Effect of prior sublethal heat shock on survival of *Salmonella spp.* during isothermal cooking

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Abstract

Exposure of *Salmonella* to sublethal stress reportedly increases the thermotolerance of the pathogen on subsequent heating. This is of importance in slow cooking of meats when pathogens may be exposed to sublethal temperatures for varying periods of time, thereby increasing the likelihood of pathogen survival. This enhanced thermotolerance may make it difficult for processors to meet target pathogen reductions outlined in USDA guidance (Appendix A).

We studied the effect of sublethal heat shock treatment on subsequent thermotolerance of *Salmonella* during isothermal cooking (54.4˚ C) of lean ground beef (6-9% fat). Six different heat shock treatment combinations of time (5 or 30 min) and temperature (47.2, 48.3, 49.4˚ C) were evaluated. An eight-strain cocktail of *Salmonella* was inoculated into 25g ground beef packages pre-warmed to the target heat-shock temperature, held for 5 or 30 min and cooked at 54.4˚ C for 2 or 4h. The control consisted of isothermal cooking (54.4˚ C for 2 or 4h) with no prior heat-shock. Viable microorganisms were enumerated every 20 min over the cooking period. Three trials were conducted for each heat shock/isothermal cooking combination and D-values were calculated.

D-value for the control was significantly lower (36.54 min) than the average D-value across all heat-shock treatments (51.65 min), indicating a heat-shock effect. D-value was not significantly affected by heat shock temperature or time (P>0.05). Previous work indicated a D-value for *E. coli* O157:H7 ranging from 25.4 to 48.6 min under similar experimental conditions (Wiegand *et al*. 2009. *J. Food Prot*. 72:1727-1731). The higher D-values seen with *Salmonella* support use of this pathogen as the target microorganism when validating cooking treatments.

Introduction

Salmonella is a Gram negative, aerobic and facultatively anaerobic, non spore forming bacterium. The optimum growth temperature is 35-37ºC, but it can grow over a wide range of temperatures (5-47ºC). It can also survive a pH range of 4.5-9 [1]. The wide adaptability and noted heat resistance of the pathogen have led the US Food Safety and Inspection Service (FSIS) to use *Salmonella* destruction as an index of process lethality for cooking meat and poultry. FSIS guidance targets a >6.5 log CFU reduction against *Salmonella* in order to ensure safety [2].

Salmonellosis, caused by *Salmonella*, is characterized by diarrhea, abdominal pain, and fever that normally lasts 8-72 h after ingestion of the contaminated food product [3]. The infection is more pronounced in children, the elderly and immuno-compromised patients [4]. The five serovars most commonly reported in human illnesses are *S. enterica* serovars Typhimurium, Enteritidis, Newport, Heidelberg and Javiana [5]: two of which are used in this study.

As with most of the pathogens, *Salmonella* is capable of producing heat shock proteins (HSP) when exposed to conditions beyond normal growth conditions [6]. The objective of this study was to study, using a ground beef system, the effects of heat shock treatments on the subsequent thermotolerance of the pathogen during isothermal cooking conditions (54.4˚ C).

Methods

Inoculum Preparation
•*Salmonella* cultures (8 strains previously isolated from beef and screened for heat-tolerance) were grown for 18-24 h on Nutrient Agar (NA; BD Difco) and suspended in Butterfield's Phosphate Diluent (BPD; Nelson Jameson) to form a combined 8-strain inoculum.

Ground Beef Preparation
•Small bags of 25g lean ground beef (94-99% lean) were pre-warmed to the heat shock temperature (Fig. 1).
•The meat was manually mixed with 1.0 mL inoculum and exposed to a specific heat-shock time / temperature combination (47.2º, 48.3º, or 49.4ºC for 5 or 30 min) in a water bath, followed immediately by isothermal heating for 2 or 4h in a pre-set 54.4ºC water bath.

Sampling
•Bags of meat were analyzed before isothermal heating (time 0), and every 20 min throughout for up to 240 min.
•After removal from the water bath, each bag was held on ice for 30s to stop cooking, surface-sterilized with 70% EtOH, aseptically cut open, and everted into a Whirl-pak filter bag (Nasco).
•The ground beef and everted bag were stomached 30s at medium speed with 99 mL of BPD.

Cell Recovery and Enumeration
•The ground beef-buffer slurry was diluted in BPD and spread-plated onto modified Eosin Methylene Blue agar (M-EMB) prepared from lactose-free EMB (Difco) with the addition of 10 g/l D-sorbitol and 5 g/l NaCl.
•After 18-24h of incubation at 35ºC, typical *Salmonella* colonies were counted. Typical colonies appear pink to purple with a green metallic sheen.

Statistical Analysis
•Statistical analysis was done with PROC MIXED of SAS version 9.2 statistical software. Least squares means analysis was done with a model accounting for unequal variances and using Tukey adjustments. D-values were calculated using DMFit 2.1 software [7].

References

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Heat Shock Results

•Reductions ranging from 2.1 to 3.9 log CFU/g were observed in the *Salmonella* inoculum during different trials of isothermal cooking (**Table 1**).
•Initial indigenous bacterial load and ground beef pH were not significant factors affecting *Salmonella* survival or death (P>0.05).
•Survival of *Salmonella* during isothermal cooking was generally enhanced by heat shock, but within the heat-shock treatments, differences in either heat shock temperature (47.2, 48.3 or 49.4ºC) or time (5 or 30 min) did not significantly (P>0.05) affect survival.
•Heat-shock treatment of 48.3ºC for 30 min was the least protective during subsequent cooking.

Table 1: Average reduction of *Salmonella* (Δ log CFU/g) after heat shock and 2 or 4h of isothermal cooking at 54.4ºC (n=3).

Heat Shock Temp (ºC)	Heat Shock Time (min)	Cooking Time (min)	Average Reduction
47.2	5	120	2.55 ± 0.05
		240	3.51 ± 0.16
47.2	30	120	2.30 ± 0.25
		240	3.41 ± 0.05
48.3	5	120	2.12 ± 0.45
		240	3.66 ± 0.15
48.3	30	120	2.48 ± 0.26
		240	3.92 ± 0.16
49.4	5	120	2.48 ± 0.87
		240	3.78 ± 0.21
49.4	30	120	2.65 ± 0.15
		240	3.76 ± 0.23

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D-value Comparison

•*Salmonella* D-values at 54.4ºC generally increased after heat-shock treatments, when compared to the D-value for *Salmonella* in ground beef that did not undergo heat shock (control) (**Table 2**).
•After heat-shock, the $D_{54.4}$ generally increased, suggesting that 5 – 30 min exposure to 47.2 – 49.4ºC can increase subsequent thermotolerance. This trend is true except in the case of a 30 min heat shock at 49.4º C, which was the most extreme of the heat-shock treatments tested (longest time, highest temperature).
•D-values from the heat-shock treatment combinations of 47.2ºC at both 5 and 30 min, and 48.3ºC at 30 min were significantly different from the control (P<0.05).

Table 2: Ranked $D_{54.4}$ values of heat-shocked *Salmonella* cells in ground beef (n=3).

HS Conditions		D-value
Temp ºC	***Time (min)***	***Mean $D_{54.4}$****
Control (no heat shock)		36.54_{b}
49.4	30	36.25_{ab}
48.3	5	49.28_{ab}
47.2	5	53.11_{a}
49.4	5	54.64_{ab}
47.2	30	58.16_{a}
48.3	30	58.58_{a}

*D-values for heat shocked cells ranked from lowest to highest. Values followed by different subscripts within the column are significantly different (P<0.05).

Figure 1: Small sample bags of 25g lean ground beef heated in hot water bath for heat-shock and isothermal heating experiments.

Conclusions

•The generally higher D-values of heat-shocked *Salmonella* compared to the control suggest that heat shock treatments have a protective effect on *Salmonella* strains during subsequent isothermal cooking.
•Predictive tools for evaluating the lethality of non-isothermal beef cooking treatments against *Salmonella* should consider the potential enhancement of cell survival resulting from heat-shock conditions existing early in some cooking processes.
•It may be necessary to revise FSIS cooking lethality requirements, to account for heat shock-induced enhancement of *Salmonella* thermotolerance.