Thermal Tolerance of O157 and non-O157 Shiga Toxigenic Strains of *Escherichia coli*, *Salmonella*, and Potential Pathogen Surrogates, in Frankfurter Batter and Ground Beef of Varying Fat Levels

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ABSTRACT

The non-O157 Shiga toxigenic *Escherichia coli* (STEC) serogroups most commonly associated with illness are O26, O45, O103, O111, O121, and O145. We compared the thermal tolerance (D$_{55\,C}$) of three or more strains of each of these six non-O157 STEC serogroups with five strains of O157:H7 STEC in 7% fat ground beef. D$_{55\,C}$ was also determined for at least one heat-tolerant STEC strain per serogroup in 15 and 27% fat ground beef. D$_{55\,C}$ of single-pathogen cocktails of O157 and non-O157 STEC, *Salmonella*, and potential pathogen surrogates, *Pediococcus acidilactici* and *Staphylococcus carnosus*, was determined in 7, 15, and 27% fat ground beef and in frankfurter batter. Samples (25 g) were heated for up to 120 min at 55°C, survivors were enumerated, and log CFU per gram was plotted versus time. There were significant differences in D$_{55\,C}$ across all STEC strains heated in 7% fat ground beef (P < 0.05), but no non-O157 STEC strain had D$_{55\,C}$ greater than the range observed for O157 STEC. D$_{55\,C}$ was significantly different for strains within serogroups O45, O145, and O157 (P < 0.05). D$_{55\,C}$ for non-O157 STEC strains in 15 and 27% fat ground beef were less than or equal to the range of D$_{55\,C}$ for O157. D$_{55\,C}$ for pathogen cocktails was not significantly different when measured in 7, 15, and 27% fat ground beef (P ≥ 0.05). D$_{55\,C}$ of *Salmonella* in frankfurter batter was significantly less than for O157 and non-O157 STEC (P < 0.05). Thermal tolerance of pathogen cocktails in ground beef (7, 15, or 27% fat) and frankfurter batter was significantly less than for potential pathogen surrogates (P < 0.05). Results suggest that thermal processes in beef validated against *E. coli* O157:H7 have adequate lethality against non-O157 STEC, that thermal processes that target *Salmonella* destruction may not be adequate against STEC in some situations, and that the use of pathogen surrogates *P. acidilactici* and *S. carnosus* to validate thermal processing interventions in ground beef and frankfurter batter would be of limited utility to processors.

In 1999, the U.S. Department of Agriculture (USDA) issued regulations that required establishments producing ready-to-eat roast beef, cooked beef, and certain ready-to-eat poultry products to meet lethality performance standards for the reduction of *Salmonella* (40). To help processors achieve the lethality standards and to establish critical limits for cooked beef roasts, the USDA published Appendix A: Compliance Guidelines for Meeting Lethality Performance Standards for Certain Meat and Poultry Products (henceforth Appendix A), which provides internal temperature and time combinations considered to be validated for achieving the mandatory 5.0-log reduction of *Salmonella* in beef products (41). In addition, the USDA has set a 5.0-log lethality-reduction standard for *Escherichia coli* O157:H7 in dry fermented sausage (30), and a similar reduction standard is assumed for other ready-to-eat beef products (43). *E. coli* O157:H7 first gained national attention in 1982 when it was isolated from stool samples from victims of a foodborne illness outbreak linked to contaminated hamburgers (35). In 2011, Scallan et al. (37) reported that there were an estimated 63,153 cases of illness each year in the United States linked to Shiga toxin–producing *E. coli* O157:H7 (O157 STEC), and they noted the emergence of non-O157 STEC, estimating an additional 112,752 cases of illness each year linked to this pathogen group. Among non-O157 STEC, six serogroups were most commonly reported to the U.S. Centers for Disease Control and Prevention’s FoodNet foodborne illness surveillance system during 2000 to 2010: O26 (26%), O103 (22%), O111 (19%), O121 (6%), O45 (5%), and O145 (4%) (14). Collectively, the emergence of STEC has been suggested to be one of the largest food safety impacts ever on the beef industry (9). On 12 June 2012, the USDA added routine verification testing of the six major non-O157 STEC serogroups to routine testing for *E. coli* O157:H7 in raw beef manufacturing trimmings (44). This change is supported by the historical
link of O157 STEC to beef products (47), the emergence of non-O157 STEC (10), and the relatively high level of mortality associated with these pathogens. Additionally, processors of beef products were required to reassess their hazard analysis and critical control point (HACCP) plans to determine whether non-O157 STEC were “reasonably likely to occur” and, if so, to implement validated interventions against this group of pathogens (46).

Since the late 1990s, studies have evaluated the thermal tolerance of Salmonella and E. coli O157:H7 in beef products (4, 16, 17, 19, 21, 23, 28, 29, 31–33, 38). However, limited research is available on the thermal tolerance of non-O157 STEC under similar conditions. A recent study compared the thermal tolerance of 19 non-O157 STEC strains to that of five O157:H7 strains in brain heart infusion (BHI) broth at 54.6, 58.0, or 63.6°C (48). Strain-to-strain variation in D- and z-values was observed across the strains, with no significant variation between O157 and non-O157 STEC when compared as groups. The authors concluded that, whereas the results suggested that thermal processing interventions that target O157 STEC may have adequate lethality against non-O157 STEC, it can be difficult to predict pathogen behavior in meat systems using data obtained in broth.

In 2012, a USDA risk assessment study evaluated whether traditional and accepted raw ground beef cooking methodology for O157 STEC would be effective against non-O157 STEC (45). The USDA cited two studies in the risk assessment, the works of Luchansky et al. and Duffy et al. (13, 25). The work of Luchansky and colleagues (25) examined the thermal resistance of O157 and non-O157 STEC inoculated into nonintent beef steaks; they observed similar heat tolerances for strains of E. coli O157:H7 and a pooled composite of non-O157 STEC strains (serogroups O45, O103, O111, O121, and O145). Duffy et al. (13) determined D55°C values for strains of E. coli O157:H7 and E. coli O26 inoculated into minced (ground) beef and found that the values were not significantly different. Whereas these studies cited in the USDA risk assessment study evaluated a similar thermal tolerance for O157 and non-O157 STEC, the USDA concluded that additional data were needed on the decimal reduction times for these pathogens in meat (45). Subsequently, Luchansky and others (27) evaluated the thermal inactivation of single strains from non-O157 STEC serogroups compared with that of one strain of O157:H7 in ground beef (7 or 30% fat) wafers; they concluded that cooking times and temperatures effective for inactivating a serotype O157:H7 strain were equally effective against the representative non-O157 STEC strains studied. The authors noted, however, that further studies were needed to comparatively evaluate the thermal tolerance of additional STEC strains.

The implementation of HACCP can be challenging for small and very small meat processing establishments. In particular, small establishments are often challenged to validate critical limits for HACCP plan critical control points. Appendix A includes validated time and temperature combinations for cooking (41), but there are few options available for establishments not willing or able to use Appendix A. Our laboratory has proposed and validated a method for in-plant validation of jerky processing lethality using a lactic acid bacterium as a pathogen surrogate (6, 7, 11). The use of a surrogate that mimics pathogen behavior could be useful for in-plant validation of thermal processes for other beef products.

The goals of the present study were, therefore, (i) to compare the thermal tolerance of representative strains from prevalent non-O157 STEC serogroups (=three strains per serogroup) to the thermal tolerance of heat-resistant strains of O157 STEC in 7, 15, and 27% fat ground beef; (ii) to evaluate the effect of fat level on the survival of single STEC strains during heating; and (iii) to compare the thermal tolerance of single-pathogen cocktails of Salmonella, E. coli O157:H7, and non-O157 STEC to the thermal tolerance of potential pathogen surrogates in ground beef of varying fat levels and in frankfurter batter.

**MATERIALS AND METHODS**

**Strain selection and maintenance.** The heat tolerance of 19 individual strains of non-O157 STEC was compared with that of five individual E. coli O157:H7 strains in 7% fat ground beef. Strain details are given in Table 1. E. coli O157:H7 strains included four beef trim isolates UWIL-BTI-1, UWIL-BTI-8, UWIL-BTI-9, UWIL-BTI-11, and an outbreak-linked strain from the American Type Culture Collection (ATCC 43895, Manassas, VA). The beef trim isolates were part of a collection of 110 E. coli O157:H7 isolates that were genetically characterized, and a subset of this collection, 23 genetically diverse strains, was screened for tolerance to cold, acid, and heat (15). Strains chosen for this research exhibited increased thermal tolerance (D34.4°C) in beef extract broth plus tryptone (pH 7.05). ATCC 43895 was implicated in an outbreak linked to hamburger and was the first sequenced E. coli O157:H7 strain (34). Three clinical isolates of each of the six epidemiologically significant non-O157 serogroups (O26, O45, O103, O111, O121, and O145) were used in single-strain trials (Table 1). One additional strain of serogroup O45, a clinical isolate from a local foodborne illness outbreak linked to contaminated bear-meat sausage and obtained from the Wisconsin State Laboratory of Hygiene, was also included. Individual O157 and non-O157 STEC strains displaying heat tolerance in 7% fat ground beef were used for subsequent single-strain experiments in 15 and 27% fat meat, at least one strain per serogroup (Table 1).

Heat tolerance for single-pathogen cocktails of O157 STEC (three strains), non-O157 STEC (seven strains), and Salmonella (four strains) was determined in 7, 15, and 27% fat ground beef and commercial frankfurter batter. Cocktail strains of O157 and non-O157 STEC were chosen based on relative heat tolerance in the single-strain experiments. The Salmonella cocktail was composed of one strain each of Salmonella Enteritidis (chicken ovary isolate, originally acquired from the New York State Department of Health, Albany, NY), Salmonella Typhimurium (a clinical isolate from the Wisconsin Laboratory of Hygiene), and Salmonella Infantis and Salmonella Hadar, the origins of which are unknown, and all of these strains have routinely been used in our laboratory for thermal processing studies (5, 6).

Stock cultures of each pathogen strain were maintained in BHI broth (pH 7.0, Difco, BD, Sparks, MD) containing 10% (vol/vol) glycerol (Fisher Scientific, Itasca, IL) and were kept frozen at −20°C. Working cultures were prepared from stock cultures by streaking for isolation on modified Levine’s eosin methylene blue (m-LEMB) agar, followed by incubation for 24 h at 37°C. m-LEMB was prepared from lactose-free EMB agar (Difco) with the...
addition of 10g/liter of D-sorbitol (Fisher) and 5g/liter NaCl (Fisher). The purity and identity of cultures were assessed by Gram reaction, cell and colony morphology, and biochemical identification (API 20E, bioMérieux, Durham, NC). Prepared working culture plates were stored at 4°C for up to 1 month.

In addition to the pathogen strains, two commercial cultures were evaluated as potential pathogen surrogates in experiments with 7, 15, and 27% fat ground beef and commercial frankfurter batter: *Pediococcus acidilactici* (Saga 200, Kerry BioScience, Rochester, MN) and *Staphylococcus carnosus* (CS-299, CHR Hansen, Milwaukee, WI). The surrogate cultures were stored in their commercial form at −20°C.

**Meat systems.** Thermal tolerance experiments were carried out in ground beef of 7, 15, and 27% fat or in commercial frankfurter batter. The fat content of the retail ground beef was determined by a commercial testing laboratory following standard USDA procedures (42) and was found to be 5.41, 14.55, and 24.05% for retail ground beef labeled 7, 15, and 27%, respectively (n = 12 samples composited for each lipid determination). Commercial frankfurter batter (all-beef) was composed of protein (9%) and fat (27%) and contained added salt (1.4%), sodium phosphate (0.2%), potassium lactate (1.2%), sodium diacetate (0.12%), and sodium nitrite (140 ppm).

Quality of the ground beef was assessed prior to each experiment by measuring the pH of beef-water slurry (1:5), as described by Wiegand et al. (50), and by measuring aerobic plate count (APC). APC was determined using Petrifilm aerobic count plates (3M Microbiology, St. Paul, MN). Ground beef was stored at 4°C and used for experiments within 2 days of purchase or was held frozen (−20°C) for longer periods of time. If frozen, ground beef was thawed at 4°C for 36 h prior to experiments. Experimental data were used only when meat had APC ≤ 4 log CFU/g and pH ≤ 5.9. For each set of experiments with commercial frankfurter batter, fresh batter was obtained on the day of manufacture and was used for experiments that day.

**Inoculum preparation.** To prepare inocula for individual STEC strains (O157:H7 and non-O157 STEC), a single colony was obtained from a working culture plate, streaked for lawn growth on BHI agar (Difco), and incubated at 37°C for 24 h. The lawn of growth was scraped from the plate using a sterile inoculating loop and was transferred to 9 ml of Butterfield’s phosphate diluent (BPD; Nelson Jameson, Marshfield, WI) to produce an inoculum of ~10^9 CFU/ml. The inoculum thus produced was vortexed for 2 min to suspend the cells and was used within 20 min.

Inocula for cocktail experiments (O157 and non-O157 STEC, *Salmonella*) were prepared by streaking individual strains for lawn
growth as described above. The lawn of growth for strains of each pathogen was then combined in 9 ml of BPD to produce a single-pathogen cocktail (three-strain O157 STEC, seven-strain non-O157 STEC, four-strain Salmonella) inoculum of \(~10^6\) CFU/ml. The inoculum thus produced was vortexed for 2 min to suspend the cells and was used within 20 min. To prepare the surrogate inoculum, 0.4 g of each frozen commercial culture was added to 5 ml of BPD and was vortexed for 2 min to suspend and thaw the culture. The surrogate inoculum was prepared before the start of each experiment and was used within 20 min of preparation. The concentration of the surrogate inoculum prepared in this way was found to be \(~10^6\) CFU/ml. At least three independent trials, each beginning with an isolated colony (pathogen experiments) or freshly prepared surrogate culture, were completed for each inoculum-meat system combination.

**Inoculation.** Meat for single-strain heat tolerance assays (7, 15, 27% fat ground beef) was inoculated as follows: samples of ground beef were prepared by weighing 25 g of meat into a Whirl-Pak bag (7.5 by 12.5 cm; Nasco, Fort Atkinson, WI); each bag of meat was inoculated (0.1 ml) and massaged for 30 s to evenly distribute the cells in the sample; the bags were flattened (7.5 cm by 7.5 cm by 3 mm), taking care to minimize entrapped air; and then the bags were heat sealed (Ziploc V101, S.C. Johnson, Racine, WI). Once all bags were sealed, they were transferred to a preheated water bath for thermal tolerance experiments. Nine bags were prepared for each experiment; this total included one uninoculated control. The uninoculated control was prepared from 25 g of meat, as described, with the addition of 0.1 ml of BPD in place of inoculum. The flattened sample bag containing the control was not sealed. The final concentration of inoculum in meat was determined at the start of each experiment and was found to be \(~10^5\) CFU/g (\(n = 130\)).

Meat (7, 15, 27% fat ground beef or commercial frankfurter batter) for experiments with pathogen cocktails or surrogates was inoculated using a modification of the protocol for single-strain experiments: a total of 1.0 ml of inoculum was added to 250 g of ground beef or commercial frankfurter batter in a sterile bag, and the mixture was massaged for 2 min. Preliminary results indicated that this method evenly dispersed inoculum throughout the meat matrix. After inoculation, aliquots of meat (25 g) were placed into WhirlPak bags, which were flattened (7.5 cm by 7.5 cm by 3 mm), sealed, and used in thermal tolerance experiments. Eight bags of meat were prepared for each experiment, plus one uninoculated control. Final concentration of inoculum cocktail in meat was \(~10^5\) CFU/g.

**Heat treatment and enumeration of surviving inocula.** Nine bags of meat were placed vertically in a wire-mesh basket and were fully submerged in a preheated circulating water bath (Phoenix II C40P, Thermo Scientific, Asheville, NC) maintained at 55°C. The water bath temperature was monitored using a data logger with digital readout (OM-CP-QUADTEMP-2000, Omega Engineering, Stamford, CT) equipped with dual T-type thermocouples (Omega Engineering) accurate to \(\pm 0.1\) C. One thermocouple at all times recorded the temperature of the meat (uninoculated control), while the other recorded the temperature of the water bath. Water circulated freely around all bags in the water bath, and the water level was at all times \(\approx 2\) cm above the top of the bags.

Prior to experiments with single-strain inocula, experiments were conducted to determine the heating pattern in meat bags in the water bath. First, to determine the cold spot in a bag of meat, thermocouples were placed at three different levels in three bags of meat, and the meat was heated at 55°C for 60 min. No difference was found in the temperature recorded by the thermocouples placed at various depths (data not shown). Therefore, subsequent temperature measurements were carried out by placing the thermocouple at the center of each meat bag. The effect of bag placement in the water bath on heating was determined. Nine bags of meat (25 g each) were prepared as described, 0.1 ml of BPD (25°C) was added to each bag as the “inoculum,” and thermocouples were placed in the center of the left-most, right-most, and center bags of meat. The bags were flattened, and all nine bags were placed in a preheated water bath (55°C). The time that it took the meat in the three bags containing thermocouples to reach the thermal death temperature (\(T_{\text{DT}}\), 55°C) was recorded. It took, on average, 67 s for the right-most and left-most bags to reach the \(T_{\text{DT}}\), whereas the center-most bag took approximately 76 s (\(n = 5\) trials). As a result, for all pathogen or surrogate experiments, the uninoculated control with thermocouple in place was positioned in the center of the line of meat bags and the experimental clock was started once the center-most bag reached the \(T_{\text{DT}}\), approximately 76 s. After the initial come-up time, the \(T_{\text{DT}}\) was consistently maintained, 55.0°C \(\pm 0.1\) C.

One bag of meat was removed from the water bath at time 0 and at regular intervals thereafter. A random number generator was used to determine the order in which bags were removed for a given set of experiments. In experiments with individual STEC strains, sampling intervals were 0, 5, 10, 20, 30, 40, 50, 60, and 75 min for 7% fat ground beef, and 0, 10, 20, 30, 40, 60, 80, and 100 min for 15 and 27% fat ground beef. Longer sampling intervals were chosen for higher fat ground beef in order to account for any potential protective effect from the fat. In experiments with pathogen cocktails and surrogates, sampling intervals were 0, 10, 20, 40, 60, 80, 100, and 120 min for ground beef and frankfurter batter.

The level of surviving inocula was determined postheating. Each bag of meat removed from the water bath was immediately chilled in ice-water slush for 4 min to halt the cooking process and allow the temperature of the meat to fall to \(\leq 4\) C. Once cooled, each bag of meat was removed from ice, surface sanitized with 70% ethanol, and aseptically opened with ethanol-sanitized scissors. The surface-sanitized bag, containing the heated meat, was everted into a WhirlPak filter bag (15 by 23 cm; Nasco), 99 ml of BPD was added, and the mixture was stomached (Seward Model 400 Stomacher; London, UK) at medium speed to recover inoculum cells. Preliminary studies compared the impact of stomaching times (30 s or 2 min) and the addition of a surfactant (TWEEN 20, Fisher) on inoculum recovery. Results showed that stomaching time did not significantly affect the recovery of pathogen from 7% fat meat (\(P \geq 0.05\); data not shown), whereas recovery of inoculum from higher fat meat was improved with an extended stomaching time (2 min; data not shown). The addition of TWEEN 20 to heat-treated 27% fat ground beef did not improve the recovery of the inoculum (data not shown). As a result, 7% fat ground beef was stomached for 30 s; higher fat ground beef (15 or 27%) and frankfurter batter were stomached for 2 min.

The stomached ground beef or frankfurter slurry was serially diluted in BPD, and 0.1 ml of each appropriate dilution was spread plated on m-LEMB for STEC (31) and Salmonella (6), on deMan Rogosa Sharpe agar (MRS; Difco) for P. acidilactici, and on BHI agar with added 5% NaCl (wt/vol; Fisher) for S. carnosus. mLEMB plates were incubated aerobically at 37°C for 24 h, and typical colonies (white to colorless for O157 STEC, purple-centered with white edges for non-O157 STEC, pink to purple with green metallic sheen for Salmonella) were enumerated. MRS and BHI agar plus salt plates were incubated at 37°C for 48 h, and
**RESULTS AND DISCUSSION**

The $D_{55\text{C}}$-value for individual non-O157 STEC strains in 7% fat ground beef ranged from 14.43 min for one strain of O26 (26C) to 29.26 min for one strain of O45 (45D) (Table 2). The range of $D_{55\text{C}}$-values in 7% ground beef for O157 STEC ranged from 17.65 min for a beef trim isolate (157-8) to 25.40 min for the reference strain 43895 (157-A).

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**TABLE 2. Thermal tolerance for individual strains of O157:H7 and non-O157 STEC in ground beef of several fat levels**

<table>
<thead>
<tr>
<th>Strain</th>
<th>7% fat</th>
<th>15% fat</th>
<th>27% fat</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$D_{55\text{C}}$ (min)</td>
<td>$D_{55\text{C}}$ (min)</td>
<td>$D_{55\text{C}}$ (min)</td>
<td></td>
</tr>
<tr>
<td>26A</td>
<td>17.17 (2.40)</td>
<td>17.77 (1.94)</td>
<td>18.43 (0.40)</td>
<td>0.112</td>
</tr>
<tr>
<td>26B</td>
<td>21.52 (3.51)</td>
<td>21.29 (1.88)</td>
<td>19.91 (0.61)</td>
<td>0.032</td>
</tr>
<tr>
<td>26C</td>
<td>14.43 (0.29)</td>
<td>19.28 (2.28)</td>
<td>23.39 (1.85)</td>
<td>0.099</td>
</tr>
<tr>
<td>45A</td>
<td>14.71 (0.44)</td>
<td>20.55 (1.31)</td>
<td>22.34 (1.88)</td>
<td>0.086</td>
</tr>
<tr>
<td>45B</td>
<td>24.83 (3.53)</td>
<td>19.28 (2.28)</td>
<td>23.39 (1.85)</td>
<td>0.099</td>
</tr>
<tr>
<td>45C</td>
<td>19.73 (0.13)</td>
<td>20.55 (1.31)</td>
<td>22.34 (1.88)</td>
<td>0.086</td>
</tr>
<tr>
<td>45D</td>
<td>29.26 (2.96)</td>
<td>22.87 (2.57)</td>
<td>21.07 (2.02)</td>
<td>0.000</td>
</tr>
<tr>
<td>103A</td>
<td>18.23 (0.92)</td>
<td>20.77 (1.78)</td>
<td>19.19 (0.79)</td>
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</tr>
<tr>
<td>103B</td>
<td>19.89 (0.67)</td>
<td>22.87 (2.57)</td>
<td>20.77 (1.78)</td>
<td>0.053</td>
</tr>
<tr>
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<td>19.28 (2.28)</td>
<td>23.39 (1.85)</td>
<td>0.099</td>
</tr>
<tr>
<td>111A</td>
<td>20.24 (2.05)</td>
<td>20.55 (1.31)</td>
<td>22.34 (1.88)</td>
<td>0.086</td>
</tr>
<tr>
<td>111B</td>
<td>16.75 (3.31)</td>
<td>19.70 (0.97)</td>
<td>19.19 (0.79)</td>
<td>0.053</td>
</tr>
<tr>
<td>111C</td>
<td>19.70 (0.97)</td>
<td>20.77 (1.78)</td>
<td>19.19 (0.79)</td>
<td>0.053</td>
</tr>
<tr>
<td>121A</td>
<td>23.79 (1.68)</td>
<td>19.20 (0.52)</td>
<td>18.94 (0.64)</td>
<td>0.000</td>
</tr>
<tr>
<td>121B</td>
<td>22.67 (1.87)</td>
<td>24.47 (2.90)</td>
<td>23.41 (0.58)</td>
<td>0.572</td>
</tr>
<tr>
<td>121C</td>
<td>21.82 (2.54)</td>
<td>22.36 (2.86)</td>
<td>23.45 (1.24)</td>
<td>0.810</td>
</tr>
<tr>
<td>145A</td>
<td>14.79 (1.22)</td>
<td>22.87 (2.57)</td>
<td>20.77 (1.78)</td>
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<td>145B</td>
<td>26.02 (2.41)</td>
<td>19.20 (0.52)</td>
<td>18.94 (0.64)</td>
<td>0.000</td>
</tr>
<tr>
<td>145C</td>
<td>19.09 (1.85)</td>
<td>24.47 (2.90)</td>
<td>23.41 (0.58)</td>
<td>0.572</td>
</tr>
<tr>
<td>157-A</td>
<td>25.40 (3.65)</td>
<td>22.36 (2.86)</td>
<td>23.45 (1.24)</td>
<td>0.810</td>
</tr>
<tr>
<td>157-B</td>
<td>17.65 (2.14)</td>
<td>22.36 (2.86)</td>
<td>23.45 (1.24)</td>
<td>0.810</td>
</tr>
<tr>
<td>157-9</td>
<td>19.75 (0.58)</td>
<td>20.32 (2.61)</td>
<td>22.09 (2.98)</td>
<td>0.043</td>
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<td>157-11</td>
<td>25.10 (0.79)</td>
<td>20.32 (2.61)</td>
<td>22.09 (2.98)</td>
<td>0.043</td>
</tr>
</tbody>
</table>

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The values are presented as means (standard deviations) of three independent trials. Values with the same upper case letters (a to c) are not significantly different across all strains tested in the system ($P \geq 0.05$). Values with the same lowercase letters (a to c) are not significantly different within each serogroup ($P \geq 0.05$) within each serogroup. Nonstatistical ranks of $D_{55\text{C}}$-values among all strains were compared using one-way analysis of variance (ANOVA; SAS Version 9.2, SAS Institute Inc., Cary, NC) and included comparisons across all strains, comparison of O157 as a group versus non-O157 STEC as a group, and $D_{55\text{C}}$ comparisons within each serogroup. For single strain experiments in 15 and 27% fat, and for experiments with pathogen cocktails and surrogates, differences in $D_{55\text{C}}$-values within each meat system across all the strains tested were compared using one-way ANOVA. In order to determine whether fat level had an effect on STEC heat resistance, contrast statements were used in PROC MIXED to obtain a two degree of freedom test for fat and a probability value ($P$). Comparisons among all means were obtained using Fisher’s least significance difference test (ANOVA) at $P = 0.05$. $D_{55\text{C}}$-values for thermal death experiments in 7% fat ground beef were assigned a nonstatistical numerical rank based on heat tolerance, with 1 = most heat tolerant and 24 = least heat tolerant.
The highest $D_{55\,C}$-value was associated with O45 isolate 45D, which was recovered from processed bear-meat sausage and was linked to a foodborne illness outbreak. Significant differences were noted in heat tolerance among strains in 7% fat ground beef across all serogroups (Table 2) ($P < 0.05$). Isolate 45D exhibited the highest $D_{55\,C}$-value across the 24 strains and was significantly more heat tolerant than all strains except for 145B and 157-A ($P < 0.05$). Isolate 26C was the least heat tolerant of all strains tested, with a $D_{55\,C}$-value of 14.43 min. Isolate 26C was significantly less heat tolerant than 17 other strains ($P < 0.05$) but was not significantly different from strains 26A, 45A, 103A, 111B, 145A, and 157-8 ($P \geq 0.05$). The heat tolerance of all non-O157 strains, considered as one group, was not different from the heat tolerance of the group of O157 strains tested ($P \geq 0.05$).

Researchers have previously evaluated the thermal tolerance of E. coli O157:H7 in beef products. Ahmed et al. (1) established a $D_{55\,C}$-value of 11.40 min for one strain of O157:H7 in 7% fat ground beef. In contrast, Orta-Ramirez et al. (32) observed a dramatically higher $D_{55\,C}$-value, $D_{55\,C}$ of 46.1 to 53.0 min, for O157:H7 strain ATCC 43894 in 3.8% fat ground beef. Our results are more consistent with those of Smith et al. (38) and Luchansky et al. (27), who observed a $D_{55\,C}$-value of 20.08 min for O157:H7 strain 204P in 4.8% fat ground beef and a $D_{54.4\,C}$-value of 23.64 min for O157:H7 strain 380-94 in 7% fat ground beef.

Limited information exists on the comparative survival of O157 and non-O157 STEC in meat products during heating. Working in a beef broth model system, Vasan et al. (48) determined that strain 103A (O103:H2) was significantly more heat tolerant than O157-A (ATCC 43895) at 54.6°C. However, this difference was not observed at higher temperatures (58 and 63.6°C) and, at 58°C and across a broad range of O157 and non-O157 strains (the same strains tested in this study), none of the non-O157 STEC strains were more heat tolerant than strains of O157 STEC. Duffy et al. (13) noted $D_{55\,C}$-values of 11.70 min and 9.73 min for E. coli O157:H7 strain 43895 and E. coli O26, respectively, in 30% fat ground beef. More recently, Luchansky et al. compared the thermal inactivation of a single strain of each of the six major non-O157 STEC serogroups, plus one strain of O104, to the thermal inactivation of one strain of O157:H7 in wafers of ground beef (27). Ground beef (7% fat) was inoculated with a single pathogen strain, formed into thin 3-g patties, and heated in a water bath at 54.4, 60, or 65.6°C. $D$-values in 7% fat ground beef ranged from 13.5 to 23.6 min at 54.4°C, from 0.6 to 1.2 min at 60.0°C, and from 0.05 to 0.08 min at 65.6°C. At each temperature, there were no significant differences observed across all strains in 7% fat ground beef (27). We observed similar $D$-values in 7% fat ground beef at 55°C; $D_{55\,C}$-values ranged from 29.26 min to 14.43 min across O157 and non-O157 STEC strains (Table 2).

This study included a broader array of non-O157 STEC strains than previously reported in heat tolerance studies in meat, and we noted significant differences in $D_{55\,C}$ among serogroups and, in some cases, among strains within a serogroup (Table 2). Significant differences in $D_{55\,C}$-values within a serogroup were noted for O26, O45, O145, and O157 ($P < 0.05$). Serogroup O45 included both the most heat-tolerant strain, 45D ($D_{55\,C} = 29.26$ min), and one of the least heat-tolerant strains, 45A ($D_{55\,C} = 14.71$ min). Similarly, serogroup O145 included one of the most heat-tolerant strains, 145B ($D_{55\,C} = 26.02$ min), and one of the least heat-tolerant strains, 145A ($D_{55\,C} = 14.79$ min). There was no significant difference in heat tolerance of strains tested within serogroups O103, O111, and O121 ($P \geq 0.05$). The heat tolerances of all 24 strains were non-statistically ranked across non-O157 and O157 serogroups, with 1 = highest $D_{55\,C}$-value and 24 = lowest $D_{55\,C}$-value (Table 2). This ranking displays the range of heat tolerances across O157 and non-O157 serogroups. There was general heterogeneity in ranking within a serogroup, with the exception of serogroup O121.

The strain from each non-O157 serogroup that displayed the greatest heat tolerance in 7% fat ground beef was further evaluated in 15 and 27% fat ground beef (Table 2). An additional strain of serogroup O45 that was outside of our original collection and that was linked to a local foodborne illness outbreak (45D) was also tested. The heat tolerance of these non-O157 strains (seven total) was compared with the heat tolerance of the three O157 strains exhibiting the highest heat tolerance in experiments with 7% fat ground beef. Heat tolerance ($D_{55\,C}$) in 15% fat ground beef ranged from 17.77 min for the strain of O26 tested to 24.47 min for the reference O157 strain (43895; 157-A) (Table 2). The $D_{55\,C}$-value of the reference O157 strain (43895; 157-A) in 15% fat ground beef was significantly higher than the $D_{55\,C}$-value of the comparison strain from serogroups O26, O103, O111, and O145, and from one of the beef trim O157 isolates (157-11) ($P < 0.05$) but was not different from comparison strains in serogroup O45 (two strains), serogroup O121, and one other O157 strain (157-1) ($P \geq 0.05$). There was no difference in thermal tolerance between serogroups O157 and non-O157 STEC in 15% fat ground beef ($P \geq 0.05$).

Heat tolerance ($D_{55\,C}$) in 27% fat ground beef ranged from 18.43 min for the strain of O26 tested to 23.45 min for an O157 isolated from beef trim (157-1) (Table 2). The $D_{55\,C}$-value of the high-heat-tolerant O157 strain (157-1) in 27% fat ground beef was not different from the heat tolerance of the two other O157 strains (157-A and 157-11), one of the strains of serogroup O45 (45D; isolate from bear-meat sausage), and strains representing serogroups O103 and O111 ($P \geq 0.05$). The $D_{55\,C}$-value of the high-heat-tolerant O157 strain (157-1) was significantly greater than the strain representing serogroups O26, O121, and O145, and one strain from O45 (45B) ($P < 0.05$). When $D$-values were grouped into non-O157 and O157 STEC, there was no difference in thermal tolerance between the two groups in 27% fat ground beef ($P \geq 0.05$). Perhaps because the strains used in trials with 15 and 27% fat ground beef were selected due to relatively high thermal tolerance in 7% fat ground beef and because a smaller number of strains were tested, the range of $D_{55\,C}$-values in 15 and 27% fat ground beef was narrower than in 7% fat beef.

A probability value was generated to understand the effect of fat on thermal tolerance (Table 2). Increasing fat
content significantly affected heat tolerance \((D_{55C})\) for strains 45B, 45D, 145B, and 157-11; as fat content increased from 7% to 15 and 27%, thermal tolerance decreased. The thermal tolerance of strains 26B, 103C, 111A, 121A, 157-A, and 157-1 was not significantly affected by fat content. Our findings are contrary to the trend observed by Ahmed et al. (1), who noted that \(D_{55C}\) increased from 11.40 min to 19.26 min for a strain of O157:H7 as fat content of ground beef increased from 7 to 20%. Similarly, Smith et al. (38) noted that a strain of \(E. coli\) O157:H7 heated at 55 °C was more heat resistant in ground beef containing 19% fat than in ground beef containing 4.8% fat. And Byrne et al. (8) noted a dramatic increase in \(D_{55C}\)-value for one strain of O157:H7, from 20.8 to 41.1 min, as ground beef fat content increased from 24 to 30%. Luchansky et al. (27) determined \(D_{54.4C}\)-C for one strain from each of the five major non-O157 STEC serogroups and one strain of O157:H7; they noted that \(D_{54.4C}\) increased for each strain as fat content increased from 7 to 30%. Stringer et al. (39) noted, however, that although cells can be more heat resistant in fatty meat than in lean meat, the effect is not always observed. Similarly, Kortola and Conner (22) found that heating cells of \(E. coli\) O157:H7 in ground turkey of varying fat content did not affect survivability. Kortola and Conner speculated that “fine grinding” of meat in their study may have so effectively dispersed fat within the lean phase that the protective effect of higher fat was obliterated. Our results also fail to support the trend of increasing heat resistance as meat fat content increases; in our study, this may be due to the fact that single strains used in higher-fat experiments were selected because they had an already established tolerance to high heat. One hypothesis that could be advanced for the lack of impact of increasing fat content on thermal tolerance is the phenomenon of regression to the mean (3), which may have occurred in the trials conducted in 15 and 27% fat ground beef. However, this explanation is not necessarily supported by our observed lack of variability with repeated measurements (low standard deviation) across all experiments.

The O157 and non-O157 strains used in experiments with 15 and 27% fat ground beef were combined in single-pathogen cocktails and, along with a cocktail of \(Salmonella\) strains, \(D_{55C}\) was determined in 7, 15, and 27% fat ground beef and in commercial frankfurter batter (Table 3). We observed \(D_{55C}\)-values of 17.65 to 25.40 min for five individual O157 strains heated in 7% fat ground beef, with an average of 22.07 min across the serogroup (Table 2). When the three most heat-tolerant O157 strains in our study were combined into a cocktail and heated in 7% fat ground beef, a \(D_{55C}\)-value of 21.31 min was observed (Table 3). \(D_{55C}\)-values for O157 and non-O157 pathogen cocktails in ground beef ranged from 21.31 to 26.32 min and were not different across fat level (7, 15, and 27%) or pathogen type (O157:H7 and non-O157 STEC \((P \geq 0.05)\) (Table 2). Similar \(D\)-values have been reported for O157 cocktails in meat. Murphy et al. (29) reported a \(D_{55C}\)-value of 21.55 min for a cocktail of \(E. coli\) O157:H7 strains heated in 34% fat ground beef, whereas Juneja et al. (21) determined a \(D_{55C}\)-value of 21.13 min for a four-strain cocktail of O157:H7 in 10% fat ground beef. Wiegand et al. (50) reported a \(D_{54.4C}\)-value of 31.5 min for a five-strain cocktail of O157:H7 in ground beef with ~7% fat. In two recent studies, Luchansky et al. (24, 25) investigated the inactivation of strains of O157 (a five-strain cocktail) and non-O157 STEC (a five-strain cocktail: one strain each of serotypes O111, O45, O103, O121, and O145) in brine-injected steaks during grilling. Luchansky et al. observed reductions in O157 STEC ranging from 0.3 to 4.1 log and, for non-O157 STEC, from 0.5 to 4.5 log, depending on endpoint temperature and study conditions. In both studies, the authors concluded that strains of O157 and non-O157 STEC behaved similarly in response to heat (24, 25).

Contrary to some published studies, we did not detect differences in thermal tolerance for O157 and non-O157 STEC cocktails due to fat content in ground beef. Luchansky and colleagues (26) studied the fate of O157:H7 and non-O157 STEC cocktails in patties prepared from 7 or 30% fat ground beef and subjected to various freeze-thaw and cooking treatments. They noted greater inactivation of \(E. coli\) O157:H7 in ground beef as fat content increased from 7 to 30% when patties were frozen and then thawed, or refrigerated, prior to cooking on a gas grill. In contrast, when cooked on a clamshell grill, there was greater inactivation of

<table>
<thead>
<tr>
<th>Inoculum(^a)</th>
<th>7% fat</th>
<th>15% fat</th>
<th>27% fat</th>
<th>Frankfurter batter</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Escherichia coli) O157:H7</td>
<td>21.31 (1.42) c a</td>
<td>22.99 (3.14) c a</td>
<td>22.64 (0.78) b a</td>
<td>48.51 (5.97) b b</td>
</tr>
<tr>
<td>Non-O157 STEC</td>
<td>24.65 (1.78) c a</td>
<td>26.32 (0.18) c a</td>
<td>22.37 (1.24) b a</td>
<td>46.44 (3.90) b b</td>
</tr>
<tr>
<td>(Salmonella)</td>
<td>21.85 (0.39) c a</td>
<td>22.48 (2.00) c a</td>
<td>23.47 (0.58) b a</td>
<td>21.48 (1.13) c a</td>
</tr>
<tr>
<td>(Pediococcus acidilactici) (Saga 200)</td>
<td>85.95 (19.08)(u) b</td>
<td>74.29 (21.51)(u) b</td>
<td>112.94 (33.24)(u) (\Lambda)</td>
<td>63.43 (0.46)(u) (\Lambda)</td>
</tr>
<tr>
<td>(Staphylococcus carnosus) (CS299)</td>
<td>152.63 (31.00)(d) (\Lambda)</td>
<td>113.57 (21.28)(d) (\Lambda)</td>
<td>102.94 (11.93)(d) (\Lambda)</td>
<td>73.45 (20.71)(d) (\Lambda)</td>
</tr>
</tbody>
</table>

\(^a\) STEC, Shiga toxin–producing \(E. coli\).

\(^b\) Three-strain (\(E. coli\) O157:H7), seven-strain (non-O157 STEC), four-strain (\(Salmonella\)) pathogen cocktails, and potential pathogen surrogates, \(P. acidilactici\) and \(S. carnosus\).

\(^c\) \(D_{55C}\)-values are means (standard deviations) of three independent trials. Values within a column with the same uppercase letters \((a\) to \(c)\) are not significantly different \((P \geq 0.05)\). Values within a row with the same lowercase letters \((a\) and \(b)\) are not significantly different \((P \geq 0.05)\).

\(^d\) \(D\)-value derived from five data points in the linear portion of the decay curve, \(R^2 \approx 0.9\); log reduction \(<2\) log.
the pathogen in lower-fat patties. For patties that were inoculated with a cocktail of non-O157 STEC, there was generally no significant effect of fat level on thermal inactivation. The absence of a protective effect afforded by higher-fat meat when cocktails of either O157 or non-O157 STEC were heated at 55 °C in our experiments was likely due to the strain-selection process: strains with established heat tolerance, and for which fat level was not found to be protective in single-strain trials, were combined into each cocktail.

The USDA has issued compliance guidelines for certain meat and poultry products (41) that outline minimum time-temperature combinations required to achieve a 6.5-log reduction of Salmonella in beef products. We compared the thermal stability of a five-strain Salmonella cocktail in 7, 15, and 27% fat ground beef with that of cocktails of O157 and non-O157 STEC and found no significant differences in pathogen survival across fat level (P > 0.05) (Table 3). \( D_{55°C} \) for Salmonella ranged from 21.85 to 23.47 min for 7 and 27% fat ground beef, respectively, and from 21.31 to 26.32 min across all three pathogen types and ground beef fat levels (Table 3). Smith et al. (38) determined a \( D_{55°C} \) of 18.66 min for an eight-strain cocktail of Salmonella strains in 19% fat ground beef and a \( D_{55°C} \) of 22.47 min for \( E. coli \) O157:H7 strain 204P under the same study conditions. Similarly, Juneja (16) determined \( D \)-values by linear regression and noted a \( D_{55°C} \) of 19.31 min for a cocktail of Salmonella strains (eight serotypes) in 25% fat ground beef and a \( D_{55°C} \) of 20.89 min for a four-strain cocktail of \( E. coli \) O157:H7. A lag phase of 8.69 min was observed upon heating of Salmonella, but none was seen for \( E. coli \) O157:H7. When Juneja used the logistic function to account for nonlinear survival data (shoulders and tails), the calculated \( D_{55°C} \)-value reached 26.44 min for Salmonella and 41.36 min for \( E. coli \) O157:H7. Significant differences in survival were also noted by Murphy et al. (29), who observed \( D_{55°C} \) of 37.05 and 21.55 min, for cocktails of Salmonella and \( E. coli \) O157:H7, respectively, in 34% fat ground beef. The presence of one notably heat-tolerant Salmonella strain of serotype Senttenberg in the cocktail used by Murphy et al. may have accounted for the higher \( D \)-value observed for this pathogen. Juneja et al. (16) suggested that the higher heat tolerance for \( E. coli \) O157:H7 in their study was due to the subpopulation of cells in the tail of the survival curve, which increased the calculated \( D_{55°C} \)-value from 20.89 min determined by linear regression to 41.36 min determined by curve fitting. A trend of increasing Salmonella thermal tolerance with increasing fat level in meat has been observed by others (17, 18, 29, 32, 38) but was not observed in our study, perhaps due to strain selection or study methodology.

When pathogen survival was evaluated in commercial frankfurter batter, there was no significant difference in \( D_{55°C} \)-value between O157 and non-O157 STEC cocktails \( (P ≥ 0.05) \), but each was significantly more heat tolerant than the cocktail of Salmonella strains \( (P < 0.05) \) (Table 3). The frankfurter batter that we used contained 27% fat, similar to one type of ground beef tested. The \( D_{55°C} \)-values for O157 and non-O157 STEC cocktails increased significantly from 22.64 to 48.51 min, and from 22.37 to 46.44 min, in studies with 27% fat ground beef and commercial frankfurter batter, respectively (Table 3). The \( D_{55°C} \)-values for Salmonella were not significantly different between 27% fat ground beef and frankfurter batter, 23.47 min and 21.48 min, respectively. The frankfurter batter used in this study contained added salt, sodium nitrite, sodium phosphates, potassium lactate, and sodium dicalcetate. Blackburn et al. (4) found that the presence of sodium chloride reduced the water activity of cells and resulted in enhanced thermal tolerance in \( E. coli \) O157:H7, and Kotrola and Conner (22) noted that thermal tolerance of \( E. coli \) O157:H7 was enhanced in turkey meat containing the additives sodium chloride, sodium lactate, and polyphosphate. In contrast, Juneja et al. (20) noted that the addition of sodium chloride and/or sodium pyrophosphate, in the presence of reduced pH, could significantly reduce thermal tolerance of \( E. coli \) O157:H7 in beef gravy. Byrne et al. (8) established that the presence of nonmeat ingredients such as seasonings and additives in “economy” burgers resulted in significantly higher \( E. coli \) O157:H7 thermal tolerance compared with survival in 100% beef patties. Wiegand et al. (51) demonstrated a dramatic increase in \( D_{54.4°C} \) for a five-strain cocktail of \( E. coli \) O157:H7 cells heated in 93% lean ground beef and in the same ground beef seasoned with a mixture of phosphates, salt, sugars, and spice to resemble a 5% “pump,” increasing from roughly 27 to 52 min, respectively. The reason for the enhanced thermal tolerance occurring with cocktails of non-O157 and O157 STEC, but not with Salmonella, heated in frankfurter batter in our study is not clear. Our results support the work of others who have noted higher heat tolerance for \( E. coli \) O157:H7 than Salmonella in meat systems (16, 38), and they emphasize the importance of strain selection and the identification of the target pathogen when establishing safe harbors for thermal meat processing operations. Our data further support the importance of using an appropriate food matrix in validating thermal processing operations.

The heat tolerance of each pathogen cocktail was compared with the heat tolerances of two potential pathogen surrogates, \( P. acidilactici \) and \( S. carnosus \), in 7, 15, and 27% fat ground beef and in commercial frankfurter batter (Table 3). The strain of \( P. acidilactici \) used in these experiments has been shown to function as an effective pathogen surrogate in the manufacture of whole-muscle and ground-and-formed beef jerky under commercial and homestyle processing conditions (5, 6, 11, 12). For a bacterial strain to function as a pathogen surrogate in a thermal processing operation, the strain should be nonpathogenic and amenable in a processing facility and should have equal or slightly enhanced thermal tolerance compared with the pathogen under identical testing conditions (7). Due to the high heat tolerance of potential surrogates, a 3-log reduction was not observed on heating; the \( D_{55°C} \)-value was determined from at least five sampling points in the linear portion of the heating curve in which there was a ±1-log reduction in strain viability. In all cases, the calculated heat tolerance of pathogen surrogates was at least 2.8-fold higher than the laboratory-determined \( D_{55°C} \)-values for pathogen...
cocktails. In 7 and 15% fat ground beef, the estimated heat tolerance of *S. carnosus* was significantly higher than for *P. acidilactici* (*P* < 0.05); in 27% fat ground beef and in frankfurter batter, the estimated heat tolerance was not different between the two cultures. In each meat system, potential surrogates were significantly more heat tolerant than any of the pathogen cocktails (*P* < 0.05) (Table 3), so much so that these two strains would not be recommended as potential pathogen surrogates in similar commercial operations.

This study provides foundational information on the comparative heat tolerance of strains of the major non-O157 STEC serogroups and O157:H7 STEC in a range of meat products. Our earlier work in broth (48), which suggested similar heat tolerances for O157 and non-O157 STEC in meat systems, is confirmed. The current study also confirms the importance of the food matrix when evaluating heat tolerance. As suggested by Murphy et al. (28) in their studies of chicken, beef, and turkey products, product formulation likely affected the thermal resistance observed in our study. Not only is the proximate composition important (the proportion of fat, protein, carbohydrate), but the presence or absence of additives and other ingredients, and the physical structure of the matrix, may also be important. We chose to evaluate heat tolerance in ground meat systems, but work by Wiegand et al. (51) and Velasquez et al. (49) suggests that thermal tolerance in ground meat systems may not mimic that in whole muscle.

We carefully selected strains for this study from across a breadth of clinical and food isolates. We evaluated heat tolerance both of single strains and of cocktails. Murphy et al. (29) noted that the USDA Food Safety and Inspection Service recommends use of a cocktail as a more realistic approach to pathogen testing. We observed that within a system, i.e., ground beef of various fat levels, the heat resistance of multistrain cocktails closely resembled the heat resistance of the individual strains. However, we had conducted screening experiments, extensively so in the case of STEC, that allowed for selection of heat-resistant pathogen strains for experiments with cocktails in order to provide robust data for our comparisons.

In this study, we standardized methods such as inoculum preparation and method of inoculation, heating, and enumeration. We noted no reduction of inoculum population during come-up time of ~76 s in any experiment. Such standardization, in this case, allows for an accurate comparison between the heat tolerances of O157:H7 and non-O157 STEC, *Salmonella*, and two nonpathogenic surrogates under experimental conditions. Juneja et al. (18) noted the presence of nonlinear survival curves for *Salmonella*, especially at the temperature used in this experiment, 55 °C. We noted some nonlinearity in pathogen survival with the presence of minimal shoulders prior to linear decay. In single-strain experiments, we noted shoulders over the first two data collection points (0, 5 min) across all fat levels. Thereafter, linear decay patterns were observed. In experiments with pathogen cocktails, we noted some shoulders extending across the first three sampling points (0, 10, 20 min), with most of the curves showing linear decay beginning at 10 min. However, as our goal was to compare *D*-values among inocula rather than to establish *D*-values for a pathogen-meat system, the occasional presence of a shoulder would not have impacted our results. Contrary to what Juneja and Eblen observed (17), there was no increase in the length of the shoulder with increased fat level in ground beef and no difference between pathogen species (data not shown).

We, therefore, conclude that heat tolerance of O157 and non-O157 STEC under the study conditions does not significantly differ in a range of beef systems, suggesting that thermal processing steps validated against O157 STEC may be considered validated against non-O157 STEC. We have identified strains that may possess higher thermal tolerance and that may prove effective in process validation under commercial conditions. We further conclude that thermal processes validated with *Salmonella* as the target organism may not, in some cases, provide sufficient lethality against STEC. We further conclude that processors could not use *P. acidilactici* (Saga 200) or *S. carnosus* (CS-299) as effective pathogen surrogates under the thermal processing conditions outlined in this study.

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