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Thermal Inactivation of *Listeria monocytogenes* in Chicken Gravy

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ABSTRACT

Heat resistance of *Listeria monocytogenes* strains V7 and Scott A in chicken gravy and changes in heat resistance during refrigerated storage were studied. After chicken gravy was made, it was cooled to 40°C, inoculated with 10^5 CFU *L. monocytogenes* per ml of gravy, and then stored at 7°C for 10 d. Gravy was heated at 50, 55, 60, and 65°C immediately after inoculation and after 1, 3, 5, and 10 d of refrigerated storage. The D values for strains Scott A and V7 in gravy heated at 50°C at day 0 were 119 and 195 min and at day 10 they were 115 and 119 min, respectively, whereas at 65°C comparable values at day 0 were 0.48 and 0.19 min and at day 10 they were 0.014 and 0.007 min. Heat resistance (expressed as D values) was greater at day 0 than at the end of refrigerated storage. The z values ranged from 3.41 to 6.10°C and were highest at the early stages of chill storage and then decreased at the later stages. Strain V7 was more heat resistant than Scott A at 50°C. Strain Scott A always had a higher z value than did strain V7 at the same storage interval. A heat treatment greater than the 4-D process recommended by the U.S. Department of Agriculture was required to inactivate the large numbers of *L. monocytogenes* that developed in chicken gravy during refrigerated storage.

Listeria monocytogenes, the cause of listeriosis, is a psychrotrophic foodborne pathogen (*35*) and thus is of major concern in the safety of refrigerated foods. In addition to illness and death, listeriosis also causes economic losses which in the United States have been estimated at 313 million dollars per year (*41*).

Since *L. monocytogenes* is a psychrotroph, it can grow in refrigerated foods, and its survival in such foods can be for extended periods (*35*). Several surveys have found *L. monocytogenes* present in retail refrigerated poultry products (all within their "sell-by" date) (*13,18,27*). From 15 to 66% of raw poultry products that have been tested contained *L. monocytogenes* (*4,5,13,15-17,19,24,29,34,39*). Several studies in Europe and southern United States have associated foodborne listeriosis with consumption of ready-to-eat chicken products (*2,28,38,42*), thus demonstrating the health hazard that sometimes can be associated with eating such foods.

One means of controlling *L. monocytogenes* in foods is the use of adequate heat followed by application of appropriate hygienic measures to prevent postheating recontamination of the product (*36*). However, *L. monocytogenes* appears to be more heat resistant than other nonsporeforming foodborne pathogens.

Most studies on heat resistance of *L. monocytogenes* in foods were done with milk or dairy products. There are conflicting reports about the ability of *L. monocytogenes* to survive the minimum high-temperature short-time pasteurization process (71.7°C for 15 s) (*7,11*). Johnson et al. (*25*) reviewed current studies on *Listeria* species and concluded that the conflicting results were caused by differences in techniques used by various researchers to study the thermal resistance of *L. monocytogenes*. Mackey and Bratchell (*32*) reviewed data on heat resistance of *L. monocytogenes* in foods and concluded that the safety margin for high-temperature short-time milk pasteurization was much lower than that for vat pasteurization (62.8°C for 30 min). They also suggested that foods should be cooked to an internal temperature of 70°C for 2 min to ensure destruction of *L. monocytogenes*.

Only few published data deal with heat resistance of *L. monocytogenes* in meat and poultry products. Karaioannoglou and Xenos (*26*) observed that *L. monocytogenes* survived in grilled meatballs cooked to an internal temperature of 78-85°C for 15 min. Smith and Zottola (*40*) found the D values of *L. monocytogenes* strain Scott A at 60 and 65°C in autoclaved ground beef were 28 and 13 min, respectively. Boyle et al. (*6*) noted that the heat resistance of *L. monocytogenes* was greater in a meat slurry than in phosphate buffer. The D value obtained for *L. monocytogenes* also was greater in the meat slurry than in milk (*7*). Glass and Doyle (*20*), however, found that heating beaker sausage containing 5 x 10^3 CFU of *L. monocytogenes* per g to an internal temperature of 62.2°C reduced the population to an undetectable level (<10 CFU/g). Harrison and Carpenter (*21,22*) and Carpenter and Harrison (*8,9*) demonstrated the ability of *L. monocytogenes* to survive on chicken breasts processed by microwave, dry, and moist heating. They found that moist heating more effectively killed this pathogen than did microwave or dry heating, but even the high cooking temperatures (73.9-

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82.2°C) were insufficient to eliminate the high population of *L. monocytogenes* from chicken products.

In another study (*23*), *L. monocytogenes* grew well in chicken gravy during cooling and refrigerated storage, and the pathogen survived reheating of the gravy to an internal temperature of 74°C. From this it is evident that the thermal resistance of *L. monocytogenes* in such cook-chill or refrigerated foods that are reheated needs further investigation. Thus, this study was designed to (a) determine the heat resistance of *L. monocytogenes* in chicken gravy and (b) study changes in heat resistance of *L. monocytogenes* in chicken gravy during refrigerated storage. These experiments comply with the recent suggestion that data on thermal resistance of this pathogen in different foods are urgently needed (*18,30,37*).

MATERIALS AND METHODS

Strains and media

Culture of *Listeria monocytogenes* strains Scott A and V7 (obtained from the Food Research Institute of the University of Wisconsin-Madison) were prepared by two consecutive transfers in tryptose broth (Difco Laboratories, Detroit, MI) and incubation at 37°C for 24 h. Samples of inoculated gravy were diluted serially with sterile peptone (0.1%) water and diluted samples were plated on tryptose agar (Difco), then plates were incubated at 35°C for 48 h. Typical colonies of *L. monocytogenes* (ca. 0.5-1.5 mm in diameter, smooth, bluish gray, slightly raised, translucent, and watery consistency) were counted using a Quebec colony counter (Scientific Instruments, Keene, NH) and numbers were expressed as CFU/ml of gravy.

Chicken gravy

Chicken stock was made from 3.7 kg chicken (skin on), 298 g onions, 149 g celery, 149 g carrots, 28 g seasoning, and 9.2 L water. Ingredients were mixed, cooked for 9 h to get a yield of 7.6 L, and the chicken stock was stored in a refrigerator (Victory, Model FS-2D-S5, Plymouth Meeting, PA) at 2°C, for 1 d before production of chicken gravy. When chicken gravy was to be prepared, the chicken stock was taken out of the refrigerator, and the layer of fat was removed from the surface. The ca. 7.6 L chicken stock together with an equal amount of water was boiled in a steam-jacketed kettle (Model TDC/2-20, Dover Corporation/Groen Division, Elk Grove Village, IL). Then 2 g thyme, 5 g white pepper, and 212 g chicken base (bouillon cubes, chicken flavor, Wyler Foods Company, Borden, Inc., Columbus, OH) were added. Finally, 1.9 L waxy corn starch (50% all purpose flour, 50% corn starch) (Karico, Inc., Elk Grove Village, IL) was added, and the mixture was simmered for 10 min to make 15 L of chicken gravy.

Inoculation with Listeria monocytogenes

Gravy (3.8 L) was poured into a stainless steel pan (Vollrath, Sheboygan, WI), 17.5 cm wide, 32.4 cm long, and 15.2 cm deep, and then placed into a refrigerator at 7°C (Lab-Line Ambi-Hi-Lo Chamber, Lab-Line Instruments, Inc., Melrose Park, IL). When the temperature of the gravy reached 40°C, gravy was divided into two equal portions and inoculated to contain ca. 10^5 CFU of strain Scott A or V7 per ml. Both treatments were stored at 7°C for 10 d. Sixteen hours were required to cool gravy from ca. 40 to 7°C. To monitor growth of *L. monocytogenes*, samples were taken initially and after every 24 h during the 10 d of chilled storage and were plated on tryptose agar which was incubated at 35°C for 48 h.

Heat treatment

Initially and after 1, 3, 5, and 10 d of refrigerated storage, 2-ml samples of inoculated gravy were taken and dispensed into a 4-ml autoclaved glass vial with a screw cap. Vials containing the gravy were completely immersed in a shaking water bath (Forma Scientific Inc., Marietta, OH) which had been adjusted to 50, 55, 60, or 65°C. Temperatures of water in the bath and of gravy in vials were monitored by copper/constantan thermocouples (TT-T-30, Omega Engineering Inc., Stamford, CT) connected to a 24 channel multiple temperature recorder (Speedomax Recorder, Leeds & Northrup Instruments Inc., Milwaukee, WI). For each heat treatment, there was a thermocouple in the center of a vial containing gravy and another thermocouple was immersed in the water bath. Time zero for each heat treatment was established when the temperature in the gravy was 0.55°C below that of the water bath. Vials were removed from the water bath at fixed heating intervals and immediately cooled in ice water.

Determination of D values and z values

Survivor curves for *L. monocytogenes* were obtained by plotting $\log_{10}$ CFU/ml versus heating time. The line of best fit for survivor plots was determined by regression analysis. The D values (negative reciprocal of the slope) were calculated from the resulting regression equations. The z values were estimated by regressing $\log_{10}$ D values versus heating temperatures. The z value is the temperature change in °C required to alter the thermal death time by an order of magnitude. Two independent trials for each strain were done. Reported data are averages of the two trials (unless indicated otherwise). Data on z values were analyzed statistically using the General Linear models of SAS statistical programs.

RESULTS AND DISCUSSION

Growth of L. monocytogenes *in chicken gravy*

Listeria monocytogenes strains Scott A and V7 grew in chicken gravy during cooling and refrigerated storage at 7°C for 10 d. A representative growth curve for strain V7 in chicken gravy appears in Fig. 1. This growth curve showed that *L. monocytogenes* grew rapidly during cooling (16 h were required to cool gravy from 40 to 7°C), and the population increased by 1.5 log during the first day of refrigerated storage. The numbers of *L. monocytogenes* reached ca. 10^9 CFU/ml of gravy after 10 d of refrigerated storage.

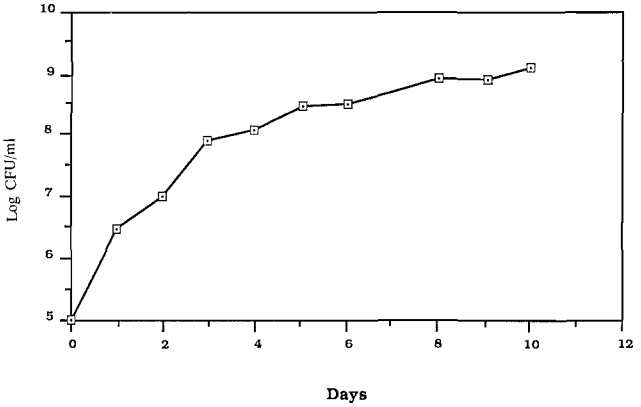

Figure 1. *Growth of* L. monocytogenes *strain V7 in chicken gravy (trial 1) during cooling and storage at 7°C for 10 d.*

Thermal inactivation of L. monocytogenes *in chicken gravy*

Figure 2 shows representative data on survival of *L. monocytogenes* strain V7 in chicken gravy stored 1 d at 7°C and then heat treated. The population of *L. monocyto-*

genes decreased linearly during the heating period. The estimated D_{60} values for strains Scott A and V7 (average of two trials) in chicken gravy after 1 d of storage at 7°C were 1.63 and 1.1 min, respectively. Conditions represented by this experiment might be encountered in a cook-chill food service system where foods usually are prepared 1 day before they are reheated and served.

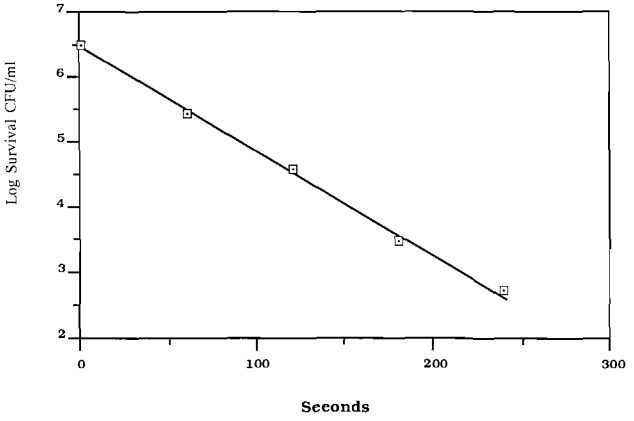

Figure 2. *Thermal death of* L. monocytogenes *Strain V7 in chicken gravy (trial 1) stored at 7°C for 1 d and then heated at 60°C.*

The D values of *L. monocytogenes* strains V7 and Scott A at day 0 when heated to 50, 55, 60, and 65°C are 195 and 119, 39.4 and 79.1, 3.13 and 7.07, and 0.19 and 0.48 min, respectively (Table 1). The D values obtained from samples immediately after inoculation (day 0) are greater than D values obtained later during refrigerated storage. Strain V7 was more heat resistant in gravy than was Scott A at 50°C, but strain V7 appeared more heat resistant at temperatures higher than 50°C (Table 1).

A thermal death time curve for strain V7 was constructed by plotting $\log_{10}$ D values versus temperature of heating (Fig. 3). The z values (Table 1) were estimated from the slope of the line of best fit. Statistical analysis indicated that the z value for *L. monocytogenes* decreased significantly ($p < 0.01$) during storage of *Listeria*-contaminated chicken gravy. The z values at days 0 and 1 were significantly greater than those at days 5 and 10 (Fisher's Least Significant Difference at $p = 0.05$). This indicates that as storage time increased, smaller changes in temperature were required to alter the D value (heat resistance) by one order of magnitude. One possible explanation for the decrease in D and z values after 1 d of refrigeration is that being in chicken gravy rather than in synthetic media made *L. monocytogenes* less heat resistant and thus less tolerant to a change in temperature. The z values for *L. monocytogenes* strain Scott A were significantly ($p < 0.01$) greater than those for strain V7, which means strain Scott A exhibited less change in heat resistance than V7 when the heating temperature was changed by a given value.

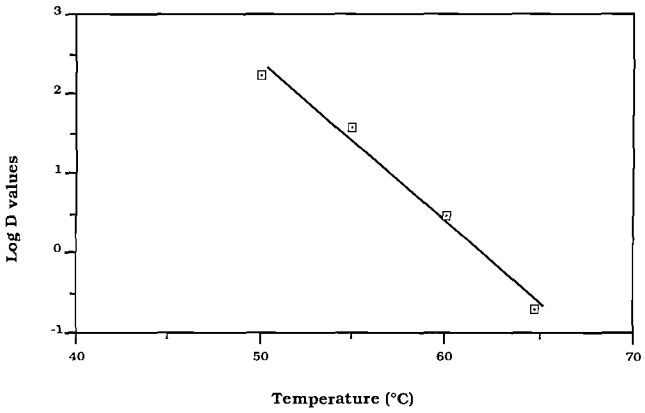

Figure 3. *Thermal death time plot (average of two trials) for* L. monocytogenes *strain V7 immediately after inoculation of the pathogen in chicken gravy.*

Farber (*12*) reported a D value at 60°C for 3.12 min and a z value of 4.92°C for 10 different strains of *L. monocytogenes* inoculated into ground meat to give a final concentration of about 1.0-2.0 x 10^7 CFU/g, then the meat was heated in sealed pouches. Lund et al. (*31*) reported that the D value of *L. monocytogenes* in nutrient broth heated at 60°C ranged from 3.6 to 5.4 min. Although these investigators used different foods, strains of the pathogen, and heating conditions, D values and z values they reported were comparable to those obtained in the present study at day 0 (immediately after inoculation of gravy).

Gaze et al. (*14*) studied the heat resistance of *L. monocytogenes* in homogenates of chicken, beef steak, and carrot at 60 to 70°C. The D values at 60°C in chicken homogenates were 5.29 and 5.02 min, and z values were 6.72 and 7.39°C for strains Scott A and NCTC 11994, respectively. The D values at 70°C ranged from 0.14 to

Table 1. *D and z values (averages of two trials) of* L. monocytogenes *strains V7 and Scott A in chicken gravy held at 7°C for 0 to 10 d and then heated to 50, 55, 60, and 65°C.*

	V7					Scott A				
	Day					Day				
Temperature	0	1	3	5	10	0	1	3	5	10
°C	D values (min)									
50	195	99.9	132	365	199	119	118	83.2	114	115
55	39.4	19.4	18.2	16.2	17.4	79.1	34.6	10.7	10.4	13.0
60	3.13	1.10	1.23	1.05	0.75	7.07	1.63	1.42	1.36	0.88
65	0.19	0.16	0.031	0.011	0.007	0.48	0.22	0.07	0.029	0.014
	z values (°C)									
	5.18	5.19	4.15	3.39	3.41	6.10	5.34	5.18	4.33	3.87

0.27 min and z values ranged from 5.98 to 7.39°C. They suggested that to achieve a 6-log reduction of *L. monocytogenes* the slowest heating point in a food product should be held at 70°C for 2 min.

Mackey et al. (*33*) studied the heat resistance of 27 strains of *L. monocytogenes* in chicken meats. They used a strain which was isolated from a prepacked chicken at a poultry processing plant and which was more heat resistant than the other isolates. According to their study, D values at 60°C were 5.6 (chicken leg) and 8.7 (chicken breast) min, and D-values at 70°C were 0.11 (chicken leg) and 0.13 (chicken breast) min. The z-values were 6.7 and 6.3°C, respectively. These D and z values were higher than those obtained in the present study, perhaps because the former investigators chose a more heat-resistant strain than those we used in this study.

The Code of Federal Regulations (*3*) states that poultry and meat products should be cooked to an internal temperature of 71.7°C. However, the Agriculture Research Service (*1*) reported that cooking frankfurters to an internal temperature of 71.1°C is "borderline" for destruction of *L. monocytogenes* because this heat treatment is only a 3-D process (68.3°C resulted in a 2-D reduction). Custer (*10*) indicated that a heat treatment of 1.48 min at 65.5°C was recommended by the U.S. Department of Agriculture to reduce the *L. monocytogenes* population by four orders of magnitude (a 4-D process). However, some researchers have reservations about the adequacy of the 4-D process to inactivate *L. monocytogenes* in foods.

According to our results, contaminated chicken gravy should be heated to an internal temperature of 65°C for at least 1.3 min to achieve a 4-D process; this is based on the assumption that only natural contamination (ca. 100 cells per g) of chicken gravy with *L. monocytogenes* occurred and that the pathogen did not grow. However, *L. monocytogenes* reached large numbers (increased about 1.5 log after 24 h) quickly in chicken gravy during cooling and refrigerated storage at 7°C. A heat treatment greater than the 4-D process is required to inactivate *L. monocytogenes* if a large population develops in chicken gravy during refrigeration. Exclusion of *L. monocytogenes* from chicken gravy by proper sanitation during handling the finished product is essential to ensure a safe product.

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