



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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ABSTRACT

This study quantified and modeled the survival of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium in soudjouk-style fermented sausage during fermentation, drying, and storage. Batter prepared from ground beef (20% fat), seasonings, starter culture, and dextrose was separately inoculated with a multi-strain mixture of each pathogen to an initial inoculum of ca. 6.5 log₁₀ CFU/g in the batter. The sausages were subsequently fermented at 24 °C with a relative humidity (RH) of 90% to 95% for 3 to 5 days to ca. pH 5.2, pH 4.9 or pH 4.6, then dried at 22 °C to a_w 0.92, a_w 0.89, or a_w 0.86, respectively, and then stored at 4, 21, or 30 °C for up to 60 days. Lethality of the three pathogens was modeled as a function of pH, a_w and/or storage temperature. During fermentation to pH 5.2 to pH 4.6, cell reductions ranged from 0 to 0.9 log₁₀ CFU/g for *E. coli* O157:H7, 0.1 to 0.5 log₁₀ CFU/g for *L. monocytogenes*, and 0 to 2.2 log₁₀ CFU/g for *S. Typhimurium*. Subsequent drying of sausages of pH 5.2 to pH 4.6 at 22 °C with 80% to 85% RH for 3 to 7 days to a_w of 0.92 to a_w 0.86 resulted in additional reductions that ranged from 0 to 3.5 log₁₀ CFU/g for *E. coli* O157:H7, 0 to 0.4 log₁₀ CFU/g for *L. monocytogenes*, and 0.3 to 2.4 log₁₀ CFU/g for *S. Typhimurium*. During storage at 4, 21, or 30 °C the reduction rates of the three pathogens were generally higher ($p < 0.05$) in sausages with lower pH and lower a_w that were stored at higher temperatures. Polynomial equations were developed to describe the inactivation of the three pathogens during fermentation, drying, and storage. The applicability of the resulting models for fermented sausage was evaluated by comparing model predictions with published data. Pathogen reductions estimated by the models for *E. coli* O157:H7 and *S. Typhimurium* were comparable to 67% and 73% of published data, respectively. Due to limited published data for *L. monocytogenes*, the models for *L. monocytogenes* would need additional validations. Results of pathogen reductions from this study may be used as a reference to assist manufacturers of soudjouk-style sausages to adopt manufacturing processes that meet the regulatory requirements. The resulting models may also be used for estimating the survival of *E. coli* O157:H7 and *S. Typhimurium* in other similar fermented sausage during fermentation and storage.

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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. 25%

to 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/United States Department of Agriculture (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_w (Barbuti and

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

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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; Riordan et al., 1998; Cosansu and Ayhan, 2000; Barbuti and Parolari, 2002; Colak et al., 2007). Some FDSS have been linked to outbreaks of foodborne illnesses. In the U.S., a dry-cured 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 a_w values, as well as storage conditions for a specific FDSS product (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 a_w 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 a_w of ca. 0.85. The acid levels (pH 5.2 to pH 4.6), water activity (a_w 0.92 to a_w 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), 101M (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 a_w reached ca. a_w 0.92, a_w 0.89, or a_w 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 stomacher bags (Spiral Biotech, Inc., Norwood, MA) using a Multivac A300/16 vacuum-packaging machine (Sepp Haggmüller KG, Wolfertschwenden, Germany). The sausages were sampled for microbial counts, pH, and a_w 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 μ l onto MacConkey sorbitol (SMAC; Difco), polymyxin B, acriflavin, lithium chloride, ceftazidime, esculin, D-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 a_w measurements

At each sampling point, the pH of sausage was measured by using a Daigger 5500 pH meter (A. Daigger and Company Inc., Wernon 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 a_w 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 (\log_{10} reductions) for each pathogen during fermentation to various pH levels from each trial were plotted versus the pH of sausage. \log_{10} 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:

$$\log_{10} \text{ reduction} = \alpha + \beta_1(\text{pH}) + \beta_2(\text{pH})^2$$

where α is the intercept, and β_1 – β_2 are estimated coefficients.

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

$$\log_{10} \text{ reduction} = \alpha + \beta_1(\text{pH}) + \beta_2(a_w) + \beta_3(\text{pH} \cdot a_w) + \beta_4(\text{pH})^2 + \beta_5(a_w)^2$$

where α is the intercept, and β_1 – β_5 are estimated coefficients.

During storage, viable counts of each pathogen (\log_{10} CFU/g) were plotted versus storage time (day) to generate survival curves. From the curves, the average reduction rates (\log_{10} CFU/day) for each pathogen were estimated by dividing the \log_{10} 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, a_w 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:

$$\text{Reduction rate}(\log_{10} \text{ CFU/day}) = \alpha + \beta_1(\text{pH}) + \beta_2(a_w) + \beta_3(\text{temperature}) + \beta_4(\text{pH} \cdot a_w) + \beta_5(\text{pH} \cdot \text{temperature}) + \beta_6(a_w \cdot \text{temperature}) + \beta_7(\text{pH})^2 + \beta_8(a_w)^2 + \beta_9(\text{temperature})^2$$

where α is the intercept, and β_1 – β_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

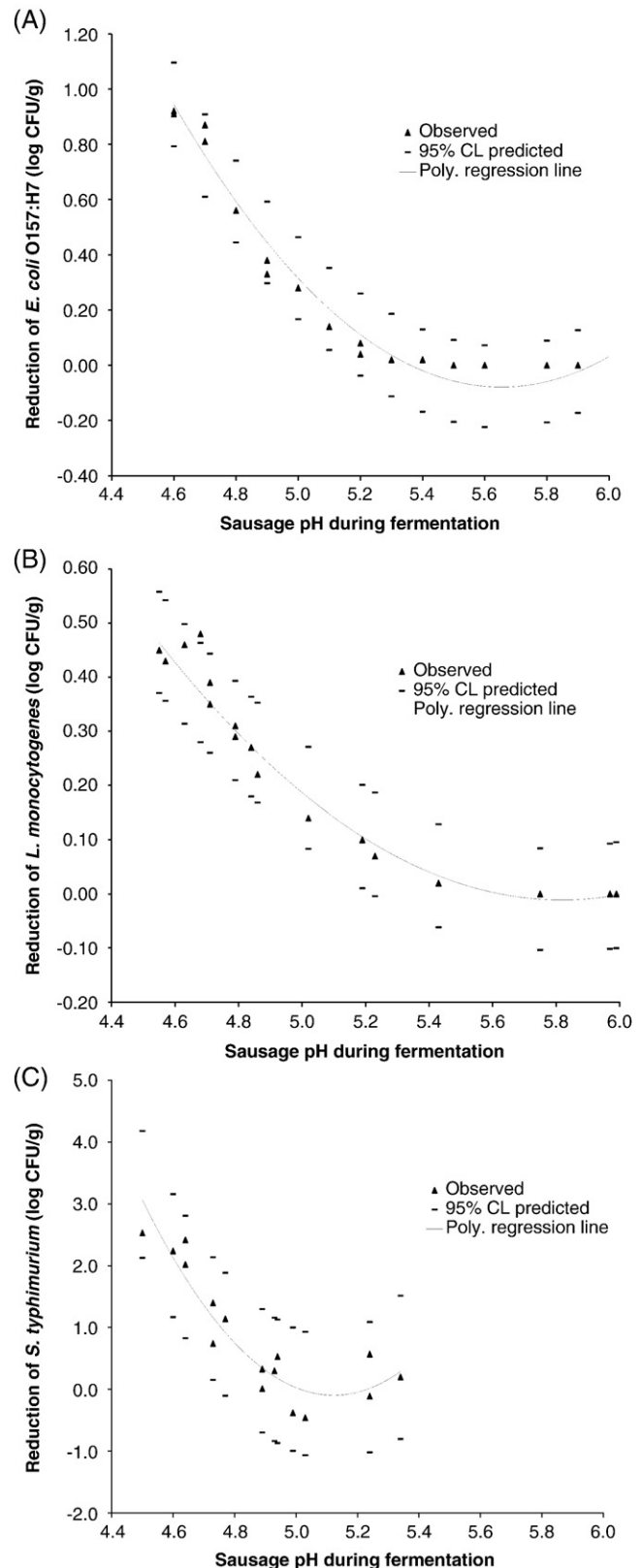


Fig. 1. Observed \log_{10} reductions, fitted regression line, and 95% CLI of values predicted from the regression equation for *E. coli* O157:H7 (A), *L. monocytogenes* (B) and *S. Typhimurium* (C) in soudjouk-type sausages after fermentation to various pH levels.

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 approximately 1080 (75 °F [24 °C]–60×72 h), which met the acceptance criterion 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 shown in Fig. 1. Reductions during fermentation were significantly higher (*p*<0.05) for *S. Typhimurium*, followed by *E. coli* O157:H7, and then by *L. monocytogenes*. Reductions of *E. coli* O157:H7, *L. monocytogenes*, and *S. Typhimurium* occurred after sausage pH values were lowered to pH 5.4, pH 5.6, and pH 5.1, respectively. The fitted models for the log₁₀ reductions of each pathogen as a function of sausage pH during fermentation are as follows:

$$E. coli \text{ O157:H7 } \log_{10} \text{ reduction (log}_{10} \text{ CFU/g)} = 29.356 - 10.412(\text{pH}) + 0.921(\text{pH})^2$$

$$L. monocytogenes \log_{10} \text{ reduction (log}_{10} \text{ CFU/g)} = 9.986 - 3.436(\text{pH}) + 0.295(\text{pH})^2$$

$$S. Typhimurium \log_{10} \text{ reduction (log}_{10} \text{ CFU/g)} = 230.949 - 90.474(\text{pH}) + 8.856(\text{pH})^2$$

A major portion of the variation of log₁₀ reductions for the three pathogens was explained by the variation in the sausage pH (*R*²=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

Parameter	<i>E. coli</i> O157:H7		<i>L. monocytogenes</i>		<i>S. Typhimurium</i>	
	Estimate	Pr> t	Estimate	Pr> t	Estimate	Pr> t
Intercept	29.356	<.0001	9.986	<.0001	230.949	<.0001
pH	-10.412	<.0001	-3.436	<.0001	-90.474	<.0001
pH*pH	0.921	<.0001	0.295	<.0001	8.856	<.0001
Model ^a	<i>F</i> value=487.41 Pr> <i>F</i> =<.0001 RMSE=0.0689 <i>R</i> ² =0.98		<i>F</i> value=225.99 Pr> <i>F</i> =<.0001 RMSE=0.04218 <i>R</i> ² =0.96		<i>F</i> value=83.27 Pr> <i>F</i> =<.0001 RMSE=0.4455 <i>R</i> ² =0.92	

^a *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. *R*² 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.

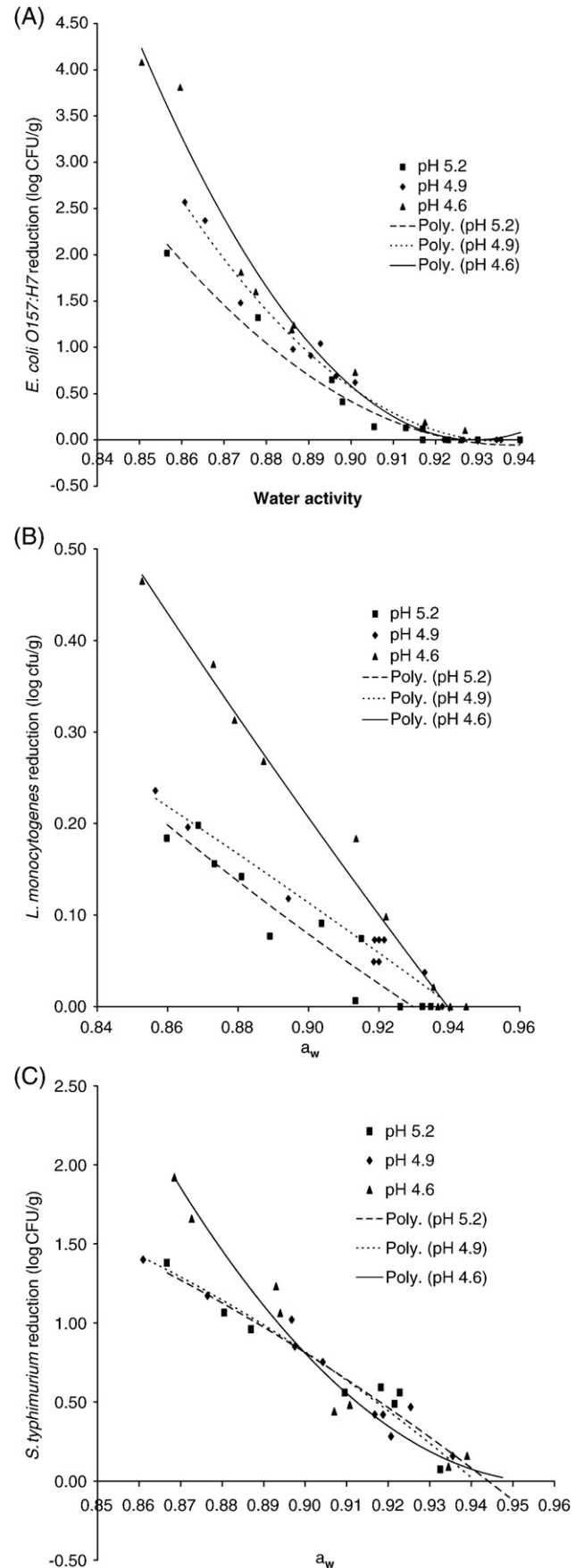


Fig. 2. Observed log₁₀ reductions and fitted regression equations of *E. coli* O157:H7 (A), *L. monocytogenes* (B) and *S. Typhimurium* (C) in soudjouk-type sausages of ca. pH 5.2, 4.9 and 4.6 during drying to various *a_w* levels.

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 sausage 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 ca. a_w 0.92 for *E. coli* O157:H7 and *L. monocytogenes*, and at ca. 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:

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

$$L. monocytogenes \log_{10} \text{ reduction } (\log_{10} \text{ CFU/g}) = 28.646 - 4.711(\text{pH}) - 33.555(a_w) + 3.472(\text{pH} * a_w) + 0.147(\text{pH})^2 + 7.292(a_w)^2$$

$$S. Typhimurium \log_{10} \text{ reduction } (\log_{10} \text{ CFU/g}) = -13.590 + 5.555(\text{pH}) - 21.414(a_w) - 11.709(\text{pH} * a_w) + 0.542(\text{pH})^2 + 8.087(a_w)^2$$

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 ($p<0.05$, Table 2) the \log_{10} reductions of each pathogen. Comparing the significance level of a_w and pH, 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

Table 2

Parameter estimates and significance levels for the \log_{10} reduction models for *E. coli* O157:H7, *L. monocytogenes*, and *S. Typhimurium* in a soudjouk-type sausage during drying

Parameter	<i>E. coli</i> O157:H7		<i>L. monocytogenes</i>		<i>S. Typhimurium</i>	
	Estimate	Pr> t	Estimate	Pr> t	Estimate	Pr> t
Intercept	376.983	<.0001	28.646	0.014	-13.590	0.7647
pH	-8.668	0.1686	-4.711	0.0012	5.555	0.1434
a_w	-757.645	<.0001	-33.555	0.1383	21.414	0.8353
pH * a_w	11.501	0.0390	3.472	0.0004	-11.709	0.0018
pH * pH	-0.216	0.5816	0.147	0.1408	0.542	0.0558
a_w * a_w	373.340	<.0001	7.292	0.5402	8.087	0.8899
Model	F value=129.91 Pr>F=<0.0001 RMSE=0.1488 R ² =0.97		F value=60.84 Pr>F=<0.0001 RMSE=0.0345 R ² =0.93		F value=69.60 Pr>F=<0.0001 RMSE=0.1297 R ² =0.95	

Table 3

The average reduction rates (\log_{10} CFU/d) of *E. coli* O157:H7 (Ec), *L. monocytogenes* (Lm) and *S. Typhimurium* (St) in soudjouk-type sausages of various pH and a_w during storage at 4, 21 and 30 °C

pH ^a	a_w	Reduction rate (\log_{10} CFU/day) during storage					
		4 °C		21 °C		30 °C	
		Ec / Lm / St	Ec / Lm / St	Ec / Lm / St	Ec / Lm / St		
5.2±0.1	0.92	0.005 / 0.029 / 0.031	0.082 / 0.029 / 0.068	0.212 / 0.052 / 0.354			
	0.89	0.019 / 0.067 / 0.021	0.075 / 0.030 / 0.051	0.470 / 0.145 / 0.423			
4.9±0.1	0.86	0.013 / 0.031 / 0.032	0.250 / 0.091 / 0.069	0.570 / 0.120 / 0.327			
	0.92	0.015 / 0.020 / 0.013	0.076 / 0.029 / 0.057	0.560 / 0.080 / 0.495			
	0.89	0.017 / 0.012 / 0.016	0.083 / 0.018 / 0.062	0.660 / 0.064 / 0.495			
4.6±0.1	0.86	0.015 / 0.023 / 0.048	0.234 / 0.019 / 0.106	0.660 / 0.111 / 0.763			
	0.92	0.012 / 0.087 / - ^b	0.055 / 0.035 / -	- / 0.105 / -			
	0.89	0.017 / 0.046 / -	0.20 / 0.096 / -	- / 0.125 / -			
	0.86	- / 0.050 / -	- / 0.025 / -	- / 0.154 / -			

^a The pH values of sausage after fermentation. For sausages with a_w 0.92, the pH remained close to the post-fermentation pH, whereas the post-drying pH values for sausages with a_w 0.89–0.86 were ca. 0.1–0.2 unit lower than the pH after fermentation.

^b Not obtained due to the pathogen numbers at the beginning of storage being below the detection limit (1.6 \log_{10} CFU/g) or rapidly falling to below the detection limit.

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 ($R^2=0.66$ to 0.86 , 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:

$$E. coli O157 : H7 \text{ reduction rate } (\log_{10} \text{ CFU/day}) = -8.6005 + 3.8091(\text{pH}) + 0.6477(a_w) - 0.0477(\text{temperature}) + 0.9298(\text{pH} * a_w) + 0.0149(\text{pH} * \text{temperature}) - 0.5050(a_w * \text{temperature}) - 0.4932(\text{pH})^2 - 3.4379(a_w)^2 + 0.0010(\text{temperature})^2$$

$$L. monocytogenes \text{ reduction rate } (\log_{10} \text{ CFU/day}) = 0.3897 - 2.3239(\text{pH}) + 11.8162(a_w) + 0.0258(\text{temperature}) - 1.1306(\text{pH} * a_w) - 0.0001(\text{pH} * \text{temperature}) - 0.0364(a_w * \text{temperature}) - 0.3370(\text{pH})^2 - 3.3240(a_w)^2 + 0.0003(\text{temperature})^2$$

$$S. Typhimurium \text{ reduction rate } (\log_{10} \text{ CFU/day}) = 49.9600 - 6.0119(\text{pH}) - 72.3786(a_w) + 0.1371(\text{temperature}) + 6.9817(\text{pH} * a_w) - 0.0247(\text{pH} * \text{temperature}) - 0.0573(a_w * \text{temperature}) - 20.9323(a_w)^2 + 0.0016(a_w)^2$$

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

Table 4

Parameter estimates and significance levels for the reduction rate models for *E. coli* O157:H7, *L. monocytogenes* and *S. Typhimurium* in a soudjouk-type sausages during storage

Parameter	<i>E. coli</i> O157:H7		<i>L. monocytogenes</i>		<i>S. Typhimurium</i>	
	Estimate	Pr> t	Estimate	Pr> t	Estimate	Pr> t
Intercept	-8.6005	0.8135	0.3897	0.9641	46.9600	0.2213
pH	3.8091	0.4299	-2.3239	0.047	-6.0119	0.1516
a_w	0.6477	0.9931	11.8162	0.5079	-72.3786	0.3378
Temp	-0.0477	0.483	0.0258	0.1149	0.1371	0.0885
pH * a_w	0.9298	0.7502	-1.1306	0.1085	6.9817	0.1383
pH * temp	0.0149	0.0285	-0.0001	0.9264	-0.0247	0.0247
a_w * temp	-0.0505	0.4475	-0.0364	0.0249	-0.0573	0.3756
pH * pH	-0.4932	0.2358	0.3370	0.0012	0.0000	
a_w * a_w	-3.4379	0.9336	-3.3240	0.735	20.9323	0.6013
Temp * temp	0.0010	0.0003	0.0003	<.0001	0.0016	<.0001
Model	F value=10.06 Pr>F=<0.001 RMSE=0.1279 R ² =0.67		F value=9.28 Pr>F=<0.001 RMSE=0.0304 R ² =0.66		F value=20.19 Pr>F=<0.001 RMSE=0.1008 R ² =0.86	

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
Comparisons of pathogen reductions reported by various studies and the model predictions

Reference	FDSS	Process ^a	pH/ a_w ^b	Microorganism ^c	Reduction (log ₁₀ CFU/g) ^d	Model prediction	Within 95% CLI ^e
Muthukumarasamy and Holley (2007)	FDS	F	4.8	Ec	1.0	0.60	N
Porto-Fett et al. (2008)	Soudjouk	F	5.2	Ec	0	0.12	Y
			5.2	Lm	0.26	0.10	N
			5.2	St	0.54	0	Y
			4.8	Ec	0.59	0.60	Y
			4.8	Lm	0.64	0.29	N
			4.8	St	1.86	0.72	N
Nightingale et al. (2006)	Salami	F	4.4	Lm	0.90	0.58	N
Riordan et al. (1998)	Pepperoni	F	5.0–5.6/<0.80	Ec	0.07	0.12 (pH 5.2)	Y
			4.7–4.9/<0.80		0.37	0.60 (pH 4.8)	N
			4.4–4.6/<0.80		0.68	0.95 (pH 4.6)	N
Ellajosyula et al. (1998)	Lebanon bologna	F	5.2	Ec	0.05	0.12	Y
			4.8		0.59	0.60	Y
			5.2	St	0.03	0	Y
			4.8		1.18	0.72	Y
Faith et al. (1997)	Pepperoni	F	4.82	Ec	1.0	0.57	N
Hinkens et al. (1996)	Pepperoni	F	4.85	Ec	0.07	0.52	N
Smith et al. (1975)	Pepperoni	F	4.6	St	1.26	2.16	Y
Muthukumarasamy and Holley (2007)	FDS	D	4.8/0.88	Ec	0.70	1.36	N
Porto-Fett et al. (2008)	Soudjouk	D	4.8/0.92	Ec	0.52	0.15	N
				Lm	0.1	0.05	Y
				St	1.77	0.40	N
				Ec	0	0.05	Y
				Lm	0	0.03	Y
				St	1.11	0.48	N
				Lm	0	0.07	Y
				Ec	0.76	0.05 (5.2/0.86)	N
					1.83	2.41 (4.8/0.86)	N
					2.07	2.58 (4.6/0.86)	N
Faith et al. (1997)	Pepperoni	D	4.82/0.90	Ec	0.9	0.60	Y
Smith et al. (1975)	Pepperoni	D	4.6/0.89	St	0.88	0.95	Y
Calicioglu et al. (2002)	Soudjouk	F + D	4.86/0.88	Ec	0.88	1.83	NA
Calicioglu et al. (2001)	Soudjouk	F + D	4.6/0.88	Ec	1.96	2.42	NA
				Ec	0.28	0.82	NA
Hinkens et al. (1996)	Pepperoni	F + D	4.85/0.87	Ec	1.2	1.81	NA
Porto-Fett et al. (2008)	Soudjouk	S	4 °C–30d	Ec	0.023/d	0.036/d	Y
				Ec	0.049/d	0/d	Y
				Lm	0.079/d	0.067/d	Y
				Lm	0.010/d	0.035/d	N
				Lm	0.011/d	0.010/d	Y
				St	0.06/d	0.016/d	N
				St	0.040/d	0/d	Y
				St	0.065/d	0/d	Y
				St	0.065/d	0.07/d	Y
				Ec	0.07/d	0.07/d	Y
				Ec	0.17/d	0.09/d	Y
Calicioglu et al. (2002)	Soudjouk	S	4 °C–21d	Ec	0.02/d	0.09/d	Y
				Ec	0.04/d	0.07/d	Y
				Ec	0.26/d	0.25/d	Y
Faith et al. (1997)	Pepperoni	S	4 °C–28d	Ec	0.03/d	0.06/d	Y
				Ec	0.14/d	0.11/d	Y

^a F: Fermentation; D: Drying; S: Storage (temperature–time).

^b pH and a_w at the end of fermentation or drying.

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

^d Log₁₀ reductions/d were calculated from the reported total log₁₀ 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) log₁₀ CFU/g for *E. coli* O157:H7, ±0.09 (0.09–0.10), 0.08 (0.08–0.09) or 0.07 log₁₀ (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) log₁₀ 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 ≥ 5 -log₁₀ 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 ≥ 5 -log₁₀ reduction was ≥ 4 days, while only a 2.5-log₁₀ 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. Typhimurium* 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 to a_w 0.86), 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 ca. 5.0 log₁₀ 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 log₁₀ CFU/g reduction), drying to a_w 0.86 (~2.5 log₁₀ CFU/g reduction) and storage at 21 °C for ≥ 10 days (~2.3 log₁₀ 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 log₁₀ CFU/g for *L. monocytogenes* and 0.5 log₁₀ 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 to 0.3) log₁₀ CFU/g for *E. coli* O157:H7, 0.1 (0 to 0.2) log₁₀ 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 log₁₀ CFU/g for *E. coli* O157:H7, 0.6 log₁₀ CFU/g for *L. monocytogenes*, and 1.9 log₁₀ CFU/g for *S. Typhimurium*, whereas the model predictions were 0.6 (0.5 to 0.85) log₁₀ CFU/g for *E. coli* O157:H7, 0.3 (0.2 to 0.4) log₁₀ CFU/g for *L. monocytogenes* and 0.7 (0 to 1.7) log₁₀ 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 (0.1–0.3 log₁₀ 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 Schillinger 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 (log₁₀ CFU/h) = $e^{(33.387)/e^{(11255/\text{temperature}[K])}}$, 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 log₁₀ CFU/d at 24 °C vs. an average of 0.2 log₁₀ CFU/d predicted by the present study's fermentation model for sausage pH 4.6–5.2, 0.2 log₁₀

CFU/d at 22 °C vs. an average of 0.2 log₁₀ for sausage with a_w 0.89 predicted by the drying model, and 0.4 log₁₀/d at 30 °C vs. an average of 0.4 log₁₀ CFU/d (0.2 to 0.6 log₁₀ 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 under-estimate 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; Chikthimma 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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