Validation of a Manufacturing Process for Fermented, Semidry Turkish Soudjouk To Control Escherichia coli O157:H7†‡

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ABSTRACT

Two soudjouk batters were prepared from ground beef (20% fat) and nonmeat ingredients and inoculated with a five-strain mixture of Escherichia coli O157:H7 to yield an initial inoculum of 7.65 log₁₀ CFU/g. One batter contained a commercial-starter culture mixture (~8.0 log₁₀ CFU/g) and dextrose (1.5%), while the other batter relied upon a natural fermentation with no added carbohydrate. Following mixing, sausage batters were held at 4°C for 24 h prior to stuffing into natural beef round casings. Stuffed soudjouk sticks were fermented and dried at 24°C with 90 to 95% relative humidity (RH) for 3 days and then at 22°C with 80 to 85% RH until achieving a product moisture level of approximately 40%. After fermentation and drying with an airflow of 1 to 1.5 m/s, the sticks were either not cooked or cooked to an instantaneous internal temperature of 54.4°C (130°F) and held for 0, 30, or 60 min. The sticks were then vacuum packaged and stored at either 4 or 21°C. For each of three trials, three sticks for each treatment/batter were analyzed for numbers of E. coli O157:H7 after inoculation, after fermentation, after cooking, and after storage for 7, 14, 21, and 28 days. Reductions in numbers of E. coli O157:H7 after fermentation and drying for sticks fermented by the starter culture (pH 4.6) and for sticks naturally fermented (pH 5.5) were 1.96 and 0.28 log₁₀ CFU/g, respectively. However, cooking soudjouk sticks produced with a starter culture and holding at 54.4°C for 0, 30, or 60 min reduced pathogen numbers from an initial level after fermentation and drying of 5.69 log₁₀ CFU/g to below a detectable level by either direct plating (<1.0 log₁₀ CFU/g) or by enrichment. In contrast, cooking soudjouk sticks produced without an added starter culture decreased pathogen numbers from an initial level after fermentation and drying of 7.37 to 5.65 log₁₀ CFU/g (54.4°C, no hold), 5.04 log₁₀ CFU/g (54.4°C, 30 min hold), and 4.67 log₁₀ CFU/g (54.4°C, 60 min hold). In general, numbers of E. coli O157:H7 within both groups of soudjouk sticks decreased faster during storage at 21°C compared to 4°C. After 28 days of storage, total reductions in pathogen numbers in soudjouk sticks produced using a starter culture but that were not subsequently cooked were 7.65 and 3.93 log₁₀ CFU/g at 21 and 4°C, respectively. For naturally fermented soudjouk, total reductions varied from 4.47 to 0.45 log₁₀ CFU/g, depending on the cooking time and storage temperature. These data provide guidelines for manufacturers of dry sausage of ethnic origin, including soudjouk, to assess the safety of their processes for control of E. coli O157:H7.

Fermented sausages are generally regarded as one of the most shelf-stable and safest meat products due to the presence or inclusion of salt and sodium nitrite, as well as the low pH and low water activity (a_w) (1). However, the ability of Escherichia coli O157:H7 to survive some dry sausage manufacturing processes (5, 10, 11, 14) and cause illness (3) has prompted regulatory changes that require sausage manufacturers to assess or validate the safety of their processes (4) relative to this bacterium (15, 16). The existing literature also suggests that each type of fermented sausage should be separately validated, because typical processing parameters and characteristics such as water content and pH vary among dry sausage types and manufacturers.

Although the processes for the more popular and higher volume types of fermented sausage such as pepperoni (11), salami (13), and summer sausage (5) have been validated for control of E. coli O157:H7, there have been comparatively few processes that have been validated for control of this pathogen in lower volume ethnic sausage.

Ethnic sausages are generally manufactured in small batches by smaller-scale establishments located on either coast of the United States, and the resulting product is typically consumed locally. However, as the immigrant population increases and spreads across the United States, a demand for greater production and broader distribution of ethnic sausage will also occur. As one example of an ethnic sausage, Turkish soudjouk is a popular fermented, spicymeat sausage in the Middle East and Balkan countries, as well as among immigrants to the United States originating from these geographical areas. Soudjouk (also known as soudjuk, soudjouk, surugu, and/or suck) is traditionally manufactured via a natural fermentation (i.e., no added starter culture) using all ruminant meat (i.e., beef, water buffalo, and/or mutton). Soudjouk-style sausage is
typically low in moisture (40%) and in acid (pH 5.0 to 5.5), and it usually does not receive any heat treatment or smoking (9, 18, 20). In recent years, the practice of using starter cultures has increased among the manufacturers of Turkish-style sausage (8). Turkish soudjouk is consumed as manufactured or it is sometimes cooked by consumers. In the present study, manufacturing procedures for a Turkish-style soudjouk were evaluated for control of E. coli O157:H7, because production parameters and product composition place this particular type of fermented sausage in a higher-risk category relative to this foodborne pathogen.

MATERIALS AND METHODS

Bacterial strains. As described previously (10), a mixture of the following five strains of E. coli O157:H7 was used to inoculate soudjouk batter: (i) C9490, a human isolate from the Western States hamburger outbreak of 1993; (ii) F-90, a sausage isolate from the Washington-California dry-cured salami outbreak of 1994; (iii) EC505B, a beef isolate from the University of Wisconsin Food Research Institute; (iv) EC204P, a pork isolate from the University of Wisconsin, Food Research Institute; and (v) C7927, a human isolate from the Massachusetts apple cider outbreak of 1991. The strains were maintained as recommended by the U.S. Department of Agriculture, Food Safety and Inspection Service (15, 16). A commercial Pediococcus acidilactici and Lactobacillus curvatus mixed starter culture (Bactoferm LCP; Chr. Hansen Inc., Milwaukee, Wis.) was maintained and propagated according to the manufacturer’s instructions.

Preparation of inoculum. Each of the five strains of E. coli O157:H7 were grown separately in 250 ml of Trypticase soy broth (Difco Laboratories Inc., Detroit, Mich.) supplemented with 1% glucose at 37°C, overnight, with shaking at 150 rpm. The cell suspensions were harvested by centrifugation and combined to yield approximately 9.0 log_{10} CFU/ml with about equal numbers from each strain, as previously described (11). A 7-ml portion of the thawed Bactoferm LCP starter culture was added to 70 ml of distilled H_2O for addition to 14.5 kg of batter.

Manufacture of Turkish soudjouk. A flow diagram of the experimental design is provided in Figure 1. About 37 kg of coarse ground beef with a fat content of about 20% was purchased from a commercial manufacturer and divided into meat blocks weighing 14.5 and 22.0 kg. The following nonmeat ingredients were added to each meat block: sodium chloride (1.9%), sodium nitrite (0.25% or 156 ppm), chopped fresh garlic (0.95%), cumin (0.95%), paprika (0.42%), black pepper (0.42%), and all spice (0.42%) (F. W. Witt and Co., Yorkville, Ill.) (17). Dextrose (1.5%) was added to the batter to which the starter culture was added. Both batters were inoculated with the five-strain mixture of E. coli O157:H7 to deliver ≥7.0 log_{10} CFU/g of batter. One batter was also inoculated with a suspension of the Bactoferm LCP starter culture to achieve about 8.0 log_{10} CFU/g of batter. The inoculated batters were mixed with nonmeat ingredients for about 3 min using a Buffalo paddle mixer (model 2VSS; John E. Smith’s and Sons Co., Buffalo, N.Y.) and held overnight at 4°C. The batters were stuffed into natural beef casings, prepared according to the manufacturer’s instructions (Vista International Packaging Inc., Kenosha, Wis.), using a hand stuffer (Koch Supplies Inc., Kansas City, Mo.) in chains of six to seven sticks, with each stick weighing about 250 g. Soudjouk sticks/chains were transferred to environmentally controlled rooms (Biotron facility, University of Wisconsin, Madison, Wis.) and hung vertically for fermentation and drying. Fermentation and drying were conducted at 24°C with 90 to 95% relative humidity (RH) for the first 3 days and then at 22°C with 80 to 85% RH for about 3 more days until achieving a product moisture content of about 40%. The moisture content was measured as described in the following section on days 3, 5, and 7 during fermentation and drying. An air-flow of 1 to 1.5 m/s, monitored daily using an anemometer (model 05951-75 LED Vane; Cole Parmer Inc., Vernon Hills, Ill.), was maintained throughout fermentation and drying. Some of the soudjouk sticks/ chains were then transferred to a smokehouse (model 1000; Vortex Inc., Beloit, Wis.) and heated at 48.8°C (120°F) with 70% RH for 1 h and then at 54.4°C (130°F) with 70% RH until achieving the target internal temperature of 54.4°C. The soudjouk sticks were held at 54.4°C for 0, 30, or 60 min. After the postfermentation cooking step, both the cooked and noncooked soudjouk sticks were air-chilled at 4°C for 2 h and then vacuum packaged in oxygen-impermeable bags (863 Saran, Curwood Inc., New London, Wis.) using a Multivac packaging apparatus (Sepp Hagenmüller KG, Germany). The vacuum-packaged sticks were stored at 4 or 21°C for up to 28 days.

Microbiological analyses of soudjouk batters and sticks. The raw meat and noninoculated batter were tested for background levels of E. coli O157:H7 and other nonsorbitol-fermenting gram-negative bacteria by spread plating macerated and diluted meat samples onto MacConkey sorbitol agar (Difco), and for numbers of total aerobic bacteria by spread plating macerated and diluted meat samples onto Trypticase soy agar (Difco). Before studding, the batter containing the starter culture was tested for numbers of lactic acid bacteria such as pediococci and lactobacilli by spread plating macerated and diluted batter samples onto deMan Rogosa Sharpe (MRS; Difco) agar plates. All plates were incubated at 37°C for 24 h, and representative colonies were counted and confirmed as previously described (11).

Soudjouk was tested for numbers of E. coli O157:H7 after...
inoculation, after fermentation and drying, after postfermentation cooking, and after storage for 7, 14, 21, and 28 days. The samples were processed and plated onto MacConkey sorbitol agar plates as previously described.

### RESULTS

**Microbiological analyses of raw meat.** Three random samples (25 g each) were taken from each of the three raw meat blocks prior to any inoculations and tested for target microorganisms. The results revealed the absence of indigenous *E. coli* O157:H7 by direct plating (<10 log₁₀ CFU/g) and a mean log count for total aerobic bacteria of 4.6 ± 0.42 log₁₀ CFU/g. After inoculation, the mean log counts for total lactic acid bacteria were 8.42 ± 0.42 log₁₀ CFU/g for soudjouk prepared with and without added starter culture, respectively. The average pH of the raw meat was pH 5.81 (range; pH 5.78 to 5.83). These data indicate that the raw materials were of good microbiological quality.

**Microbiological analyses of soudjouk sticks fermented with a commercial starter culture.** Overall, using a starter culture was significantly more effective for inactivating *E. coli* O157:H7 than a natural fermentation (*P > 0.05*). Numbers of the pathogen decreased from initial levels of 7.65 to 5.69 log₁₀ CFU/g after fermentation and drying (Table 1). Average pH values at the end of fermentation and drying were pH 4.55 ± 0.026. However, postfermentation cooking to 54.4°C (130°F) instantaneous, as well as holding at this temperature for 30 or 60 min, decreased the numbers of the pathogen to below detection by direct plating, and no viable cells were recovered following enrichment. Soudjouk samples that were cooked were not tested.
during storage. Although numbers of the pathogen in soudjouk sticks that were not cooked gradually decreased during storage at 21°C and decreased to below detection by direct plating on day 28, viable E. coli O157:H7 were recovered following enrichment. A 5.0-log10 CFU/g reduction of the pathogen was achieved after storage at 21°C for 14 days. In contrast, counts of the pathogen in the soudjouk sticks decreased at a significantly slower rate (P < 0.05) during refrigerated storage; a total reduction of 3.9 log10 CFU/g was achieved after fermentation, drying, and storage for 28 days at 4°C for the noncooked product.

Microbiological analyses of soudjouk sticks fermented naturally. Fermentation and drying to a final pH of pH 5.48 ± 0.11 only decreased the pathogen numbers from an initial level of 7.65 to 7.37 log10 CFU/g over an 8-day period (Table 1). None of the heat treatments applied produced the desired 5-log10 CFU/g reduction in numbers of E. coli O157:H7. However, during storage at either 4 or 21°C, pathogen numbers within soudjouk sticks that were cooked to an internal temperature of 54.4°C was also significantly more destructive on viability of the pathogen for all treatments than storage at 4°C.

Chemical analyses of soudjouk sticks. Chemical analyses of the sticks revealed that there were no appreciable differences in proximate composition among treatments due to cooking (Table 2). However, there were appreciable differences in proximate composition among sticks produced using an added starter culture and naturally fermented sticks.

**DISCUSSION**

The present study was undertaken to establish guidelines for manufacturing Turkish soudjouk relative to survival of E. coli O157:H7. Our findings revealed that the use of a starter culture increased the safety of soudjouk by appreciably lowering the level of E. coli O157:H7. Our results are in agreement with those of other investigators, including Yaman et al. (19), relative to the utility of using lactic acid bacteria as starter cultures to produce a commercially viable product with enhanced quality and safety attributes. Consistent with previous reports (5, 9, 10, 13), in the presence of high acid a mild degree of postfermentation cooking reduced pathogen numbers from an initial level of 7.65 to <1.0 log10 CFU/g. However, manufacturing soudjouk without an added starter culture or added dextrose to mimic the practice of some small processors located on the East Coast of the United States required longer cooking and/or extended storage at 21°C to deliver an appreciable reduction of E. coli O157:H7. The slight pH decline in that latter group can be explained by the fermentation of residual carbohydrates in the meat fermentable sugars and spices (2).

The microbiological safety of traditional Turkish soudjouk evaluated by other workers is in general agreement

| TABLE 2. Microbiological analyses of soudjouk sticks after storage for 28 days at 4°C. |
|--------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                      | **Treatment**   | **Protein (%)** | **Moisture (%)** | **Salt (%)** | **Tn** |
|                                      |                 |                 |                 |               |       |
|                                      | Fermented with added starter culture | 6.48 ± 0.05 | 0.98 ± 0.20 | 1.28 ± 0.09 | 1.28 ± 0.06 |
|                                      | Naturally fermented | 5.40 ± 0.05 | 0.98 ± 0.20 | 1.28 ± 0.09 | 1.28 ± 0.06 |
|                                      | Cook, no hold | nt | nt | nt | nt |
|                                      | Cook, hold for 30 min | nt | nt | nt | nt |
|                                      | nt, not tested | nt | nt | nt | nt |
with the results of the present study. Erol and Hildebrandt (9) reported that numbers of Staphylococcus aureus, Salmonella Typhimurium, and Listeria monocytogenes increased or remained unchanged during the ripening period of soudjouk sticks naturally fermented at 25 or 20°C, respectively. However, these same researchers also reported that controlled fermentation of soudjouk with a mixed starter culture comprised of Staphylococcus carnosus and Lactobacillus plantarum provided a significant reduction of all three pathogens. Similarly, Yurtyeri et al. (20) reported that counts of Enterobacteriaceae and total coliforms survived longer in naturally fermented Turkish soudjouk during a 21-day fermentation and drying period compared to soudjouk sticks prepared using a starter culture. Cosansu and Ayhan (7) evaluated the survival of E. coli O157:H7 (5.5 log_{10} CFU/g) in Turkish soudjouk during an 8-day natural fermentation and drying and a 90-day vacuum-packaged storage at 4°C. Their results indicated that fermentation at 22 to 24°C to pH 4.86 within 8 days provided over a 3-log_{10} unit reduction of the pathogen, and that numbers of E. coli O157:H7 declined to below detection after 2 months of vacuum-packaged storage. In general, these results are in agreement with the results of the present study. However, one difference that should be noted is that the natural fermentation referred to by other investigators was aided by supplementation of the batter with a fermentable carbohydrate, which is a common practice (9, 18). Although the use of a starter culture and inclusion of carbohydrate in the batter resulted in a product of pH 4.6 in the present study, other investigators reported a 10-fold greater reduction in the number of E. coli O157:H7 in product of pH 4.86 prepared without a starter culture but with added carbohydrate. This difference, in part, might be explained by the variation in acid resistance among individual strains of E. coli O157:H7 used separately compared to a mixture of five different strains as used in the present study.

Despite the limited number of samples tested for proximate analyses, results of the present study indicated that there was an appreciable difference between starter culture-added and naturally fermented soudjouk sticks relative to pH, aw, moisture, moisture and protein ratio (M/Pr), and protein levels. These differences can largely be explained by the higher acid levels in soudjouk sticks that were prepared using a starter culture, presumably due to more drying. Moreover, low pH is more likely to have a stronger effect on survival of E. coli O157:H7 in combination with postfermentation cooking and/or with storage at ambient temperature than the effect of aw, moisture, and protein levels (5, 10, 11, 14). Similarly, Ünlütürk and Turantaş (18) evaluated the fate of the indigenous coliforms in Turkish soudjouk during natural fermentation with higher (1%) and lower (<0.5%) levels of added dextrose, and during the subsequent vacuum-packaged storage at 20 or 4°C. Their results revealed that total and fecal coliforms survived significantly better in soudjouk manufactured with a low level of dextrose and a final pH of 5.3 than with a higher level of dextrose and a final pH of 4.5. E. coli Biotype 1 levels dropped to below detection within 7 days of manufacturing at pH 4.5, whereas numbers gradually decreased and remained detectable until day 21 within soudjouk of pH 5.3. They also reported that storage at 20°C was more destructive on coliforms than storage at 4°C.

In summary, our data establish that the following processing procedures of Turkish soudjouk validate a 5-D reduction of E. coli O157:H7: (i) controlled (i.e., added starter culture and carbohydrate) fermentation to pH ≤ 4.6 and subsequent cooking to an internal temperature of 54.4°C; (ii) controlled fermentation, no postfermentation cooking, and vacuum-packaged storage at 21°C for at least 14 days; and (iii) natural fermentation to pH ≤ 5.4, cooking to an internal temperature of 54.4°C and holding for 60 min, and storage at 21°C for at least 14 days. The impact of low pH and postfermentation cooking on sensory characteristics requires further study, because the procedures evaluated herein are not common for traditional manufacture of Turkish soudjouk. Our data validate for manufacturers of Turkish soudjouk or similar products that the practice of controlled fermentation, drying, cooking, and storage can assure the safety of their products, as well as provide guidelines for establishing effective limits at critical control points for hazard analysis.

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