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# Viability of multi-strain mixtures of *Listeria monocytogenes*, *Salmonella typhimurium*, or *Escherichia coli* O157:H7 inoculated into the batter or onto the surface of a soudjouk-style fermented semi-dry sausage ☆

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ABSTRACT

The fate of *Listeria monocytogenes*, *Salmonella typhimurium*, or *Escherichia coli* O157:H7 were separately monitored both in and on soudjouk. Fermentation and drying alone reduced numbers of *L. monocytogenes* by 0.07 and 0.74 $\log_{10}$ CFU/g for sausages fermented to pH 5.3 and 4.8, respectively, whereas numbers of *S. typhimurium* and *E. coli* O157:H7 were reduced by 1.52 and 3.51 $\log_{10}$ CFU/g and 0.03 and 1.11 $\log_{10}$ CFU/g, respectively. When sausages fermented to pH 5.3 or 4.8 were stored at 4, 10, or 21 °C, numbers of *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 decreased by an additional 0.08–1.80, 0.88–3.74, and 0.68–3.17 $\log_{10}$ CFU/g, respectively, within 30 days. Storage for 90 days of commercially manufactured soudjouk that was sliced and then surface inoculated with *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 generated average *D*-values of ca. 10.1, 7.6, and 5.9 days at 4 °C; 6.4, 4.3, and 2.9 days at 10 °C; 1.4, 0.9, and 1.6 days at 21 °C; and 0.9, 1.4, and 0.25 days at 30 °C. Overall, fermentation to pH 4.8 and storage at 21 °C was the most effective treatment for reducing numbers of *L. monocytogenes* (2.54 $\log_{10}$ CFU/g reduction), *S. typhimurium* ($\geqslant$5.23 $\log_{10}$ CFU/g reduction), and *E. coli* O157:H7 (3.48 $\log_{10}$ CFU/g reduction). In summary, soudjouk-style sausage does not provide a favorable environment for outgrowth/survival of these three pathogens.

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## 1. Introduction

Although the market for ethnic foods in the US increased from ca. $37 billion in 1997 to ca. $75 billion in 2005 (Agriculture and Agri-Food Canada, 2005), there is a scarcity of information about the safety of such unique/specialty products. Many ethnic products, including ready-to-eat (RTE) sausages, are made primarily by small and very small producers in relatively small batches. Consequently, producers may lack adequate resources to validate the lethality of their processes towards food borne pathogens, provide adequate/timely documentation for their HACCP programs, and/or appeal United States Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) decisions and requirements.

In recent years, there has been an intense demand for ethnic/specialty sausages in the US, primarily as a result of the continued expansion of our various immigrant populations (Calicioglu et al., 2002; Agriculture and Agri-Food Canada, 2005). As one example, soudjouk is a highly spiced, Mediterranean-style, semi-dry sausage typically produced by regional/local manufacturers on a small-scale using traditional processing methods. Soudjouk-style sausage is made by mixing ground meat, spices, and curing salts, and then stuffing the resulting batter into a natural or artificial casing that is flattened and subsequently fermented and dried at ambient temperature over several days (Saricoban et al., 2006; Siriken et al., 2006). The fermentation is usually performed by the indigenous meat microflora, followed, or not, by a heat treatment that may appreciably affect the sensory attributes of the product from batch-to-batch (Vural, 1998; Bozkurt and Erkmen, 2002; Calicioglu et al., 2002). According to the USDA, the standard of identity for soudjouk requires it to have a moisture-to-protein ratio (M:Pr) value of $\leqslant$2.04:1.0 (USDA, 2005), whereas to be shelf-stable, like other semi-dry sausage, soudjouk must be pH 5.2 and have a water activity ($a_w$) value of $<$0.95 or only a pH of $<$5.0 or only an $a_w$ of $<$0.91, assuming typical levels of the other hurdles such as salt, curing agents, etc. (http://www.fsis.usda.gov/PDF/FSRE_SS_7Principles.pdf). As such, soudjouk is shelf-stable for up to 2 years when vacuum packaged and stored at ambient temperature (Karl Ayanian, personal communication).

☆ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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Although soudjouk-style sausage has not caused any documented illnesses in the US, in Turkey, the consumption of soudjouk was linked to an outbreak that resulted in 42 cases of salmonellosis (Ulutan et al., 1988). Furthermore, between 1997 and 1998, the USDA/FSIS recalled ca. 385 lbs. of soudjouk-style sausage due to potential contamination with *Listeria monocytogenes* (www.usda.fsis.gov/FSIS_Recalls/index.asp). In addition, Çon et al. (2001) reported that 16.7% (7 of 30) of soudjouk-style sausages collected from retail markets in Turkey were contaminated with *L. monocytogenes*. Siriken et al. (2006) also conducted a survey of local shops and retail markets in Turkey and reported an incidence of 7% (7 of 100) for both *L. monocytogenes* and *Salmonella* spp. in soudjouk sausage that ranged from pH 6.5 to 4.7; none of these sausages tested positive for *Escherichia coli* O157:H7. More recently, Colak et al. (2007) reported a prevalence of 21% (63 of 300) for *Listeria* spp. and 11.6% (35 of 300) for *L. monocytogenes* in soudjouk-style sausages obtained from retail outlets in Istanbul between February of 2004 and January of 2005. These reports substantiate that soudjouk may harbor food borne pathogens, but do not provide sufficient insight on their types, levels, persistence, and/or potential pathogenicity.

The shelf-stability of fermented sausage depends primarily on a reduction of both pH and $a_w$ during fermentation and drying. However, food borne pathogens such as *L. monocytogenes*, *Salmonella*, and/or *E. coli* O157:H7 are highly tolerant to acidic and/or dry conditions (Clavero and Beuchat, 1996; Duffy et al., 2000; Greenacre et al., 2003; Bonnet and Montville, 2005) and, thus, may survive during manufacture and maturation of many types of RTE sausage. At present, the USDA/FSIS requires manufacturers of red meat to validate that their processes deliver a 5.0 $\log_{10}$ reduction for *E. coli* O157:H7 and a 6.5 $\log_{10}$ reduction of *Salmonella* and/or that the finished product satiates the "zero tolerance" policy and meets the compliance guidelines for *L. monocytogenes* (Shank et al., 1996; USDA, 2001; Anonymous, 2003). The processes and formulations used to prepare ethnic/specialty sausages may differ markedly among types and processors. Thus, to ensure that each process and formulation is adequate to reduce and/or eliminate food borne pathogens, targeted processes and formulations should be validated, and subsequently modeled, to assure that the lethality standards for specific pathogens in RTE meats are achieved. Although previous studies have been published on the viability of *E. coli* O157:H7 during manufacture and storage of soudjouk (Calicioglu et al., 2001; Calicioglu et al., 2002), currently, there is relatively little scientifically validated information published on the viability of *L. monocytogenes* (Erol and Hildebrandt, 1992) and/or *Salmonella typhimurium* (Turantaş and Ünlütürk, 1993) in soudjouk. Thus, the objective of the present study was to evaluate the viability of *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 inoculated either into the batter prior to stuffing or onto the surface of slices of a retail soudjouk-style sausage that were subsequently stored at different temperatures to validate process lethality from manufacture through shelf-life.

## 2. Material and methods

### 2.1. Bacterial strains and preparation of inoculum

The five isolates of *L. monocytogenes* (MFS2, MFS102, MFS104, MFS105, and MFS110), the six isolates of *S. typhimurium* (H3278, G7601, H3402, H2662, H3380, and G8430), and the three isolates of *E. coli* O157:H7 (EC505B, C7927, and SLH21788) used in this study were confirmed, cultured, and maintained as described previously (Porto-Fett et al., 2008). To prepare the multi-strain mixtures for each pathogen, equal volumes of each cell suspension were separately combined to yield ca. 9.0 $\log_{10}$ CFU/ml for each pathogen. A bacteriocin-producing pediococcal starter culture (Lactacel 115; Kerry Bio-Science, Rochester, MN) was maintained and prepared according to the manufacturer's directions.

### 2.2. Inoculation of the batter and manufacture of soudjouk-style sausage

The eight batches of raw, ground beef (~20% fat) were purchased at a local supermarket and maintained at 0 °C for up to 15 days. In addition to ground beef, the batter contained the following final concentrations of non-meat ingredients: dextrose (0.25% or 0.60%), sodium chloride (1.9%; Morton International Inc., Chicago, IL), sodium nitrite (0.25% or 156 ppm), chopped fresh garlic (0.95%), and various spices (cumin (0.95%), paprika (0.42%), black pepper (0.42%), and all-spice (0.42%); Atlantic Spice Co., North Truro, MA). The raw ground beef was separately inoculated with each pathogen mixture to obtain a final concentration of ca. 6.5 $\log_{10}$ CFU/g and mixed using a commercial countertop mixer (Univex SRM12; Salem, NH) for 5 min at room temperature (22 ± 1 °C or 71.6 ± 1.8 °F). Next, the commercial pediococcal starter culture (final concentration of ca. 8.0 $\log_{10}$ CFU/g of batter) and the non-meat ingredients were added to the inoculated ground beef and mixed for an additional 10 min. The batter was stuffed manually using a commercial stuffer (Dick D-73779; Deizisau, Germany) into 25 mm diameter collagen casings (Nippi, Tokyo, Japan). After stuffing, the links (ca. 100 g each; 9.0 cm L × 2.5 cm W × 0.8 cm H) were flattened with the aid of a rectangular-stamped, chrome-plated, steel steak press (11.4 cm W × 22.9 cm L) (Model 47708, Vollrath Company, Sheboygan, WI) and then hung vertically in an environmentally controlled incubator (EJS Systems Inc., Chagrin Falls, OH) with an air flow of 1.0–1.5 m/s. The relative humidity (RH) and air temperature were controlled and measured using the Dynamist 2000 System and the Partlow MRC5000 chart recorder (EJS Systems Inc.). The sausages were fermented at 24 ± 0.5 °C (75.2 ± 0.9 °F) with a RH of 90–95% for 72 h and then dried at 22 ± 0.5 °C (71.6 ± 0.9 °F) for an additional 72 h with 80–85% RH. After 3 days of fermentation and 3 days of drying, each chub was individually vacuum packaged (950 mBar) in sterile polyethylene bags (Nasco, Modesto, CA) using a Multivac A300/16 vacuum-packaging unit (Sepp Haggmüller KG, Wolfertschwenden, Germany) and stored at 4, 10, or 21 °C for up to 30 days. In each of the two trials, three packages/links were sampled at each sampling interval. Bacterial numbers were expressed as $\log_{10}$ CFU/g.

### 2.3. Surface inoculation of soudjouk-style sausages obtained from a commercial manufacturer

The three batches (ca. 2.3 kg each; ca. 18 packages with two links of ca. 128 g each per package) of freshly processed, vacuum-sealed packages of soudjouk-style sausage (beef, garlic, salt, spices, and paprika stuffed into collagen casings) were prepared specifically for this study by a local manufacturer. The sausages were transferred aseptically from the original packages onto styrofoam trays (1012S, Genpak, Glens Falls, NY) and sliced (ca. 13 g each slice; 1.5 cm L × 2.0 cm W × 0.8 cm H) with the aid of an ethanol-sterilized knife. Individual slices were aseptically weighed, placed onto styrofoam trays (Genpak), and separately inoculated by pipeting 10 μl of the multi-strain mixtures of *L. monocytogenes*, *S. typhimurium*, or *E. coli* O157:H7 onto the flattened top surface of each piece; the face that was cut/sliced was not the portion inoculated. The inoculum was distributed over the entire surface with the aid of a sterile L-shaped plastic

cell spreader (Midsci; St. Louis, MO). The trays were placed into a laminar-flow hood and held for 15 min at ambient temperature ($22 \pm 1$ °C or $71.6 \pm 1.8$ °F) to allow for the bacteria to attach to the slices. The slices were then flipped over and the inoculation procedure was repeated on the opposite side. As such, the final concentration of each pathogen was ca. 5.5 $\log_{10}$ CFU/slice. The slices were then re-packaged (one slice/package) into sterile polyethylene bags and vacuum-sealed to 950 mBar. The packages were stored at 4, 10, 21, and 30 °C for up to 90 days. Slices of commercial soudjouk were stored for up to 90 days rather than 30 days as for the experimental chubs because we had more product available for the former compared to the amount of product we were able to manufacture ourselves. In each of the three trials, three packages/slices were sampled at each sampling interval. Bacterial numbers were expressed as $\log_{10}$ CFU/slice.

### 2.4. Microbiological analyses

When pathogens were inoculated into soudjouk batter, they were recovered at each sampling interval by transferring a 5-g portion of the sausage into a filter stomacher bag, adding 15 ml of sterile peptone water (0.1%), and macerating for 2 min (Seward Stomacher 400, Cincinnati, OH). The resulting fluid was transferred to a sterile 50-ml screw-capped conical centrifuge tube with the aid of a sterile pipette. When inoculated onto the surface of slices of soudjouk-style sausage, the pathogens were recovered using the package rinse method (Luchansky et al., 2002). Briefly, the outside surface of the bag was wiped with an ethanol-soaked (70%, v/v) paper towel, and the bag was opened with the aid of ethanol-sterilized scissors. Five milliliters of sterile peptone water were added to the bag, the slices were massaged by hand for 1 min, and the resulting rinsate was transferred to a sterile 15-ml screw-capped conical centrifuge tube with the aid of a sterile pipette. Pathogen numbers were enumerated by serial diluting the macerated fluid or the package rinsate in sterile peptone water as needed. Pathogens were enumerated by spread plating 250 μl of the macerated fluid or 500 μl of the rinsate onto two separate polymyxin B, acriflavin, lithium chloride, ceftazidine, esculin, D-mannitol (PALCAM; Difco Laboratories Inc., Detroit, MI), xylose-lysine-tergitol-4 (XLT4; Difco), or MacConkey sorbitol (SMAC; Difco) agar plates for *L. monocytogenes*, *S. typhimurium*, or *E. coli* O157:H7, respectively. The XLT4 and SMAC agar plates were incubated at 37 °C for 24 h and the PALCAM agar plates were incubated at 37 °C for 48 h. Following incubation, typical colonies of each pathogen on representative plates were counted manually. When levels of the three pathogens decreased to below detection by direct plating, that being $\leqslant 1.20$ $\log_{10}$ CFU/g or $\leqslant 0.7$ $\log_{10}$ CFU/slice, the presence or absence of each pathogen were determined by enrichment essentially as described previously (Porto-Fett et al., 2008). Total aerobic bacteria were enumerated by spread plating 100 or 250 μl of the macerated fluid or rinsate onto brain heart infusion (BHI; Difco) agar plates and incubating for 72 h at 30 °C. For enumeration of lactic acid bacteria (LAB), 100 or 250 μl of the fluid or rinsate were spread plated onto De Man, Rogosa and Sharpe (MRS; Difco) agar plates and incubated anaerobically (10.1% carbon dioxide, 4.38% hydrogen and balance nitrogen; Bactron IV Anaerobic/Environmental Chamber, Sheldon Manufacturing Inc., Cornelius, OR) for 48 h at 37 °C. Bacterial numbers were expressed as $\log_{10}$ CFU/g or CFU/slice.

### 2.5. Physical– chemical analyses of soudjouk

For each of the two trials, the $a_w$ and the pH of three experimentally manufactured sausages were measured at each sampling point using an electronic water activity meter (Decagon Aqualab Model Series 3; Decagon Devices, Pullman, WA) and a model 6000P pH/temperature electrode and a model 5500 pH meter (Daigger, Vernon Hills, IL), respectively. Samples for $a_w$ measurement were prepared according to the manufacturer's instructions. For pH measurements, a 5-g portion of sausage was transferred to a filter stomacher bag containing 15 ml of peptone water and then macerated for 2 min. The pH of the resulting fluid/slurry was determined at each sampling point with a model 6000P pH/temperature electrode and a model 5500 pH meter. For the commercially manufactured sausage, the pH was determined from two sausages from two of the three batches of soudjouk obtained directly from the manufacturer. The proximate composition of soudjouk was determined by methods approved and described by the Association of Official Analytical Chemists (McNeal, 1990) as conducted by a commercial testing laboratory. Proximate composition analyses on sausages manufactured experimentally were performed on a composite sample (ca. 60 g total) using 30 g from each of two sausage links from one trial taken directly after fermentation/drying. For sausages manufactured commercially, proximate composition analyses were performed on a composite sample (ca. 60 g total) from two sausages (ca. 30 g from each) from two of the three batches of soudjouk obtained directly from the manufacturer.

### 2.6. Statistical analyses

Data were analyzed using version 9.1.3 of the SAS statistical package (SAS Institute Inc., Cary, NC). When the three pathogens tested were inoculated into soudjouk batter, analysis of variance (ANOVA) was performed to evaluate the effects and interactions of the fermentation and drying steps, formulation/pH (0.25% (pH ~5.3) or 0.60% (pH ~4.8) dextrose), and storage temperatures on pathogen reduction over time. When bacterial counts decreased to below the threshold of detection ($\leqslant 1.20$ $\log_{10}$ CFU/g), a value of zero was used for determination of the arithmetic mean. Means and standard deviations of pathogen numbers in soudjouk-style sausage fermented to ca. pH 5.3 or 4.8 for each storage temperature were determined from the average of three samples at each sampling interval for each of the two trials. Mean separations were performed using the Bonferroni LSD method. The lethality/*D*-values, that being the time required in days to achieve a 1.0 $\log_{10}$ CFU/g reduction at a given storage temperature, were determined for pathogen lethality on slices of soudjouk using the DMFit curve fitting software kindly provided by J. Baranyi (Institute of Food Research, Norwich, UK). The *D*-value is the absolute value of the inverse of the linear inactivation rate of the surviving cell fraction. The *z*-values, that being the temperature required for the thermal inactivation curve to decrease one log cycle, were calculated as the absolute value of the inverse of the linear regression of the *D*-value versus the corresponding storage temperatures. Means and standard deviations of *L. monocytogenes*, *S. typhimurium*, or *E. coli* O157:H7 numbers in sausage slices at each of the four storage temperatures were determined from the average of three samples at each sampling interval for each of the three trials.

## 3. Results

### 3.1. Levels of indigenous flora and pH of raw ground beef

Analyses of each of the eight batches of raw ground beef prior to inoculation with the pathogens and starter culture revealed the absence of any indigenous *L. monocytogenes*, *S. typhimurium*, or *E. coli* O157:H7 by both direct plating ($\leqslant 1.20$ $\log_{10}$ CFU/g) and by

enrichment (data not shown). The average levels of total aerobic bacteria and indigenous LAB in the meat were 6.69 $\log_{10}$ CFU/g (range of 5.43–8.44 $\log_{10}$ CFU/g) and 5.70 $\log_{10}$ CFU/g (range of 4.35–7.44 $\log_{10}$ CFU/g), respectively. The average pH of the raw ground beef was pH 6.26 $\pm$ 0.25 (range of pH 6.06–6.86) and the average $a_w$ was 0.992 $\pm$ 0.01 (range of $a_w$ 0.994–0.980). The average pH and $a_w$ of the experimentally manufactured soudjouk formulated with 0.25% of added dextrose was pH 5.27 $\pm$ 0.14 (range of pH 5.04–5.39) and $a_w$ 0.923 $\pm$ 0.06 (range of $a_w$ 0.917–0.926). The average pH and $a_w$ of the experimentally manufactured soudjouk formulated with 0.60% of added dextrose was pH 4.81 $\pm$ 0.26 (range of pH 4.67–5.0) and $a_w$ 0.915 $\pm$ 0.03 (range of $a_w$ 0.90–0.93). The average pH and $a_w$ of the commercially manufactured soudjouk was pH 4.97 $\pm$ 0.02 (range of pH 4.95–4.98) and $a_w$ 0.845 $\pm$ 0.03 (range of $a_w$ 0.0.821–0.869).

### 3.2. Proximate composition of soudjouk-style sausages

Results of the proximate composition analyses did not show any noticeable differences in the levels of ash, fat, moisture, protein, or M:Pr ratio among the treatments that being experimentally manufactured sausages fermented to ca. pH 5.3 or 4.8 and the commercially manufactured sausage (Table 1). However, a difference of ca. 2.2% and ca. 1% in the carbohydrate level and salt content, respectively, as well as a difference of ca. 0.5-unit in pH, were observed between the experimentally manufactured sausages fermented to ca. pH 5.3 and 4.8. In addition, differences of ca. 4.2% in the carbohydrate level, ca. 0.5% in the salt content, ca. 0.3-unit in pH, and a ca. 0.07-unit in $a_w$ were observed between the commercially manufactured and experimentally manufactured sausages fermented to ca. pH 5.3. When experimentally manufactured sausages fermented to ca. pH 4.8, differences of ca. 2% in the carbohydrate level, ca. 1.5% in the salt content, ca. 0.15-unit in pH, and a ca. 0.07-unit in $a_w$ were observed when compared with those sausages that were commercially manufactured. However, these data confirmed that the soudjouk experimentally manufactured fermented to pH 4.8 and the commercially manufactured sausages achieved the USDA/FSIS recommended M:Pr ($\leqslant$2.04:1.0) (USDA, 2005) to be named soudjouk, and the pH ($<$5.2) and $a_w$ (0.85–0.93) combination values to be designated by USDA/FSIS as shelf-stable. The results of fat, protein, moisture, M:Pr ratio, salt, pH, and $a_w$ values obtained for soudjouk-style sausages manufactured either experimentally or commercially were similar to those reported in previous studies (Calicioglu et al., 2002; Saricoban et al., 2006).

### 3.3. Viability of pathogens in soudjouk during fermentation, drying, and storage

As expected, there was a noticeable difference in pH values between sausages formulated with 0.25% (pH 5.27) versus 0.60% (pH 4.81) of added dextrose after fermentation and drying (Table 1). For sausage formulated with 0.25% added dextrose, the pH after 30 days of storage at 4, 10, or 21 °C was pH 5.22, 5.18, and 5.39, respectively (data not shown). For sausage formulated with 0.60% added dextrose, the pH after 30 days of storage at 4, 10, or 21 °C was pH 4.78, 4.87, and 4.81, respectively (data not shown). The $a_w$ value of sausages with 0.25% or 0.60% added dextrose decreased from an initial value of ca. $a_w$ 0.99 to a value of ca. $a_w$ 0.92 after fermentation and drying (Table 1) and to a value on average of ca. $a_w$ 0.90 (range of $a_w$ 0.91–0.88) after 30 days of storage at 4, 10, or 21 °C (data not shown).

Data on behavior of *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 during fermentation, drying, and storage are shown in Table 2. In general, the lower the pH and the higher the storage temperature, the greater the inactivation for all the three pathogens. However, *S. typhimurium* decreased to a greater extent after 30 days in soudjouk than either *L. monocytogenes* or *E. coli* O157:H7 for both dextrose/pH levels and at all storage temperatures tested. Regardless of pH, there were significant ($P\leqslant0.05$) differences in viability of *L. monocytogenes* compared to viability of *S. typhimurium* in soudjouk when stored at 4 or 10 °C for up to 30 days. However, there were no significant differences in viability of *E. coli* O157:H7 when compared to viability of either *L. monocytogenes* or *S. typhimurium* in soudjouk stored at 4 or 10 °C for up to 30 days. When sausages were fermented to ca. pH 5.3 and stored at 21 °C for up to 30 days, inactivation of *S. typhimurium* and *E. coli* O157:H7 was significantly ($P\leqslant0.05$) greater than inactivation of *L. monocytogenes*, whereas there were no significant ($P\geqslant0.05$) differences in viability among these three pathogens when sausages were fermented to ca. pH 4.8 and stored at 21 °C for up to 30 days.

Fermentation and drying alone reduced numbers of *L. monocytogenes* by 0.07 and 0.74 $\log_{10}$ CFU/g for sausages fermented to ca. pH 5.3 and 4.8, respectively. Numbers of *L. monocytogenes* decreased by an additional 0.08–1.8 $\log_{10}$ CFU/g during storage. With regards to *S. typhimurium*, fermentation and drying alone reduced pathogen numbers by 1.52 and 3.51 $\log_{10}$ CFU/g for sausages fermented to pH 5.27 and 4.81, respectively. Numbers of *S. typhimurium* decreased by an additional 0.88 to $\geqslant$3.74 $\log_{10}$ CFU/g during storage. After 30 days of storage at 21 °C, the pathogen was detected in sausages fermented to pH

**Table 1**
Proximate composition of soudjouk

| Analysis | Experimentally manufactured fermented (pH 5.3) and dried[a] | Experimentally manufactured and fermented (pH 4.8) and dried[a] | Commercially manufactured[b] |
|---|---|---|---|
| Ash (g/100 g) | 4.46 | 4.69 | 5.17 $\pm$ 0.16[c] |
| Carbohydrates (g/100 g) | 2.24 | 4.41 | 6.44 $\pm$ 0.54 |
| Fat (g/100 g) | 30.7 | 29.5 | 29.15 $\pm$ 3.32 |
| Moisture (forced draft oven) (g/100 g) | 32.7 | 33.0 | 29.35 $\pm$ 3.46 |
| Protein (Kjedahl) (g/100 g) | 29.9 | 28.4 | 29.90 $\pm$ 0.57 |
| Salt (g/100 g) | 3.02 | 2.08 | 3.55 $\pm$ 0.32 |
| Moisture–protein ratio (M:Pr) | 1.09 | 1.16 | 0.97 $\pm$ 0.10 |
| pH | 5.27 | 4.81 | 4.97 $\pm$ 0.02 |
| Water activity ($a_w$) | 0.923 | 0.915 | 0.850 $\pm$ 0.03 |

[a] Proximate composition analyses conducted on experimentally manufactured sausages were performed on a composite sample (ca. 60 g total) from two sausages (ca. 30 g from each link) from one trial taken directly after fermentation and drying.
[b] Proximate composition analyses conducted on commercially manufactured sausages were performed on a composite sample (ca. 60 g total) from two sausages (ca. 30 g from each link) from each of the two trials obtained directly from the local manufacturer.
[c] Means of two trials $\pm$ S.D.

**Table 2**
Viability of *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 during fermentation, drying, and storage of experimentally (10 log CFU/g ± S.D.) and commercially manufactured (10 log CFU/slice ± S.D.) soudjouk-style sausage

| Process | Experimentally manufactured fermented (pH 5.3) and dried | | | Experimentally manufactured fermented (pH 4.8) and dried | | | Commercially manufactured | | |
|---|---|---|---|---|---|---|---|---|---|
| | *L. monocytogenes* | *S. typhimurium* | *E. coli* O157:H7 | *L. monocytogenes* | *S. typhimurium* | *E. coli* O157:H7 | *L. monocytogenes* | *S. typhimurium* | *E. coli* O157:H7 |
| After inoculation | 6.72[a] ± 0.38 | 6.66 ± 0.12 | 5.86 ± 0.02 | 6.80 ± 0.36[a] | 6.43 ± 0.20 | 6.00 ± 0.08 | NA[b] | NA | NA |
| After fermentation | 5.41 ± 0.32 | NA | NA | NA | | 6.46 ± 0.65 | 6.25 ± 0.17 | 6.22 ± 0.60 | 6.16 ± 0.32 |
| 4.69 ± 0.69 | | | | | | | | | |
| After drying | 6.65 ± 0.43 | 5.14 ± 0.57 | 5.83 ± 0.15 | 6.06 ± 0.50 | 2.92 ± 0.01 | 4.89 ± 0.56 | 5.77[c] ± 0.16 | 5.09 ± 0.53 | 5.55 ± 0.48 |
| Storage day 2 | | | | | | | | | |
| 4 °C | 6.35 ± 0.47 aA[d,e] | 4.98 ± 0.06 aA | 5.61 ± 0.20 aA | 5.37 ± 0.79 aA | 3.26 ± 0.68 aA | 4.68 ± 1.06 aA | 4.88 ± 0.07 aA | 3.85 ± 0.48 aA | 4.61 ± 0.63 aA |
| 10 °C | 6.30 ± 0.45 aA | 4.83 ± 0.11 aA | 5.64 ± 0.01 aA | 6.07 ± 0.87 aA | 2.97 ± 0.54 aA | 4.40 ± 0.62 aA | 4.77 ± 0.66 aA | 3.99 ± 0.44 aA | 4.49 ± 0.13 aA |
| 21 °C | 6.33 ± 0.33 aA | 4.03 ± 0.23 aA | 5.20 ± 0.64 aA | 4.33 ± 1.99 aA | 2.13 ± 0.43 aA | 4.34 ± 1.25 aA | 3.46 ± 0.5 aA | 1.83 ± 1.05 aA | 2.12 ± 0.97 aA |
| 30 °C | ND[f] | ND | ND | ND | ND | ND | 1.10 ± 0.36 | 1.61 ± 0.80 | 0.90 ± 0.35 |
| Storage day 7 | | | | | | | | | |
| 4 °C | 6.15 ± 0.66 aA | 5.02 ± 0.10 aA | 5.74 ± 0.10 aA | 5.00 ± 1.48 aA | 2.62 ± 0.88 bA | 4.91 ± 1.00 aA | 4.10 ± 0.41 aA | 2.98 ± 0.41 bA | 3.61 ± 0.65 aA |
| 10 °C | 6.38 ± 0.78 aA | 4.03 ± 0.17 aA | 5.52 ± 0.05 aA | 5.73 ± 1.28 abA | 2.26 ± 1.34 aA | 4.75 ± 0.92 aA | 3.02 ± 0.88 bA | 2.64 ± 0.74 aA | 2.99 ± 0.67 aA |
| 21 °C | 6.21 ± 0.74 aA | 3.19 ± 0.95 aB | 4.58 ± 0.89 aAB | 5.55 ± 0.36 aA | 1.20 ± 0.03 abB | 4.56 ± 1.57 aAB | 0.95 ± 0.3 bA | ⩽0.70 ± 0.0 bA | 0.95 ± 0.33 bA |
| 30 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| Storage day 12 | | | | | | | | | |
| 4 °C | 6.13 ± 0.57 aA | 4.59 ± 0.33 aA | 5.48 ± 0.16 aA | 5.91 ± 0.16 aA | 2.50 ± 1.09 aA | 4.82 ± 1.04 aA | 3.89 ± 0.11 aA | 2.53 ± 0.43 aA | 2.33 ± 1.13 aA |
| 10 °C | 6.06 ± 0.64 aA | 4.12 ± 0.21 aA | 5.42 ± 0.33 aA | 6.25 ± 0.47 aA | 2.02 ± 0.71 abB | 4.30 ± 0.84 abAB | 2.54 ± 0.89 bA | 1.70 ± 0.91 bA | 1.21 ± 0.37 bA |
| 21 °C | 6.11 ± 0.54 aA | 2.28 ± 0.24 aB | 4.25 ± 1.29 aAB | 4.96 ± 1.56 aA | 1.20 ± 0.03 aA | 3.73 ± 1.88 aA | ⩽0.70 ± 0.0 bA | ⩽0.70 ± 0.0 aA | ⩽0.70 ± 0.0 bA |
| 30 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| Storage day 14 | | | | | | | | | |
| 4 °C | 6.40 ± 0.73 aA | 4.61 ± 0.51 aA | 5.76 ± 0.17 aA | 6.16 ± 0.08 aA | 3.08 ± 1.29 abA | 4.78 ± 1.01 abA | 3.75 ± 0.30 aA | 2.08 ± 0.75 bA | 2.33 ± 1.13 bA |
| 10 °C | 6.31 ± 0.69 aA | 4.31 ± 0.34 aA | 5.72 ± 0.09 aA | 6.47 ± 0.40 aA | 2.03 ± 1.11 abB | 4.28 ± 0.70 abAB | 2.26 ± 1.72 bA | ⩽0.70 ± 0.0 bA | 1.01 ± 0.43 bA |
| 21 °C | 6.28 ± 0.79 aA | 2.85 ± 1.03 aB | 4.32 ± 1.08 aAB | 5.82 ± 0.64 aA | ⩽1.20 ± 0.0 abB | 2.98 ± 2.55 abAB | ⩽0.70 ± 0.0 bA | ⩽0.70 ± 0.0 bA | ⩽0.70 ± 0.0 bA |
| 30 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| Storage day 22 | | | | | | | | | |
| 4 °C | 6.44 ± 0.99 aA | 4.04 ± 0.14 aA | 5.43 ± 0.03 aA | 6.02 ± 0.01 aA | 2.33 ± 0.65 abB | 4.63 ± 1.08 abAB | 3.59 ± 0.37 aA | 1.51 ± 0.32 bB | 1.66 ± 0.52 bB |
| 10 °C | 6.42 ± 1.03 aA | 3.44 ± 0.33 aB | 5.43 ± 0.15 aAB | 6.57 ± 0.47 aA | 1.73 ± 0.61 abB | 3.69 ± 0.85 abAB | 1.87 ± 0.97 bA | ⩽0.76 ± 0.10 bA | 1.40 ± 0.05 bA |
| 21 °C | 6.24 ± 0.81 aA | 1.46 ± 0.0 aB | 3.46 ± 0.81 aB | 4.45 ± 2.14 aA | ⩽1.20 ± 0.0 aA | 2.79 ± 2.27 abA | ⩽0.70 ± 0.0 bA | ⩽0.70 ± 0.0 aA | ⩽0.70 ± 0.0 bA |
| 30 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| Storage day 30 | | | | | | | | | |
| 4 °C | 6.55 ± 0.84 aA | 3.64 ± 0.16 aB | 4.88 ± 0.39 aAB | 5.80 ± 0.13 abA | 2.04 ± 0.45 abB | 4.21 ± 1.42 abAB | 3.17 ± 0.68 bA | 1.26 ± 0.81 bB | 1.46 ± 0.67 bAB |
| 10 °C | 6.31 ± 0.33 aA | 2.91 ± 0.25 aB | 5.27 ± 0.07 aAB | 6.46 ± 0.63 aA | ⩽1.20 ± 0.0 aB | 3.42 ± 1.06 abAB | 1.91 ± 0.61 bA | ⩽0.70 ± 0.0 aA | 0.79 ± 0.08 bA |
| 21 °C | 6.58 ± 0.86 aA | 1.40 ± 0.0 aB | 2.66 ± 1.45 aB | 4.26 ± 1.53 aA | ⩽1.20 ± 0.0 aA | 2.52 ± 1.89 abA | ⩽0.70 ± 0.0 bA | ⩽0.70 ± 0.0 aA | ⩽0.70 ± 0.0 bA |
| 30 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| Storage day 45 | | | | | | | | | |
| 4 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | 0.97 ± 0.34 | ⩽0.70 ± 0.0 |
| 10 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| 21 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| 30 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| Storage day 60 | | | | | | | | | |
| 4 °C | ND | ND | ND | ND | ND | ND | 1.73 ± 0.97 | 0.81 ± 0.19 | ⩽0.70 ± 0.0 |
| 10 °C | ND | ND | ND | ND | ND | ND | 0.83 ± 0.18 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| 21 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| 30 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| Storage day 75 | | | | | | | | | |
| 4 °C | ND | ND | ND | ND | ND | ND | 1.24 ± 0.76 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| 10 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| 21 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| 30 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| Storage day 90 | | | | | | | | | |
| 4 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| 10 °C | ND | ND | ND | ND | ND | ND | ⩽0.75 ± 0.07 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| 21 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |
| 30 °C | ND | ND | ND | ND | ND | ND | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 | ⩽0.70 ± 0.0 |

[a] Mean of two trials (±S.D.) ($N = 2$ trials, $n = 3$ sausages per trial) for each pathogen. Detection limit ⩽ 1.18 $\log_{10}$ CFU/g.
[b] NA: not applicable.
[c] Mean of three trials (±S.D.) ($N = 3$ trials, $n = 3$ sausages per trial) for each pathogen. Detection limit ⩽ 0.70 $\log_{10}$ CFU/g.
[d] For a given pathogen, means with different lower case letters in common within rows are significantly ($P \leqslant 0.05$) different.
[e] For a given treatment, that being experimentally manufactured fermented to pH 5.3, experimentally manufactured fermented to pH 4.8, and commercially manufactured, pathogen means with different upper case letters in common within rows are significantly ($P \leqslant 0.05$) different.
[f] ND: not determined.

5.27 only by enrichment (83.3%; five of six samples tested), whereas in sausages fermented to pH 4.81, the pathogen was not detected even by enrichment after 22 days of storage at 21 °C. For sausages fermented to pH 5.27 and 4.81 that were inoculated with *E. coli* O157:H7, fermentation and drying alone reduced pathogen numbers by 0.03 and 1.11 $\log_{10}$ CFU/g, respectively. Numbers of *E. coli* O157:H7 decreased by an additional 0.95–3.17 $\log_{10}$ CFU/g during storage.

Regardless of pH and storage temperature, it was not possible to calculate *D*-values for *L. monocytogenes*, *S. typhimurium*, or *E. coli* O157:H7 during extended storage of the experimentally manufactured sausages due to insufficient lethality and/or substantial variability among trials.

### 3.4. Viability of pathogens on the surface of soudjouk during storage

In general, when separately inoculated onto the surface of slices, the viability of all the three pathogens was significantly ($P \leqslant 0.05$) less than their viability when inoculated into soudjouk batter that was fermented to ca. pH 5.3 (Table 2), except for *S. typhimurium* in sausages that were stored at 21 °C. Regardless of the storage temperature, when separately inoculated onto the surface of slices, viability of *S. typhimurium* and *E. coli* O157:H7 was not significantly ($P \geqslant 0.05$) different from results obtained when these same pathogens were inoculated into the batter that was fermented to pH 4.81 (Table 2). However, there were significant ($P \leqslant 0.05$) differences in viability of *L. monocytogenes* between the experimentally manufactured sausages fermented to ca. pH 4.8 (~0.3–1.8 $\log_{10}$ CFU/g reduction) and the commercially manufactured sausages (pH 4.97; (~2.6–5.07 $\log_{10}$ CFU/g reduction) after 30 days of storage at 4, 10, or 21 °C. Collectively, all the three pathogens were inactivated to a greater extent on slices (pH ~4.97) when compared to inoculation of batter that was subsequently fermented to pH 4.8 or 5.3.

As expected, examination of *D*-values revealed that the higher the storage temperature, the greater the level and rate of inactivation for all three pathogens tested. With the possible exception of sausages surface inoculated with *E. coli* O157:H7 that were stored at 10 °C, storage of surface-contaminated soudjouk-style sausage at 21 or 30 °C was significantly ($P \leqslant 0.05$) more lethal for all three pathogens than storage at 4 or 10 °C (Table 3). In addition, cells of *E. coli* O157:H7 were inactivated at a significantly ($P \leqslant 0.05$) greater rate than *L. monocytogenes* when sausages were stored at 4 or 10 °C. However, there were no significant ($P \geqslant 0.05$) differences in inactivation rates of *E. coli* O157:H7 compared with *S. typhimurium* when sausages were stored at 4 or 10 °C. These *D*-values were plotted against the various storage temperatures to calculate the corresponding *z*-values (Table 3). Based on these results, in the event that soudjouk becomes surface contaminated with *L. monocytogenes*, to achieve an estimated 5 $\log_{10}$ reduction it would be necessary to store it for 50.5–4.4 days at 4–30 °C. Likewise, to achieve the required 5 $\log_{10}$ reduction for *E. coli* O157:H7 on the surface of soudjouk, it would be necessary to store it for 29.7–1.2 days at 4–30 °C. For *S. typhimurium*, to achieve the required 6.5 $\log_{10}$ reduction, it would be necessary to store surface-contaminated soudjouk for 50 to 4.4 days of storage at 4–30 °C.

**Table 3**
Inactivation of *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7 inoculated onto the surface of slices of retail soudjouk

| | *D*-values[a] (days) ($r^2$) | *z*-values[b] (°C) ($r^2$) |
|---|---|---|
| *L. monocytogenes* | | |
| 4 °C | 10.12 ± 1.79 a[c,d] (0.88) | 22.61 (0.97) |
| 10 °C | 6.46 ± 1.79 b,c (0.90) | |
| 21 °C | 1.37 ± 0.22 ef (0.92) | |
| 30 °C | 0.87 ± 0.88 f (0.82) | |
| *S. typhimurium* | | |
| 4 °C | 7.63 ± 1.30 a,b (0.89) | 30.74 (0.78) |
| 10 °C | 4.28 ± 0.51 c,d,e (0.95) | |
| 21 °C | 0.93 ± 0.60f (0.95) | |
| 30 °C | 1.37 ± 1.77 e,f(0.49) | |
| *E. coli* O157:H7 | | |
| 4 °C | 5.93 ± 2.01 b,c,d (0.93) | 20.07 (0.93) |
| 10 °C | 2.91 ± 0.27 d,e,f (0.95) | |
| 21 °C | 1.59 ± 0.40 e,f, (0.86) | |
| 30 °C | 0.24 ± 0.14 f (0.93) | |

[a] *D*-values were determined using DMFit curve fitting software and represent the absolute value of the inverse of the linear inactivation rate of the surviving cell fraction. It was not possible to calculate *D*-values for experimentally manufactured sausages due to insufficient lethality and/or substantial variability among trials.

[b] *z*-values were calculated as the absolute value of the inverse of the linear regression of *D*-value versus the corresponding storage temperatures.

[c] Mean of three trials (±S.D.) ($N = 3$ trials, $n = 3$ sausages per trial) for each pathogen.

[d] Means with the different letters within columns are significantly ($P \leqslant 0.05$) different.

## 4. Discussion

The prevalence and levels of *L. monocytogenes* in RTE meats are well documented (Gianfransceschi et al., 2006; Angelidis and Koutsoumanis, 2006; Gombas et al., 2003; Wallace et al., 2003). Although the meat industry has appreciably reduced the incidence of this pathogen in RTE meats, the USDA/FSIS reported that between 1990 and 1999, the prevalence of *L. monocytogenes* in fermented meats in Federally inspected plants was 3.3% (27 of 830 samples) (Levine et al., 2001). Our results are consistent with other reports showing that *L. monocytogenes* was inhibited during fermentation and drying of soudjouk formulated with a starter culture and that pathogen numbers continued to decrease during subsequent storage of the finished product. For example, Erol and Hildebrandt (1992) studied the viability of *L. monocytogenes* in soudjouk that was naturally fermented/matured at 20 °C for up to 14 days and reported that pathogen numbers increased by ca. 1.0 $\log_{10}$ CFU/g during the fermentation/maturation step, whereas in sausages containing a mixture of starter cultures (*Staphylococcus carnosus* and *Lactobacillus plantarum*), pathogen numbers decreased by ca. 1.0 $\log_{10}$ CFU/g. Likewise, Hampikyan and Ugur (2007) reported that *L. monocytogenes* numbers increased by ca. 2.0 $\log_{10}$ CFU/g during fermentation of soudjouk at 24–18 °C (95–75% RH) for up to 5 days, whereas in sausages containing 50 or 100 μg/g of nisin, pathogen numbers decreased by 0.9 and 2.1 $\log_{10}$ CFU/g, respectively, during fermentation under otherwise similar conditions. The policies established by the USDA/FSIS to control *L. monocytogenes* on RTE meats and poultry products requires manufacturers to validate that their processes achieve "zero tolerance" and to include a post-process lethality treatment and/or suppress outgrowth during shelf life (Anonymous, 2003). Our results established that it was possible to achieve a total reduction of ca. 2.5 $\log_{10}$ CFU/g of *L. monocytogenes* inoculated into batter that was subsequently fermented to pH 4.8 and stored at 21 °C for 30 days. In contrast, when soudjouk was fermented to ca. pH 5.3 and stored at 21 °C for 30 days, pathogen numbers decreased by ⩽0.1 $\log_{10}$ CFU/g, presumably due to the lower acid content of the finished product. Based on the *D*-values calculated in this study, in case of a surface contamination, to achieve a 5-$\log_{10}$ reduction of *L. monocytogenes*, it would be necessary to store soudjouk for ca. 50.5, 32.5, 7, and 4.5 days at 4, 10, 21, and 30 °C, respectively.

Fermented meats have also been epidemiologically implicated as the vehicle of infection from *Salmonella* spp. (Cowden et al., 1989; Pontello et al., 1998; Bremer et al., 2004; Hjertqvist et al., 2006). In the USA, Levine et al. (2001) reported a 1.4% (10 of 698 samples) prevalence of *Salmonella* spp. in fermented sausages collected in Federally inspected plants between 1990 and 1999. Results published to date suggest that fermentation and drying alone may not be sufficient to achieve the recommended 6.5-$\log_{10}$ reduction of *Salmonella* in RTE red meat. For example, Turantaş and Ünlütürk (1993) reported ca. a 2.0-$\log_{10}$ decrease in numbers of *S. typhimurium* after fermentation of soudjouk at 24 °C (>90% RH) for 2 days and ca. a 3.0-$\log_{10}$ reduction after subsequent drying at 22 °C (80–90% RH) for 2 days. Ihnot et al. (1998) reported ca. a 1.3-$\log_{10}$ decrease in numbers of *S. typhimurium* in pepperoni after fermentation to pH⩽4.8 at 36 °C (92% RH) and ca. a 1.6-$\log_{10}$ reduction after drying at 13 °C (65% RH). Similar results were observed by Smith et al. (1975) who reported that fermentation to pH 4.5 at 35 °C (85% RH) and drying at 12 °C (65% RH) for 22 days resulted in ca. a 1.3- and 3.0-$\log_{10}$ reduction, respectively, of *S. typhimurium* in pepperoni. Overall, these results are in general agreement with the results obtained in the present study.

As a result of an outbreak of *E. coli* O157:H7 illnesses associated with the consumption of salami in the early 1990s, the USDA/FSIS requires producers to achieve a 5.0-$\log_{10}$ reduction (options 1, 2, and 4) of this pathogen or implement additional measures, such as a hold-and-test program for finished product or raw batter testing and achieve a 2-$\log_{10}$ reduction in pathogen numbers during processing (options 3 or 5, respectively), to assure the safety of fermented meats (Reed, 1995). In agreement with our results, other validation studies have demonstrated that fermentation and drying alone were not sufficient to deliver a 5.0-$\log_{10}$ reduction of *E. coli* O157:H7 in fermented sausage, including soudjouk. For example, Calicioglu et al. (2001) assessed the survival of *E. coli* O157:H7 in soudjouk-style sausage and reported that use of a commercial pediococcal starter culture and fermentation to ca. pH 4.6 at 24 °C (90–95% RH) for 3 days and drying at 22 °C (80–85% RH) until the sausage achieved about a 40% moisture content delivered ca. a 2.0-$\log_{10}$ CFU/g reduction. Likewise, Cosansu and Ayhan (2000) reported a 3.5-$\log_{10}$ reduction of *E. coli* O157:H7 after spontaneous, that being without the addition of a defined starter culture, fermentation of soudjouk to ca. pH 4.9 at 24 °C (90–95% RH) for 3 days and drying at 22 °C (80–90% RH) for 5 days. Faith et al. (1998) reported that regardless of how the batter for salami was "pre-conditioned", fermentation at 24 °C (90% RH) to pH⩽4.8 and drying at 13 °C (65% RH) to a M:Pr of ⩽1.9:1 resulted in ca. 1.1–2.1 $\log_{10}$ CFU/g reduction in numbers of *E. coli* O157:H7. As a final example, Hinkens et al. (1996) reported a 1.2 $\log_{10}$ CFU/g reduction of *E. coli* O157:H7 in pepperoni formulated with the addition of a pediococcal starter culture that was fermented to pH 4.7 at 36 °C (85% RH) and then dried at 13 °C (65% RH) to a M:Pr of ⩽1.6:1. Our data are also in general agreement with previous studies (Faith et al., 1998; Ihnot et al., 1998; Nightingale et al., 2006) reporting that *S. typhimurium* was less viable than *E. coli* O157:H7 or *L. monocytogenes* following manufacture and storage of fermented sausages.

In general, colder storage temperatures (4–15 °C) supported viability of *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 in fermented sausages better than warmer storage temperatures (⩾25 °C) (Faith et al., 1998; Calicioglu et al., 2002; Uyttendaele et al., 2001; Ihnot et al., 1998). Our data also revealed that storage of soudjouk-style sausage at ⩾21 °C was significantly ($P \leqslant 0.05$) more deleterious to *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 inoculated either into the batter or onto the surface of soudjouk than storage at 4 or 10 °C. However, when sausages were stored at 30 °C, considerable untoward changes in color and texture of the product were observed after ca. 3 days of storage. In general, reductions of *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 were greater when these pathogens were inoculated onto the surface of soudjouk slices compared to when they were inoculated into the batter. It should also be noted that the gradual decrease in pH that occurs during fermentation and drying could trigger an acid adaptation response of *L. monocytogenes*, *S. typhimurium*, and/or *E. coli* O157:H7, consequently enhancing the ability of these pathogens to survive better during subsequent storage of the final product. Previous studies reported that acid tolerance alters cellular resistance of all three of these pathogens to the low pH environment found in fermented meats and strengthens their subsequent viability at colder storage temperatures (Lin et al., 1996; Chikthimmah and Knabel, 2001). Although Gram-negative bacteria are generally more resistant to acidic environments than Gram-positive bacteria due to their defined acid tolerance resistance (ATR) mechanisms (Bearson et al., 1997), our findings showed that *S. typhimurium* and *E. coli* O157:H7 were inactivated to a greater extent than *L. monocytogenes* during storage of soudjouk-style sausage. Although further research is warranted to gain insight as to why cells of *L. monocytogenes* were more recalcitrant than cells of *S. typhimurium* or *E. coli* O157:H7, this observation may be attributed, at least in part, to differences in osmolarity-sensing mechanisms, and/or sensitivity to some ingredients such as garlic and spices, and/or differences in membrane fluidity. Regardless, maximum reductions for all three pathogens inoculated either into or onto soudjouk-style sausage were obtained by the combined interactions of the relatively low pH and/or relatively low $a_w$ of the sausage coupled with the intrinsic antimicrobial potency of the formulation/ingredients and/or relatively higher storage temperatures (⩾21 °C). Further studies are warranted to determine the effect of the competitive flora and/or intrinsic strain-to-strain variation of these pathogens on both the rate and extent of their inactivation in and on soudjouk. As stated previously herein, variability in the composition and processing parameters among producers can also have an appreciable influence on the fate of pathogens in soudjouk.

The addition of a starter culture(s) in the production of fermented meats is a common practice worldwide. However, many ethnic/specialty meats traditionally produced by small and very small manufacturers continue to rely solely on spontaneous fermentation, resulting in substantial variations in the physical, chemical, and/or microbiological composition of the resulting products. In the present study, the addition of a pedicococcal starter culture and added dextrose decreased the pH to ca. 5.3 or 4.8 following fermentation and drying and the lower pH resulted in a quantifiable greater lethality. Previous studies have shown that the use of a starter culture resulted in 1.0 and 4.0 $\log_{10}$ CFU/g greater reduction of *E. coli* O157:H7 and *Yersinia enterocolitica*, respectively, than otherwise similar soudjouk manufactured without a starter culture (Ceylan and Fung, 2000; Calicioglu et al., 2002). In the present study, however, the use of a pediocin-producing starter culture did not appreciably affect the viability of *L. monocytogenes* compared to the antilisterial contribution of pH alone in comparison to the fate of *E. coli* O157:H7 and *Salmonella*. Regardless, it is strongly suggested that manufacturers use a starter culture to improve the quality, consistency, and safety of their products.

In summary, our results showed that fermentation to ca. pH 4.8 followed by storage at 21 or 30 °C was appreciably more effective in reducing pathogen numbers than storage at 4 or 10 °C for 30 days. In general, regardless of the pH or storage temperature, all three pathogens were inactivated to a greater extent when inoculated onto the surface of soudjouk rather than when directly added to the batter. Our data also validate that soudjouk-style sausage does not provide a favorable environment for outgrowth of *L. monocytogenes*, *S. typhimurium*, and *E. coli*

O157:H7 under the conditions tested herein. In a companion study (Hwang et al., 2007), we modeled the fate of these three pathogens at specific pH (pH 5.2, 4.9, and 4.6) and $a_w$ ($a_w$ 0.92, 0.89, and 0.86) values to better predict their behavior in soudjouk-style sausage. Additional research should also focus on optimization of a post-process heating step and/or the inclusion of selected antimicrobials into the batter, onto the surface of the product, and/or into the inside of the container to satisfy lethality guidelines and enhance product safety. Such studies would greatly assist small and very small producers to validate their products/processes, leading to a more wholesome product.

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