Synergistic Effect of Heat and Elevated Hydrostatic Pressure for Inactivation of *Listeria monocytogenes*

Abimbola Allison, Eleonora Troyanovskaya, Shahid Chowdhury, and Aliyar Fouladkhah

Public Health Microbiology Laboratory, Tennessee State University, Nashville, TN

ABSTRACT

Recent epidemiological investigations derived from CDC active surveillance data indicates 99% of illnesses caused by *Listeria monocytogenes* are foodborne in nature, leading to hospitalizations in 94% of episodes, and are collectively responsible for estimated 266 annual deaths of American adults. Current study investigates effects of elevated hydrostatic pressure on cell reduction and inactivation rates of *Listeria monocytogenes* at 4 and 55ºC. Various times (0 to 10 minutes) and intensity levels (0 to 380 MPa) of elevated hydrostatic pressure were investigated for inactivation of *Listeria monocytogenes* inoculated into phosphate-buffered saline at target population of 7.5 log CFU/mL. Temperature was monitored, and maintained at 4 and 55 ºC by a circulating water bath and a stainless steel water jacket surrounding the chamber. The experiment was conducted in two biologically independent repetitions, as blocking factors of a randomized complete block design, containing three repetitions per time/temperature/pressure within each block. Experiment was analyzed by GLM procedure of SAS using Tukey- and Dunnett-adjusted ANOVA. The inactivation K_{max} and D-values were calculated using best-fitted (maximum R^2) model obtained by GInaFiT software. At 380 MPa (0 to 10 minutes), D-value of 2.81 min and inactivation K_{max} of 1.60±0.41 1/min were observed at 4 ºC. At 55 ºC, these values were 1.59, and 3.94±0.96, respectively. At 4 ºC, the pathogen were reduced (P< 0.05) by 3.84, 2.44, and 1.05 log CFU/mL after exposure to 10 minutes of hydrostatic pressure at 380, 310, and 240 MPa, respectively. These reductions (P< 0.05) were >7.13, 6.36, and 4.53 for 10-minute treatments at 55 ºC, respectively. Treatments below two minutes were less efficacious ($P \geq$ 0.05) against the pathogen in vast majority of the tested time, temperature, and pressure combinations. Results of this study could be incorporated as part of a risk assessment modeling and predictive microbiology for reducing the public health burden of listeriosis.

Figure 1. Sensitivity of four-strain mixture of (ATCC® numbers 13932, 51779, 51772, BAA-2658) *Listeria monocytogenes* exposed to elevated hydrostatic pressure (Barocycler Hub440, Pressure BioScience Inc., South Easton, MA) for various time intervals. Columns of each time interval followed by different Uppercase letters are resenting log CFU/mL values that are statistically (P<0.05) different (Tukey-adjusted ANOVA). Uppercase letters followed by † sign are statistically (P<0.05) difference than the control (Dunnett-adjusted ANOVA).

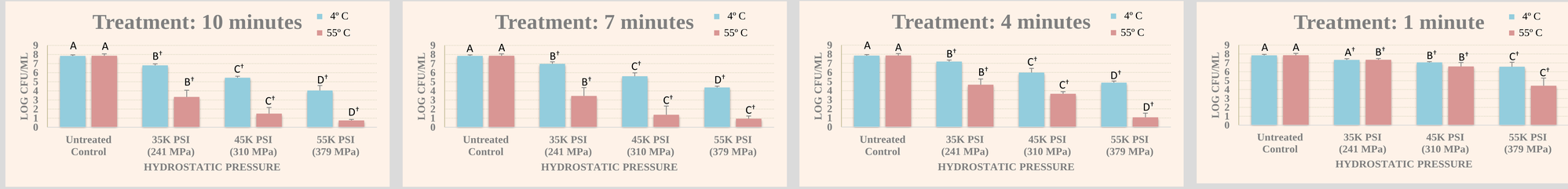
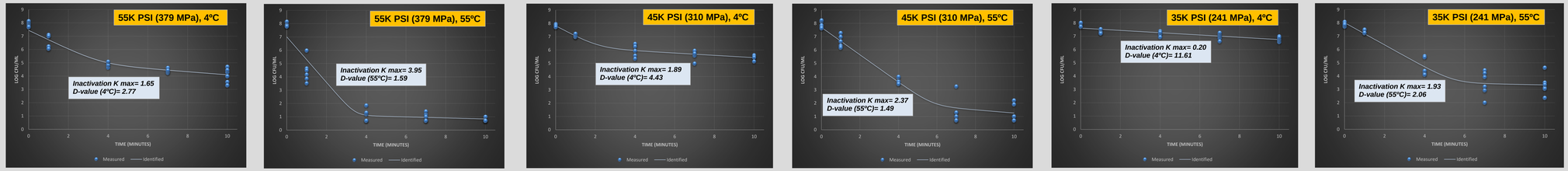


Figure 2. Figure 1. Inactivation rates for four-strain mixture (ATCC® numbers 13932, 51779, 51772, BAA-2658) *Listeria monocytogenes* exposed to elevated hydrostatic pressure (Barocycler Hub440, Pressure BioScience Inc., South Easton, MA). Kmax values are selected from best fitted model (goodness-of-fit indicator of R2 value, α=0.05) among Biphasic or Log-linear regression with or without shoulder or tail, using GInaFiT software (Greeraerd et al., 2005). Kmax values are expression of number of log cycles of reduction in 1/min unit, thus larger values indicate less time required for microbial cell reductions in each tested level of hydrostatic pressure. D-values provided, indicate time required for one log (90%) microbial cell reductions of the habituated microbial mixture.



Temperature and pH Recordings

	35,000 PSI (241 MPa)	45,000 PSI (310 MPa)	55,000 PSI (379 MPa)
4°C Treatment			
10 minute	7.43 ± 0.2	7.37 ± 0.1	7.39 ± 0.1
7 minutes	7.37 ± 0.1	7.51 ± 0.1	7.36 ± 0.1
4 minutes	7.35 ± 0.1	7.41 ± 0.1	7.38 ± 0.1
1 minutes	7.41 ± 0.1	7.39 ± 0.1	7.38 ± 0.1
0 minutes	7.39 ± 0.1	7.37 ± 0.1	7.44 ± 0.2
55 °C Treatment			
10 minute	7.43 ± 0.2	7.42 ± 0.2	7.47 ± 0.1
7 minutes	7.43 ± 0.1	7.46 ± 0.1	7.37 ± 0.1
4 minutes	7.42 ± 0.1	7.51 ± 0.1	7.45 ± 0.1
1 minutes	7.44 ± 0.1	7.42 ± 0.2	7.40 ± 0.2
0 minutes	7.44 ± 0.2	7.43 ± 0.1	7.52 ± 0.2

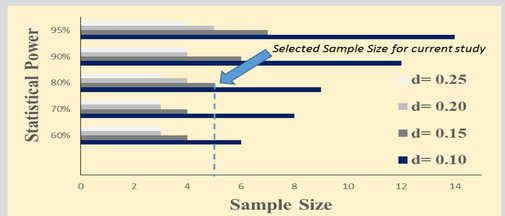
	Treatments					
	35,000 PSI (241 MPa)		45,000 PSI (310 MPa)		55,000 PSI (379 MPa)	
	before	After	before	After	before	After
4°C Treatment						
10 minute	3.95 ± 0.1	3.97 ± 0.2	3.78 ± 0.4	3.80 ± 0.3	3.65 ± 0.3	3.47 ± 0.2
7 minutes	3.72 ± 0.2	3.63 ± 0.2	3.80 ± 0.2	3.80 ± 0.2	3.83 ± 0.3	3.88 ± 0.2
4 minutes	3.62 ± 0.2	3.65 ± 0.3	3.62 ± 0.1	3.58 ± 0.1	3.92 ± 0.1	4.08 ± 0.2
1 minutes	3.50 ± 0.1	3.58 ± 0.3	3.63 ± 0.1	3.72 ± 0.2	4.02 ± 0.2	4.02 ± 0.2
0 minutes	4.00 ± 0.0	4.00 ± 0.0	4.00 ± 0.0	4.00 ± 0.0	4.00 ± 0.0	4.00 ± 0.0
55 °C Treatment						
10 minute	54.82 ± 0.1	55.13 ± 0.3	54.47 ± 0.2	54.87 ± 0.5	54.05 ± 0.6	54.43 ± 0.9
7 minutes	55.05 ± 0.3	55.46 ± 0.3	55.00 ± 0.2	55.40 ± 0.3	54.73 ± 0.2	55.05 ± 0.2
4 minutes	55.28 ± 0.1	55.62 ± 0.2	55.07 ± 0.3	55.53 ± 0.3	54.70 ± 0.3	55.33 ± 0.1
1 minutes	55.02 ± 0.4	54.89 ± 0.2	54.75 ± 0.5	54.93 ± 0.3	54.53 ± 0.3	54.96 ± 0.3
0 minutes	55.00 ± 0.0	55.00 ± 0.0	55.00 ± 0.0	55.00 ± 0.0	55.00 ± 0.0	55.00 ± 0.0

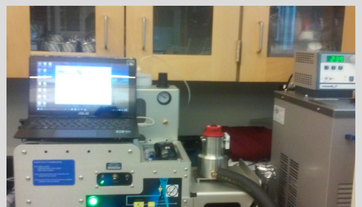
Listeria monocytogenes

- ❑ *L. monocytogenes* is widely distributed in many environments. Although rare in occurrence, as compared to other major foodborne diseases, Listeriosis is a severe illness, being responsible for 3.8 % of foodborne hospitalization and 27.4% of foodborne disease deaths in the United States.
- ❑ The very young, elderly, pregnant women, and the immunocompromised are among the most susceptible groups.
- ❑ Due to the presence of *L. monocytogenes* in a wide array of environments, its halophilic nature, its potential to form biofilms, and its ability to survive and multiply at refrigeration temperatures it has been of special interest in academic and industrial research.
- ❑ *Listeria monocytogenes* is the third etiological agent contributing to foodborne illnesses resulting in about 260 deaths per year (Scallan et al, 2011).
- ❑ In addition to its ubiquitous nature, *L. monocytogenes* can grow at standard refrigeration temperature (4ºC/40ºF) and in anaerobic conditions, making it a particular problem in food safety.
- ❑ A very hardy organism, it can withstand a wide range of conditions which includes freezing, drying, heat, and relatively high levels of acid.

Design, Methods, and Analyses

- ❑ Two biologically independent repetitions (i.e., two blocking factor).
- ❑ Each block, containing three instrumental replications.
- ❑ Each instrumental replication had two microbiological repetitions.
- ❑ Four strain (ATCC® numbers 13932, 51779, 51772, BAA-2658) *Listeria monocytogenes* were used for inoculation of Phosphate Buffer Saline.
- ❑ Inoculation, microbiological analyses, and enumeration of the bacteria were based on Bacteriological Analytical Methods (BAM) of the U.S. Food and Drug Administration (FDA).
- ❑ Information pertaining to outbreaks were obtained from Centers for Diseases Control and Prevention (CDC), Foodborne Outbreak Online Database (CDC FOOD tool).
- ❑ Hydrostatic pressure (Barocycler Hub440, Pressure BioScience Inc., South Easton, MA) of 35,000 to 55,000 PSI (241 to 379 MPa) were applied at various time internal for decontamination of the inoculated pathogen.
- ❑ Analysis of Variance (ANOVA) followed by Tukey- and Dunnett-adjusted mean separations were conducted at type I error level of 5% using the Generalized Linear Model of SAS (SAS Inst., Cary, NC). Values were log-transformed prior to the analysis.





Study Highlights and Summary

- ❑ The inactivation K_{max} and D-values were calculated using best-fitted (maximum R^2) model obtained by Excel (Linear regression) and GInaFiT software (biphasic log-linear model).
- ❑ At 380 MPa (0 to 10 minutes), D-value of 2.77 min and inactivation K_{max} of 1.65 1/min were observed at 4 ºC. At 55 ºC, these values were 1.59, and 3.95, respectively.
- ❑ At 310 MPa (0 to 10 minutes), D-value of 4.43 min and inactivation K_{max} of 1.89 1/min were observed at 4 ºC. At 55 ºC, these values were 1.49, and 2.37, respectively.
- ❑ At 240 MPa (0 to 10 minutes), D-value of 11.61 min and inactivation K_{max} of 0.20 1/min were observed at 4 ºC. At 55 ºC, these values were 2.06, and 1.93, respectively.
- ❑ At 4 ºC, the pathogen were reduced (P< 0.05) by 3.84, 2.44, and 1.05 log CFU/mL after exposure to 10 minutes of hydrostatic pressure at 380, 310, and 240 MPa, respectively.
- ❑ These reductions (P< 0.05) were >7.13, 6.36, and 4.53 for 10-minute treatments at 55 ºC, respectively.
- ❑ Treatments below two minutes were less efficacious ($P \geq$ 0.05) against the pathogen in vast majority of the tested time, temperature, and pressure combinations.
- ❑ Results of this study could be incorporated as part of a risk assessment modeling and predictive microbiology for reducing the public health burden of listeriosis.

Photo Courtesy

High Pressure Processing Unit (Barocycler Hub440, Pressure BioScience Inc., South Easton, MA) equipped with water jacket and circulating water bath for precise application of hydrostatic pressure at controlled temperature. Public Health Microbiology Laboratory, Tennessee State University.

Acknowledgements

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