Modeling the survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella Typhimurium* during fermentation, drying, and storage of soudjouk-style fermented sausage

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1. Introduction

Fermented dry or semi-dry sausages (FDSS) are produced by fermenting and drying a raw meat batter containing sugar, seasonings/spices, and/or curing agents. The fermentation is conducted by natural microflora in the ingredients and/or by added starter cultures. In the United States, dry sausages are manufactured with chopped or ground meat that is fermented to ≤pH 5.3 and dried to remove ca. 50% of the moisture, whereas semi-dry sausages are fermented to ≤pH 5.3 and dried to remove ca. 15% of the moisture, resulting in a moisture/protein ratio (MPR) complying with the Federal requirements. Guidance from the Food Safety and Inspection Service (FSIS/USDA) requires that shelf-stable semi-dry and dry sausage be nitrite cured, fermented, and smoked, and have MPR of ≤3.1:1 and ≤1.9:1, respectively, with a final pH of ≤pH 5.0 (American Meat Institute Foundation, 1997). Soudjouk (soudjuk, soudjouk, surugu, or sucuk), chorizo, frizzes, pepperoni, Lola or Lolita, and Lyons sausages, and Genoa salami are examples of dry sausages, whereas summer sausages, Lebanon bologna, and mortadella are examples of semi-dry sausages (FSIS, 2003). The FDSS are generally considered as stable, ready-to-eat (RTE) meat products due to the relatively low pH and low a<sub>W</sub> (Barbuti and...
Parolari, 2002). However, foodborne pathogens such as Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella spp. may contaminate these products via contaminated raw meat, ingredients and/or processing equipment, and/or from post-processing contamination. These pathogens have also been detected in raw meat and have also been shown to survive certain sausage manufacturing processes (Glass and Doyle, 1989; Glass et al., 1992; Hinkens et al., 1996; Farber et al., 1993; Calicioglu et al., 1997; Faith et al., 1997; Nissen and Holck, 1998; Riordian et al., 1998; Cosansu and Ayhan, 2000; Barbutti and Parolari, 2002; Colak et al., 2007). Some FDSS have been linked to outbreaks of foodborne illnesses. In the U.S., a dried salami product was implicated in 20 cases of illnesses caused by E. coli O157:H7 in California in 1994 (CDC, 1995), and in 1995 a salmonellosis outbreak was epidemiologically linked to the consumption of Lebanon bologna (Sauer et al., 1997). In Australia in 1995, dry fermented sausages (mettwurst) contaminated with shiga-like toxin-producing E. coli were implicated in an outbreak causing 21 illnesses and one death (Paton et al., 1996), and in 2001 in Germany an outbreak of salmonellosis was linked to consumption of fermented sausage (Bremer et al., 2004). The potential health hazards associated with FDSS prompted the FSIS/USDA to require sausage manufacturers to adopt at least one of the five "validated manufacturing processes" to ensure the safety of their products with respect to E. coli O157:H7 (Reed, 1995). In addition, pathogens such as L. monocytogenes and Salmonella spp. should be absent from RTE products. Among the five validated processes, heat is effective for achieving the required reduction of E. coli O157:H7 in FDSS (Hinkens et al., 1996; Calicioglu et al., 2002). However, a post-process heat treatment may not be applicable to some products because the sensory quality would be adversely affected. Calicioglu et al. (2002) reported that heating soudjouk-style sausage to an internal temperature of 63 °C (145.4 °F) achieved a ≥6.0-log10 reduction of E. coli O157:H7; however, the heating resulted in unacceptable product quality.

As a result of outbreaks and recalls due to contamination with E. coli O157:H7, Listeria monocytogenes, and/or Salmonella in FDSS, the FSIS/USDA requires that FDSS manufacturers verify that their manufacturing processes meet existing regulatory guidelines. While large FDSS manufacturers have resources to conduct microbiological studies to validate their processes, small and very small producers may not be able to determine whether their processes meet the regulatory requirements. Manufacturing processes for FDSS vary significantly among FDSS varieties and among manufacturers of the same variety. The uses of different ingredients, formulations, starter cultures, and fermentation, drying, and storage conditions for FDSS lead to different characteristics of the final product. In addition, most validation studies that have been published were conducted for selected pH and aw values, as well as storage conditions for a specific FDSS process (McNeal, 1990; Nickelson et al., 1996; Calicioglu et al., 1997). Therefore, results from a validation study for a FDSS are only applicable to that specific product or related products. The objectives of this study were to quantify the survival of E. coli O157:H7, L. monocytogenes, and Salmonella Typhimurium in a soudjouk-style sausage during fermentation and drying to various pH and aw values and at various storage temperatures, and to describe the survival using mathematical equations to estimate the survivability of these three pathogens in other FDSS products. Soudjouk is a Mediterranean-style fermented sausage, which is made by mixing ground meat, spices, curing salts, and with or without a starter culture. The batter is stuffed into casings to form sausage links, and the links are fermented and dried for several days (Saricoban et al., 2006). Soudjouk samples obtained from the market place had a pH of ca. 5.0 and aw of ca. 0.85. The acid levels (pH 5.2 to pH 4.6), water activity (aw 0.92 to aw 0.86), and storage temperatures (4, 21, and 30 °C) evaluated in this study were typical for several FDSS. The applicability of the models to other types of FDSS was evaluated by comparing model predictions with published data.

### 2. Materials and methods

#### 2.1. Bacterial strains

Three strains of E. coli O157:H7 [EC204P (a beef isolate), C7927 (a clinical isolate from the 1991 Massachusetts outbreak linked to apple cider), and SLH21788 (a clinical isolate from the 1994 Wisconsin daycare-linked outbreak)], five strains of L. monocytogenes [MFS 2 (serotype 1/2a, an environmental isolate from a pork processing plant), H7776 (4b, frankfurter isolate), Scott A (4b, a clinical isolate from a 1983 Massachusetts outbreak linked to pasteurized milk), 1011M (4b, beef and pork sausage isolate), and F6854 (1/2a, turkey frankfurter isolate)], and 6 strains of S. Typhimurium [H3278, G7601, H3402, H2662, H3380, and G8430 (all clinical isolates)] were used in this study. These bacterial cultures were confirmed, cultured, and maintained as described previously by Porto-Fett et al. (2008).

#### 2.2. Preparation and inoculation of sausage

Raw ground beef (20% fat) was obtained from a local retail store and kept frozen until used. Sausage batter was prepared by mixing 5 kg of raw ground beef, 1.9% sodium chloride (Morton International Inc., Chicago, IL), 0.25% sodium nitrite (Sigma Chemical Co., St. Louis, MO), 0.95% chopped fresh garlic, 0.95% cumin, 0.42% paprika, 0.42% black pepper, 0.42% all spice (Atlantic Spice Company, North Truro, MA), and 0.25%, 0.50%, or 0.70% dextrose (Difco Laboratories Inc., Detroit, MI) with the aid of a commercial countertop mixer (Univex SRM12; Salem, NH) for 5 min. Following the mixing, the batter was separately inoculated with the multi-strain mixture of E. coli O157:H7, L. monocytogenes, or S. Typhimurium to achieve a cell concentration of ca. 6.5 log10 CFU/g of batter. A commercial Pediococcus acidilactici and Staphylococcus carnosus starter culture (Formula 102; Trumark Inc., Linden, NJ) was prepared as per the manufacturer's instruction and added into the batter (6.0 to 7.0 log10 CFU/g). The batter was then mixed for an additional 10 min. The batter was stuffed into 25 mm diameter collagen casings (Nippi Co., Tokyo, Japan) using a manual stuffer (Dick D-73779; Deizisau, Germany), and the sausages were hand tied with cotton strings at ca. 15-cm intervals. Each sausage link was ca. 100 g. Sausages were hung vertically in an environmentally-controlled incubator (EJS Systems Inc., Changrin Falls, OH) for fermentation at 24 °C (75.2 °F) with a relative humidity (RH) of 90% to 95% and an air flow speed of 1.0 to 1.5 m/s until the pH of sausage reached ca. pH 5.2, pH 4.9, or pH 4.6 (corresponding to dextrose concentrations of 0.25%, 0.5%, or 0.7%, respectively). The sausages were then dried at 22 °C (71.6 °F) with 80 to 85% RH until aw reached ca. aw 0.92, aw 0.89, or aw 0.86. The air temperature and RH during fermentation and drying were controlled and measured using the Dynamist 2000 System and the Partlow MRC5000 chart recorder (EJS Systems). After drying, two sticks of sausage were vacuum-packed in stomach bags (Spiral Biotech, Inc., Norwood, MA) using a Multivac A300/16 vacuum-packaging machine (Sepp Hagmüller KG, Wolfratshausen, Germany). The sausages were sampled for microbial counts, pH, and aw daily during fermentation and drying, and at day 0, 5, 10, 20, 30, 40, 50, and 60 during storage at 4, 21, or 30 °C for each of the two trials conducted in this study, two sausage links were analyzed at each sampling time.

#### 2.3. Microbiological analyses

At each sampling interval, a 5-g portion of the sausage from the middle of each stick was removed for the enumeration of cell counts of lactic acid bacteria (LAB) and pathogens. The 5-g sample represented about 10% (w/w) of the sausage quantity available for each sampling. While a 25-g portion is the sample size normally used for pathogen testing in commercial food production, the ratio of the sample size to
the size of product from which samples are withdrawn seldom exceeds 10%. The 5-g sample size was deemed to be representative of the sample. The sample was transferred to a stomacher bag, added with 45 ml of 0.1% sterile peptone water, and mixed in a BagMixer 400 stomacher (Interscience, St Nom, France) for 2 min. Pathogen populations were enumerated by serial dilution in 0.1% sterile peptone as needed and spread-plating 100 or 250 µl onto MacConkey sorbitol (SMAC; Difco), polymyxin B, acriflavin, lithium chloride, ceftazidine, esculin, p-mannitol (PALCAM; Difco), xylose-lysine-tergitol-4 (XLT-4; Difco), and deMann Rogosa Sharpe (MRS; Difco) agar plates for E. coli O157:H7, L. monocytogenes, S. Typhimurium, and LAB, respectively. All plates were incubated aerobically at 37 °C for 24 to 48 h, except for MRS plates that were incubated anaerobically (10.1% carbon dioxide, 4.38% hydrogen, and balance nitrogen; Bactron IV Anaerobic/Environmental Chamber, Sheldon Manufacturing Inc., Cornelius, OR), before typical colonies were counted. Presumptive E. coli O157:H7 colonies recovered from the samples were further confirmed since non-E. coli O157:H7 microorganisms were able to grow on SMAC, albeit the growth was slower than that of E. coli O157:H7.

2.4. pH and aw measurements

At each sampling point, the pH of sausage was measured by using a Daigger 5500 pH meter (A. Daigger and Company Inc., Vernon Hills, IL). Five grams of sausage were macerated with 15 ml of peptone water in a filter stomacher bag for 2 min, and the pH of resulting slurry was analyzed. The aw of sausage was measured by placing 2 to 3 g of sample in an AquaLab CX-2 water activity meter (Decagon Devices, Inc., Pullman, WA).

2.5. Data analyses

The values of reduction in viable counts (log10 reductions) for each pathogen during fermentation to various pH levels from each trial were plotted versus the pH of sausage. Log10 reductions as a function of pH were analyzed using the Regression procedure of the Statistical Analysis System (SAS) version 9.1 software for Windows (SAS Institute Inc., Cary, NC) fitted with the following polynomial equation:

\[
\text{Log}_{10} \text{ reduction} = \alpha + \beta_1 (\text{pH}) + \beta_2 (\text{pH})^2
\]

where \(\alpha\) is the intercept, and \(\beta_1, \beta_2\) are estimated coefficients.

A polynomial regression was performed to fit the \(\text{Log}_{10}\) reductions of each pathogen in soudjouk sausage during drying as a function of pH at the beginning of drying (at the end of drying):

\[
\text{Log}_{10} \text{ reduction} = \alpha + \beta_1 (\text{pH}) + \beta_2 (\text{aw}) + \beta_3 (\text{pH} \times \text{aw}) + \beta_4 (\text{pH})^2 + \beta_5 (\text{aw})^2
\]

where \(\alpha\) is the intercept, and \(\beta_1, \beta_2, \ldots, \beta_5\) are estimated coefficients.

During storage, viable counts of each pathogen (log10 CFU/g) were plotted versus storage time (day) to generate survival curves. From the curves, the average reduction rates (log10 CFU/day) for each pathogen were estimated by dividing the log10 reduction values during storage by the length of time of storage (days). The reduction rates of each pathogen in the soudjouk sausage during storage as a function of sausage pH at the beginning of drying, aw at the end of drying, and storage temperature were analyzed using the General Linear Model (GLM) of SAS 9.1 and fitted to the following quadratic equation:

\[
\text{Reduction rate (log10 CFU/day)} = \alpha + \beta_1 (\text{pH}) + \beta_2 (\text{aw}) + \beta_3 (\text{temperature}) + \beta_4 (\text{pH} \times \text{aw}) + \beta_5 (\text{pH} \times \text{temperature}) + \beta_6 (\text{aw} \times \text{temperature}) + \beta_7 (\text{pH})^2 + \beta_8 (\text{aw})^2 + \beta_9 (\text{temperature})^2
\]

where \(\alpha\) is the intercept, and \(\beta_1, \ldots, \beta_9\) are estimated coefficients.

For each regression analysis, the 95% confidence limit interval (95% CLI) of the predictions for each observed value used in model development was also obtained. The 95% CLI provides the upper and lower boundaries of the predictions, which accommodate the variability in the predicted values and the variability in the observed

![Fig. 1.](image) Observed log10 reductions, fitted regression line, and 95% CLI of values predicted from the regression equation for E. coli O157:H7 (A), L. monocytogenes (B) and S. Typhimurium (C) in soudjouk-type sausages after fermentation to various pH levels.
values. When compared to data that were not included in the model development, predicted values obtained from the models were considered acceptable when the outside data were within the 95% CLI of the predicted values. Mean comparisons were conducted using Tukey mean comparison test (SAS 9.1) at a significance level of 95%.

3. Results

3.1. Reduction of pathogens during fermentation

The initial numbers of E. coli O157:H7, L. monocytogenes, and S. Typhimurium in the sausages were ca. 6.5 log10 CFU/g. The pH of the sausage was pH 5.9±0.1 before fermentation. During fermentation at 24 °C, the pH of sausage reached ca. pH 5.2 after 3 days, ca. pH 4.9 after 4 days, and ca. pH 4.6 after 5 days in sausages formulated with 0.2%, 0.5%, and 0.7% of added dextrose. The pH of sausage reached pH 5.3 within 72 h of fermentation. The fermentation process had a degree-hours of 1200 degree-hours when the fermentation temperature is less than 32.2 °C (90 °F) (American Meat Institute, 1997). After fermentation, the total LAB counts increased from the initial 7.0 log10 criterion of fermentation. The fermentation process had a degree-hours of 1200 degree-hours when the fermentation temperature is less than 32.2 °C (90 °F) (American Meat Institute, 1997). After fermentation, the total LAB counts increased from the initial 7.0 log10 CFU/g to 9.0 log10 CFU/g. The reductions in viable cell counts in log10 CFU/g (log10 reduction) for each pathogen in soudjouk sausage during fermentation are as follows:

E. coli O157: H7 log10 reduction (log10 CFU/g) = 29.356 − 10.412(pH) + 0.921(pH)²
L. monocytogenes log10 reduction (log10 CFU/g) = 9.986 − 3.436(pH) + 0.295(pH)²
S. Typhimurium log10 reduction (log10 CFU/g) = 230.949 − 90.474(pH) + 8.856(pH)²

A major portion of the variation of log10 reductions for the three pathogens was explained by the variation in the sausage pH (R² = 0.92 to 0.98), and the p value for each estimated coefficient was < 0.001 (Table 1), indicating that the pH of sausage significantly affected the log10 reductions of each pathogen during fermentation; i.e., the log10 reductions increased as sausages were fermented to lower pH. The estimated log10 reductions obtained from the models and the 95% CLI of the predictions for each observed reduction are shown in Fig. 1. Comparing the observed and predicted values, the models appear to closely describe the observed values (Fig. 1 and Table 1).

Table 1 Parameter estimates and significance levels for the log10 reduction models for E. coli O157:H7, L. monocytogenes and S. Typhimurium in a soudjouk-type sausage during fermentation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>E. coli O157:H7</th>
<th>L. monocytogenes</th>
<th>S. Typhimurium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>29.356 &lt; .0001</td>
<td>9.986 &lt; .0001</td>
<td>230.949 &lt; .0001</td>
</tr>
<tr>
<td>pH</td>
<td>−10.412 &lt; .0001</td>
<td>−3.436 &lt; .0001</td>
<td>−90.474 &lt; .0001</td>
</tr>
<tr>
<td>pH²</td>
<td>0.921 &lt; .0001</td>
<td>0.295 &lt; .0001</td>
<td>8.856 &lt; .0001</td>
</tr>
<tr>
<td>Model</td>
<td>F value = 487.41</td>
<td>F value = 225.99</td>
<td>F value = 83.27</td>
</tr>
<tr>
<td></td>
<td>Pr &gt; F = &lt; .0001</td>
<td>Pr &gt; F = &lt; .0001</td>
<td>Pr &gt; F = &lt; .0001</td>
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<tr>
<td>RMSE</td>
<td>0.0689</td>
<td>0.04218</td>
<td>0.4455</td>
</tr>
<tr>
<td>R²</td>
<td>0.98</td>
<td>0.96</td>
<td>0.92</td>
</tr>
</tbody>
</table>

* F value was used to test that the estimates were not equal to zero. If the associated p value was < 0.05, it indicated that at least one of the estimates was not zero. RMSE (square root of the error mean square) estimated the standard deviation of the random error term in the model. R² indicated the portion (in percentage) of the variation of the log10 reduction that was explained by the variation in the independent variable in the model.

Fig. 2. Observed log10 reductions and fitted regression equations of E. coli O157:H7 (A), L. monocytogenes (B) and S. Typhimurium (C) in soudjouk-type sausages of ca. pH 5.2, 4.9 and 4.6 during drying to various aw levels.
3.2. Reductions of pathogens during drying

Before fermentation, the \( a_w \) of sausage batter was ca. \( a_w = 0.97 \), whereas after 3 to 5 days of fermentation the \( a_w \) decreased to \( a_w = 0.94 \). During drying, the \( a_w \) decreased to \( a_w = 0.92 - 0.86 \) after 3 to 5 days in sausage with pH 5.2, after 4 to 6 days in sausages with pH 4.9, and after 5 to 7 days in sausage with pH 4.6. The log\(_{10}\) reductions of \( E. coli \) O157:H7, \( L. monocytogenes \), and \( S. Typhimurium \) in sausage during drying are shown in Fig. 2. Significant lethality was observed at \( a_w = 0.92 \) for \( E. coli \) O157:H7 and \( L. monocytogenes \), and at \( a_w = 0.94 \) for \( S. Typhimurium \). As expected, the log\(_{10}\) reductions of \( E. coli \) O157:H7 and \( L. monocytogenes \) during drying increased as sausage final \( a_w \) decreased, and higher log\(_{10}\) reductions occurred in sausage with lower pH. The reductions of \( S. Typhimurium \) also increased as the \( a_w \) decreased; however, the log\(_{10}\) reductions were similar in sausage with pH 5.2 and pH 4.9 during drying. The log\(_{10}\) reductions of each pathogen during drying as a function of sausage pH and \( a_w \) are as follows:

\[
\text{E. coli O157 : H7 log}_{10} \text{reduction (log}_{10}\text{CFU/g)} = 376.983 + 8.668(\text{pH}) - 757.685(a_w) + 11.501(\text{pH}a_w - 0.216)(a_w)^2 + 373.340(a_w)^2
\]

\[
L. monocytogenes \text{ log}_{10} \text{reduction (log}_{10}\text{CFU/g)} = 28.646 - 4.711(\text{pH}) - 35.555(a_w) + 3.472(\text{pH}a_w) + 0.147(a_w)^2 + 7.292(a_w)^2
\]

\[
S. Typhimurium \text{ log}_{10} \text{reduction (log}_{10}\text{CFU/g)} = -13.590 + 5.555(\text{pH}) - 21.414(a_w) - 11.709(\text{pH}a_w) + 0.542(a_w)^2 + 8.087(a_w)^2
\]

The variation of the log\(_{10}\) reductions for the three pathogens was contributed largely by the changes of sausage pH and \( a_w \) \((R^2 = 0.93 \) to 0.97), and the interaction of sausage pH and \( a_w \) significantly affected \((p < 0.05, \text{Table 2})\) the log\(_{10}\) reductions of each pathogen. Comparing the significance level of \( a_w \) and \( a_w \), \( a_w \) was a more significant factor than pH in \( E. coli \) O157:H7 reduction, while pH was a more significant factor than \( a_w \) in \( L. monocytogenes \) reduction, and \( a_w \) and pH were not different in \( S. Typhimurium \) reduction.

3.3. Reductions of pathogens during storage at 4, 21, and 30 °C

The observed reduction rates (log\(_{10}\) CFU/day) of each pathogen in sausages with different pH and \( a_w \) values during storage at 4, 21, and 30 °C are presented in Table 3. In general, the reduction rates of each pathogen were higher in sausage stored at 30 °C, and the reduction rates of \( S. Typhimurium \) in sausage during storage were higher than those of \( E. coli \) O157:H7 and \( L. monocytogenes \). Although the reduction rates of \( E. coli \) O157:H7, \( L. monocytogenes \), or \( S. Typhimurium \) were higher in most sausage samples with low pH or \( a_w \), the reduction rates in some sausages with lower pH or \( a_w \) were not higher than those in sausages with higher pH or \( a_w \). It is possible that, since the reduction rates were for microbial populations that survived fermentation and drying, the survivors may be more resistant to low pH and/or \( a_w \) values, and hence had lower reduction rates during storage. The irregular trend of reduction rates resulted in a low portion of the variation in reduction rates for the three pathogens being contributed by the sausage pH, \( a_w \), and storage temperature \((R^2 = 0.66 \text{ to } 0.86, \text{Table 4})\). The quadratic equations to describe the reduction rates of each pathogen as a function of sausage pre-drying pH, post-drying \( a_w \), and storage temperature are as follows:

\[
E. coli O157 : H7 \text{ reduction rate (log}_{10}\text{CFU/g/day)} = -8.6005 + 3.8091(\text{pH}) + 0.0477(\text{temperature}) + 0.9298(\text{pH}a_w) + 0.0149(\text{temperature}a_w) - 0.4932(\text{pH})^2 - 3.4379(a_w)^2 - 0.0010(\text{temperature})^2
\]

\[
L. monocytogenes \text{ reduction rate (log}_{10}\text{CFU/g/day)} = -3.8977 - 2.3239(\text{pH}) + 1.1812(a_w) + 0.0258(\text{temperature}) - 1.1306(\text{pH}a_w) + 0.0001(\text{temperature}a_w) + 0.3370(\text{pH})^2 - 3.3240(a_w)^2 + 0.0003(\text{temperature})^2
\]

\[
S. Typhimurium \text{ reduction rate (log}_{10}\text{CFU/g/day)} = 49.9600 - 6.0119(\text{pH}) + 72.3786(a_w) + 0.1371(\text{temperature}) + 6.9817(\text{pH}a_w) - 0.2471(\text{temperature}a_w) + 0.573(a_w)^2 - 0.1823(a_w)^2 - 0.0016(a_w)^2
\]

The quadratic term of storage temperature (temperature temperature) was a significant factor \((p < 0.05)\) in all three models. In addition, the interaction effects of pH and temperature were significant.
in the *E. coli* O157:H7 model, the interaction of pH, \(a_w\), and temperature and the quadratic term of pH (pH* pH) were significant in the *L. monocytogenes* model, and the interaction of temperature and pH and the quadratic term of temperature (temp* temp) were significant in the *S. Typhimurium* model (Table 4).

### 4. Discussion

Studies have been conducted to examine the survival of *E. coli* O157:H7, *L. monocytogenes* or *S. Typhimurium* in pepperoni (Hinkens et al., 1996; Faith et al., 1998, 1997; Riordan et al., 1998), soudjouk-style sausage (Calicioglu et al., 2001, 2002; Porto-Fett et al., 2008), Lebanon bologna (Chickthimmah and Knabel, 2001) and other types of fermented sausages (Farber et al., 1993; Nissen and Holck, 1998; Benkerroum et al., 2003) during fermentation/drying/maturation/aging or storage. Reductions of pathogens reported in some of these studies (Table 5) are in general agreement with the present study in that *E. coli* O157:H7, *L. monocytogenes* and *S. Typhimurium* were inactivated during the soudjouk-type sausage manufacturing processes. The reported pathogen reduction values based on sausage pH/\(a_w\)/storage temperature and time varied from study to study; it is difficult to generalize the levels of pathogen reduction that would be achieved during fermentation,

### Table 5

<table>
<thead>
<tr>
<th>Reference</th>
<th>FDSS</th>
<th>Process</th>
<th>(\text{pH}/a_w^b)</th>
<th>Microorganism</th>
<th>Reduction (log_{10} CFU/g)^c</th>
<th>Model prediction</th>
<th>Within 95% CLI^d</th>
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<td>Muthukumarasamy and Holley (2007)</td>
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<td>St</td>
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<td>Lm</td>
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<td>D</td>
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<td>Muthukumarasamy and Holley (2007)</td>
<td>FDS</td>
<td>D</td>
<td>4.8/0.88</td>
<td>Ec</td>
<td>0.52</td>
<td>0.15</td>
<td>N</td>
</tr>
<tr>
<td>Porto-Fett et al. (2008)</td>
<td>Soudjouk</td>
<td>D</td>
<td>4.8/0.82</td>
<td>Lm</td>
<td>0.1</td>
<td>0.05</td>
<td>Y</td>
</tr>
<tr>
<td>Smith et al. (1975)</td>
<td>Pepperoni</td>
<td>D</td>
<td>4.6/0.89</td>
<td>St</td>
<td>1.77</td>
<td>0.40</td>
<td>N</td>
</tr>
<tr>
<td>Calicioglu et al. (2002)</td>
<td>Soudjouk</td>
<td>F + D</td>
<td>4.86/0.88</td>
<td>Ec</td>
<td>0.88</td>
<td>1.83</td>
<td>NA</td>
</tr>
<tr>
<td>Calicioglu et al. (2001)</td>
<td>Soudjouk</td>
<td>F + D</td>
<td>4.6/0.88</td>
<td>Ec</td>
<td>1.96</td>
<td>2.42</td>
<td>NA</td>
</tr>
<tr>
<td>Hinkens et al. (1996)</td>
<td>Pepperoni</td>
<td>F + D</td>
<td>4.85/0.87</td>
<td>Ec</td>
<td>1.2</td>
<td>1.81</td>
<td>NA</td>
</tr>
<tr>
<td>Porto-Fett et al. (2008)</td>
<td>Soudjouk</td>
<td>S (4 °C–30d)</td>
<td>4.8/0.82</td>
<td>Ec</td>
<td>0.037/d</td>
<td>0.067/d</td>
<td>Y</td>
</tr>
<tr>
<td>Calicioglu et al. (2001)</td>
<td>Soudjouk</td>
<td>S (4 °C–28d)</td>
<td>4.6/0.88</td>
<td>Ec</td>
<td>0.07/d</td>
<td>0.07/d</td>
<td>Y</td>
</tr>
<tr>
<td>Calicioglu et al. (2002)</td>
<td>Soudjouk</td>
<td>S (4 °C–21d)</td>
<td>4.8/0.88</td>
<td>Ec</td>
<td>0.02/d</td>
<td>0.09/d</td>
<td>Y</td>
</tr>
<tr>
<td>Faith et al. (1997)</td>
<td>Pepperoni</td>
<td>S(4 °C–28d)</td>
<td>4.8/0.89</td>
<td>Ec</td>
<td>0.03/d</td>
<td>0.06/d</td>
<td>Y</td>
</tr>
</tbody>
</table>

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*a*: Fermentation; *d*: Drying; *S*: Storage (temperature–time).

*b*: \(\text{pH} / a_w\) at the end of fermentation or drying.

*c*: Ec: *E. coli* O157:H7; Lm: *L. monocytogenes*; St: *S. Typhimurium*.

*d*: log_{10} reductions/d were calculated from the reported total log_{10} reduction over the storage period.

*e*: 95% confidence limits are the predicted values ± the average confidence ranges of 0.15 (0.15–0.17), 0.34 (0.32–0.38) or 0.28 (0.27–0.29) log_{10} CFU/g for *E. coli* O157:H7, ±0.09 (0.09–0.10), 0.08 (0.08–0.09) or 0.07 log_{10} (0.065–0.069) CFU/g for *L. monocytogenes*, and ±1.0 (0.99–1.16), 0.30 (0.28–0.35) or 0.23 (0.22–0.24) log_{10} CFU/g for *S. Typhimurium* for fermentation, drying or storage, respectively. These values were the means of differences between the predicted values and 95% CLI for each of the observed values used in model development. Y: within 95% CLI; N: not within 95% CLI, and NA: not applicable.
drying, and storage of these products. It is generally recognized that the lower the pH and/or $a_w$ of FDSS and/or the higher the storage temperatures, the greater the reduction of pathogens in FDSS that would be obtained. Nickelson et al. (1996) reported that fermenting small-casing sausage at 90 °F (32.2 °C) to pH 4.6 and holding them for ≥ 6 days resulted in a 2.5-log$_{10}$ reduction of E. coli O157:H7. At a higher fermentation temperature of 100 °F (37.8 °C) to pH 4.6, the holding time necessary to achieve a 2.5-log$_{10}$ reduction was ≥ 4 days, whereas only a 2.5-log$_{10}$ reduction was achieved when the sausages were fermented at 90 °F (32.2 °C) to pH 5.3 and held for 7 days. Nissen and Holck (1998) reported that E. coli O157:H7, L. monocytogenes, and S. Kentucky in Norwegian fermented, dry sausages (pH 4.8, $a_w$ 0.89) were inactivated to a greater extent when the sausages were stored at 20 °C than at 4 °C. Chikthimmah and Knabel (2001) also reported that E. coli O157:H7 and L. monocytogenes levels were reduced to lower levels in Lebanon bologna stored at 13 °C than at 3.6 °C. The present study examined wider ranges of sausage acidity (pH 5.2 to pH 4.6), moisture ($a_w$ ≥ 0.92), and storage temperatures (4, 21, and 30 °C) and the results allowed for predictions of the survival of E. coli O157:H7, L. monocytogenes and S. Typhimurium in soudjouk-type sausages of various product pH/$a_w$ and during storage at various temperatures. Manufacturers of soudjouk-type sausage may use the results from this study as a reference to select the sausage pH, $a_w$, and storage temperature/time that would achieve the desired pathogen reductions or estimate the levels of pathogen reductions for their processing protocols and product characteristics that meet one of the five USDA/FSIS “validated manufacturing processes.” For example, the present study showed that a reduction of 5.0 log$_{10}$ of E. coli O157:H7 in the soudjouk-type sausage could be achieved by a combination of fermenting sausage to pH 4.9 (−0.4 log$_{10}$ S. Typhimurium reduction), drying to $a_w$ 0.86 (−2.5 log$_{10}$ CFU/g reduction) and storage at 21 °C for ≥ 10 days (−2.3 log$_{10}$ CFU/g reduction).

To expand the application of results obtained from this study to soudjouk-type sausages made with different processing steps and/or other formulations and to other types of fermented sausage, we proposed the development and use of mathematical models to describe our results and predict the reductions of E. coli O157:H7, L. monocytogenes, and S. Typhimurium during fermentation, drying, and storage in other FDSS as affected by the pH, $a_w$, and storage temperature. The applicability of these models was evaluated by comparing the 95% CLI of the predicted values obtained from these models with data from published reports (Table 5). A model prediction is considered acceptable, and the model therefore applicable, if an observed value is within the 95% CLI of the predicted value. For example, Porto-Fett et al. (2008) reported no reduction for E. coli O157:H7, and reductions of 0.3 log$_{10}$ CFU/g for L. monocytogenes and 0.5 log$_{10}$ CFU/g for S. Typhimurium when a soudjouk-type sausage was fermented to pH 5.2, whereas the models predicted a reduction (95% CLI) of 0.1 (0.0 to 0.3) log$_{10}$ CFU/g for E. coli O157:H7, 0.1 (0 to 0.2) log$_{10}$ CFU/g for L. monocytogenes, and no reduction (0 to 1.0) for S. Typhimurium. When the sausage was fermented to pH 4.8, the reductions were 0.6 log$_{10}$ CFU/g for E. coli O157:H7, 0.6 log$_{10}$ CFU/g for L. monocytogenes, and 1.9 log$_{10}$ CFU/g for S. Typhimurium, whereas the model predictions were 0.6 (0.5 to 0.85) log$_{10}$ CFU/g for E. coli O157:H7, 0.3 (0.2 to 0.4) log$_{10}$ CFU/g for L. monocytogenes and 0.7 (0 to 1.7) log$_{10}$ CFU/g for S. Typhimurium. The model predictions therefore seemed accurate for the fermentation step in making soudjouk-type sausage, with observed reductions either falling within the predicted CLI or a small amount of $0.1–0.3$ log$_{10}$ CFU/g above it. The model predictions were comparable to the reported reductions of E. coli O157:H7 and S. Typhimurium in Lebanon bologna fermented to pH 5.2 (Ellajosyula et al., 1998). The predicted reduction for sausage with pH 4.8 was acceptable in comparison to the reported reduction for E. coli O157:H7 (Ellajosyula et al., 1998), but was lower than the reported E. coli O157:H7 reduction in a model system simulating commercial processing of Lebanon bologna (Chikthimmah et al., 2001). For S. Typhimurium, the predicted reduction was acceptable in comparison to the reported reduction in Lebanon bologna fermented to pH 4.8 (Ellajosyula et al., 1998). Therefore, the model is conservatively applicable to evaluating the process lethality for the fermentation of Lebanon bologna. The model predictions were appreciably higher than the reported reductions for E. coli O157:H7 in pepperoni fermented to pH between 5.0 and 5.6, 4.7 and 4.9, and 4.4 and 4.6, respectively (Riordan et al., 1998). The model predictions were acceptable for E. coli O157:H7 and S. Typhimurium in various types of fermented sausages during fermentation to different pH values (Table 5).

The predicted reductions of E. coli O157:H7, L. monocytogenes, and S. Typhimurium during drying sausage (pH 4.8) to $a_w$ 0.92 were lower than those reported by Porto-Fett et al. (2008). Compared to results reported by Calicioglu et al. (2001, 2002), the models predicted higher reductions of E. coli O157:H7 in a soudjouk-style sausage at pH 4.9 and 4.5 ($a_w$ 0.88). The model predictions of E. coli O157:H7 reduction were higher than those in pepperoni reported by Hinkens et al. (1996) and Riordan et al. (1998) (Table 5). The models over-predict pathogen reductions for E. coli O157:H7 due to the combination of fermentation and drying, although some of the observed values were within the 95% CLI of the predicted values.

During storage of FDSS, pathogens continue to be exposed to the unfavorable environment created by fermentation and drying, hence the inactivation of pathogens continues during storage (Faith et al., 1997, 1998; Chikthimmah and Knabel, 2001). E. coli O157:H7 survived better than L. monocytogenes in fermented sausages during fermentation and maturation (Glass et al., 1992), and during storage of Lebanon bologna (Chikthimmah and Knabel, 2001). However, Nissen and Holck (1998) reported that L. monocytogenes survived storage better than E. coli O157:H7 in Norwegian fermented dry sausage. In the present study, the reduction rates for E. coli O157:H7 were higher than for L. monocytogenes at 21 and 30 °C. The differences in the resistance of E. coli O157:H7 and L. monocytogenes in fermented sausages during storage may be due to the different starter cultures (lactic acid bacteria), and potentially the different bacteriocins and secondary metabolites produced during fermentation. In the present study, S. Typhimurium had the highest reduction rates among the three pathogens during storage. The relatively rapid reduction of S. Typhimurium in fermented sausages during storage has also been reported by Schilling and Lucke (1989) and Nissen and Holck (1998). The model predictions for reduction rates of E. coli O157:H7, L. monocytogenes, or S. Typhimurium in sausages of different pH and/or $a_w$ during storage at different temperatures are listed along with the reported reductions from other studies in Table 5. The model predictions of the reductions of E. coli O157:H7 were acceptable for 4, 15, and 21 °C storage when compared to results from a study using semidry soudjouk (Calicioglu et al., 2002). The models predictions were also acceptable when compared to the reduction rates of E. coli O157:H7, L. monocytogenes, or S. Typhimurium in soudjouk sausages reported by Porto-Fett et al. (2008). The model predictions seemed relatively accurate for the drying step in making soudjouk-type sausage (Table 5).

A model for E. coli O157:H7 inactivation in uncooked fermented meat products (UCFM) was proposed by Ross and Shadbolt (2001). They obtained inactivation rates of E. coli O157:H7 in UCFM from a variety of published and unpublished sources, and fitted the rates as a function of temperature with a simple Arrhenius model. The Arrhenius model, inactivation rate ($log_{10}$ CFU/h) = $e^{-33.387/([112.95/temperature[K]])}$, predicts the inactivation rates of E. coli O157:H7 in UCFM at temperatures that the UCFM are fermented/matured, regardless of product pH and/or $a_w$. The different parameters in the models of Ross and Shadbolt and the present study permitted comparisons between these two models only for temperatures of 22, 24, and 30 °C that can be obtained from models in the present study. The Arrhenius model predicted reduction rates of 0.2 log$_{10}$ CFU/d at 24 °C vs. an average of 0.2 log$_{10}$ CFU/d predicted by the present study’s fermentation model for sausage pH 4.6–5.2, 0.2 log$_{10}$
CFU/d at 22 °C vs. an average of 0.2 log_{10} for sausage with a_{w} 0.89 predicted by the drying model, and 0.4 log_{10} at 30 °C vs. an average of 0.4 log_{10} CFU/d (0.2 to 0.6 log_{10} CFU/d for pH 5.2–4.6 and a_{w} 0.92–0.86) predicted by the storage model. The predictions by both models seemed to be comparable. Although both models use different parameters in predicting reductions of E. coli O157:H7 in fermented sausage, the present study’s pH and a_{w} parameters are directly affected by fermentation/drying temperature and time, i.e., to achieve lower pH and a_{w} in sausage requires higher temperatures and longer time, which are in agreement with the Arrhenius model’s temperature/time as the main factors contributing to the inactivation of E. coli O157:H7 during fermentation/drying/maturation of fermented sausage.

Reductions of E. coli O157:H7 and S. Typhimurium predicted by fermentation and storage models developed from this study were acceptable (within 95% CLI) to data reported in Table 5. The predictions from fermentation and storage models were acceptable in 50% and 100% of cases, respectively, for E. coli O157:H7, and in 80% and 100% of cases for S. Typhimurium, when compared to published data. The comparisons for L. monocytogenes were limited due to the sparse published data. Overall, the model predictions were acceptable to 63% (17/27) of reported data for E. coli O157:H7, 29% (2/7) for L. monocytogenes, and 73% (8/11) for S. Typhimurium, and the models tended to underestimate the reductions during fermentation and storage, and over-estimate the reductions during drying. In comparison to other studies, there were variations among reported pathogen reductions during fermentation, drying, and storage of FDSS based on product pH and/or a_{w}. Acidity (pH), a_{w}, presence of curing salts and the competitive microorganisms (background microflora and/or added starter culture of lactic acid bacteria) in FDSS, and storage temperature all contributed to the inactivation of foodborne pathogens in FDSS (Glass et al., 1992; Hugas et al., 1995; Chikthimmah and Knabel, 2001; Gonzalez and Diez, 2002). Effects of these individual and combined hurdles have not been examined thoroughly, and are likely the sources of the discrepancy among the reported pathogen reductions. In addition, pathogens subjected to the stress of changing pH during fermentation would have complex and varying patterns of cell damage and cell death during drying/maturation/storage (Smith et al., 1975; Riordan et al., 1998), and injured cells were not fully recovered by a direct counting method when compared to an enrichment method (Nightingale et al., 2006). These suggest that the strains of pathogens used for testing FDSS processes and the enumeration method would also affect the reported reduction/survival patterns of pathogens. Soudjouk sausages in this study were produced with an original diameter of 25 mm, which was smaller than some of the FDSS such as pepperoni (30 to 55 mm), salami (55 to 60 mm), and chorizo (34 to 40 mm). The drying time for smaller diameter sausage is likely to be different from those of larger diameter sausage. Therefore, the inactivation of pathogens may also be affected by the size of the sausage. Sausage pH and a_{w} are the most commonly used factors associated with reductions of pathogens during fermentation, drying, and storage of FDSS. It is reasonable to assume that sausage formulation, starter culture, size, and fermentation/drying conditions also contribute to pathogen reductions in addition to sausage pH and a_{w}. While the models reported in the present study may be used to estimate the survival of E. coli O157:H7 or S. Typhimurium in FDSS during fermentation and storage, additional studies are warranted to establish the correlation between the model predictions and the observed pathogen reductions for a particular FDSS product.

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