

Research Note

Heat Inactivation of *Listeria monocytogenes* and *Salmonella enterica* Serovar Typhi in a Typical Bologna Matrix during an Industrial Cooking-Cooling Cycle

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ABSTRACT

The heat resistance of *Salmonella enterica* serovar Typhi PF-724 and *Listeria monocytogenes* 2812 was determined in a commercial bologna batter. The heat inactivation of the two bacterial species was also studied in a semiautomatic pilot smokehouse under cooking conditions that reproduced an industrial bologna process. *S. enterica* serovar Typhi PF-724 was less heat resistant than *L. monocytogenes* 2812. The *D*-values (times required to reduce the population by 1 logarithmic cycle) for *S. enterica* serovar Typhi PF-724 ranged from 10.11 to 0.04 min for temperatures of 50 to 70°C, while for *L. monocytogenes* 2812, the *D*-values were 2.5-, 4.9-, 3.8-, 3.3-, and 2-fold higher at 50, 55, 60, 65, and 70°C, respectively, than for *S. enterica* serovar Typhi PF-724. However, the *z*-value (temperature required to reduce log *D* by 1 logarithmic cycle) for *S. enterica* serovar Typhi PF-724 (5.72°C) was not significantly different from the *z*-value for *L. monocytogenes* 2812 (7.04°C), indicating that a given increase in temperature would have a similar effect on the decimal reduction time for both bacterial species in that meat emulsion. Our data on experimentally inoculated batter also showed that processing bologna at a cooking-cooling cycle commonly used in the industry resulted in a minimum 5-log reduction for both *S. enterica* serovar Typhi PF-724 and *L. monocytogenes* 2812.

Heat treatments are commonly used in the food processing industry, not only to produce the desired organoleptic qualities but also to control pathogenic and spoilage microorganisms. Inadequately processed meats have been linked to foodborne outbreaks (2, 28), causing serious and even fatal illnesses (9, 10, 34). The efficacy of thermal processing of food is expressed by the *D*-value (time required to reduce the population by 1 logarithmic cycle) and the *z*-value (temperature required to reduce log *D* by 1 logarithmic cycle) and is considerably affected by factors related to the contaminating microorganisms themselves as well as by environmental conditions (31). For example, previous reports indicate that gram-positive bacteria are more resistant to heat treatments than are gram-negative bacteria, while *Clostridium perfringens* spores are more resistant than vegetative cells (26). *Listeria monocytogenes*, for example, is less sensitive to heat treatment than *Salmonella* and *Escherichia coli* O157:H7, according to several comparative studies of food matrices (21–23).

The environmental factors that affect the efficacy of heat treatments can be divided into two main groups: (i) those related to the growth conditions (e.g., temperature, pH, composition of the growth medium) (18) and (ii) those

related to the properties of the food matrix (e.g., composition, water activity, pH, heat transfer properties) (3, 12, 15, 16, 23). Constituents of the heat-treated food (e.g., fatty materials, additives, glycerol, sucrose) also affect the heat resistance of microorganisms. Fatty materials, for example, protect the microflora by reducing the water activity and thermal conductivity of the matrix (1, 8). Recently, Doyle et al. (7) observed four to eight times higher *D*-values for *L. monocytogenes* in foods with a high fat content than in foods with a low fat content. The pH of the heating medium has a remarkable role in determining the efficacy of heat treatments. Heat resistance is generally greater when the pH of the matrix is close to neutrality (27, 35). The growth temperature, the pH, and the acidulate type used to adjust the pH also influence the heat resistance (14). Hence, for process validation, it is best to evaluate the *D*- and *z*-values in real food matrices.

Several methods have been developed to determine the heat inactivation of food-associated microorganisms. The most frequently used methods are the determination of thermal death time in sealed containers (tube, can, flask, or tank) and the thermoresistometer method (33). Unsealed tubes and capillary tubes have also been used. Thorough process validation for industrial meat cooking-cooling operations requires pilot plant facilities where pathogens can

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be handled safely, and equipment setups must mimic industrial processing lines accurately.

The present study was undertaken to determine the *D*- and *z*-values for *L. monocytogenes* and *Salmonella enterica* serovar Typhi in a fine commercial bologna emulsion and to evaluate the level of destruction of these microorganisms under real industrial conditions in a pilot smokehouse.

MATERIALS AND METHODS

Commercial bologna emulsion. A fine commercial bologna emulsion was obtained from a local meat processing plant. It was transported and maintained under refrigerated conditions overnight for subsequent use in the validation experiment or was kept frozen at -20°C for a maximum of 2 months when used for the *D*- and *z*-value determinations. The frozen emulsions were thawed at 4°C , 24 h prior to inoculation. According to the manufacturer's specifications, the emulsions contained 62% water, 20% fat, 2.4% salt, 12% protein, and 2.4% carbohydrate.

Bacteria and growth conditions. *S. enterica* serovar Typhi PF-724, a pork carcass strain recently isolated, and *L. monocytogenes* 2812, a salami isolate implicated in human illness, were kindly provided by Dr. S. Quessy (Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Quebec, Canada) and Dr. J. M. Farber (Health Canada, Ottawa, Ontario, Canada), respectively. These field isolates were maintained at -80°C in brain heart infusion broth (Difco, Becton Dickinson, Sparks, Md.) supplemented with 10% (vol/vol) glycerol.

Cells were subcultured twice (1%) and incubated overnight at 35°C with agitation (150 rpm) prior to being used in experiments. They were harvested by centrifugation ($5,000 \times g$, 4°C , 5 min), washed twice, and resuspended in 10 ml of peptone water (0.1% [wt/vol]; Difco, Becton Dickinson) for *L. monocytogenes* 2812 or buffered peptone water (2% [wt/vol]; Oxoid Ltd., Basingstoke, UK) for *S. enterica* serovar Typhi PF-724 (11); isolation method MFHPB-30 was used for *L. monocytogenes*, and isolation method MFHPB-20 was used for *Salmonella*.

All meat samples (25 g) were homogenized with a Lab Blender 80 Stomacher (Seward Medical, London, UK) for 1 min in 225 ml of sterile peptone water (0.1% [wt/vol]) and buffered peptone water (2% [wt/vol]) for *L. monocytogenes* 2812 and *S. enterica* serovar Typhi PF-724, respectively. Serial dilutions (10-fold) were prepared with the same buffers, plated on tryptic soy agar (TSA), and incubated for 2 h at 35°C to allow the recovery of process-injured cells before replication on selective media by the method of Naim et al. (25). The TSA plates were replicated by direct contact (2 min) on PALCAM agar base (Oxoid) or brilliant green sulfa pyridine (Difco, Becton Dickinson) plates, which were incubated at 35°C for 72 h for *L. monocytogenes* 2812 and 24 h for *S. enterica* serovar Typhi PF-724.

Determination of *D*- and *z*-values in the commercial bologna emulsion. Samples (25 ± 0.5 g) of meat emulsion were aseptically weighed in 18-oz sterile Whirl-Pak sampling bags measuring 11.4 by 22.8 cm (Nasco, Newmarket, Ontario, Canada) and were inoculated with either *L. monocytogenes* 2812 or *S. enterica* serovar Typhi PF-724 at a final concentration of 10^7 CFU/g. The inoculated emulsions were hand massaged to ensure an even distribution of the cells. The samples were then flattened into a thin layer (2 mm thick and 10 cm high in the bag) for maximum heat transfer. The bags were heated, one at a time, in a circulating water bath adjusted with a programmable controller (1167P circulator model, VWR International, Mississauga, Ontario, Canada) at temperatures ranging from 50 to $70 \pm 0.1^{\circ}\text{C}$ for various times

TABLE 1. Heat treatments of a bologna batter for the determination of *D*- and *z*-values

Strain	Temp ($^{\circ}\text{C}$)	Time (min)
<i>L. monocytogenes</i> 2812	50	10, 15, 20, 25, 30
	55	10, 15, 20, 25, 30
	60	2, 5, 8, 10, 12, 14
	65	0.25, 0.75, 1, 1.25, 1.5, 1.75
	70	0.06, 0.1, 0.13, 0.16, 0.25
<i>S. enterica</i> serovar Typhi PF-724	50	2, 3, 4, 5, 6, 8, 10, 12, 14
	55	2, 3, 4, 5, 6, 7, 8
	60	1, 1.5, 2, 2.5, 3
	65	0.25, 0.5, 0.75, 1.08, 1.25
	70	0.03, 0.06, 0.08, 0.1, 0.13, 0.16, 0.33, 0.42

(Table 1). To determine the thermal profile in the emulsion, the temperatures of uninoculated samples were monitored with a HotMux Thermocouple Data Logger (TempoTech Controls Inc., Oakville, Ontario, Canada). A come-up time of 35 s was necessary to reach 50 and 55°C , while only 30 s was required to reach 60, 65, and 70°C (data not shown). The treated samples were immediately cooled in an ice water bath to 37°C (which took 10 s) and homogenized immediately in the appropriate buffer for cell counts to avoid cold shock. The time spent at the target temperatures was used for the determination of the *D*-values.

D- and *z*-values were determined according to the method of Murphy et al. (22). For each temperature studied, logarithm survivors were plotted against heating times, and *D*-values were calculated as the negative inverse of the slope of the regression line. The *z*-value was determined as the negative inverse of the slope when the logarithm of *D* was plotted against the temperature of treatment. Because the decrease in cell counts was limited for certain couples of time and temperature treatments, only those meaningful with respect to industrial cooking-cooling cycles were taken into account for the calculation of the *D*- and *z*-values.

Process validation. The bologna was processed in a biosafety level II pilot plant for meat processing (Faculté de médecine vétérinaire, Université de Montréal). The production steps were similar to those used industrially. To ensure an even distribution of the test microorganisms, the inocula were first thoroughly mixed into a small quantity of emulsion (1 kg) that was then thoroughly mixed with the remaining 19 kg to reach a final concentration of 10^7 CFU/g. The mixing was performed for 3 min in a 45-kg capacity double-action paddle mixer (DMX 100 model, Daniels Food Equipment, Parkers Prairie, Minn.). Impermeable casings supplied by the emulsion manufacturer were filled with the batter by a 25-kg capacity Ramon 25 electrical piston stuffer (Talleres Ramon, Villasar de Dalt, Spain). Uninoculated samples served as negative controls and were prepared before the inoculated batter to avoid cross-contamination. Samples were cooked in a semiautomatic pilot smokehouse (model SC-40, Sipromac Inc., St-Germain-de-Grantham, Quebec, Canada) that can cook up to 32 bologna units. Each production run consisted of eight inoculated samples, four uninoculated controls, and 20 filling units used in order to fill the smokehouse to its maximum capacity and better simulate commercial conditions. The filling units consisted of commercial bologna provided and prepared by the processed meat manufacturer. To track the temperature, three thermocouples connected to a HotMux Thermocouple Data Logger (TempoTech)

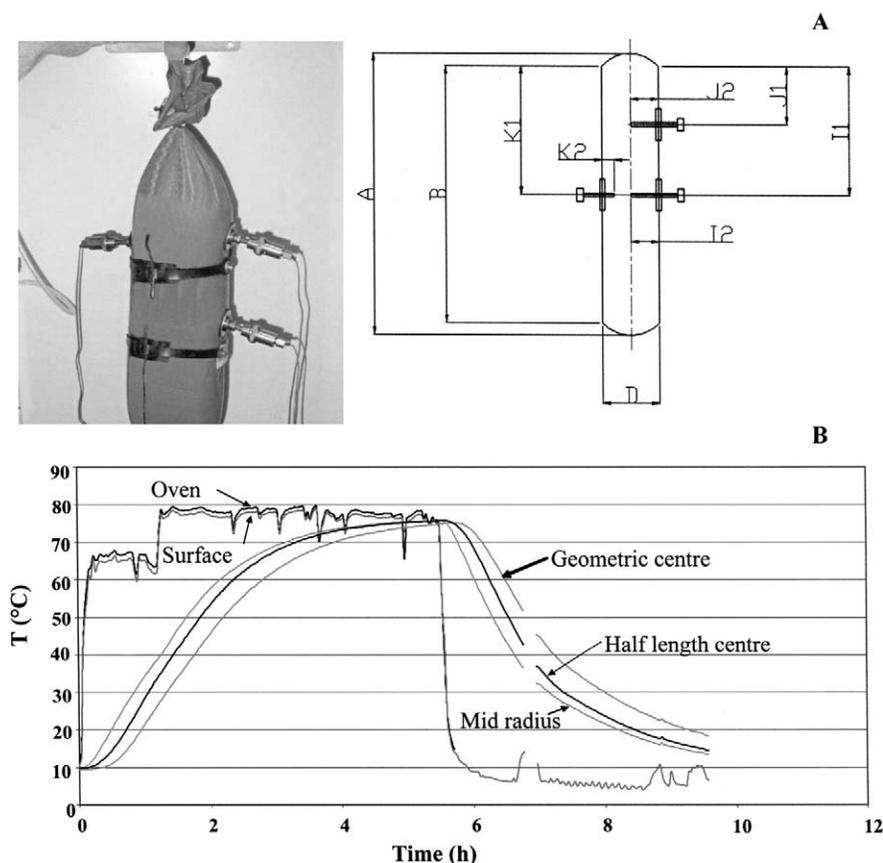


FIGURE 1. Location of thermocouples (A) and temperature profile in bologna (B) during heat treatment in the pilot smoke-house.

were placed at specific locations (geometric center, mid-radius, and 2 mm from the surface) (Fig. 1) in the four uninoculated controls. The cooking-cooling profile consisted of average industrial cooking-cooling conditions. Typically, for bologna stuffed in impermeable casings, the time-temperature profile consists of three steps: 60 min at 68°C and 100% relative humidity, 240 min at 78°C and 100% relative humidity (until the internal temperature reaches 73°C), and 45 min in a shower with cold water (5°C). For microbiological analysis, two 25-g samples were taken from two different bologna units at the geometrical center of the products (where the heating rate is slowest during a thermal process) for three different sampling times (i.e., before cooking, before the cooling period, and after the entire process was completed). Viable counts were done as previously described.

Statistical analyses. Experiments for D - and z -value determinations were repeated at least three times. Process validation was repeated twice, and all cell counts were performed in duplicate. Significant differences between treatments were evaluated by an analysis of variance. Treatment comparisons among means were performed with the Fisher least significant difference test by STATGRAPHICS plus 4.1 software (Manugistics Inc., Rockville, Md.) (32). The level of significance was set at $P < 0.05$. The large heterogeneity of variance between temperatures failed to reveal any significant differences. To eliminate the influence of variance heterogeneity, a nonparametric procedure (Kruskal-Wallis test) was used. The Kruskal-Wallis test is performed by the RANKS and GLM procedures of the SAS system (29). The least significant difference option was used to compare mean ranks.

RESULTS AND DISCUSSION

D - and z -values in a meat product. The D - and z -values in commercial bologna emulsions are summarized

in Table 2. *S. enterica* serovar Typhi PF-724 appears more heat sensitive than *L. monocytogenes* 2812. D -values for *S. enterica* serovar Typhi PF-724 were significantly smaller ($P < 0.05$) than those for *L. monocytogenes* 2812 at 50 and 55°C but not at 60°C and higher. With respect to temperature, the D -values at 55, 60, 65, and 70°C were not significantly different for *S. enterica* serovar Typhi PF-724, and the D -values for *L. monocytogenes* 2812 were not significantly different above 60°C. The analysis of variance failed to reveal significant differences between those temperatures because of the large heterogeneity of variance (standard deviation). The experiments were performed with commercial batters produced in large quantities, and the variability observed may very well represent the true variation for industrially processed deli meats. To eliminate the influence of variance heterogeneity, a nonparametric procedure (Kruskal-Wallis test) (29) was performed, and the results of mean ranks are presented in Table 3. These results indicate that D -values were significantly different ($P < 0.0001$) for all temperatures tested with *S. enterica* serovar Typhi PF-724. For *L. monocytogenes* 2812, D -values were significantly smaller with increasing temperatures, except at temperatures of 50 and 55°C, where no significant difference was observed. Hence, this suggests that a threshold temperature of 55°C is required to destroy *L. monocytogenes* 2812 effectively.

At 60, 65, and 70°C, our results are in agreement with those reported by Murphy et al. (21), who did not observe any significant differences between D -values for cocktails of six *L. monocytogenes* and six *Salmonella* strains during

TABLE 2. Heat resistance of target organisms in a bologna batter

Strain	Repetition	D-values (min) at temp (°C):					z-value (°C)
		50	55	60	65	70	
<i>L. monocytogenes</i> 2812	1	17.72	16.72	1.72	0.51	0.04	6.22
	2	31.15	17.54	5.97	0.93	0.12	5.89
	3	27.78	17.64	9.03	1.36	0.09	5.07
	($\bar{x} \pm \delta$) ^a	25.21 ± 7.54 C b	17.30 ± 0.50 B b	5.57 ± 3.67 A a	0.93 ± 0.42 A a	0.08 ± 0.04 A a	5.72 ± 0.59 a
<i>S. enterica</i> serovar Typhi PF-724	1	9.10	2.78	1.09	0.37	0.07	8.32
	2	14.77	4.19	1.86	0.28	0.04	5.96
	3	6.47	3.52	ND ^b	0.20	0.02	6.86
	($\bar{x} \pm \delta$)	10.11 ± 4.24 B a	3.49 ± 0.70 A a	1.47 ± 0.54 A a	0.28 ± 0.08 A a	0.04 ± 0.02 A a	7.04 ± 1.19 a

^a Mean ± standard deviation; different lowercase letters in the same column or capital letters within the same row are significantly different ($P < 0.05$).
^b ND, not determined.

TABLE 3. Mean ranks of D-values at various temperatures obtained by the Kruskal-Wallis analysis

Strain	Mean rank at temp (°C):				
	50	55	60	65	70
<i>L. monocytogenes</i> 2812	28.0 A	25.0 A	18.0 B	11.3 C	4.5 D
<i>S. enterica</i> serovar Typhi PF-724	21.7 A	17.0 B	13.5 C	8.0 D	2.5 E
Both strains	24.8 A	21.0 B	16.2 C	9.7 D	3.0 E

^a Mean ranks with different letters in the same row are significantly different ($P < 0.05$).

heat treatments of ground pork. These authors reported D-values varying from 5.07 to 0.083 min and from 5.61 to 0.085 min at temperatures ranging from 60 to 70°C for the cocktails of *Salmonella* and *L. monocytogenes* strains, respectively. The significant difference obtained for the D_{50} - and D_{55} -values for the two organisms reported in the present study is most likely due to differences between species but may also be due to differences in the bacterial stress response to heat treatments at low temperatures. Seyer et al. (30) reported that more intracellular DnaK heat shock protein is produced by *E. coli* when cells are treated at 50°C than at 55°C for a similar process lethality value (F_{70}^{10}). Furthermore, when DnaK is induced at a higher level prior to a lethal heat treatment, the resistance of the cells improves significantly. Doyle and Mazzotta (6) reported that for similar heat treatments, the D-values of different serotypes of *Salmonella* vary considerably. Such differences were also observed among 35 strains of *Salmonella* studied by Juneja et al. (13). De Jesús and Whiting (5) also observed significant differences in the heat resistance (D-values) of 25 strains of *L. monocytogenes* belonging to three distinct genotypic lineages.

Despite differences in D-values, the z-values for *S. enterica* serovar Typhi PF-724 (5.72°C) and *L. monocytogenes* 2812 (7.04°C) were not significantly different (Table 2). This indicates that, in a bologna mix, a given increase in temperature will have a similar effect on the D-value of both bacterial species. Similar results were reported by Murphy et al. (23) for fully cooked poultry products, where z-values from 4.9 to 7.0°C were observed for *Salmonella*, *Listeria innocua*, and *L. monocytogenes* after heat treatments ranging from 55 to 70°C. In another study, Muriana et al. (20) reported a z-value of 7.9°C for *L. monocytogenes* in roast ham. The rather limited variations in reported z-values might have been due to differences in the strains used and the characteristics (e.g., pH, fat) of the food matrix (23, 24).

Experimental process validation. The processing target of the industrial cooking procedure under validation in this study was to reach a temperature of 73°C at the coldest spot in the bologna. The survival data of both organisms in inoculated bologna after cooking in the pilot smokehouse are presented in Table 4. Both strains were very sensitive to the applied heat treatments. The initial inoculum was 7

TABLE 4. Effect of heat treatments in a pilot smokehouse on the survival of *L. monocytogenes* 2812 and *S. enterica* serovar Typhi PF-724 in an inoculated bologna batter

	Bacterial counts (log CFU/g) ^a	
	<i>L. monocytogenes</i> 2812	<i>S. enterica</i> serovar Typhi PF-724
Sampling period		
Before cooking	7.42 ± 0.21	7.04 ± 0.05
Before cooling	BDL ^b	BDL ^c
After cooling	BDL ^b	BDL ^c
Destruction level (log CFU/g)	5.72	5.04

^a Results are expressed as the mean ± standard deviation from two repetitions.

^b BDL, below the detection level of 50 CFU/g.

^c BDL, below the detection level of 100 CFU/g.

log CFU/g, and the survivor counts were below the detection limit of 2 log CFU/g before cooling and at the end of the process. Hence, cooking reduced the cell population by at least 5 log CFU/g (Table 4). Our data are in agreement with those of Carlier et al. (4) and Mazzotta and Gombas (19), who reported a 5-log reduction of *L. monocytogenes* Scott A after cooking brined ham (71.1°C for 23.5 s) and hot dogs (71.1°C for 30 s). Preliminary results suggest that cell counts do not increase between the time of inoculation and the beginning of the cooking cycle. Inoculated batters held at 4°C for 24 h and incubated for up to 2 h at 35°C had similar cell counts (data not shown). However, additional experiments are needed to determine the behavior of the target organisms at different stages of the process. More intermediate sampling points during the heat treatment would be necessary to accurately monitor the kinetics of bacterial destruction. These results (*D*- and *z*-values) would help our research team develop a generic model and the software required to monitor the cooking-cooling cycles encountered in the meat and poultry industries (17).

The present article demonstrated that *L. monocytogenes* and *S. enterica* serovar Typhi have different patterns of heat resistance. According to the *D*-values obtained, *S. enterica* serovar Typhi PF-724 was more temperature sensitive than *L. monocytogenes* 2812, even though their respective *z*-values were not significantly different. Process validation experiments confirmed a minimum 5-log reduction for both target organisms by the industrial cooking process tested.

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