# High Pressure Processing for Decontamination of Orange Juice from Natural Flora and *Salmonella* serovars


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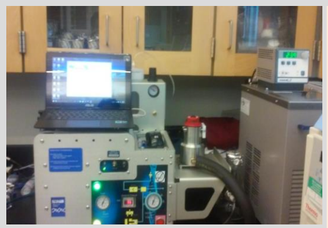
## ABSTRACT


Despite advancements in public health interventions for over a century after identification of *Salmonella* serovars, foodborne non-typhoidal *Salmonella* are currently leading etiological agent for foodborne hospitalizations and deaths in the United States. With recent improvements in commercial feasibility of high pressure processing units, the technology is gaining rapid acceptability across various sectors of food manufacturing, thus requiring extensive validation studies for effective adoption. Various times (1 to 10 minutes) and intensity levels (0 to 380 MPa) of elevated hydrostatic pressure (Pressure BioScience Inc) were investigated in two separate experiments for decontamination of background microflora and inoculated *Salmonella* in fresh-press, and sterilized fresh-press orange juice, respectively. Unit and sample temperatures were maintained precisely at 4ºC by a circulating water bath and stainless steel jacket surrounding the chamber. Each experiment was conducted in two biologically independent repetitions, as blocking factors of a randomized complete block design, containing three repetitions per time/treatment within each block. For *Salmonella*-inoculated experiment, a five-strain habituated mixture of the pathogen were prepared at target level of 7.5 log CFU/ml. Results were analyzed by GLM procedure of SAS using Tukey- and Dunnett-adjusted ANOVA. The $K_{max}$ and D-values were calculated using best-fitted (maximum $R^2$) model obtained by the GInaFit software.

At 380 MPa, for treatments of 1 to 10 minutes, D-value of 1.35, 4-D reduction of 3.4, and inactivation $K_{max}$ of 3.34 were observed for *salmonella* serovars. D-values were 5.90 and 14.68 for treatments of 241 and 103 MPa, respectively. Up to 1.01 and >7.22 log CFU/mL reductions ($P$<0.05) of habituated *Salmonella* serovars at planktonic stages were achieved using application of pressure at 380 MPa for 1 and 10 minutes, respectively. Similarly, background microflora counts were reduced ($P$<0.05) by 1.68 to 5.29 log CFU/mL after treatment at 380 MPa for 1 and 10 minutes, respectively. Treatments below two minutes were less efficacious ($P$ ≥0.05) against the pathogen and background microflora, in vast majority of time and pressure combinations. Results of this study could be incorporated as a part of risk-based food safety management systems and risk assessment analyses for mitigation of public health burden of non-typhoidal *Salmonella* serovars.


## Industry Relevance

With recent improvements in engineering and commercial feasibility of high pressure processing units, and consumer acceptability of pressure-treated products, the technology is gaining rapid adoption across various sectors of food manufacturing, thus requiring extensive public health microbiological validation studies for efficacious adoption of the technology. Results of this study could be incorporated as a part of predictive microbiological modeling and risk assessment analyses for prevention of Salmonellosis episodes.



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.

## Sensitivity of five-strain habituated *Salmonella* serovars exposed to elevated hydrostatic pressure

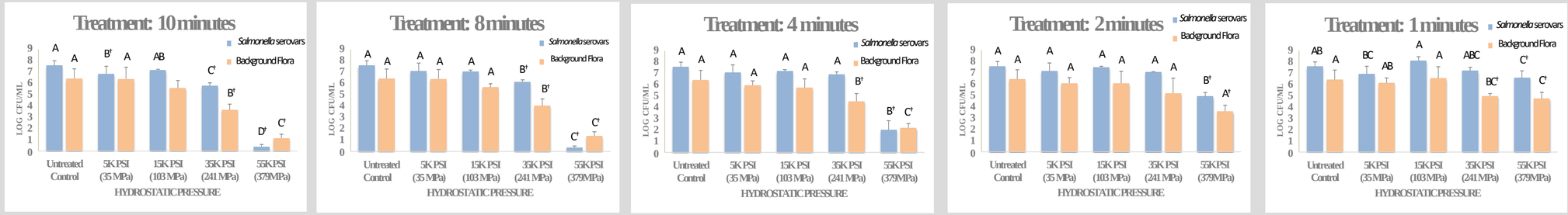



**Figure 1.** Sensitivity of five-strain mixture of (ATCC ® numbers 13076, 8387, 6962, 9270, 14028) habituated *Salmonella* serovars exposed to elevated hydrostatic pressure (Barocycler Hub 440, Pressure BioSciences Inc., South Easton, MA) for various time intervals. Columns of each time interval followed by different uppercase letters are representing log CFU/mL values that are statiscally (P<0.05) difference (Turkey-adjusted ANOVA). Uppercase letters followed by * sign are statistically (P<0.05) difference than the control( Dunnett-adjusted ANOVA).

## Inactivation rates for five-strain habituated *Salmonella* serovars exposed to elevated hydrostatic pressure

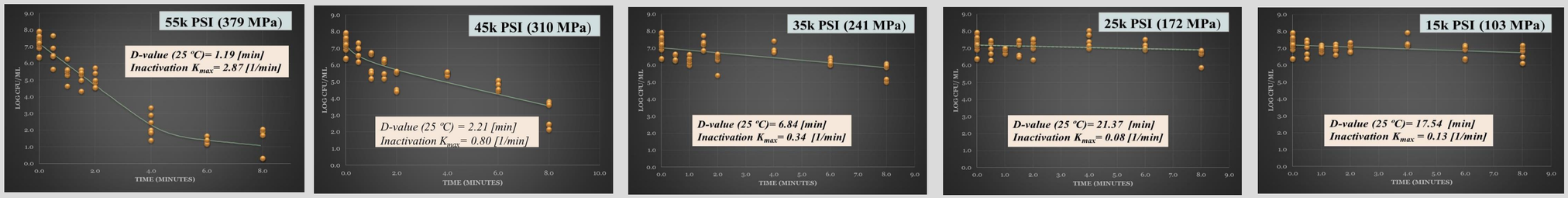



**Figure 2.** Inactivation rates for five-strain mixture (ATCC ® numbers 13076, 8387, 6962, 9270, 14028) habituated *Salmonella* serovars exposed to elevated hydrostatic pressure (Barocycler Hub 440, Pressure BioSciences Inc., South Easton, MA). $K_{max}$ values are selected from best fitted model (goodness-of-fit indicator of R2 values, 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.

## Power Analysis

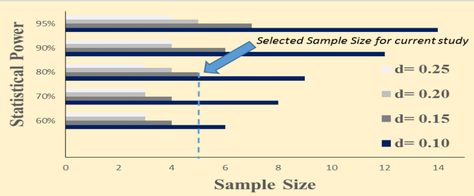



**Figure 3.** Statistical power and sample size calculation at *type I* error level of 5% (α= 0.05) for various detectable differences (d, log CFU/mL). Calculations derived from *Proc Power* command of $SAS_{9.4}$ (SAS Inst., Cary, NC), based on preliminary trials, Public Health Microbiology Laboratory of Tennessee State University.

## Recovery of Injured Cells

| | | Recovery Media | | | | |
|---|---|---|---|---|---|---|
| | Pressure (Time) | TSA | TSA+YE | TSA+PY | TSA+YE+PY | Selective* |
| *Salmonella* serovars | 55k PSI (5 min) | 4.48 ± 0.4 A | 4.40 ± 0.4 A | 4.51 ± 0.5 A | 4.50 ± 0.4 A | <1.96 ± 0.5 B |
| *Salmonella* serovars | 0K PSI (5 min) | 7.07 ± 0.3 A | 7.07 ± 0.3 A | 7.13 ± 0.3 A | 7.06 ± 0.3 A | 6.84 ± 0.3 A |
| *Listeria monocytogenes* | 55k PSI (5 min) | 4.49 ± 0.4 A | 4.35 ± 0.3 A | 4.40 ± 0.4 A | 4.36 ± 0.4 A | <2.32 ± 0.6 B |
| *Listeria monocytogenes* | 0K PSI (5 min) | 7.08 ± 0.2 A | 7.02 ± 0.1 A | 7.04 ± 0.2 A | 7.09 ± 0.2 A | 5.61 ± 0.3 B |

**Figure 4.** Preliminary trial for selection of a medium for recovery of injured cells.* XLD for *Salmonella* serovars and PALCAM for Listeria monocytogenes.

## Pressure Treatment

- The challenge experiment were conducted in **Barocycler Reaction PULSE Tubes**, enabling the precise application of pressure and control temperature.
- Internal pressure, temperature, compression rate were monitored every 3 seconds using **Barocycler HUB PBI 2.3.11** Software.

## Material and Methods


- Two biologically independent repetitions (i.e., two blocking factor).
- 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 orange juice.
- The strains were grown individually into Tryptic Soy Agar at 37ºC for 22-24 hours and sub-cultured at 37ºC for 22-24 hours.
- Strains were then individually washed and harvested with Phospate Buffered Saline (PBS) at 6000 RPM for 15 minutes.
- In order to improve the external validity of the challenge study, each strain were then individually habituated in sterile orange juice for 72 hours prior to experiment.
- On the day of experiments, a five-strain composite of the habituated strains were prepared for inoculation of the product.
- For experiments involving background microflora, a product without any thermal and non-thermal treatment were used.
- Prior to microbiological analysis each sample was neutralized using D/E broth.
- Medium was selected based on preliminary trial to enhance the growth of the injured cells.
- Microbiological analyses, incubation, 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.
- 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.

## Highlights and Conclusions

- At 380 MPa, for treatments of 1 to 10 minutes, D-value of 1.35, 4-D reduction of 3.4, and inactivation $K_{max}$ of 3.34 were observed for *salmonella* serovars.
- D-values were 5.90 and 14.68 for treatments of 241 and 103 MPa, respectively.
- Up to 1.01 and >7.22 log CFU/mL (i.e. > **99.99999**% )reductions ($P$<0.05) of habituated *Salmonella* serovars at planktonic stages were achieved using application of pressure at 380 MPa for 1 and 10 minutes, respectively.
- Similarly, background microflora counts were reduced ($P$<0.05) by 1.68 to 5.29 log CFU/mL after treatment at 380 MPa for 1 and 10 minutes, respectively.
- Treatments below two minutes were less efficacious ($P$ ≥0.05) against the pathogen and background microflora, in vast majority of time and pressure combinations.
- Results of this study could be incorporated as a part of risk-based food safety management systems and risk assessment analyses for mitigation of public health burden of non-typhoidal *Salmonella* serovars.

## Cited Literature


- Scallan, E. et al., (2015). An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years.
- WHO estimates of the global burden of foodborne diseases. (n.d.). Retrieved April 13, 2017.
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