Survival of *Salmonella* and the surrogate *Enterococcus faecium* in cooking of moisture enhanced reconstructed comminuted chicken patties by double pan-broiling

Wentao Jiang, Carly Waldman, KaWang Li, Jacek Jaczynski, and Cangliang Shen\(^1\)

Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, WV 26506, USA

**ABSTRACT** This study compares kinetic parameters of *Salmonella* and *Enterococcus faecium* in moisture enhanced, reconstructed comminuted chicken patties prepared with different pump rates during double pan-broiling with various set-up temperatures. Fresh 1.5-kg chicken breast meat was course grounded, inoculated with *S. Typhimurium* and *Tennessee*, or *E. faecium*, followed by adding NaCl (2.0%) + Na-tripolyphosphate (0.5%) solutions to achieve pump rates of 1%, 5%, or 11.1%. Meat samples were manually manufactured into patties with the thickness of 2.1 cm and diameter of 10.4 cm. Patties were packaged with polyvinyl chloride films in the foam-tray stored at 4°C for 42 h before double pan-broiling set at 200°, 300°, or 425°F for 0 to 420 s. Counts of pathogens were analyzed on xylose-lysine-Tergitol-4 and bile esculin agars with tryptic soy agar layers. Microbial data and kinetic parameters (n = 9, USDA-Integrated-Predictive-Modeling-Program/USDA-Global-Fit software) were analyzed by the Mixed Model Procedure (SAS). Double pan-broiling reduced >5-log\(_{10}\) CFU/g (\(P < 0.05\)) of *Salmonella* after 360 (200°F), 180 to 225 (300°F), and 150 to 165s (425°F), and of *E. faecium* after 270 s (300°F), and 180 s (425°F) across all samples. D-values (Mafart-Weibull model) of *Salmonella* and *E. faecium* in 1% moisture enhanced samples cooked at 200 to 425°F (102.7−248.2 and 115.5−271.0 s) were lower (\(P < 0.05\)) than 11.1% samples (119.8−263.7 and 122.5−298.3 s). *Salmonella* were more susceptible (\(P < 0.05\)) to heat than *E. faecium*. “Shoulder-time” (Buchanan-Two-Phase model) of *Salmonella* cooking at 200° to 425°F increased (\(P < 0.05\)) from 82.3−229.0 to 116.6−246.2 s as pump rate increased from 1 to 11.1%, whereas this phenomenon was not shown for *E. faecium*. Results indicate that *Salmonella* were resistant to heat in chicken patties with greater pump rate. *E. faecium* can be used as a surrogate for *Salmonella* to validate thermal inactivation in chicken products.

**Key words:** reconstructed chicken product, *Salmonella*, *Enterococcus faecium*, moisture enhancement, cooking temperature

**INTRODUCTION**

*Salmonella* is a Gram-negative, rods shape, non-endospore forming, facultative foodborne pathogen which caused 905 outbreaks in the United States in 2018 (U.S.-CDC, 2020). Chicken products are the number 1 food category (>100) of outbreaks based on new surveillance data published by the U.S. Centers for Disease Control and Prevention in December 2020 (U.S.-CDC, 2020). An early study of Morris et al. (2011) also confirms that *Salmonella* is responsible for approximately 35% of the foodborne illnesses associated with poultry products. In February 2016, the United States Department of Agriculture-Food Safety and Inspection Service established a new performance standard in response to national surveillance baseline data from 2012 to 2015 (USDA-FSIS, 2016). The new standard allowed the maximum acceptable positive rate of *Salmonella* up to 25% in comminuted chicken (325 g sample) and up to 15.4% in chicken parts (4 lb. sample).

Raw chicken carcasses are usually further processed through reduction of raw chicken particle size, extraction of meat proteins, binding meat pieces with salt and/or phosphate, and marination with commercial or domestic marinades. These techniques are followed by grinding, tumbling, or chopping for further manufacturing into retail chicken products such as ground chicken, chicken steaks, or bags of chicken roasts. Reconstructed, comminuted chicken meat is often mixed with brine solutions containing various salt and polyphosphate...
concentrations to increase water-holding capacity, decrease cooking losses, improve sensory tasting scores, and to maintain good quality of completed chicken products (Gill et al., 2004). Applying appropriate concentrations of salt and tri-polyphosphate into the chicken meat products can generate an optimal water-holding capacity value for solubilizing muscle myofibrillar proteins to form a stable and desired final product shape as shown in commercial retail packages (Young et al., 1988; Young and Lyon, 1997). Recently, new nationwide sampling results showed high prevalence of *Salmonella* (36.7–83.5%) in comminuted chicken products, representing 1.6 to 2.3-fold increase of *Salmonella* prevalence compared to bone-in chicken parts and carcasses (USDA-FSIS, 2015). These data raised microbiological safety concerns of foodborne pathogens. The mild heat generated during grinding and possibly translocation of foodborne pathogens from the surface to internal tissues during restructuring, moisture enhancement and marination could add to the microbial safety risk, especially if the final products are undercooked (Shen et al, 2010).

Cooking raw chicken to 74°C internal target temperature is expected to produce a 7-log reduction of *Salmonella* (NACMCF, 2007). However, studies on chicken breast fillets observed unexpected heat resistance to *Salmonella* (WHO, 2009). The presence of chemical ingredients, size of the product, cooking method, water activity, fat content, and product pH are factors that affect pathogen heat resistance (WHO, 2009). Furthermore, *Salmonella* may survive during the cooking of comminuted chicken manufactured products and cause subsequent illness in consumers, especially if the chemical ingredients interfere with thermal inactivation or increase the heat resistance of the pathogens. To date, there are no published studies that show the thermal inactivation activity of *Salmonella* in moisture enhanced reconstructed chicken products during common cooking practices. The lack of quantitative data relating chicken cooking practices for with the reduction of *Salmonella* in chicken products remain a large, unaddressed problem in food safety guidelines (WHO, 2009).

The common cooking practices to inactivate foodborne pathogens in chicken products including pan-broiling, double pan-broiling, and roasting (American Meat Science Association, 2015) should be evaluated in real commercial cooking settings, because that environment is expected to be much less controlled and much more dynamic than a laboratory setting. Almost no commercial chicken meat processors are willing to use a microbial foodborne pathogen in their cooking practices to determine the critical control points and critical limits of cooking temperatures in their Hazard-Analysis-Critical-Control-Point plan. Therefore, choosing a surrogate of pathogen and including that surrogate in laboratory validation studies before moving onto pilot plant or commercial testing is an appropriate method (Hu and Gurtler, 2017). *Enterococcus faecium*, is a Gram-positive, cocci with chain shape arrangement, non-endospore forming, and facultative bacteria. Previous studies at West Virginia University have included *E. faecium* as a *Salmonella* surrogate in the steaming of (Boney et al., 2018) and standard or aggressive thermal pelleting of chicken feeds (Boltz et al., 2019). Our previous study also confirmed that *E. faecium* is a promising *Salmonella* surrogate in antimicrobial dip testing for broiler carcasses (Lemonakis et al., 2017). However, *E. faecium* has not been studied on chicken meat products during cooking to verify that it is an ideal surrogate for *Salmonella*.

Therefore, this study aims to conduct side-by-side comparison cooking studies of *Salmonella* versus *E. faecium* to compare their thermal inactivation kinetics in reconstructed, comminuted chicken patties moisture enhanced (MH) with various pump rates and double pan-broiled with various set-up temperatures.

### MATERIALS AND METHODS

#### Bacteria Strains

Bacterial cultures used in this study include *Salmonella Typhimurium* American Type Culture Collection (ATCC) 14028, *Salmonella Tennessee* ATCC 10722, and the *Salmonella* surrogate bacteria *Enterococcus faecium* ATCC 8459. These same strains were used in our previous validation studies of antimicrobials on broiler carcasses (Lemonakis et al., 2017). Individual strains of *Salmonella* and *Enterococcus* was stored as frozen culture at −80°C freezer and activated by streak-plating a loop of bacteria lawn onto xylose-lysine-Tergitol-4 (XLT-4) (Hardy Diagnostics, MD) and bile esculin agar (BEA, Hardy Diagnostics) followed by incubating at 35°C for 48 h to obtain the single colonies of *Salmonella* and *E. faecium*, respectively. The XLT-4 agars of *Salmonella* were stored at 4°C ready for the preparation of the experimental inoculum. Since natural background bacteria of chicken meat can be grown on bile esculin agar which interferes with the enumeration of inoculated *E. faecium* (unpublished data), a nalidixic acid (NaL)-resistant strain of *E. faecium* was prepared prior to the experiment.

##### Preparation of NaL-Resistant *E. Faecium* Strain

Two single colonies from the BEA were transferred into 10 mL of tryptic soy broth (TSB, Hardy Diagnostics) and incubated at 35°C for 24 h, followed by spread plating 0.3 mL of the 24 h culture solution onto a BEA containing 100 ppm of NaL (BEA-NaL, Hardy Diagnostics) and incubated at 35°C for 48 h. A single colony from BEA-NaL was transferred into fresh TSB with 100 ppm NaL (TSB-NaL) and incubated for 24 h. Then, 100 μL of the 24 h solution was continuously subcultured into fresh TSB-NaL 5 times. The final subculture solution was streak-plated onto BEA-NaL and incubated at 35°C for 48 h to create a NaL-resistant *E. faecium*. Since this NaL-resistant *E. faecium* was created by “point-mutation”, the NaL-resistant *E. faecium* used
in this study was cultured with media containing 100 ppm Nal, both broth and agar.

**Preparation of Bacterial Inoculum**

Two single colonies from the XLT-4 (*Salmonella*) or BEA-NaL (*E. faecium*) agars were picked-up by a sterilized plastic loop and transferred into a 10 mL of TSB and TSB-NaL followed by incubating at 35°C for 24 h, respectively. The fresh 24 h culture broth were then washed twice in 0.1% buffered peptone water (BPW, Hardy Diagnostics) by centrifuging for 15 min at 5,000 × g, resuspended in 10 mL of sterilized 0.1% BPW, centrifuging again, and resuspending again in a fresh sterilized 0.1% BPW. After the washing process, the two *Salmonella* strains were mixed and spread plated onto TSB-NaL agars with 100-fold serial dilution in 0.1% BPW to determine the concentration of inoculum (∼7.4 log₁₀ CFU/mL). The NaL-resistant *E. faecium* solution was also enumerated on BEA-NaL to calculate the concentration of inoculum (∼8.0 log₁₀ CFU/mL).

**Manufacturing of Chicken Patties and Inoculation**

Frozen bone-less chicken breasts used in this study purchased from a local market in Bridgeport, West Virginia, and shipped to the West Virginia University Food Science Core Lab. The frozen chicken meat was thawed overnight at 4°C. On the day of experiment, the thawed meat was manually cut into small slices with knives and distributed into 1.5 kg batches. Each batch was then coarse grounded in a small benchtop scale meat grinder with a kidney plate (0.95 cm diameter) followed by the addition of 30 mL of the prepared inoculum of either *Salmonella* or *E. faecium* to reach the initial bacterial concentration of ∼6.0 ± 0.4 log CFU/g. The inoculation process was conducted by mixing the chicken meat (1.5 kg) and the prepared inoculum (30 mL) thoroughly by stirring for 2 min in a bowl-lift standard mixer (KitchenAid, St. Joseph, MI) at the slowest speed. Then, the inoculated chicken meat was MH to reach 1, 5 and 11.1% of pump rates by adding 15, 75, or 150 mL of a NaCl (2.0%) plus Na-tripolyphosphate (0.5%) solution (BK Giulini Corporation, Simi Valley, CA) into the meat, respectively, followed by mixing at the same speed for another 2 min. Therefore, the MH chicken meat with the final pump rates of 1, 5, and 11.1% containing 0.2 and 0.05%, 1.0 and 0.25%, 2.0 and 0.50% of NaCl and Na-tripolyphosphate (wt/wt), respectively. The chicken meat portion was weighed (120 ± 1.0 g) and manually manufactured into a chicken patty using a hamburger patty maker (Mainstays 6-ounce-patty maker, Walmart, Bentonville, AR). Each chicken patty was 2.1 cm thick with a 12.4 cm diameter with a total number of 14 patties were formed. Two chicken patties were placed into a foam tray (20 × 25 cm, Pactiv, Lake Forest, IL) containing absorbent pads, packaged manually with polyvinyl chloride films (Omni-film, Pliant Corporation, OH) using a film dispenser and stored in a refrigerated incubator at 4.2° ± 0.3°C for 42 h.

**Cooking of Nonintact Chicken Patties**

After 42 h storage, chicken patties were aseptically removed from the tray under a biosafety hood and cooked on a grill (Farberware 4-in-1 Grill, Fairfield, CA) for 0, 30, 60, 90, 120, 150, 180, 210, 240, 300, 330, 360, 390, and 420 s, respectively. The grill was set at “grill” referred as double pan-broiling with heated top and bottom plates touching meat samples and pre-heated with the temperatures set at 200°, 300°, and 425°F, respectively. This procedure was used to determine the microbial populations of *Salmonella* or *E. faecium* and their related thermal dynamic parameters including D-values and “shoulder time” for each temperature. The internal temperature of each patty during cooking was monitored and recorded using PicoLog software (Pico Technology Ltd., Cambridge, UK) after insertion of a type-K thermocouple into the geometric center of each patty with temperatures automatically recorded at 10 s intervals.

**Microbiological Analyses**

After cooking, chicken samples were immediately placed individually into sterile WhirlPak food sample filter bags (19 × 30 cm, Nasco, Modesto, CA) containing 100 mL of refrigerated TSB plus 0.1% sodium pyruvate (Fisher Scientific, Fair Lawn, NY) for enumeration of bacteria survival populations including heat injured cells. The sample bags with chicken meat were homogenized in a blender (Microbiology International, Frederick, MD) for 2 min. The liquid solution from the filtered side of sample bags was then 10- or 100-fold serial diluted in 9.0 or 9.9 mL of 0.1% BPW. One tenth mL of this solution was spread-plated onto XLT-4 and BEA-NaL agars for *Salmonella* and *E. faecium*, respectively. After spread plating, 12 mL of tempered, melted trypsic soy agar (Hardy Diagnostics) was overlaid onto the surface of each plate before incubating at 35°C for 48 h. After incubation, colonies were manually counted to determine the recovery of heat injured cells. All bacterial counts were transformed to log₁₀ CFU/g with the detection limit of 0.3 log₁₀ CFU/g.

**Statistical Analysis**

After preliminary tests, 3 replicates with 3 chicken patties (120 g per sample unit) in each treatment generating a total of 9 samples was conducted. Experimental design was a completely randomized (3) × (3) × (6-14) factorial structure with 3 different pump rates, 3 different set-up temperatures, and 6 to 14 different cooking times. Survival and reduction data of the two bacterial cells were first analyzed using the SAS mixed model procedure (version 9.2, SAS Institute, Cary, NC) with individual factors and interactions between them. Thermal
kinetic parameters of "shoulder-time" and D-values for each cooking treatment were calculated using the United States Department of Agriculture (USDA)-Integrated-Predictive-Modeling-Program (IPMP) and the USDA-Global-Fit software according to the procedures described in Huang (2014) and Huang (2017), respectively. Finally, calculated “shoulder-times” and D-values of each treatment were analyzed with the same mixed model procedure of SAS and a pair-wised t test was used to compare parameter differences between Salmonella and its surrogate E. faecium. The differences of each individual comparison were determined by Tukey’s HSD with the significance level at α = 0.05.

**RESULTS**

**Temperature Changes of the Geometric Center**

Figure 1 shows the temperature changes at the geometric center of chicken patties cooked at different set-up temperatures. Preliminary investigation indicated that various pump rates (1, 5, and 11.1%) did not affect (P > 0.05) temperature of chicken samples during cooking, therefore Figure 1 depicts the average values of 6 cooked samples across the three pump rates. After aerobic storage at 4.2°C for 42 h, the initial temperatures were ranged from 2.3° to 3.6°C among all chicken samples before cooking (Figure 1). Double pan-broiling chicken patties with the griller temperatures set at 200°, 300°, and 425°F took 300, 255, and 165 s, respectively, to reach the geometric mean temperature of 73.8°C, the target internal temperature of cooked chicken meat products to prevent microbial safety risks (USDA-FSIS, 2013). Internal temperatures of chicken samples reached as high as 84.7°, 80.4°, and 86.5°C with set-up cooking temperatures at 200°, 300°, and 425°F, respectively, by the end of the cooking period (Figure 1).

**Survivals of Microbial Population During Cooking**

Survival curves of Salmonella and E. faecium cell populations in MH reconstructed comminuted chicken patties under isothermal cooking conditions set at 200°, 300°, and 425°F were shown in Figures 2 and 3, respectively. Among all chicken samples, cooking did not reduce significantly (P < 0.05) Salmonella or E. faecium at the early period (0–150 s). Cellular reductions accelerated after the early period. Under isothermal conditions, as expected, cooking chicken samples by double pan-broiling gradually reduced (P < 0.05) the bacterial cells with increased cooking times (Figures 2 and 3) with higher temperatures reducing cells at a faster rate (Figures 2 and 3).

For Salmonella, double pan-broiling decreased (P < 0.05) cell counts from 5.97 to 6.33 log10 CFU/g to below the detectable limit (0.3 log10 CFU/g) or achieved reductions of >5.5 log10 CFU/g after 360, 180 to 225, and 150 to 165 s after cooking chicken patties at 200°, 300°, and 425°F, respectively, regardless of pump rates (Figure 2). For E. faecium, double pan-broiling chicken patties across all pump rates at 200°, 300°, and 425°F reduced the cell counts by 3.71 to 4.73, 4.67 to 5.48, and 5.56 to 6.14 log10 CFU/g, respectively, by the end of the cooking period (Figure 3). Compared to Salmonella, the surrogate E. faecium in chicken samples was resistant (P < 0.05) to heat treatments because no sample was reduced >5.5 log10 CFU/g when cooked at 200° and 300°F (Figures 2 and 3).

For Salmonella, less (P < 0.05) time was required to achieve the reduction of 5.5 log10 in chicken patties MH.
with 1.0% pump rate compared with those of 5.0 and 11.1% pump rates, as shown by the 180 vs. 210 and 225 s, and 150 vs. 165 s times cooking at 300°F and 425°F, respectively (Figure 2). A greater ($P < 0.05$) reduction in *E. faecium* was shown in chicken samples with 1.0% pump rate compared with those from the 5.0 and 11.1% ones, as shown as 4.73 vs. 4.29 and 3.71 log$_{10}$ CFU/g, 5.48 vs. 4.74 and 4.67 log$_{10}$CFU/g, and 6.14 vs. 5.56 and 5.99 log$_{10}$ CFU/g, when cooked at 200°, 300°, and 425°F, respectively (Figure 3).
Modeling of Bacterial Survivals During Cooking

The USDA-IPMP software (Huang, 2014), containing 4 survival mathematical models, were used in this study to calculate “shoulder-times” (Buchanan Two-phase Model) and D-values (Mafart-Weibull model) of Salmonella and E. faecium in chicken patties prepared with three different pump rates. The IPMP-Global fit software (Mafart-Weibull model, Huang, 2017) was also used to compare the D-values of Salmonella and E. faecium in chicken samples cooked at three different set-up temperatures using a single pump rate (1.0, 5.0, or 11.1%) simultaneously.

Figure 3. Survival-temperature profiles of the surrogate Enterococcus faecium in reconstructed comminuted chicken patties moisture-enhanced with 1.0, 5.0, and 11.1% pump rate during double pan-broiling at 200°, 300°, and 425°F.
As expected, the calculated values of “shoulder-time” of Salmonella and E. faecium in chicken patties decreased ($P < 0.05$) with increasing set-up temperatures (Table 1). When the set-up temperatures increased from 200° to 425°F, the “shoulder-time” of Salmonella and E. faecium in chicken samples across all pump rates decreased ($P < 0.05$) from 229.0−247.8 to 82.3−118.0 s and 234.8−259.4 to 128.3−130.9 s (Table 1), respectively. For Salmonella, the pump rates had a significant effect on ($P > 0.05$) the “shoulder-times” in chicken patties during cooking. When cooked at 300°F, the “shoulder-times” of samples with 1.0% and 5.0% pump rate were 128.0 and 133.4 s, respectively, which were shorter ($P < 0.05$) than the 11% samples (158.6 s, Table 1). When the set-up temperature was increased to 425°F, a “shoulder-time” in samples with 1% pump rate (82.3 s) was significantly shorter ($P < 0.05$) than those of the 5.0 (118.0 s) and 11.1% pump rates (116.6 s, Table 1). In contrast to Salmonella, “shoulder-times” of E. faecium in chicken patties did not differ significantly ($P > 0.05$) regardless of various pump rates. The “shoulder-times” of chicken patties with 1.0% pump rate were 235.6, 136.2, and 128.3 s, which were similar ($P > 0.05$) to the 5.0% samples (259.4, 130.1, and 128.6 s) and the 11.1% samples (234.8, 151.5, and 130.9 s) when cooked at 200°, 300°, and 425°F, respectively (Table 1).

The D-values of Salmonella and E. faecium (Table 2) in chicken patties were significantly affected by the set-up temperatures ($P < 0.05$) and pump rates ($P < 0.05$) but the interaction was not significant ($P = 0.05$ to 0.06). The E. faecium D-values of chicken patties with 1.0% pump rate cooked at 200°, 300°, and 425°F were 248.2, 127.0, and 102.7 s, respectively, which were lower ($P < 0.05$) than the 5.0% samples (260.3, 157.7, and 115.3 s) and the 11.1% samples (263.7, 156.7, and 119.8 s) (Table 2). The Salmonella D-values of E. faecium in chicken samples with 1.0% pump rate of 200°, 300°, and 425°F were 271.0, 168.0, and 115.5 s, respectively, which were similar ($P > 0.05$) to the 5.0% samples (284.7, 172.7, and 119.3 s), but lower ($P < 0.05$) than the 11.1% samples (298.3, 185.0, 122.5 s). Figure 4 shows the pairwise comparisons between the D-values of Salmonella and E. faecium in all samples with all combinations of set-up temperatures and pump rates. D-values of Salmonella were lower ($P < 0.05$) than the surrogate E. faecium in almost all cooked chicken patties except for the samples with 5 and 11.1% pump rates cooked at 425°F, which showed similar D-values between the two bacteria (Figure 4).

**DISCUSSION**

Studies related to thermal inactivation of Salmonella in chicken products were initiated about 2 decades ago. In 2 early studies, Murphy et al. (1999; 2000) reported that heating ground chicken breast meat in a 70°C water bath reduced Salmonella by 7-log10 CFU/g after approximately 2.1 min (126 s). In the current study, the manufacturing, packaging, storage and cooking of MH reconstructed comminuted chicken patties stimulated the retail commercial processing. Results indicated that double pan-broiling with the set-up temperature of 425°F achieved > 5.5 log10 CFU/g reduction after cooking for 2 to 3 min, suggesting that double pan-broiling with two heating plates, employed by most fast food restaurant kitchens, is a very efficient approach for thorough cooking of chicken patties.

For double pan-broiling of chicken patties at 200°, 300°, and 425°F, Salmonella and E. faecium did not decrease significantly in the early stage of cooking indicating a “shoulder effect,” which agrees with the previous studies of Huang (2009), Li et al. (2017) and Jiang et al. (2020). The internal temperatures of the chicken patties did not increase rapidly enough to kill bacterial cells at the early stage due to the geometry of the chicken patties (Huang, 2009). The “shoulder effect” observed in this study was expressed as “shoulder time”.

---

**Table 1.** Buchanan Two-phase Model calculated “shoulder-times” (mean ± standard deviation) of Salmonella Typhimurium and Enterococcus faecium in reconstructed comminuted chicken patties moisture-enhanced with 1.0, 5.0, and 11.1% pump rate and double pan-broiling at 200, 300, and 425°F.

| Temperature (°F) | Pump rate (%) | 20% NaCl + 0.5% Na-tripolyphosphate | 5.0% NaCl + 0.5% Na-tripolyphosphate |
|------------------|---------------|-------------------------------------|-------------------------------------|
| 200              |               | 229.0 ± 36.4%A                      | 247.8 ± 28.9%B                      |
| 300              |               | 128.0 ± 13.6%A                      | 133.4 ± 16.3%A                      |
| 425              |               | 82.3 ± 16.0%A                       | 118.0 ± 6.8%B                       |

**Table 2.** Mafart-Weibull model calculated D-values (mean ± standard deviation) of Salmonella Typhimurium and Enterococcus faecium in reconstructed comminuted chicken patties moisture-enhanced with 1.0, 5.0, and 11.1% pump rate of double pan-broiling at 200°, 300°, and 425°F.

| Temperature (°F) | Pump rate (%) | 20% NaCl + 0.5% Na-tripolyphosphate | 5.0% NaCl + 0.5% Na-tripolyphosphate |
|------------------|---------------|-------------------------------------|-------------------------------------|
| 200              |               | 248.2 ± 12.7%A                      | 260.3 ± 6.0%B                       |
| 300              |               | 127.0 ± 8.4%A                       | 157.7 ± 5.0%B                       |
| 425              |               | 102.7 ± 5.6%A                       | 115.3 ± 6.9%B                       |

Mean values with different letters within a column differ significantly ($P < 0.05$).

Mean values with different capital letters within a row differ significantly ($P < 0.05$).
for each cooked sample calculated from the Buchanan Two-phase Model in the USDA-IPMP software (Huang, 2014). The “shoulder-times” of Salmonella in chicken patties decreased with increasing pump rates at each cooking temperature. In these samples, the higher concentration of phosphate immobilized more water in the muscle myofibril lattices which decreased the rate of heat transfer inside of the chicken patties during cooking (Offer and Trinick, 1983).

The D-value, defined as the time required to kill 90% (1.0-log) of the organism at a specific heating temperature, is used commonly to measure the death rate of an

Figure 4. Pair-wised comparison of D-values of Salmonella Typhimurium and Tennessee and the surrogate Enterococcus faecium in reconstructed comminuted chicken patties moisture-enhanced with 1.0, 5.0, and 11.1% pump rate during double pan-broiling at 200°, 300°, and 425°F. Different letters indicate significant difference ($P < 0.05$).
organism during the thermal inactivation process (Jay et al., 2005). Juneja et al. (2001) reported that the Salmonella D-values ranged from 7.08 to 0.59 min (424.8 –35.4 s) in ground chicken with 3% fat heated between 58° and 65°C. Murphy et al. (2002) found that the D-values of Salmonella at 60° to 70°C in a commercially manufactured ground chicken patties (5% fat) were 8.09 to 0.32 min (485.4–19.2 s). In a related study, Murphy et al. (2003) also reported that the D-values of Salmonella in ground chicken breast meat at 60° to 70°C ranged from 3.83 to 0.10 min (229.8–6 s). Comparing the current D-values with previous findings is limited by three factors. First, the current study used commercial size MH chicken patties rather than 10 to 100 g ground chicken meat. Second, the cooking method was commercial double pan-broiling compared with immersion heating in a circulated water bath. Third, D-values were calculated using the Mafart-Weibull model which includes the “shoulder-effect” of the cooking process in this study instead of linear or linear regression models used in the previous studies. The current D-values calculated for Salmonella are similar to the previous studies even with the above limitations.

In this study, Salmonella cells in chicken patties MH with 1.0% pump rate were more susceptible to heating as shown by shorter cooking times to reach >5.5 log10 reduction, shorter “shoulder times” and lower D-values compared with the samples with higher pump rates. Our most recent study (Jiang et al., 2020) also found that Campylobacter jejuni is more heat sensitive in chicken patties with a 1.0% pump rate cooked at 400° and 425°F compared with those sample having an 11% pump rate. These results could be explained by the following 2 reasons, (1) compared to the 11% pump rate samples, chicken samples MH with 1% pump rate demonstrated higher moisture loss during cooking increasing the fat content; and (2) compared to the 1.0% pump rate samples, the 11% samples higher levels of sodium chloride and tripolyphosphate protect the bacterial cells from heating by stabilizing bacterial cell membranes (Mukherjee et al., 2008). Kotrola and Conner (1997), reported that the D-values of Escherichia coli O157:H7 in ground turkey breast (8% salt and 0.5% polyphosphate) with 11% fat heated at 55° and 57°C were smaller than the samples with 3% fat, 17.9 vs. 23 s and 6.1 vs. 10.8 s, respectively. The same study also found that E. coli O157:H7 D-values in the ground turkey with 8% salt heated at 55 (25.1–27.2 vs. 7.7–11.0 s), 57 (11.0–12.7 vs. 2.7–3.4 s) and 60°C (2.9–4.8 vs. 0.7 s) were greater than the samples without salt ingredients (Kotrola and Conner,1997). These results indicate that cooking protocols for chicken products need to consider salt content.

Evaluating the behavior of surrogate bacteria in food processing treatments has become more popular in recent years (Hu and Gurtler, 2017). An ideal surrogate organism should be non-pathogenic, easy to prepare, generally stable, survive in various environmental conditions, and behave equally well or resistant to interventions (i.e., antimicrobials or thermal treatments) compared with its target pathogen (Liu and Schaffner, 2007; Hu and Gurtler, 2017). E. faecium fulfills these requirements as a surrogate for Salmonella due to its survival at the wide temperature ranges of 5° to 65°C, pH ranges of 4.5 to 10.0, and high salt concentrations (6.5%) (Fisher and Phillips, 2009). For chicken products, our previous study found that unstressed or cold-stressed E. faecium on chicken carcasses had a similar or more resistant response to four different antimicrobial solutions (peroxyacetic acid, lactic acid, lactic/citric acid blend, and chlorine water) than Salmonella (Lemonakis et al., 2017). Results of this study indicated that E. faecium is less susceptible to heat treatment than Salmonella in MH chicken patties because of fewer reductions during the same cooking period, longer “shoulder times,” and greater D-values. Bianchini et al. (2014) found that E. faecium is more resistant to heat than Salmonella in a complex carbohydrate-protein meal by showing a higher temperature requirement to reach a 5-log reduction (73.7° vs. 60.6°C) and complete elimination of bacterial cells (80.3° vs. 68°C). Ceylan and Bautista (2015) also reported that D-values of E. faecium in thermally processed pet food with 9% moisture were greater than the seven (7) Salmonella strains tested at 76.7 (11.7 vs. 6.5 min), 82.2 (4.1 vs. 2.7 min), and 87.8°C (1.7 vs. 1.1 min). The thermal resistance of E. faecium is mainly associated with its growth phase, membrane structure, amount of lipids and fatty acids, and sigma factors. First, E. faecium was grown at 35°C in this study, compared to the growth at 40° and 45°C, this relatively low temperature may increase saturated fatty acids, decreasing unsaturated fatty acids, further decreasing the fluidity of the cell membrane, and therefore elevating thermal resistance (Martinez et al., 2003; Fisher and Phillips, 2009). Second, similar to previous studies (Bianchini et al., 2014; Ceylan and Bautista, 2015), E. faecium was at the stationary phase and might initiate an alternative sigma factor mediated programming adaptation which directing the RNA polymerases to transcribe many genes that can be translated into proteins that protect bacterial cells from thermal treatments (Martinez et al., 2003).

In conclusion, results of this study suggested that increasing the pump rates of MH reconstructed comminuted chicken patties could cause Salmonella heat resistance during double pan-broiling. E. faecium could be an appropriate surrogate for Salmonella to be used in the thermal validation studies of chicken meat products. Further studies are needed to validate the behavior of E. faecium versus Salmonella in different formulations with various chemical ingredients such as antimicrobials or antioxidants.

ACKNOWLEDGMENTS

This work is supported by the United States Department of Agriculture-National Institute of Food and Agriculture (NIFA, Grant # WVA00684 and WVA00736) and the West Virginia Agricultural and Forestry Experiment Station (Scientific Article No. 3399), and partially
supported by the USDA-NIFA-AFRI-Critical Agricultural Research and Extension (CARE) Program (Grant # 2019-68008-29828).

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

American Meat Science Association. 2015. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. Accessed March 2021. https://meatscience.org/docs/default-source/publications-resources/amsa-sensory-and-tenderness-evaluation-guidelines/research-guide-2015-amsa-sensory-guidelines-1-0.pdf?sfvrsn=6.

Bianchini, A., J. Stratton, S. Weier, T. Harter, B. Plattner, G. Rokey, G. Hertzl, L. Gompa, B. Martinez, and K. M. Eskridge. 2014. Use of Enterococcus faecium as a surrogate for Salmonella enterica during extrusion of a balanced carbohydrate-protein meal. J. Food Prot. 77:75–82.

Boltz, T. P., J. W. Boney, C. Shen, J. Jacyzynski, and J. S. Moritz. 2019. The effect of standard pelleting and more thermally aggressive pelleting utilizing a hygieniser on feed manufacture and reduction of enterococcus faecium, a Salmonella surrogate. J. Appl. Poult. Res. 28:1226–1233.

Boney, J. W., J. Jacyzynski, J. L. Weidhaas, A. N. Bergeron, and J. S. Moritz. 2018. The effects of steam conditioning and antimicrobial inclusion on feed manufacturing and inactivation of Enterococcus faecium, a Salmonella surrogate. J. Appl. Poult. Res. 27:472–482.

Ceylan, E., and D. A. Bautista. 2015. Evaluating pediococcus acidilactici and Enterococcus faecium NRRL B-2354 as thermal surrogate microorganisms for Salmonella for in-plant validation studies of low-moisture pet food products. J. Food Prot. 78:934–939.

Fisher, K., and C. Phillips. 2009. The ecology, epidemiology and virulence of Enterococcus. Microbiology (Reading, England) 155:1749–1757.

Gill, C. O., J. C. McGinnis, S. Barbut, D. Young, N. Lee, and K. Rahn. 2004. Microbiological conditions of moisture-enhanced chicken breasts prepared at a poultry packing plant. J. Food Prot. 67:2675–2681.

Hu, M., and J. B. Gurltler. 2017. Selection of surrogate bacteria for use in food safety challenge studies: a review. J. Food Prot. 80:1506–1536.

Huang, L. 2017. IPMP Global Fit

Jiang, W., K. Li, Y.-C. Chiu, C. Waldman, and C. Shen. 2020. Inactivation of Enterococcus faecium: effect of growth temperature and physiological state of microbial cells. Lett. Appl. Microbiol. 37:475–481.

Li, K., A. G. McKeith, C. Shen, and R. McKeith. 2018. A comparison study of quality attributes of ground beef and veal patties and thermal inactivation of Escherichia coli O157:H7 after double pan-broiling under dynamic conditions. Foods 7:1.

Liu, B., and D. W. Schaffner. 2007. Mathematical modeling and assessment of microbial migration during the sprouting of alfalfa in trays in a nonuniformly contaminated seed batch using Enterobacter aerogenes as a surrogate for Salmonella Stanley. J. Food Prot. 70:2620–2629.

Martínez, S., M. López, and A. Bernardo. 2003. Thermal inactivation of Enterococcus faecium: effect of growth temperature and physiological state of microbial cells. Lett. Appl. Microbiol. 37:475–481.

Morris, J. G., J. H., Sandra and B. Batz. 2011. Ranking the risks: the 10 pathogen-food combinations with the greatest burden on public health emerging pathogens Institute at University of Florida.

Mukherjee, A., Y. Yoon, K. E. Belk, J. A. Scanga, G. C. Smith, and J. N. Sofos. 2008. Thermal inactivation of Escherichia coli O157:H7 in beef treated with marination and tenderization ingredients. J. Food Prot. 71:1349–1356.

Murphy, R. Y., L. K. Duncan, B. L. Beard, and K. H. Driscoll. 2003. D and z values of Salmonella, Listeria innocua, and Listeria monocytogenes in fully cooked poultry products. J. Food Sci. 68:1443–1447.

Murphy, R. Y., L. K. Duncan, E. R. Johnson, M. D. Davis, and J. N. Smith. 2002. Thermal inactivation D- and z-values of Salmonella serotypes and listeria innocua in chicken patties, chicken tenders, franks, beef patties, and blended beef and turkey patties. J. Food Prot. 65:53–60.

Murphy, R. Y., B. P. Marks, E. R. Johnson, and M. G. Johnson. 1999. Inactivation of Salmonella and Listeria in ground chicken breast meat during thermal processing. J. Food Prot. 62:980–985.

Murphy, R. Y., B. P. Marks, E. R. Johnson, and M. G. Johnson. 2000. Thermal inactivation kinetics of Salmonella and Listeria in ground chicken breast meat and liquid medium. J. Food Sci. 65:706–710.

National Advisory Committee on Microbiological Criteria for Foods. 2007. Analytical utility of Campylobacter methodologies. J. Food Prot. 70:241–250.

Offer, G., and J. Trinick. 1983. On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. Meat Sci. 8:245–281.

Shen, C., J. M. Adler, I. Geornaras, K. E. Belk, G. C. Smith, and J. N. Sofos. 2010. Inactivation of Escherichia coli O157:H7 in non-intact beefsteaks of different thicknesses cooked by pan broiling, double pan broiling, or roasting by using five types of cooking appliances. J. Food Prot. 73:461–469.

U.S. Centers for Disease Control and Prevention (U.S.-CDC). 2020. The Interagency Food Safety Analytics Collaboration. Foodborne Illness Source Attribution Estimates for 2018 for Salmonella, Escherichia coli O157, Listeria Monocytogenes, and Campylobacter Using Multi-Year Outbreak Surveillance Data. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Food and Drug Administration, U.S. Department of Agriculture’s Food Safety and Inspection Service, United States GA and D.C. Accessed March 2021. https://www.cdc.gov/foodsafety/ifasac/pdf/PI9-2018-report-TriAgency-508.pdf.

United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS). 2013. Salmonella questions and answers. Accessed March 2021. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-factsheets/foodborne-illness-and-disease/salmonella-questions-and-answers/.

United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS). 2016. New Performance Standards for Salmonella and Campylobacter in Not-Ready-to-Eat Comminuted Chicken and Turkey Products and Raw Chicken Parts and Changes to Related Agency Verification Procedures: Response to Comments and Announcement of Implementation Schedule The Daily Journal of the United States Government. Accessed March 2021. https://www.federalregister.gov/documents/2016/02/11/2016-02586/new-performancestandards-for-salmonella-and-campylobacter-in-not-ready-to-eat-comminuted-chicken#print.

United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS). 2015. DRAFT FSIS compliance
guideline for controlling *Salmonella* and *Campylobacter* in raw poultry. Accessed March 2021. http://www.fsis.usda.gov/wps/wcm/connect/6732c082-af40-415e-9b57-90533ea4e252/Controlling-Salmonella-Campylobacter-Poultry-2015.pdf?MOD=AJPERES.

World Health Organization. Food and Agriculture Organization of the United Nations. 2009. *Salmonella* and *Campylobacter* in chicken meat: meeting report.

Young, L. L., D. Hamm, C. Y. W. Ang, R. Wilson, and C. E. Lyon. 1988. Research note: effect of carcass size and sodium pyrophosphate on moisture absorption and retention by marinated broiler halves. Poult. Sci. 67:510–512.

Young, L. L., and C. E. Lyon. 1997. Effect of postchill aging and sodium tripolyphosphate on moisture binding properties, color, and Warner-Bratzler shear values of chicken breast meat. Poult. Sci. 76:1587–1590.