Thermal Inactivation Studies of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in Ready-to-Eat Chicken-Fried Beef Patties

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**ABSTRACT**

Thermal inactivation studies were used to determine the D- and z-values of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in ready-to-eat chicken-fried beef patties. Inoculated meat was packaged in sterile bags, which were immersed in a circulated water bath and held at 55, 57.5, 60, 62.5, 65, 67.5, and 70°C for different lengths of time. D- and z-values were determined with a linear regression model. Average D-values at temperatures 55 to 70°C were 27.62 to 0.04 min for *E. coli* O157:H7, 67.68 to 0.22 min for *Salmonella*, and 81.37 to 0.31 min for *L. monocytogenes*. The z-values were 5.2°C for *E. coli* O157:H7, 6.0°C for *Salmonella*, and 6.1°C for *L. monocytogenes*. The results of this study can be used by food processors to validate their processes and help eliminate pathogenic bacteria associated with chicken-fried beef products.

Foodborne pathogenic bacteria are responsible for over 76 million cases of related illnesses in the United States. Every year, 325,000 victims are hospitalized and more than 5,000 die (25). Some bacterial pathogens responsible for these illnesses are *Escherichia coli* O157:H7 (5, 20, 23) and various *Salmonella* serotypes (6, 33, 51). These two foodborne pathogens are commonly associated with the intestinal tracts of livestock and poultry to be processed (1, 3, 14, 26). However, *Listeria monocytogenes* is a ubiquitous pathogen that is associated with foodborne illnesses and is present in the environment; it can be found in food processing operations on floors and equipment (7, 12, 14, 19, 27). *L. monocytogenes* is a very difficult pathogen to control because it is relatively thermostable and salt tolerant and has the ability to grow at refrigeration temperatures (2, 11, 14, 27, 36). *L. monocytogenes* has been associated with food-related illnesses for more than 20 years and still causes problems (7). From 18 July to 30 September 2002, *L. monocytogenes*-related illnesses originating from ready-to-eat (RTE) meat products occurred in a multistate region, affecting more than 46 people, with seven deaths and three stillbirths or miscarriages (4). This outbreak led to a recall of more than 295,000 lb (133,930 kg) of fresh and frozen RTE chicken and turkey products (43).

Because of continuing consumer health problems, the federal government set regulations addressing adulteration of animal products for human consumption in its hazard analysis and critical control point (HACCP) plan (47). Hazard analysis for *E. coli* O157:H7 was addressed in this federal regulation to control microbial contamination that produces natural toxins. Federal regulations have also addressed *Salmonella* contamination of fully cooked meat and poultry products (42), calling for a 6.5-log reduction of the pathogen in beef and a 7-log reduction in poultry or an alternative control program that eliminates viable organisms in the finished product. New regulations to include RTE meat and poultry products have been drafted by the Risk Assessment Division of the U.S. Food Safety and Inspection Service (FSIS) (49). The U.S. Department of Agriculture (USDA) has also addressed *L. monocytogenes* contamination in RTE products exposed to the pathogen after lethality treatment (48) and has released updated compliance guidelines (44, 45). Even though these regulations have been in place, *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* can still be found in RTE products (7, 46).

Thermal processing of RTE products remains the standard commercial method for producing quality foods that are free of foodborne pathogens. Evaluation of the thermal inactivation of pathogenic bacteria in plain meat is not sufficient for processors of RTE meat products to validate their processes. Both the composition of the meat product and additives used to formulate these products can affect the thermal inactivation of pathogens (1, 8, 9, 15–17, 21, 24, 34, 52, 53). Murphy et al. (29) reported that thermal inactivation of *Salmonella* and *Listeria innocua* was significantly different among various commercially formulated meat and poultry products, such as chicken breast meat, chicken patties, chicken tenders, franks, beef patties, and blended beef and turkey patties. Although quantitative data on thermal inactivation of foodborne pathogens in different meat products have been published, there are no reports on the thermal inactivation of *E. coli*, *Salmonella*, and *L. monocytogenes* in chicken-fried beef.

Quantitative data on the thermal inactivation of pathogens are required for every formulated meat product to...
determine whether it meets the lethality performance standards (42, 47). Therefore, thermal inactivation studies of \textit{E. coli} O157:H7, \textit{Salmonella}, and \textit{L. monocytogenes} in chicken-fried beef patties were performed to calculate $D$- and $z$-values for these pathogenic bacteria in this product. These data will allow processors to accurately monitor thermal processing of chicken-fried beef patties to ensure safety and quality for consumers and to comply with federal regulations.

\section*{MATERIALS AND METHODS}

\textbf{Meat samples.} Chicken-fried beef patties were shipped frozen from the processor. The 100-g patties arrived uncooked, formed, and individually frozen. The product was kept at $-20^\circ$C until 1 day before the experiment, at which time the product was thawed overnight in a 4$^\circ$C refrigerator.

\textbf{Product composition.} Chicken-fried beef patties contained 56% moisture, 14.6% fat, and 13.1% protein.

\textbf{Bacterial strains.} According to FSIS recommendations, a mixture of temperature-resistant bacterial strains should be used to reflect worst-case scenarios of foodborne bacterial pathogens that may be present in the processed product (47). Six isolates of \textit{E. coli} O157:H7 were obtained from Dr. M. Johnson (Department of Food Science, University of Arkansas, Fayetteville): human isolate FS01, human isolate FS85, pork isolate 204P, beef isolate 50SB, human isolate 932, and beef isolate 933.

Six nalidixic acid–resistant \textit{Salmonella} serotypes were used in this study. \textit{Salmonella} Senftenberg (ATCC 43845) was purchased from the American Type Culture Collection (Rockville, Md.), and the other five \textit{Salmonella} cultures (Typhimurium, Heidelberg ATCC 8326, Mission, Montevideo ATCC 8387, and California ATCC 23201) were originally obtained from Dr. Amy Waldroup (formerly of the Department of Poultry Science, University of Arkansas, Fayetteville). Six strains of \textit{L. monocytogenes} were used in this study. Five strains (ARS V67, ARS V72, ARS V113, ARS V125, and ARS 105) were obtained from Dr. M. E. Berrang (USDA, Agricultural Research Service, Athens, Ga.), and strain LCDC 81-861 (4b) was obtained from Dr. M. Johnson.

\textbf{Bacterial cultures.} \textit{E. coli} isolates were grown individually in tryptic soy broth (TSB; Becton Dickinson, Sparks, Md.). \textit{Salmonella} serotypes were grown individually in TSB with 200 ppm nalidixic acid (Sigma-Aldrich Chemical Co., St Louis, Mo.). \textit{L. monocytogenes} strains were grown individually in TSB plus 0.6% yeast extract (Becton Dickinson). Bacterial cultures were incubated at 37$^\circ$C for 24 h to obtain an inoculum concentration of more than or equal to 10$^7$ CFU/ml.

\textbf{Sample preparation and thermal inactivation.} Cocktails of \textit{E. coli} O157:H7, \textit{Salmonella}, and \textit{L. monocytogenes} were mixed with meat samples at a ratio of 1 ml of culture per 75 g of meat. After mixing for 5 min, inoculated meat samples were kept at 4$^\circ$C for 30 min. Inoculated product was then weighed in 5-g aliquots (5 g per inoculum cocktail), placed in Seward stomacher bags (BA6040, Seward Ltd., Norfolk, UK), and sealed at $-1$ bar with a vacuum sealer (KOMET Maschinenfabrik GmbH, Plochingen, Germany). Vacuum-sealed samples were rolled thin until evenly flattened to approximately 1-mm thickness, placed separately in metal cages, and dropped into a heated circulating water bath (Lauda Co., Lauda-Konigshofen, Germany) at a set temperature. Samples were removed from the water bath at set times and immediately immersed in ice water for 1 h. Samples from the bags were removed for culture within 4 h.

\textbf{Bacterial culture and enumeration.} Sample bags were opened aseptically, and 15 ml of 0.1% peptone water was added to each sample to achieve a 1:4 dilution. The resulting samples were stomached for 2 min and further diluted in a 10$^{-1}$ dilution series, and dilutions were plated on various agars and incubated at 37$^\circ$C for 72 h. Cultures were grown on sorbitol MacConkey agar (Becton Dickinson) to select for \textit{E. coli} O157:H7, on tryptic soy agar with 200 ppm nalidixic acid for \textit{Salmonella}, and on modified Oxford medium supplemented with modified Oxford antimicrobial supplement (Becton Dickinson) for \textit{L. monocytogenes}. Cultures were incubated at 37$^\circ$C for 72 h, and surviving colonies were enumerated.

\textbf{Data analysis.} Data were entered into a Microsoft Excel spreadsheet (Microsoft, Redmond, Wash.) and analyzed for $D$- and $z$-values with both Microsoft Excel and Sigma Plot 9.0. The common logarithms of the number of surviving \textit{E. coli} O157:H7, \textit{Salmonella}, and \textit{L. monocytogenes} cells in chicken-fried beef after each heat treatment were plotted against the heating time. The $D$-value for each microorganism at each temperature was calculated from the linear regression model for the log of surviving bacterial cells and heating time. The $D$-value is the negative inverse slope of the plot:

$$\log(N) = \log(N_0) - \frac{t}{D}$$

where $N$ is the number of survivors at time $t$ and $N_0$ is the number of survivors at time 0.

The $z$-values for \textit{E. coli} O157:H7, \textit{Salmonella}, and \textit{L. monocytogenes} were calculated by determining the linear regression of the log of $D$-values and temperatures ($T$). The $z$-value is the negative inverse slope of the plot:

$$\log(D) = \log(D_0) - \frac{T}{z}$$

The $z$-values of \textit{E. coli} O157:H7, \textit{Salmonella}, and \textit{L. monocytogenes} were calculated from the inverse negatives slopes from the graph of the log $D$-values versus temperatures.

\section*{RESULTS AND DISCUSSION}

\textbf{$D$-values.} No obvious shoulders or concavities were observed in the survivor curves for each studied microorganism. Representative survivor curves are shown in Figure 1 for \textit{E. coli} O157:H7 at 62.5$^\circ$C, Figure 2 for \textit{Salmonella} at 65$^\circ$C, and Figure 3 for \textit{L. monocytogenes} at 57.5$^\circ$C. Similar figures for \textit{E. coli} O157:H7, \textit{Salmonella}, and \textit{L. monocytogenes} were also obtained at temperatures of 55, 57.5, 60, 62.5, 65, 67.5, and 70$^\circ$C (data not shown). The determination coefficient $R^2$ of the regression curves was always more than 0.85 at temperature of 55 to 70$^\circ$C.

Average $D$-values for the three bacterial cocktails at temperatures of 55 to 70$^\circ$C in chicken-fried beef patties (Table 1) were 27.62 to 0.04 min, 67.68 to 0.22 min, and 81.37 to 0.31 min for \textit{E. coli} O157:H7, \textit{Salmonella}, and \textit{L. monocytogenes}, respectively. $D$-values differ for different meat products depending on composition and processing conditions. Product composition, such as moisture, fat, protein content, and food additives, has an inconsistent effect on the thermal resistance of organisms. Murphy et al. (31) evaluated the same strains of \textit{E. coli} O157:H7 that were used in the present study to determine their $D$-values in ground turkey (5.4% fat) and ground beef (34.4% fat).
Their $D$-values were lower in ground turkey at temperatures 55, 57.5, 60, 62.5, 65, 67.5, and 70°C and higher in ground beef at temperatures 62.5, 65, 67.5, and 70°C than those reported in this study. Murphy et al. (28), using the same strains of $E.\ coli$ O157:H7, reported higher $D$-values in ground pork (40.2% fat) at temperatures of 55, 60, 62.5, 65, and 70°C than those reported in this study. The differences between the $D$-values in this study and those previously reported can be explained by the differences in product fat content. Higher fat concentrations result in increased thermal resistance of microorganisms. Ahmed et al. (1) found that increasing the fat content from 3 to 11% resulted in higher $D$-values for $E.\ coli$ O157:H7 in meat and poultry products. Line et al. (23) observed that $E.\ coli$ O157:H7 had higher $D$-values in fatty ground beef (30%) than in lean meat (2% fat). Smith et al. (40) found that $E.\ coli$ O157: H7 was more heat resistant in beef containing 19.1% fat than in beef containing 4.8% fat. The protective effect on microorganisms of fat in food can be explained by low thermal conductivity or reduced water activity of fat (38, 39, 50).

Juneja et al. (17) studied the thermal resistance of Salmonella at temperatures ranging from 58 to 65°C in beef (12.5% fat), turkey (9% fat), and chicken (7% fat) and found that higher fat concentrations resulted in higher $D$-values for Salmonella in these products. The $D$-values of Salmonella in the present study were higher than those reported by Juneja et al. (17), probably because of the high fat concentration in the chicken-fried beef patties (14.6%) and the variations in thermal resistance of the individual bacterial strains in the cocktails. At temperatures of 55 to 70°C, Murphy et al. (30) found lower $D$-values for a cocktail of Salmonella in chicken breast meat (0.12% fat). Their values ranged from 30.1 to 0.24 min; this difference can be also explained by the high fat concentration in the chicken-fried beef.

In addition to product differences, variations in bacterial strains and experimental methodologies may partially account for $D$-value differences. For accurate comparisons among the studies, it is better to evaluate studies in which different bacteria were tested under the same conditions. To accurately compare results from different studies, it is recommended to use the same bacterial strains and identical conditions.

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>$E.\ coli$ O157:H7</th>
<th>Salmonella</th>
<th>L. monocytogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>27.62 ± 3.16</td>
<td>67.68 ± 9.95</td>
<td>81.37 ± 5.91</td>
</tr>
<tr>
<td>57.5</td>
<td>11.35 ± 0.13</td>
<td>27.31 ± 0.77</td>
<td>40.49 ± 0.00</td>
</tr>
<tr>
<td>60</td>
<td>2.50 ± 0.20</td>
<td>15.51 ± 3.82</td>
<td>22.98 ± 5.97</td>
</tr>
<tr>
<td>62.5</td>
<td>0.75 ± 0.01</td>
<td>5.61 ± 0.70</td>
<td>7.15 ± 0.36</td>
</tr>
<tr>
<td>65</td>
<td>0.23 ± 0.035</td>
<td>2.00 ± 0.06</td>
<td>2.81 ± 0.16</td>
</tr>
<tr>
<td>67.5</td>
<td>0.11 ± 0.026</td>
<td>0.50 ± 0.049</td>
<td>0.93 ± 0.001</td>
</tr>
<tr>
<td>70</td>
<td>0.04 ± 0.007</td>
<td>0.22 ± 0.026</td>
<td>0.31 ± 0.016</td>
</tr>
</tbody>
</table>

$^a$ Values are the means from two to four replications ± standard deviations.
TABLE 2. Pairwise comparisons of D-values for E. coli O157: H7, Salmonella, and L. monocytogenes in chicken-fried beef patties at thermal processing temperatures of 55 to 70°C

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Pairs</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>Salmonella vs L. monocytogenes</td>
<td>0.1098</td>
</tr>
<tr>
<td></td>
<td>Salmonella vs E. coli O157:H7</td>
<td>0.0133a</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes vs E. coli O157:H7</td>
<td>0.0014a</td>
</tr>
<tr>
<td>57.5</td>
<td>Salmonella vs L. monocytogenes</td>
<td>0.0017a</td>
</tr>
<tr>
<td></td>
<td>Salmonella vs E. coli O157:H7</td>
<td>0.0012a</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes vs E. coli O157:H7</td>
<td>&lt;0.0001a</td>
</tr>
<tr>
<td>60</td>
<td>Salmonella vs L. monocytogenes</td>
<td>0.1416</td>
</tr>
<tr>
<td></td>
<td>Salmonella vs E. coli O157:H7</td>
<td>0.196a</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes vs E. coli O157:H7</td>
<td>0.193a</td>
</tr>
<tr>
<td>62.5</td>
<td>Salmonella vs L. monocytogenes</td>
<td>0.0082a</td>
</tr>
<tr>
<td></td>
<td>Salmonella vs E. coli O157:H7</td>
<td>0.0008a</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes vs E. coli O157:H7</td>
<td>&lt;0.0001a</td>
</tr>
<tr>
<td>65</td>
<td>Salmonella vs L. monocytogenes</td>
<td>0.0203a</td>
</tr>
<tr>
<td></td>
<td>Salmonella vs E. coli O157:H7</td>
<td>&lt;0.0001a</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes vs E. coli O157:H7</td>
<td>0.0002a</td>
</tr>
<tr>
<td>67.5</td>
<td>Salmonella vs L. monocytogenes</td>
<td>0.0064a</td>
</tr>
<tr>
<td></td>
<td>Salmonella vs E. coli O157:H7</td>
<td>0.0097a</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes vs E. coli O157:H7</td>
<td>0.0005a</td>
</tr>
<tr>
<td>70</td>
<td>Salmonella vs L. monocytogenes</td>
<td>0.0563</td>
</tr>
<tr>
<td></td>
<td>Salmonella vs E. coli O157:H7</td>
<td>0.0012a</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes vs E. coli O157:H7</td>
<td>&lt;0.0001a</td>
</tr>
</tbody>
</table>

a Means are significantly different (P < 0.05).

The same strains and same methodologies are used for thermal inactivation experiments. Murphy et al. (31) employed the same strains of Salmonella and L. monocytogenes that were used in the present study and the same methodology to determine the thermal resistance of these organisms in turkey (5.4% fat) and beef (34.4% fat). For all meat types, they reported lower D-values for all bacteria than those reported in the present study, although the beef used in their study had a higher fat concentration. The reported D-values for Salmonella and L. monocytogenes in the present study were also higher than those reported by Murphy et al. (28) for the same bacterial strains in ground pork (40.2% fat). The difference between these previously reported D-values and those reported here can be explained by the effect of salts, which were part of the formulation of chicken-fried beef but were not added to the turkey, beef, or pork in previous studies.

In our study, the chicken-fried beef patty formulation included sodium compounds such as sodium chloride (1.32%), monosodium glutamate (0.47%), sodium phosphate (0.35%), and disodium inosinate–guanylate (0.01%). These additives are mixed with the meat to enhance flavor, add tenderness and juiciness, and increase water retention. Addition of salts decreases water activity and increases the thermal resistance of bacteria. As the water activity of the heating medium decreases, the thermal resistance of vegetative cells and spores increases (10, 16, 22, 41). Kotrola and Conner (21) explained this phenomenon by the ability of salt to bind water in the heating menstruum. Binding of water to salts leads to poor heat penetration through the heating menstruum during cooking; therefore, more bacteria will survive in the heating medium (15, 21). Line et al. (23) reported that when ground pork mixed with 1 or 2% sodium chloride was cooked to an internal temperature of 60°C, a 6.75- or 6.36-log reduction of L. monocytogenes was obtained compared with a 7.11-log reduction for the control sample. Juneja and Eblen (16) found that sodium chloride (1.5, 3, 4.5, and 6%) increased the thermal resistance of L. monocytogenes in beef gravy. Oste-Triantafyllou et al. (35) and Saraiva et al. (37) reported that the addition of sodium phosphate increased the thermal stability of horseradish peroxidase and chymotrypsin, respectively.

The addition of sodium phosphate to chicken-fried beef patties may also have an effect on the thermal stability of the bacterial strains. Pathogenic bacteria respond differently to food additives. For example, sodium lactate at up to 4.5% of the total formulation had no effect on the thermal resistance of E. coli O157:H7 in beef (13), but a similar concentration increased the heat resistance of L. monocytogenes in meat (15, 32). Further research is needed to determine possible effects of various food additives on pathogenic bacteria.

Table 2 shows paired comparisons of D-values between E. coli O157:H7, Salmonella, and L. monocytogenes in the chicken-fried beef patties. The D-values for Salmonella were significantly lower than those for L. monocytogenes at temperatures 57.5, 62.5, 65, and 67.5°C but were not significantly lower at temperatures 55, 60, and 70°C. These results indicate that Salmonella is less heat resistant than L. monocytogenes. At all temperatures, the D-values for E. coli O157:H7 were significantly lower than those for Salmonella and L. monocytogenes, which indicates that E. coli O157:H7 is more heat sensitive than Salmonella and L. monocytogenes.

The z-values for E. coli O157:H7, Salmonella, and L. monocytogenes were 5.2, 6.0, and 6.1°C, respectively, in the chicken-fried beef patties (Figs. 4 through 6). These values obtained are similar to those reported for the same
bacterial strains used by Murphy et al. (28) in ground pork, by Murphy et al. (31) in ground turkey and beef products (Table 3), and in other formulated products that we have evaluated (data not shown). Juneja et al. (18) reported a higher \( z \)-value (6.0°C) for \( E.\ coli \) O157:H7 in 90% lean ground beef than that found in the present study. Line et al. (23) obtained \( z \)-values of 4.6 and 4.7°C for \( E.\ coli \) O157:H7 in lean and fatty beef, respectively. Juneja et al. (17) reported that the \( z \)-values of eight serotypes of \( \text{Salmonella} \) in ground beef (12.5% fat), ground turkey (9% fat), and ground chicken (7% fat) were 6.0, 6.9, and 6.1°C, respectively. Mazzotta (24) obtained a \( z \)-value of 5.7°C for a cocktail of \( \text{Salmonella} \) in chicken breast meat. A higher \( z \)-value (7.6°C) was reported by Murphy et al. (30) for a cocktail of \( \text{Salmonella} \) in commercially formulated chicken patties. Farber et al. (9) reported \( z \)-values of 4.9 and 3.5°C for \( \text{L. monocytogenes} \) in beef gravy and cured beef gravy, respectively. Juneja (15) reported \( z \)-values of 5.93 to 7.36°C for \( \text{L. monocytogenes} \) in ground beef mixed with different concentrations of NaCl, sodium lactate, or sodium diacetate. The differences in thermal resistance parameters (\( D \)- and \( z \)-values) between this study and other studies are possibly due to the differences in product composition and bacterial strains.

The \( z \)-value of a pathogen in each formulated meat or poultry product is needed to calculate process lethality (\( F \)), which is important for validating thermal processes and for meeting one aspect of the HACCP plan (47). Process lethality can be calculated from the integration of the time-temperature relationship during cooking and cooling of the product:

\[
F = \int_0^t \frac{10^{(T(t) - T(\text{ref})}/dt
\]

To achieve a 6.5-D reduction of \( E.\ coli \) O157:H7, \( \text{Salmonella} \), and \( \text{L. monocytogenes} \), process lethality at a reference temperature of 70°C must be 0.26 min (6.5 × 0.04 min) or higher, 1.43 min (6.5 × 0.22 min) or higher, and 2.02 min (6.5 × 0.31) or higher, respectively. These results would be useful for RTE meat processors to help them meet performance standards, validate the microbial lethality of their processes, and fulfill one aspect of the farm-to-table (HACCP) program requirements.

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