Reducing a Pathogen of Public Health Concern through Application of Elevated Hydrostatic Pressure

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ABSTRACT

With tremendous ability of plethora of microorganisms to move towards fitness through vertical and horizontal gene transfer mechanisms, prevention of natural and anthropogenic pathogens of public health concern is a daunting task and a moving target. Elevated hydrostatic pressure is a non-thermal procedure that exposes pathogens to pressures of up to 80,000 PSI (>550 MPa). Various serovars of *Salmonella* are one of the leading causes of diarrheal diseased both nationally and internationally. In 2013, Centers for Disease Control and Prevention had also categorized non-typhoidal *Salmonella* as a "serious threat" to the public health due to persistence of various multidrug resistant phenotypes of the bacterium in environmental and healthcare facilities. The current study discusses laboratory challenge studies for inactivation of the pathogen exposed to various time and intensity levels of elevated hydrostatic pressure. Studies are randomized complete block designs, analyzed statistically using GLM procedures of $SAS_{9.2}$ software at type one error level of 5%. A Barocycler Hub440 unit (Pressure BioScience Inc., South Easton, MA), equipped with a water jacket and circulating water bath for precise application of hydrostatic pressure and controlled temperature was utilized. Five outbreak associated strains of *Salmonella* were used in this study. Up to 0.7 and 6.2 log reductions (P<0.05) of habituated *Salmonella* serovars at planktonic stages were achieved using application of pressure at 379 MPa for 30 seconds and 8 minutes, respectively. Similar reductions were observed for lower intensity levels of 310 MPa, and 241 MPa with 0.5 to 4.2 and 0.8 to 1.6 log reductions, respectively. Results of this study could be incorporated as a part of predictive public health microbiology modeling and risk assessment analyses for prevention of Salmonellosis episodes.

Elevated Hydrostatic Pressure: An Industrial Reality

- ❑ Elevated hydrostatic pressure, commonly known as High Pressure Processing, is a non-thermal procedure that exposes the final packaged food products to pressures of up to 80,000 PSI.
- ❑ The pressure of 80,000PSI (550 Megapascal) are over 5,000 times higher that atmospheric pressure and five times higher that the pressure in deepest part of the oceans (Marianas Trench).
- ❑ Since the product is processed inside final package there is no risk of cross-contamination and no need to add other preservation methods.
- ❑ With recent advancements in the engineering of machines, and consumer acceptability of pressure-treated products, this technology is gaining rapid adoption across many sectors of the food industry.

Design, Methods, and Analyses

- ❑ Two biologically independent repetitions (i.e., two blocking factor).

Complete Randomized Block Design

Biologically Independent Repetition A	Biologically Independent Repetition B
I	I
II	II
III	III

- ❑ Each block, containing three instrumental replications.
- ❑ Each instrumental replication had two microbiological repetitions.
- ❑ Five strain habituated Salmonella serovars (ATCC® numbers 13076, 8387, 6962, 9270, 14028) were used for inoculation of fresh-press Apple Cider.
- ❑ 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 15,000 to 55,000 PSI (103 to 380 MPa) were applied at various time internal for decontamination of the inoculated pathogen.
- ❑ ANOVA-based analyses 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).

Inactivation Rate of Five-strain Mixture of Habituated *Salmonella* Serovars Exposed to Elevated Hydrostatic Pressure

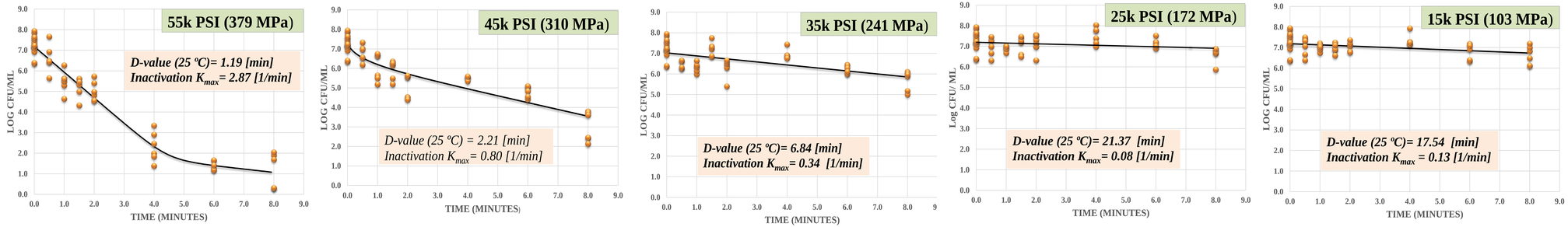


Figure 1. Inactivation rates for five-strain mixture (ATCC® numbers 13076, 8387, 6962, 9270, 14028) of habituated Salmonella serovars exposed to elevated hydrostatic pressure (Barocycler Hub440, Pressure BioScience Inc., South Easton, MA). Kmax values are selected from best fitted model (goodness-of-fit 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.

Sensitivity of Five-strain Mixture of Habituated *Salmonella* Serovars Exposed to Elevated Hydrostatic Pressure

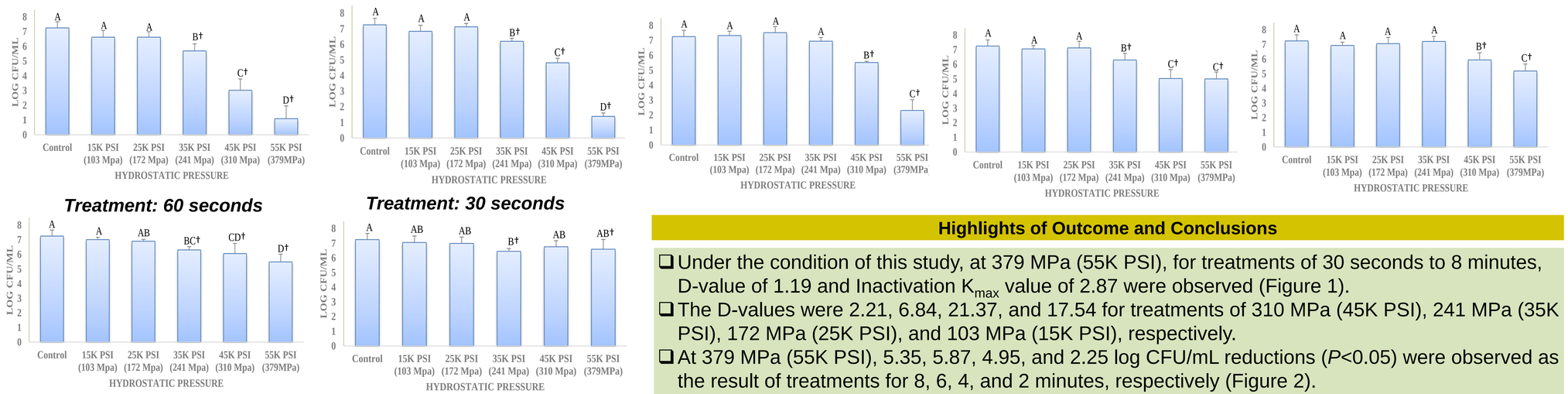
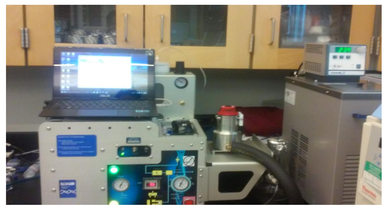


Figure 2. Sensitivity of five-strain mixture of (ATCC® numbers 13076, 8387, 6962, 9270, 14028) habituated Salmonella serovars exposed to elevated hydrostatic pressure (Barocycler Hub440, Pressure BioScience Inc., South Easton, MA) for various time intervals. Columns of each time interval followed by different uppercase letters are resenting log CFU/mL values that are statistically (P<0.05) difference (Tukey-adjusted ANOVA). Uppercase letters followed by † sign are statistically (P<0.05) difference than the control (Dunnett-adjusted ANOVA).



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 of Tennessee State University.

Acknowledgements

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Highlights of Outcome and Conclusions

- ❑ Under the condition of this study, at 379 MPa (55K PSI), for treatments of 30 seconds to 8 minutes, D-value of 1.19 and Inactivation K_{max} value of 2.87 were observed (Figure 1).
- ❑ The D-values were 2.21, 6.84, 21.37, and 17.54 for treatments of 310 MPa (45K PSI), 241 MPa (35K PSI), 172 MPa (25K PSI), and 103 MPa (15K PSI), respectively.
- ❑ At 379 MPa (55K PSI), 5.35, 5.87, 4.95, and 2.25 log CFU/mL reductions (P<0.05) were observed as the result of treatments for 8, 6, 4, and 2 minutes, respectively (Figure 2).
- ❑ Similar results were observed for treatments at 310 MPa (45K PSI) with 4.23, 2.43, 1.73, and 2.22 log CFU/mL reductions (P<0.05) at 8, 6, 4, and 2 minutes, respectively (Figure 2).
- ❑ Treatments below 2 minutes were less efficacious (P>0.05) for microbial cell reductions, in vast majority of tested time and pressure combinations (Figure 2).
- ❑ Overall, a 5-log reduction (P<0.05) i.e. 99.999% inactivation of the habituated 5-strain *Salmonella* serovars was achieved through application of elevated hydrostatic pressure at 25° C.

Cited Literature

CDC Food Tools. Available at: https://www.google.com/webhp?sourceid=chrome-instant&ion=1&espv=2&ie=UTF-8#q=CDC+food+tools

Bacteriological Analytical Methods of Food and Drug Administration. Available at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

Geeraerd, A. H., Valdramidis, V. P., & Van Impe, J. F. (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. International journal of food microbiology, 102(1), 95-105.