ENHANCED THERMAL SENSITIVITY OF *LISTERIA MONOCYTOGENES* BY COLD SHOCK.

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Background:

Listeria monocytogenes is a Gram positive food borne pathogen that can grow at temperatures to -0.5°C (Walker et al., 1990). It is widespread throughout the environment and frequently contaminates foods. Human infections can result in severe invasive disease having a mortality rate of nearly 30%. While infants, the elderly and immunocompromised individuals are at high-risk for infection, the incidence of human listeriosis in the U.S. has declined by over 50% since 1989. Yet, the U.S. regulatory requirement for ready-to-eat foods to have no *L. monocytogenes* in a twenty-five gram sample continues to impart considerable economic loss to both the domestic industry, as well as to companies who export products to the U.S. For example, between 1989 and 1995 USDA surveillance programs triggered 74 *Listeria*-related product recalls (Tompkin, 1996). The severity of the problem of *Listeria* contamination in ready-to-eat foods could be overcome by a post-processing lethal step. However the use of heat for this purpose is hampered by the comparatively high decimal reduction time (D-value) for *L. monocytogenes*. A cost-effective method is required to lower potential *Listeria* levels on pre-cooked foods without affecting product quality attributes.

The literature on *Escherichia coli* suggests that a novel post-processing intervention strategy may be developed by exploiting the antagonistic nature of the heat and cold shock responses (Jones and Inouye, 1994). Heat shock is a well studied phenomenon, whereby a sublethal heat stress induces a biochemical response in cells to permit survival during subsequent, and normally lethal, heat stress. Cold shock is less well characterized, and permits enhanced survival after temperature down-shifts. Both share the common response that a set of inducible proteins is synthesized that stabilize physiological proteins and the protein synthesis machinery, and thus help ensure cell survival. A hallmark of the cold shock response in *E. coli* is the reduction of levels of heat shock proteins. We hypothesized that this phenomenon may be exhibited by *Listeria monocytogenes*, and would, therefore, result in heightened thermal sensitivity.

Objectives: The objectives of this study were: 1) to determine if *Listeria monocytogenes* is more vulnerable to heating after receiving a cold shock, and 2) to evaluate the potential for this phenomenon to be used as a mild post-processing treatment in a ready-to-eat product such as frankfurters.

Methods: *Listeria* strains, including *L. monocytogenes* Scott A (serotype 4b,), a clinical isolate, V7 (serotype 1), a milk isolate, and *L. innocua* were grown in Lauria's broth at 37°C to stationary phase. In the model system, cultures were cold shocked by a temperature down-shift from 37° to 15° or 0°C for 0, 1, and 3 hrs. Cold shocked and control samples were then evaluated for thermal resistance at 60°C using a submerged coil heating apparatus (Cole and Jone, 1990). Heated samples were collected in 1 ml portions, plated onto a non-selective medium (brain heart infusion agar) to detect heat-injured cells, then enumerated after a 36 h incubation at 37°C. For experiments on frankfurters, commercial post cooking products were collected immediately prior to chilling. Frankfurters were formulated with 67% pork, 20% water and 10% beef. After transporting to the laboratory, surfaces were aseptically removed using a sterile scalpel and forceps. Skins were sterilized by dipping into a 70% ethanol solution for 5 min, then dried for 30 min in a laminar flow hood. One hundred µl of a stationary phase culture ($\log_{10}$ 9 cfu) of *L. monocytogenes* Scott A was applied directly to the surface of a 9 cm^2 piece of sterile frankfurter skin (ca. 1 g). Inoculated skin samples were allowed to dry for 30 min in a laminar flow hood before placement into a filtered stomacher bag which was then evacuated and heat sealed. Frankfurter samples were cold shocked at 0°C for 3 hr by immersion in an ice slush, then heated in 60°C water for up to 10 min. Samples were removed periodically then immediately cooled in an ice bath. Sample bags were opened, diluted with 9 ml of 0.1% peptone (pH 6.8), then blended with a laboratory stomacher. Diluted samples were plated and incubated as above. D-values were estimated using linear regression analysis, then analyzed using the SAS General Linear Model.

Results: Cells grown at 37°C to stationary phase, cold shocked, then heated at 60°C using the heating coil apparatus, exhibited decreased decimal reduction times, compared to control cells that were not cold shocked (Fig. 1). D_{60}-values fell from 1.25 min, in non-cold shocked controls, to a mean of 0.73 min (42% reduction), for 3 h cold shocked cells ($P<0.01$), and to a mean of 0.80 min (36% reduction), for cells that were cold shocked at 0°C ($P<0.05$). Cold shock duration was observed to be more important than the temperature differential, as evidenced by the nearly identical D_{60}-values obtained after 3 h of cold shock, regardless of temperature differential.

In a second experimental series the D_{60}-values of 37°C cells in either lag, exponential, or stationary phase were determined and the effect of cold shock on thermal resistance was determined (Fig 2). Stationary cells were over 50% more thermally resistant (D_{60}=1.27 min), compared to lag and exponential cells, which had D_{60} -values of 0.83 and 0.79, respectively. When these cells were cold shocked prior to heating at 60°C D-values were lowered by 42%, 30%, and 8% compared to non-shocked controls for stationary, lag and exponential cells, respectively.

The enhanced thermal sensitivity after a 37° to 0°C (for 3 h) temperature down-shift was demonstrated in two serotypes (Table 1) of *L. monocytogenes*, strains Scott A (serotype 4b) and V7 (serotype 1). Moreover, altered D-values after cold shock were demonstrated in *L. innocua.*

Figure 3 shows the enhanced thermal sensitivity of *L. monocytogenes* Scott A on inoculated frankfurter skins. D_{60} -values decreased 25%, from 2.22 (±0.05) min, for non-shocked controls, to 1.67 (±0.12) min, for inoculated skins that were cold shocked.

Discussion: Previous research showed *L. monocytogenes* Scott A to be more sensitive to heating at 52°C if grown below 28°C, than if grown above 28°C (Smith et al., 1991). The present study extends this by showing that decimal reduction time for *L. monocytogenes* inactivation was lowered using a temperature down-shift prior to heating. Maximum effect was in stationary phase cells, which most simulates cells contaminating food. Efficacy was demonstrated for two *L. monocytogenes* serotypes and for *L. innocua*, suggesting genus sensitivity.

Bayles et al. (1996) showed that temperature down-shift produced arrested growth and protein synthesis, in *L. monocytogenes,* followed by renewed protein synthesis, which included specific cold-induced proteins, and growth resumption. Prior research on *E. coli* indicated that cold shock proteins appeared to function to reassociate ribosomal subunits, which reinitiated translational activity. The mechanism of the present observation, similarly, may be inferred from *E. coli*, where cold shock inhibited heat shock protein synthesis (Jones and Inouye, 1994). Most likely, this renders the organism more heat sensitive.

The observation that *L. monocytogenes* inoculated onto frankfurter skins and vacuum packaged under film were 25% more heat sensitive after receiving a cold shock, demonstrates the practical application of this observation. For example, steam pasteurization of beef frankfurters was shown to inactivate *L. innocua* (Cygnarowicz-Provost et al., 1994). Employing steam pasteurization in fully processed product after a temperature down-shift may serve as a pre- or post-packaging listeriocidal treatment. This concept needs to be further explored to assure that product quality is not compromised.

Key References

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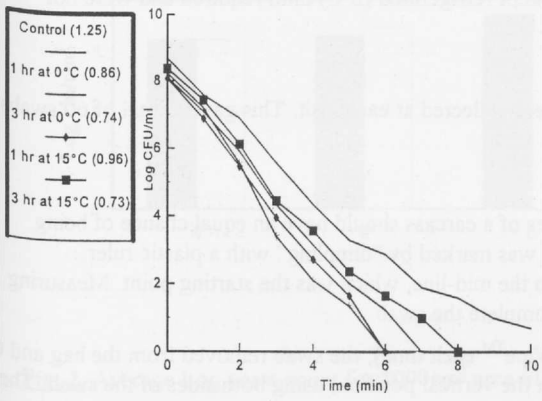
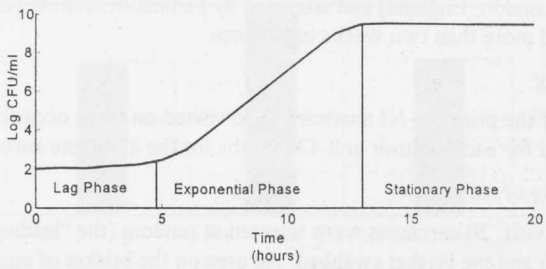


Fig 1. *Listeria monocytogenes* (Scott A) grown to stationary phase at 37°C, then heated at 60°C after a 0 or 15°C cold shock for 1 and 3 hours. Each symbol is the mean of 2 trials. Numbers in parenthesis in the legend are D_{60}-values, estimated using linear regression analysis



Cold Shock Temperature	Growth Phase of 37°C Cells		
	Lag	Exponential	Stationary
Control	0.83 (0.05)	0.79 (0.04)	1.27 (0.09)
15°C	0.60 (0.08)	0.74 (0.04)	0.75 (0.05)
0°C	0.58 (0.09)	0.73 (0.03)	0.74 (0.00)

Fig 2. Post cold shock thermotolerance at 60°C of *Listeria monocytogenes* (Scott A) in different 37°C cell phases. Each value represents the mean (SD) D_{60}-value (min) of 2 trials.

		D-Values (min)					
		Control		0°C Cold Shock			
Strain	Serotype	mean	std dev	mean	std dev	%D_{60}-Value Decrease	Trials
L. innocua (2340)	—	1.44	0.06	1.08	0.03	25	2
L.monocytogenes (scott A)	4	1.27	0.09	0.76	0.05	40	27
L. monocytogenes (V7)	1	1.31	0.03	0.88	0.02	33	2

Table 1. Cold shock effects on thermal inactivation values at 60°C of different *Listeria* strains grown at 37°C to stationary phase.

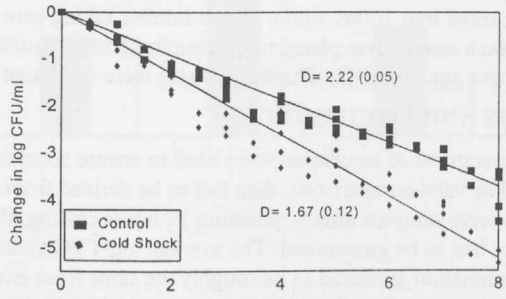


Fig 3. D-values at 60°C for *Listeria monocytogenes* (Scott A) inoculated onto frankfurter skins then cold shocked at 0°C for 3 hours. Cells were grown at 37°C to stationary phase prior to inoculation (4 trials).