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

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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, United States Department of Agriculture [**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

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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

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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 temperatures 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* verse *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 numeration 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 XLT-4 agars with 100-fold serial dilution in 0.1% BPW to determine the concentration of inoculum (~7.4 $\log_{10}$ CFU/mL). The NaL-resistant *E. faecium* solution was also enumerated on BEA-NaL to calculate the concentration of inoculum (~8.0 $\log_{10}$ 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 tryptic 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 $\log_{10}$ CFU/g with the detection limit of 0.3 $\log_{10}$ 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 $\alpha = 0.05$.

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 $\log_{10}$ CFU/g to below the detectable limit (0.3 $\log_{10}$ CFU/g) or achieved reductions of >5.5 $\log_{10}$ 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 $\log_{10}$ 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 $\log_{10}$ 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 $\log_{10}$ in chicken patties MH

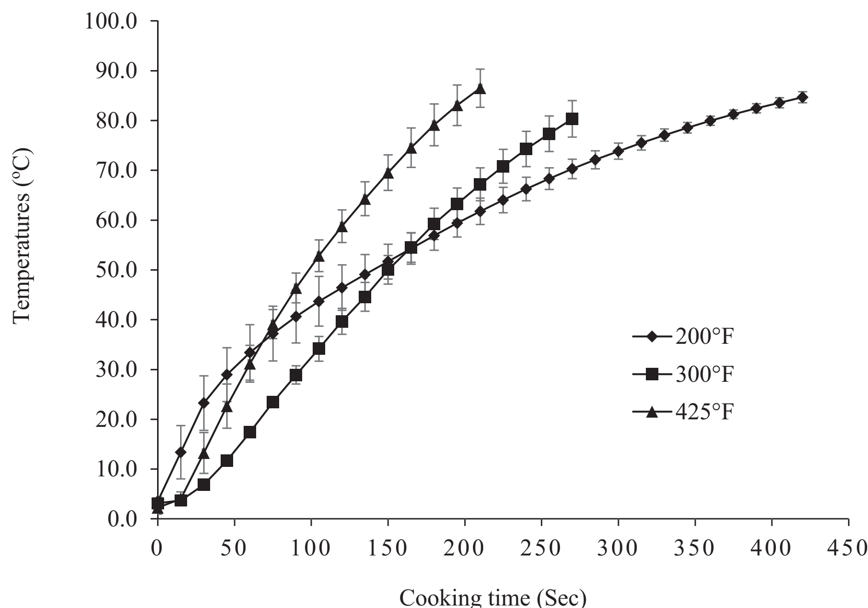

Figure 1. Time-temperature profiles of the geometric center of moisture-enhanced reconstructed comminuted chicken patties during double pan-broiling set at 200, 300, and 425°F. Each data point is the average value across all pump rates.

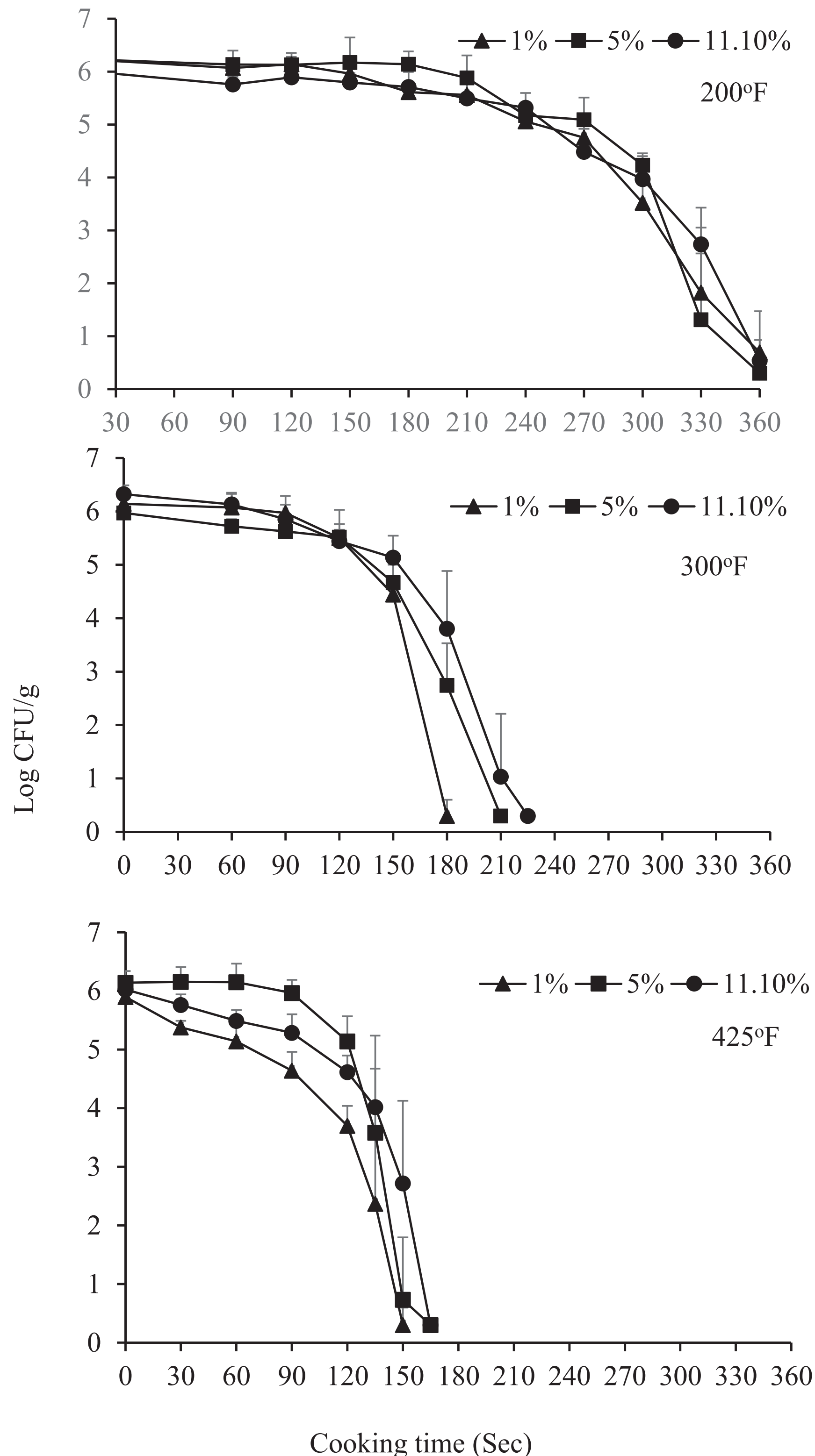

Figure 2. Survival-temperature profiles of *Salmonella* Typhimurium and Tennessee in reconstructed comminuted chicken patties moisture-enhanced with 1.0, 5.0, and 11.1% pump rate during double pan-broiling at 200°, 300°, and 425°F.

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 and 165 s times cooking at 300° 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).

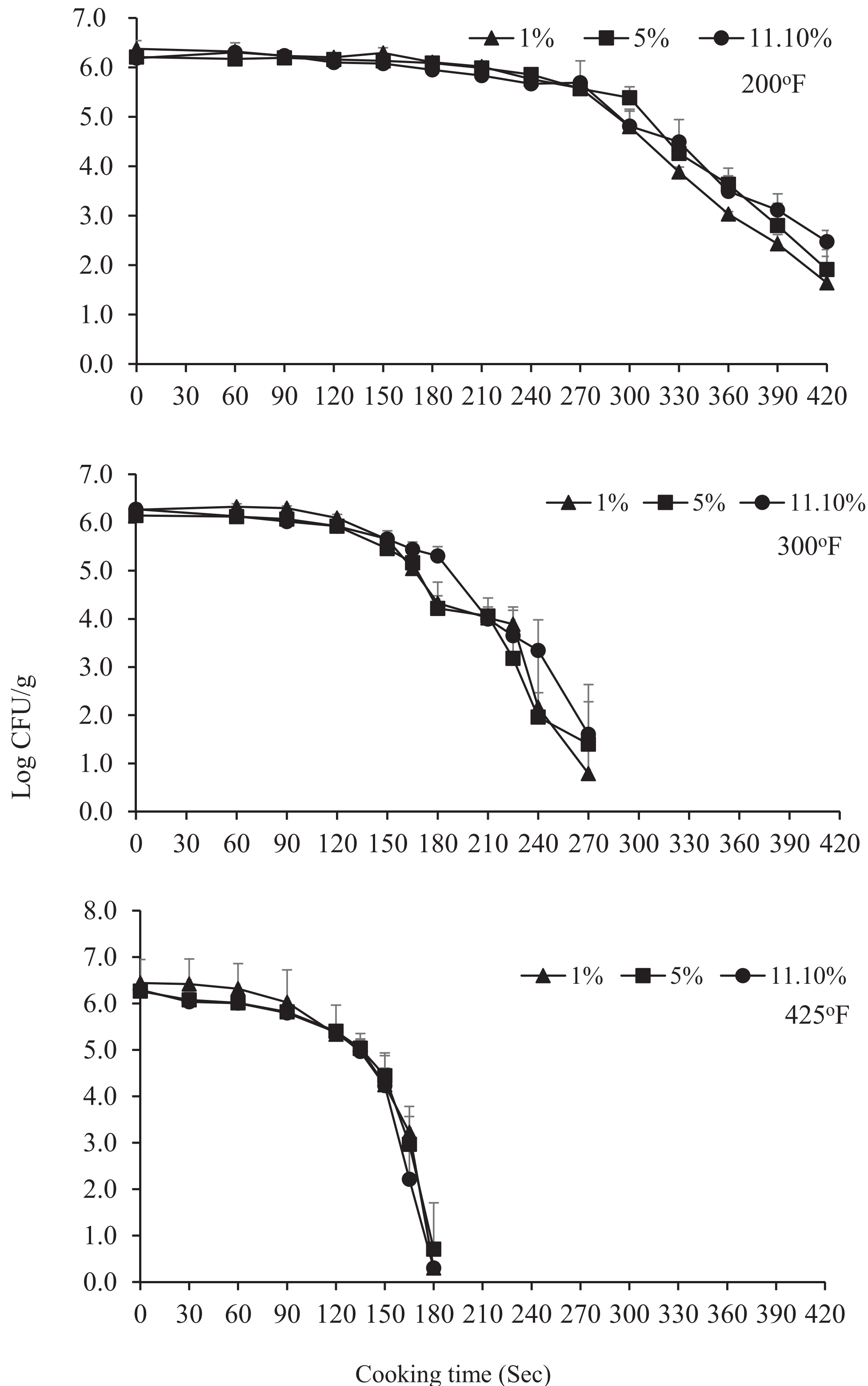

Figure 3. Survival-temperature profiles of the surrogate *Enterococcus faecium* in reconstructed comminuted chicken patties moisture-enhanced with 1.0, 5.0, and 11.1% pump rate during double pan-broiling at 200°, 300°, and 425°F.

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 S*almonella* 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.

Table 1. Buchanan Two-phase Model calculated "shoulder-times" (mean ± standard deviation) of *Salmonella Typhimurium* and *Tennessee* 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.

	2.0% NaCl + 0.5% Na-tripolyphosphate		
	Pump rate (%)		
Salmonella			
Temperature (°F)	1	5	11.1
200	229.0 ± 36.4^{aA}	247.8 ± 28.2^{aB}	246.2 ± 12.4^{aB}
300	128.0 ± 13.6^{bA}	133.4 ± 16.3^{bA}	158.6 ± 27.4^{bB}
425	82.3 ± 16.0^{cA}	118.0 ± 6.8^{cB}	116.6 ± 17.8^{cB}
Enterococcus			
Temperature (°F)			
200	235.6 ± 9.7^{aA}	259.4 ± 6.0^{aB}	234.8 ± 29.5^{aA}
300	136.2 ± 10.8^{bA}	130.1 ± 14.9^{bA}	151.5 ± 13.6^{bB}
425	128.3 ± 8.5^{cA}	128.6 ± 10.6^{bA}	130.9 ± 7.0^{cA}

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

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

Table 2. Mafart-Weibull model calculated D-values (mean ± standard deviation) of *Salmonella Typhimurium* and *Tennessee* 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.

	2.0% NaCl + 0.5% Na-tripolyphosphate		
	Pump rate (%)		
Salmonella			
Temperature (°F)	1	5	11.1
200	248.2 ± 12.7^{aA}	260.3 ± 6.0^{aB}	263.7 ± 9.6^{aB}
300	127.0 ± 8.4^{bA}	157.7 ± 5.6^{bB}	156.7 ± 10.8^{bB}
425	102.7 ± 5.6^{cA}	115.3 ± 6.9^{cB}	119.8 ± 6.7^{cB}
Enterococcus			
Temperature (°F)			
200	271.0 ± 10.1^{aA}	284.7 ± 8.8^{aB}	298.3 ± 16.5^{aC}
300	168.0 ± 6.8^{bA}	172.7 ± 10.1^{bA}	185.0 ± 10.4^{bB}
425	115.5 ± 5.1^{cA}	119.3 ± 7.5^{cA}	122.5 ± 5.4^{cA}

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

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

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% 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 to 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-$\log_{10}$ 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 $\log_{10}$ 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"

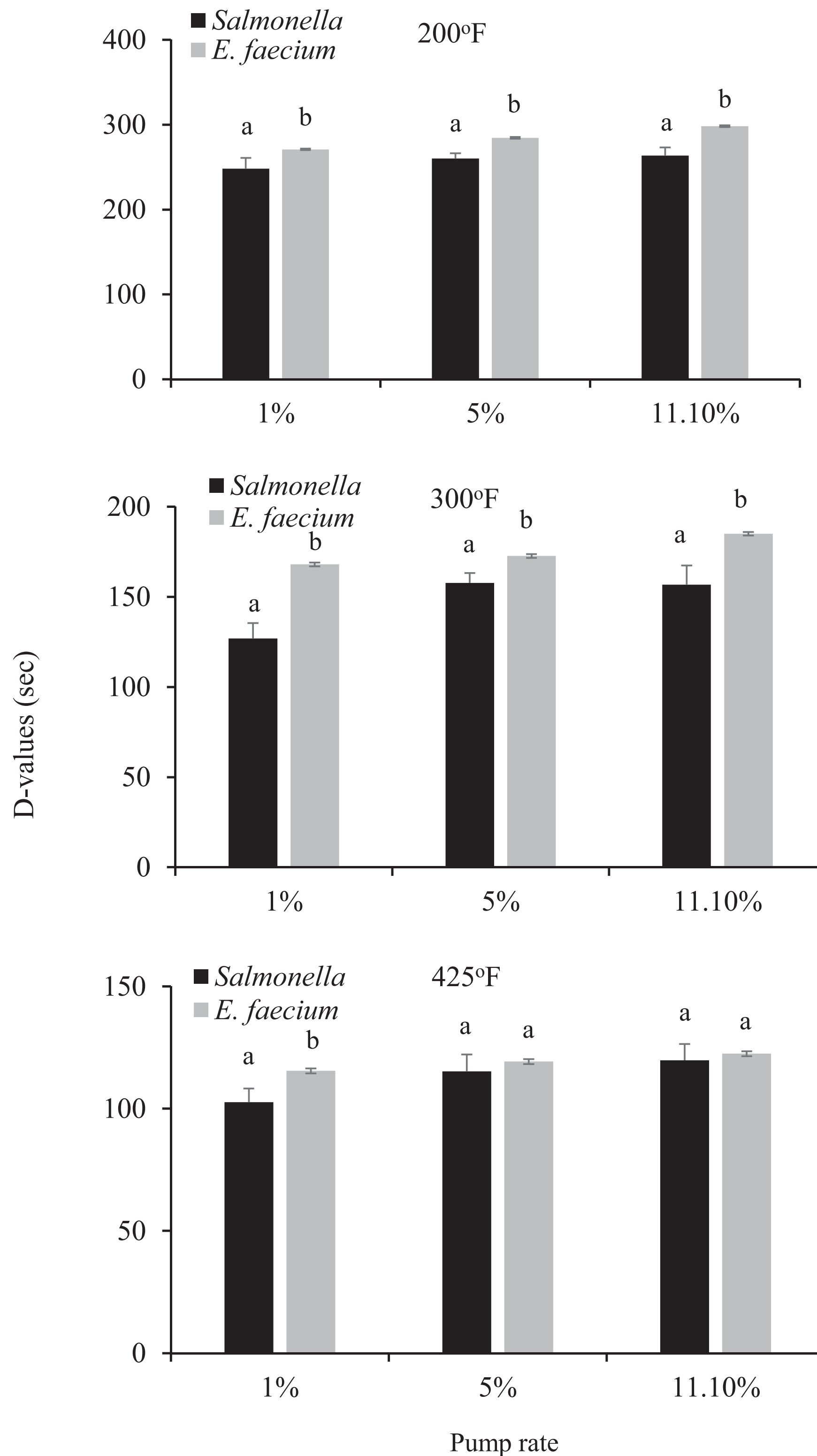

Figure 4. Pair-wised comparison of D-values of *Salmonella* Typhimurium and Tennessee and the surrogate *Enterococcus faecium* in reconstructed comminuted chicken patties moisture-enhanced with 1.0, 5.0, and 11.1% pump rate during double pan-broiling at 200°, 300°, and 425°F. Different letters indicate significant difference ($P < 0.05$).

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

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 $\log_{10}$ 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* verse *Salmonella* in different formulations with various chemical ingredients such as antimicrobials or antioxidants.

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DISCLOSURES

The authors declare no conflicts of interest.

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