food Codex (6) for minced meat. According to these standards acceptable levels of total viable counts (TVC), *E. coli* and *S. aureus* are 5×10^6, 5×10^2, and 5×10^3 log cfu/g, respectively. Total plate counts were also found higher than previously reported (1,7-10). Sachindra et al. (1) found that TVC in minced buffalo meat was 5.41 log cfu/g. In previous research studies, TVC of minced meat were found as 7.9×10^6 (7), 1.11×10^6 (8), 4.7 cfu/g (9), and 2.19×10^4 (10). The most probable reason of high microbial count in minced meat might be the poor hygienic quality of raw meat, inadequate storage and thawing conditions, contamination from grinder, and the time between mincing and mixing.

Minced beef poses more risk compared to intact muscle tissue because it can be contaminated throughout due to increased surface area and mixing during the mincing operation (9). For raw meat products, potential safety and quality can be estimated with the use of indicator microorganisms including aerobic plate count and *E. coli* count. High *E. coli* generally correlate with higher levels of food-borne pathogens originating from fecal origin (11-12).

E. coli and yeast-mold counts in sausage batter were higher than those of minced meat. The most probable reason for these increases in *E. coli* and yeast-mold is addition of ingredients into the batter. Sachindra et al. (1) reported similar increases in batter samples and also stated that these increases do not have much relevance from the point of microbial safety, since the cooking process follows. Total plate counts in the batter of cooked ring sausages ranged between 5.0 and 8.2 log cfu/g (13). Borch et al. (14) found TPC of 5.2 log cfu/g in a sausage emulsion.

Cooking process significantly affected microbial counts. Total plate and *S. aureus* counts decreased, *E. coli* and yeast-molds were not found after cooking. Sachindra et al. (1) and Borch et al. (14) reported similar decreases in TPC after cooking of sausages. The results show that there were no contaminations during cooling and peeling operations. All values found for peeled sausages comply with standard values (15,16). Pasteurization also affected microbial count. Total plate and *S. aureus* counts significantly decreased after pasteurization. This result is important to reveal the effectiveness of pasteurization on inhibition of *S. aureus* that can survive or is present due to contamination after cooking. *E. coli* and yeast-mold were not detected in pasteurized sausages. Since *E. coli,* yeast, and mold were already inhibited due to cooking process, these microorganisms were not present after pasteurization.

Total plate count of equipment surfaces was below the standard limit value (Table 2). However, some researchers (17-19) have found lower values than those observed in this study. One of the most significant risk factors identified is cross contamination, particularly between food and preparation surfaces (20,21). Generally microbial load of equipment surfaces depends on microbial quality of food and cleaning and sanitation program in the plant (22). High microbial count causes an increase in the microbial count of the product at processing stages. Our results have confirmed this assertion.

One of the major risks of food contamination originates from the working practices of food handlers and disease-causing microorganisms present in or on the food handler's body are subsequently transported from the food handler to the food during the handling process (23). Personnel hygiene is very important in food processing because a major source of contamination in food poisoning caused by *S. aureus* is human. Poor personal hygiene practices, such as negligence to wash hands after visiting the bathroom may result in up to 10^7 pathogens under the fingernails of the food handler (24).

Spice, which has an important role in meat products, can be contaminated with bacteria, molds, and yeasts. Microbial load of spice depends on its variety, processing method, granule size, and moisture content (25). The microbial load of the spice mix affected the microbial load of sausages. In this connection, the microbial load of sausage batter was found higher than those found in minced meat. A major reason for this increase in the microbial load of batter is the addition of spice mix into the batter. Microbial counts of spice mix were found higher at sampling days that microbial load of sausages was

found higher as well. There were significant ($P < 0.01$) correlations in *S. aureus* ($r = 0.96$) and yeast-mold ($r = 0.95$) counts between spice mix and batter.

Total plate count of ice water was found below the standard value. This result showed that microbial quality of water and ice in the plant was appropriate for use in production.

In conclusion, microbial loads of minced meat and spice mix were found above standard limit values. Minced meat and spice mix were determined as the primary contamination sources in sausage manufacturing. The most probable reason for minced meat for having high microbial load is the poor hygienic quality of raw meat. Microbial quality of raw meat and non-meat ingredients affects the quality of the final product.

Microbial loads of equipment surfaces and personnel hands were found below the acceptable standard values. Significant correlations existed between microbial counts from equipment, personnel, and sausages showed that hygiene of plant and personnel was very important to produce safer sausage. Equipment surfaces and personnel hands were determined as secondary contamination sources. Cooking was effective in reducing microbial counts and had a positive effect on the microbial quality of the final product.

Finally to ensure the microbiological quality of the final product, raw meat and ingredients must be inspected prior to entering the plant. Certified suppliers must be selected. Strong criteria for hygienic quality of raw meat must be set for suppliers. After receiving, raw meat and ingredients must be stored in appropriate conditions until use. Effective cleaning and sanitation program must be performed in the plant. Personnel should follow the standard hygienic procedures and personnel health conditions must be monitored regularly. Proper time and temperature settings for cooking should be selected.

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