



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 $\geq 6.0\text{-log}_{10}$ 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 log_{10} 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 log_{10} 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, Wolfertschwend, 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

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