Validation of Baking To Control *Salmonella* Serovars in Hamburger Bun Manufacturing, and Evaluation of *Enterococcus faecium* ATCC 8459 and *Saccharomyces cerevisiae* as Nonpathogenic Surrogate Indicators

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**ABSTRACT**

This study was conducted to validate a simulated commercial baking process for hamburger buns to destroy *Salmonella* serovars and to determine the appropriateness of using nonpathogenic surrogates (*Enterococcus faecium* ATCC 8459 or *Saccharomyces cerevisiae*) for in-plant process validation studies. Wheat flour was inoculated (~6 log CFU/g) with three *Salmonella* serovars (Typhimurium, Newport, or Senftenberg 775W) or with *E. faecium*. Dough was formed, proofed, and baked to mimic commercial manufacturing conditions. Buns were baked for up to 13 min in a conventional oven (218.3°C), with internal crumb temperature increasing to ~100°C during the first 8 min of baking and remaining at this temperature until removal from the oven. *Salmonella* and *E. faecium* populations were undetectable by enrichment (>6-log CFU/g reductions) after 9.0 and 11.5 min of baking, respectively, and ≥5-log-cycle reductions were achieved by 6.0 and 7.75 min, respectively. D-values of *Salmonella* (three-serovar cocktail) and *E. faecium* ATCC 8459 in dough were 28.64 and 133.33, 7.61 and 55.67, and 3.14 and 14.72 min at 55, 58, and 61°C, respectively. A high level of thermal lethality was observed for baking of typical hamburger bun dough, resulting in rapid elimination of high levels of the three-strain *Salmonella* cocktail; however, the lethality and microbial destruction kinetics should not be extrapolated to other bakery products without further research. *E. faecium* demonstrated greater thermal resistance compared with *Salmonella* during bun baking and could serve as a conservative surrogate to validate thermal process lethality in commercial bun baking operations. Low thermal tolerance of *S. cerevisiae* relative to *Salmonella* serovars limits its usefulness as a surrogate for process validations.

Key words: Baking; D-value; *Enterococcus faecium*; *Salmonella*; Yeast

Approximately 2,800 commercial bakeries and 6,000 retail bakeries operate in the United States, with a market value of nearly $30 billion per year. Baked breads account for ~40% of commercial bakery sales, followed by rolls, buns, muffins, and bagels (~20% of sales) (5). Title I (Sec. 103) of the Food Safety Modernization Act calls for food processors to conduct and document product- and process-specific hazard analyses and to institute risk-based preventative controls to ensure product safety (39). A wide array of baked products are manufactured, characterized by notable differences in processing parameters and compositional components; thus, product-specific analysis of hazards and of the adequacy of processing steps is necessary to mitigate these risks.

Although not directly linked to improper processing practices, there were 30 disease outbreaks and 706 illnesses linked to bread products between 1998 and 2007 (9). *Salmonella* can be introduced into bakery products prior to thermal processing, through contaminated ingredients such as eggs (7, 36), milk products (1, 15), flour (2, 12, 33), milk chocolate (13), coconut (16), peanut butter (34), fruits (18), spices (20), and yeast flavorings (22). *Salmonella* cells that have survived desiccation (e.g., in stored dry ingredients) tend to exhibit greater thermal resistance during processing (19). *Salmonella* Typhimurium has been the most prevalent food-associated serovar since 1997, and *Salmonella* Newport has been reported to be the third most common serovar associated with foodborne disease outbreaks (10). *Salmonella* Senftenberg 775W is a notable heat-resistant serovar in liquid culture media (31) and in high-moisture foods (32), but it does not demonstrate remarkable heat resistance in low-moisture conditions (27). Although *Salmonella* cannot
grow in foods or ingredients with a water activity (aw) <0.93, it can survive for months and then grow when favorable conditions occur, such as rehydration of flour (15). Between October 2008 and January 2009, 67 Salmonella Typhimurium phage type 42 cases, including 12 hospitalizations, were reported in New Zealand and were traced to consumption of a contaminated uncooked baking mixture containing flour (28).

To validate in-plant food safety processes without risking facility contamination, nonpathogenic surrogates for specific pathogens are often identified and characterized through laboratory studies. Surrogates should demonstrate similar growth and/or survival characteristics to the specific pathogen(s) of interest in a similar food matrix (24). Enterococcus faecium NRRL B-2354 (deposited at the American Type Culture Collection [ATCC], Manassas, VA, as Biosafety Level-1 Micrococcus freudenreichii Guillebeau ATCC 8459) has been used by the food industry for over 60 years for a variety of purposes, including use as a surrogate for Salmonella in thermal processing (24). Kopit et al. 2014 (23) reported that E. faecium NRRL B-2354 has relatively high acidic and thermal resistances and does not possess virulence or antibiotic resistance genes and, therefore, suggested its use for food process thermal validation studies. E. faecium NRRL B-2354 has been recommended for in-plant validation of thermal processes for dry and moist roasting of almonds to control Salmonella (3, 21).

Although most bakery products undergo a kill step at the point of production, such as baking or cooking, studies validating these control points are generally lacking in the literature. Bakeries need such validation documentation to support their food safety plans under requirements of the Food Safety Modernization Act; hence, a laboratory-based kill-step validation protocol for hamburger buns is hereby presented that supports the baking industry’s compliance mandate with the preventive controls rule outlined in the act. The objectives of this study were to validate a simulated commercial baking process for hamburger buns to control Salmonella serovar contamination introduced via raw ingredients, to determine the appropriateness of using nonpathogenic surrogates (E. faecium ATCC 8459 or Saccharomyces cerevisiae) for in-plant process validation studies, and to determine thermal inactivation parameters (D- and z-values) of Salmonella serovars and the potential surrogates in a dough matrix.

MATERIALS AND METHODS

Design of studies and statistical analysis. Three separate studies were conducted to compare target microbial population (Salmonella, E. faecium 8459, and Saccharomyces cerevisiae) survival characteristics during baking of hamburger buns and to compare thermal lethality kinetics for these target organisms in heated dough. Specifically, the first study determined the microbial lethality associated with a standard commercial bun baking protocol utilizing baking times that provided finished buns with acceptable quality. The second baking study determined times that yielded a minimum reduction in cell numbers of Salmonella and E. faecium by 5 log cycles and times that resulted in no recoverable target organism by enrichment plating (subsequently referred to as the “breakpoint”) in undercooked buns. Finally, a study was conducted to establish D- and z-values for Salmonella (three- serovar cocktail), E. faecium 8459, and S. cerevisiae in heated dough.

The first baking study was designed as a randomized complete block (replications being blocks) with seven treatment times at one oven temperature: flour inoculation, preproof dough formation, postproof dough formation, 9-min bake, 11-min bake, 13-min bake, and 13-min bake with 30-min ambient temperature cooling (B+C). Analysis of variance for the surviving target microbial populations (log CFU per gram) was conducted using SAS version 9.3 (SAS Institute, Cary, NC). Three independent replications were conducted for each target organism, and all microbial enumerations were done using duplicate samples and plating in duplicate. For the second baking study, the same procedure was followed, using shorter baking times and analyzing only single samples collected for each baking pull time.

The D- and z-value study was designed as a randomized complete block, with replications as blocks. Three independent replications (as represented by new inoculum preparation, different lots of inoculated flour, and separate days of preparation and water bath heating) were conducted utilizing three target dough temperatures, and all microbial enumerations were done in duplicate. Linear regression graphs were plotted using Microsoft Excel 2011 (Microsoft Corp., Redmond, WA), and the D- and z-values were calculated.

Cultures and inoculum preparation. Salmonella Senftenberg 775W (widely regarded as an unusually heat-resistant strain in high-moisture matrices), and the two Salmonella serovars (Typhimurium and Newport) most frequently associated with foodborne disease, which have not been reported to be as heat tolerant, were chosen as inoculants in the present study (4, 32). Salmonella enterica serovars Typhimurium (ATCC 14028), Newport (ATCC 6962), and Senftenberg (ATCC 43845) and Enterococcus faecium (ATCC 8459) were obtained from the ATCC (Manassas, VA). In a preliminary study, the thermal resistance of these three Salmonella ATCC cultures in a muffin batter matrix was compared to six additional Salmonella isolates that were collected from various dry food products (dry pet food, dry animal protein powder, and peanut butter; provided by Dr. Edward Richter, Richter International, Inc., Columbus, OH). The cocktail of ATCC strains used in the current study was more heat tolerant at 58°C than the six isolates from low-aw matrices (D-values of 32.8 and of 30.0 to 30.7 min, respectively).

All bacterial cultures were propagated in tryptic soy broth (Difco, BD, Sparks, MD) and were stored at ~80°C on protectant beads in glycerol (Pro-Lab Diagnostics Microbiobacterial Preservation System, Round Rock, TX). Working cultures were activated from the frozen state by transferring one bead into 10 ml of brain heart infusion broth (BHI; Oxoid Thermo Scientific, Basingstoke, Hampshire, UK) and incubating it for 24 h at 37°C. Individual cultures from BHI broth were then propagated as lawns on BHI agar plates for 24 h at 37°C (eight plates per organism). Lawns were harvested by washing each plate twice with 1 ml of 0.1% peptone water, using a disposable L-spreader to dislodge cells from the agar surface, and pipetting the resultant fluid into a 50-ml conical vial (providing ~16 ml of concentrated inoculum). When Salmonella serovars were used as a cocktail in the D- and z-value study, all three serovars were mixed in equal proportions before the flour inoculation procedure.

Inoculation of flour. Flour (400 g) was evenly spread in a shallow plastic tub (35.6 by 21.6 by 14.0 cm), which was placed within a large biohazard bag inside a Class II Type A2 biosafety
**Table 1. Dough recipe for hamburger buns**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread flour</td>
<td>700 g</td>
</tr>
<tr>
<td>Sugar (granulated)</td>
<td>84 g</td>
</tr>
<tr>
<td>Salt</td>
<td>14 g</td>
</tr>
<tr>
<td>Sodium stearoyl lactylate</td>
<td>3.5 g</td>
</tr>
<tr>
<td>Calcium propionate</td>
<td>1.8 g</td>
</tr>
<tr>
<td>Yeast food, no oxidants*</td>
<td>0.7 g</td>
</tr>
<tr>
<td>Shortening, all-purpose</td>
<td>42 g</td>
</tr>
<tr>
<td>Yeast, compressed</td>
<td>17 g</td>
</tr>
</tbody>
</table>

**Liquid**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid solution (1.6%)</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Water</td>
<td>478.5 ml</td>
</tr>
</tbody>
</table>

* Contains calcium sulfate, ammonium sulfate, wheat flour, and salt.

The dough was hand rolled into balls (71 ± 0.5 g) and was allowed to rest for 10 min at room temperature. Dough balls were then molded into compartments of two standard greased bun pans (eight buns per pan), which were placed into a proofing cabinet at 81% relative humidity and 43.3°C for approximately 60 min, or until the dough rose to a height of 32 mm above the top of the pan. Upon achieving this height, two samples were removed from one pan and were analyzed to establish target microbial populations in the postproof dough (immediately before baking). One pan of buns was then placed into the preheated oven for baking.

**Hamburger bun baking.** In preliminary studies, trained baking technicians established an optimum test oven (Amana model ACR3130BAW0, Whirlpool Corporation, Benton Harbor, MI) temperature of 218.3°C by comparing commercially obtained bun heating curves to those from the test oven. Further, four bun pull times for microbiological analyses in the first study were determined based on the minimum baking time that provided a near-acceptable quality (slightly underbaked) bun and the time that yielded buns of standard commercial bakery quality (9, 11, and 13 min of baking, and B+C).

The oven was preheated to 218.3°C for 30 min prior to use to ensure equilibration, which was confirmed using an eight-channel data logging system (USB-TG with MCC DAQ software, Measurement Computing, Norton, MA) and fine-gauge type T thermocouples (Omega Engineering Inc., Stamford, CT). One pan containing eight buns was used for each baking treatment replication for each target organism. Two of the eight numbered bun positions on the tray were randomly assigned to each of the four sampling times. Before placement of the pan into the oven, thermocouples were inserted into the geometric center (the cold spot) of the two buns that were designated to receive the B+C treatment, and one thermocouple was affixed to the side of the pan to monitor oven air temperature, with readings logged at 1-s intervals over the defined baking and cooling period. At each pull time, two buns were removed from the oven and analyzed as duplicate samples to determine surviving target organism population. One of the two buns designated for the B+C treatment was used to measure postbake bun pH and aw, and the other was analyzed for surviving target bacterial population. At each sampling time, the oven door was opened and the two buns were quickly removed using sanitized tongs, placed into separate stomacher bags containing 125 ml of prechilled (4°C) peptone water, and hand-massaged to minimize further thermal lethality of the treatment.

For the second hamburger bun baking study to determine breakpoint times, the bun dough was prepared, proofed, baked, and sampled as previously described. However, only one bun was sampled at regular time intervals during baking (every 30 s, starting at 4.0 min for Salmonella Newport and at 5.5 min for Salmonella Senftenberg and Salmonella Typhimurium; and every 45 s starting at 7.0 min for E. faecium). These sampling points were selected based on preliminary experiments and the results obtained from the first baking study.

**D- and z-value determinations.** Flour was inoculated with the three-serovar Salmonella cocktail or with E. faecium ATCC 8459 to achieve a target level of ~6 log CFU/g, and dough was prepared as described previously. Polyethylene Whirl-Pak filter bags (catalog no. 01-812-5, Thermo Fisher Scientific, Waltham, MA) were trimmed (13 by 20.5 cm), and edges were sealed using a FoodSaver Vacuum Sealing System (Sunbeam Products, Boca Raton, FL). Small lead fishing line weights were attached to the bottom edge of each sample bag to fully submerge samples in the temperature-controlled water bath and ensure adequate water...
circulation space around all bags. After proofing, 25 g of inoculated dough was transferred into the prepared bags, the dough inside the bag was pressed to a uniform thickness of ~0.5 cm, and the bags were vacuum sealed using the FoodSaver Vacuum Sealing System. The aforementioned data logging system was used to monitor temperatures of the hot water baths and the dough (measured by inserting probes in the center of sealed dough samples dedicated to temperature monitoring only). Once target time-temperature parameters were achieved in the dough, a sample bag was quickly removed from the water bath and was submerged into ice water for rapid cooling. Sampling time intervals at 55, 58, and 61°C were 25, 6, and 2 min, respectively, for Salmonella, and 85, 23, and 10 min, respectively, for E. faecium. Sampling time intervals for S. cerevisiae at 52, 55, and 58°C were 10, 2.5, and 0.5 min, respectively. A lower-temperature series was used for S. cerevisiae because a complete destruction of the yeast population was observed during the come-up time for 61°C, and D-value calculations were not possible.

Microbiological analyses. Duplicate samples of inoculated flour (10 g), preproof dough (10 g), postproof dough (71 g), and baked whole buns (71 g) in 125 ml of chilled peptone water were homogenized for 1 min in a lab blender (AES CHEMUNEX Smasher, bioMérieux Inc., Hazelwood, MO). Serial dilutions of each sample were spread plated in duplicate on selective and injury-recovery media. For selective plating, Salmonella serovars were enumerated on xylose lysine desoxycholate agar (XLD; Difco, BD) incubated at 35°C for 6 h, overlaid with Parafilm M (American National Can Group Inc., Chicago, IL), and analyzed within 30 min. For each replication, one bun from the B+C treatment was used to determine final product pH and aw. For pH measurements, 15 g of the interior bun crumb was added to 100 ml of deionized water, and the mixture was stirred continuously with a spatula until a stable pH reading (Oakton Instruments, Vernon Hills, IL) was obtained. Proximate analyses of postproof dough and bun samples during baking (9, 11, and 13 min, and B+C) were determined. Buns collected at each baking time were separated by hand into crumb and crust components and were stored in airtight plastic bags at 4°C. Samples were sent to the analytical laboratory at Kansas State University (Department of Animal Sciences and Industry) for the analyses.

RESULTS AND DISCUSSION

Oven selection and optimization to mimic industry baking. The wholesale baking industry typically uses ovens that utilize a combination of heat transfer mechanisms (radiant, convection, and conduction) to bake hamburger buns. These ovens are well suited for large-scale production because they accommodate up to several hundred full-sized pans at one time, with the large batch size leveling out the transfer of heat (232.2°C for 10 min being a common industry practice). In this investigation, a standard electric kitchen oven was chosen to bake hamburger buns. A series of preliminary hamburger bun baking trials were carried out at different temperatures and time periods to determine the optimum hamburger bun bake profile simulating the common baking industry practice. The buns baked at 218.3°C (425°F) for 13 min in the kitchen oven were found to be optimum, matching the baking industry’s hamburger bun end-use quality parameters such as crust color, appearance, size, texture, and internal crumb temperature.

For the D- and z-value study, heat-treated (and subsequently chilled) postproof dough samples (25 g) were diluted with 75 ml of chilled peptone water and were spread plated as previously described. D- and z-values were calculated as described by Michael et al. (30). D-values were calculated as absolute values of the inverse of the slopes of the regression lines of the log of viable bacterial cells versus time. The z-value for each target microorganism was calculated as the absolute value of the inverse of the slope of the regression line of the log of D-value versus temperature.

a_w, pH, and proximate analyses of hamburger buns. The a_w of the crumb and crust of baked buns were determined separately at 25°C, using an AquaLab Series 4TEV a_w meter (Decagon Devices, Inc., Pullman, WA). a_w were determined in the first study for pre- and postproof dough, along with separate crumb and crust measurements during baking (2, 4, 6, 8, 10, 12, and 14 min) and after baking (treatment B+C) buns. At each sampling point, and within 10 s of removal from the oven, the crumb and crust of buns were separated by hand, placed into separate a_w cups, sealed with Parafilm M (American National Can Group Inc., Chicago, IL), and analyzed within 30 min. For each replication, one bun from the B+C treatment was used to determine final product pH and a_w. For pH measurements, 15 g of the interior bun crumb was added to 100 ml of deionized water, and the mixture was stirred continuously with a spatula until a stable pH reading (Oakton Instruments, Vernon Hills, IL) was obtained. Proximate analyses of postproof dough and bun samples during baking (9, 11, and 13 min, and B+C) were determined. Buns collected at each baking time were separated by hand into crumb and crust components and were stored in airtight plastic bags at 4°C. Samples were sent to the analytical laboratory at Kansas State University (Department of Animal Sciences and Industry) for the analyses.

**TABLE 2. Proximate analyses of hamburger dough and bun during the 13 min of baking at 218.3°C oven temperature and after 30 min of postbake cooling**

<table>
<thead>
<tr>
<th>Sample</th>
<th>% moisture</th>
<th>% crude protein</th>
<th>% crude fat</th>
<th>% starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dough</td>
<td>46.94</td>
<td>8.48</td>
<td>3.79</td>
<td>40.75</td>
</tr>
<tr>
<td>9 min</td>
<td>43.71</td>
<td>7.91</td>
<td>2.47</td>
<td>39.9</td>
</tr>
<tr>
<td>11 min</td>
<td>43.58</td>
<td>7.4</td>
<td>3.12</td>
<td>38.05</td>
</tr>
<tr>
<td>13 min</td>
<td>24.61</td>
<td>10.63</td>
<td>2.93</td>
<td>49.59</td>
</tr>
<tr>
<td>B+C</td>
<td>44.38</td>
<td>7.78</td>
<td>3.2</td>
<td>39.55</td>
</tr>
</tbody>
</table>

a After 30 min of postbake cooling.
cooled for 30 min. These aw measurements of the breadcrumb and crust were similar to those reported by Czuchajowska et al. (11) for finished bread loaves. Percent moisture, crude protein, crude fat, and starch in buns during baking and after 30 min of cooling are presented in Table 2. Similar to aw results, moisture content of internal crumb was greater than that of crust during baking and after cooling. However, as expected, the observed percentages of protein, fat, and starch contents of crust were greater than those of crumb due to the corresponding moisture loss from the external crust during the baking process.

The 0.97 aw and 42.7% moisture of the crumb at the observed 5.46 pH of the finished bun suggests that Salmonella serovars can potentially grow inside the buns during storage at ambient temperature. Salmonella may continue to grow at aw as low as 0.94 (35). This suggests that achieving an adequate thermal treatment is vital during baking of hamburger buns to eliminate the food safety risk due to potential Salmonella contamination of raw bun ingredients. The large Salmonella population reductions determined in this study during short baking times that yielded unacceptably undercooked buns would indicate that there is a very low likelihood of any contamination surviving a commercial bun baking process, and the higher crumb aw of baked hamburger buns would only be a food safety risk if postbaking recontamination were to occur.

**Validation of the bun baking process.** This study sought to verify that a standard hamburger bun baking process delivers an adequate lethal effect to ensure destruction of pathogenic microorganisms that may be introduced via raw ingredients. The internal heating profile (average of 15 baking runs) of hamburger buns during 13 min of baking in a 218.3°C conventional oven, followed by 30 min of ambient temperature cooling, is shown in Figure 1. Crumb temperatures increased rapidly to ~100°C during the first 8 min of baking and remained at this temperature for the next 5 min while buns were in the oven and for ~1 min after removal from the oven. Internal bun temperatures decreased to 50°C during the first 9 min of ambient air-cooling. Population levels of Salmonella serovars, E. faecium 8459, and S. cerevisiae during proofing, baking, and cooling as enumerated on injury-recovery or nonselective and selective agars are presented in Figure 2A and 2B, respectively. Surviving Salmonella serovar populations were similar (P > 0.05) throughout the process at the specified sampling times, with no viable cells enumerated by direct plating on selective or injury-recovery media (detection limit of 0.22 log CFU/g) after the minimum 9 min of baking in the first baking study. All samples that tested negative by direct plating were also negative for all three Salmonella serovars after enrichment (indicating >6-log CFU/g reductions). In a similar study, Lathrop et al. (25) inoculated peanut butter cookie dough with a five-serovar cocktail of Salmonella (Tennessee FSL-R8-5221, Tornow FSL-R8-5222, Hartford FSL-R8-5223, Typhimurium FSL-W1-030, and Agona FSL-S5-517) and baked it at 177°C for up to 15 min. They reported that Salmonella serovars were detectable in the peanut butter cookies (after the enrichment) when baked for 14 min; however, Salmonella serovars were not detected in the cookies after 15 min of baking. The longer Salmonella serovar inactivation time observed by Lathrop et al. (25) compared with this study (9 min) is likely owing to differences in the food matrices used in the respective studies (peanut butter cookies versus bread dough; specifically, differences in fat content and aw values).

E. faecium demonstrated greater thermal resistance compared with the Salmonella serovars. Although the population levels of Salmonella serovars and E. faecium were similar (P > 0.05) until postproofing, survival of E. faecium on both selective and injury-recovery media was observed up to 11 min of baking compared with no detectable Salmonella serovars at 9 min of baking (Fig. 2A
and 2B). The S. cerevisiae population during the first baking study process was similar (P > 0.05) to that of Salmonella serovars (Fig. 2A); however, this reflects the similar population levels in prebaked dough (~7 log CFU/g) and the fact that no viable cells of either organism were detected after 9 min of baking. It is likely that yeast cells were inactivated to populations below the detection level within a short baking time.

Microbial counts on injury-recovery and selective media during baking to determine the breakpoints for the three Salmonella serovars and E. faecium are presented in Figure 3A and 3B, respectively. Low microbial counts, approaching the plating limit of detection on injury-recovery media, were observed up to 7.5, 6.0, 8.5, and 10.0 min for Salmonella Senftenberg (0.5 log CFU/g), Salmonella Newport (0.6 log CFU/g), Salmonella Typhimurium (0.85 log CFU/g), and E. faecium (1.3 log CFU/g), respectively (Fig. 3A). Counts on selective media decreased to <0.49 log CFU/g (detection limit) by 5.5 min of baking for all Salmonella serovars and 10.0 min for E. faecium (Fig. 3B). For validation of commercial food manufacturing processes as preventive control steps, a 5-log reduction of a specified pathogen population is often targeted (8, 37, 38). Microbial reductions of ≥5 log CFU/g on the injury-recovery media were achieved by 6.0, 5.0, 5.0, and 7.75 min for Salmonella serovars Senftenberg, Newport, and Typhimurium, and for E. faecium, respectively. Therefore, a typical commercial hamburger bun baking process, as depicted in the current study, should achieve a 5-log-cycle reduction in Salmonella serovar population after 6 min of baking at an oven temperature that results in an internal crumb temperature of ~100°C within 8 min of baking. Moreover, the longer baking time required to reduce the E. faecium 8459 population by ≥5 log CFU/g suggests that this culture can be used as a conservative, nonpathogenic surrogate to validate commercial hamburger bun baking processes.

Although the time required to achieve a 5-log CFU/g reduction in the buns during baking was slightly longer for Salmonella Senftenberg (6.0 min) compared with Salmonella Typhimurium (5.0 min), the occasional detection of very low levels of viable cells of each serotype using an enrichment protocol revealed that Salmonella Typhimurium could be detected at up to 8.5 min of baking, whereas Salmonella Senftenberg could only be detected up to 7.5 min. In a heat inactivation study of Salmonella Senftenberg 775W and Salmonella Typhimurium in milk chocolate having a low αw, Goepfert and Biggie (17) reported that Salmonella Typhimurium had much greater D-values (D70 = 1,050, D80 = 222, and D90 = 78 min) compared with the D-values of Salmonella Senftenberg 775W (D70 = 480, D80 = 144, and D90 = 42 min). Several researchers have documented the uniquely high thermal tolerance of Salmonella Senftenberg 775W in high moisture conditions. In the current dough baking study, the 775W strain did not show this high thermal tolerance, even though it was expected due to the relatively high αw (0.97) of the dough and bun crumb. The design of the current study does not allow a determination as to why 775W did not demonstrate its notable high-moisture thermal resistance; however, it is possible that the proximate composition and included ingredients (e.g., ascorbic acid, sodium stearoyl lactylate, salt) of the dough system, and the interaction of rapidly metabolizing yeast and yeast by-products during dough proofing, could significantly alter the thermal characteristics of Salmonella Senftenberg 775W. A potential impacting factor, and one that seems to have not been previously investigated, is the physiological impact of desiccation of cells during inoculation, drying, and 48-h storage of flour, followed by rapid rehydration of these cells during doughmaking, with subsequent exposure to growth temperatures (i.e., 25 to 43°C) for approximately 90 min prior to rapid temperature increases during the initial phases of baking. With the possibility of these contributing factors in mind, and until further research can be conducted on diverse bakery products, it is important that thermal process lethality be determined on a product by product basis (or on product groups that are compositionally similar and manufactured using similar parameters).

**Determination of D- and z-values in bun dough.** Whereas Salmonella Senftenberg 775W is generally regarded as one of the most thermally resistant Salmonella strain, a cocktail of relevant Salmonella serovars is often used to generate D- and z-values, as it better represents the range of contamination possibilities in the industry (14). Furthermore, some Salmonella strains react to heat differently in different food matrices. For example, in most experiments with eggs, Salmonella Enteritidis shows greater heat resistance than Salmonella Typhimurium, whereas in molten...
milk chocolate, *Salmonella* Typhimurium had greater heat tolerance compared with *Salmonella Senftenberg* 775W (14).

After thermal inactivation of *Salmonella* (three-serovar cocktails), viable cells were enumerated on selective and injury-recovery media (Fig. 4A and 4B, respectively); *E. faecium* populations on selective and injury-recovery media are presented in Figure 5A and 5B, respectively. For *Salmonella*, \( D_{55} \), \( D_{58} \), and \( D_{61} \)-values were 21.30 ± 2.61 and 28.64 ± 5.19, 7.53 ± 0.61 and 7.61 ± 0.61, and 2.29 ± 0.21 and 3.14 ± 0.32 min on selective and injury-recovery media, respectively, and \( D_{55} \), \( D_{58} \), and \( D_{61} \)-values of *E. faecium* were 87.21 ± 4.74 and 133.33 ± 0.00, 45.33 ± 6.79 and 55.67 ± 9.00, and 6.14 ± 0.47 and 14.72 ± 4.11 min for *E. faecium* on selective and injury-recovery media, respectively (Table 3). The log \( D \)-values versus temperature (°C) curves for *Salmonella* serovars, *E. faecium*, and *S. cerevisiae* on injury-recovery and selective media are presented in Figure 6. The calculated \( z \)-values (on selective and injury-recovery media, respectively) were 6.22 ± 0.32 and 6.58 ± 0.96 °C for *Salmonella* and 5.20 ± 0.05 and 6.25 ± 0.80 °C for *E. faecium*.

The greater \( D \)- and \( z \)-values (\( P \leq 0.05 \)) of *Salmonella* and *E. faecium* on the injury-recovery media compared to the corresponding \( D \)-values on the selective media confirm that a subpopulation of injured bacterial cells is able to survive heating in dough at the three temperatures studied in the current study. Injured pathogenic cells can recover when

![FIGURE 4. Thermal inactivation of *Salmonella* serovars at 55, 58, and 61°C in hamburger bun dough: (A) viable cells enumerated on xylose lysine desoxycholate (XLD) agar; (B) viable cells enumerated on brain heart infusion (BHI) agar with XLD agar overlay.](image)

![FIGURE 5. Thermal inactivation of *Enterococcus faecium* at 55, 58, and 61°C in hamburger bun dough: (A) viable cells enumerated on m-Enterococcus (m-E) agar; (B) viable cells enumerated on brain heart infusion (BHI) agar with m-E agar overlay.](image)

**TABLE 3.** \( D \)-values and \( z \)-values with standard errors of a three-serovar *Salmonella* cocktail, *Enterococcus faecium* ATCC 8459, and *Saccharomyces cerevisiae* during heating of hamburger bun dough

<table>
<thead>
<tr>
<th>Item</th>
<th>Salmonella serovars</th>
<th><em>E. faecium</em></th>
<th><em>S. cerevisiae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BHI/XLD</td>
<td>XLD</td>
<td>BHI/mEA</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>55</td>
<td>28.64 ± 5.19 A</td>
<td>21.30 ± 2.61 B</td>
<td>133.33 ± 0.00 A</td>
</tr>
<tr>
<td>58</td>
<td>7.61 ± 0.61 A</td>
<td>7.53 ± 0.61 A</td>
<td>55.67 ± 9.00 A</td>
</tr>
<tr>
<td>61</td>
<td>3.14 ± 0.32 A</td>
<td>2.29 ± 0.21 A</td>
<td>14.72 ± 4.11 A</td>
</tr>
<tr>
<td>( z )-value (°C)</td>
<td>6.58 ± 0.96 A</td>
<td>6.22 ± 0.32 B</td>
<td>6.25 ± 0.80 A</td>
</tr>
</tbody>
</table>

\( a \) \( D \)-values are presented in minutes. BHI/XLD, injury-recovery media, brain heart infusion agar with xylose lysine desoxycholate agar overlay; XLD, xylose lysine desoxycholate agar; BHI/mEA, injury-recovery media, BHI agar with m-Enterococcus agar overlay; mEA, m-Enterococcus agar; PDA, potato dextrose agar plus chloramphenicol; ND, not determined.

\( b \) Values within a row and under respective microorganism followed by different letters are different (\( P \leq 0.05 \)).
favorable environmental conditions are available and may pose a foodborne illness risk.

McCormick et al. (29) reported the D-value of Salmonella Typhimurium in low-fat ready-to-eat turkey bologna as 4.63 and 0.95 min at 57 and 60°C, respectively. They also reported the z-value of Salmonella Typhimurium as 5.56°C. D- and z-values reported by McCormick et al. (29) are different from values determined in this study because of differences in the composition of bologna and bun dough, along with differences in Salmonella strains used. Bianchini et al. (6) reported that, during extrusion of balanced carbohydrate-protein meal, the minimum temperature required to achieve a 5-log reduction for a five-screw extrusion of Salmonella enterica (Branderup NVSL 96-12528, Oranienburg NVSL 96-12608, Typhimurium ATCC 14028, Enteritidis IV/NVSL 94-13062, and Heidelberg/Sheldon 3347-1) was 60.6°C compared with 73.7°C for E. faecium NRRL B-2354. The authors stated that E. faecium can be used for in-plant thermal inactivation validation studies for Salmonella during extrusion because the inactivation temperature for E. faecium was higher compared to that for Salmonella.

Populations of S. cerevisiae versus time during thermal inactivation at 52, 55, and 58°C are presented in Figure 7. The D-values of S. cerevisiae in hamburger bun dough were 18.73, 5.67, and 1.03 min at 52, 55, and 58°C, respectively, and the z-value of S. cerevisiae was 4.74 (Table 3). These D- and z-values for S. cerevisiae were considerably lower than those for Salmonella and E. faecium. In comparison, the D50-values of the three microorganisms (7.61 ± 0.61 for Salmonella, 55.67 ± 9.0 for E. faecium, and 1.03 ± 0.21 for S. cerevisiae) point out the challenges for using E. faecium or S. cerevisiae as surrogates for Salmonella during baking validation studies. In such instances, the differences in D-values should be taken into consideration. López-Malo et al. (26) reported that the D-values of S. cerevisiae in Sabouraud glucose 2% broth were 18.3, 4.8, and 2.7 at 50, 52.5, and 55°C, respectively. The D-values reported by López-Malo et al. (26) were lower than those reported in this study because of the differences in the heating medium (liquid versus solid matrix). However, these researchers reported z-values (4.2°C) similar to those reported in the current study, indicating that the thermal sensitivity of S. cerevisiae to the change in the temperature was similar in Sabouraud broth and hamburger buns.

The present study demonstrated that the typical hamburger bun baking process will eliminate Salmonella serovar populations (>6-log CFU/g reductions) utilizing oven temperatures ≥218.3°C (425°F) and baking for at least 9 min. Also, considering the internal temperature versus time recorded in this study, it is clear that all the Salmonella cells were destroyed in the buns within 9 min, prior to the optimum bake time (as determined and utilized in these studies). The thermal resistance of S. cerevisiae in hamburger bun dough is substantially lower than that of Salmonella, and therefore, S. cerevisiae is not recommended as a surrogate for Salmonella in thermal inactivation studies. The greater survival of E. faecium during bun baking and the higher D-values of E. faecium compared with those of Salmonella suggest that E. faecium can be used as a conservative surrogate for Salmonella for baking studies in processing facilities.

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