P2-59 Interactions of Carvacrol, Caprylic Acid, Habituation, and Mild Heat for Pressure-based Inactivation of O157 and Non-O157 Serogroups of Shiga Toxin-producing *Escherichia coli* in Low-Acid Environments

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ABSTRACT

From 1998 to 2017 at least 599 foodborne outbreaks in the United States were associated with contaminated food products with O157 and non-O157 serogroups of Shiga toxin-producing *Escherichia coli*. Current study investigated synergism of elevated hydrostatic pressure, habituation, mild heat, & antimicrobials for inactivation of O157 & non-O157 serogroups of *Escherichia coli*. Various times (0, 1, 3, 5, and 7 minutes) at pressure intensity level of 450 MPa (*e.g.* 65K PSI) were investigated at 4 and 45 ºC with and without presence of carvacrol, Y caprylic acid (100 to 500 ppm) before & after 7-day aerobic habituation of O157 and non-O157 serogroups of Shiga toxin-producing *Escherichia coli* inoculated in blueberry juice. Experiments were conducted in three biologically independent repetitions each consist of 2 replications. The study was analyzed as a randomized complete block design using GLM procedure of SAS followed by Tukey-adjusted mean separation. Under the condition of this experiment, habituation of the microbial pathogen played an influential ($P < 0.05$) role on inactivation rate of the pathogen. As an example, O157 and non-O157 serogroups were reduced ($P < 0.05$) by 1.41 and 1.63 Log CFU/ml after a 450 MPa treatment at 4 ºC, respectively, before habituation. The corresponding log reductions after 7-day aerobic habituation were 2.64, and 3.31, respectively. Carvacrol and caprylic acid both augmented the decontamination efficacy of the treated samples. As an example, *Escherichia coli* O157 were reduced ($P < 0.05$) by 2.64 and 4.17 log CFU/ml after a 7-minute treatment at 450 MPa without, & with presence of Carvacrol, respectively. Results of current study indicate an optimized pressure-based intervention with mild heat & antimicrobial agents could be efficacious for inactivation of >99.9% of the pathogens. Current experiment also exhibits critical role of habituation on increasing external validity of a challenge study.

DESIGN, METHODS, & ANALYSES

- ❑ Two biologically independent repetitions (i.e., two blocking factors).
- ❑ Each block, containing three instrumental replications.
- ❑ Each instrumental replication had two microbiological repetitions.
- ❑ Inoculation, microbiological analyses, and enumeration of the bacteria were based on Bacteriological Analytical Methods (BAM) of the U.S. Food and Drug Administration Hydrostatic pressure (Barocycler Hub880, Pressure BioScience Inc., South Easton, MA) of 55,000 PSI (379 MPa) were applied at various time internal for decontamination of the inoculated pathogen.
- ❑ After habituation and prior to microbial analysis samples were neutralized using D/E neutralizing broth.
- ❑ Shiga toxigenic *E. coli* O157:H7 (six strain):

  ATCC Strains: BAA 460, 43888, 43894, 35150, 43889, 43890
- ❑ Non-O157 toxigenic *Escherichia coli* (six strains):

  ATCC Strains: BAA 2196, BAA 2193, BAA 2215, BAA 2440, BAA 2219, BAA 2192
- ❑ Analysis of Variance (ANOVA) followed by Tukey- and Dunnett-adjusted mean separations were conducted at type I error level of 5%

Acknowledgements

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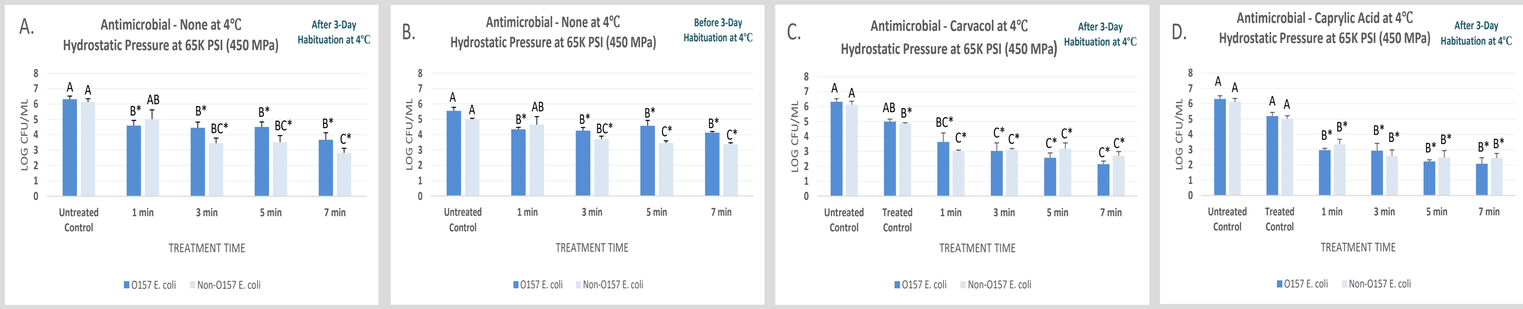

Figure 1. Inactivation of six-strain cocktail of habituated and non-habituated *E. coli* O157:H7 (ATCC® numbers BAA 460, 43888, 43894, 35150, 43889, 43890) and the 'Big Six' non-O157 STEC habituated strain serogroups mixtures (ATCC® numbers BAA 2196, BAA 2193, BAA 2215, BAA 2440, BAA 2219, BAA 2192) in sterilized blue berry juice, treated by carvacol (0.5%), caprylic acid (0.5%) and elevated hydrostatic pressure at 450 MPa (Barocycler Hub 880, Pressure Bioscience Inc., South Easton, MA, USA) for 0, 1, 3, 5, and 7 minutes at 4 ºC. In each graph, and for each pathogen separately, columns of each time interval followed by different uppercase letters are representing log CFU/ml values (mean ± SE) that are statistically ($p < 0.05$) different (Tukey-adjusted ANOVA). Uppercase letters followed by * sign are statistically ($p < 0.05$) different than the untreated control (not treated with antimicrobial) (Dunnett-adjusted ANOVA). (**A**) After 3 day habituation, treated by no antimicrobial at 4 ºC ; (**B**) Before 3 days habituation, treated by no antimicrobial at 4 ºC; (**C**) After 3 day habituation, treated by carvacol at 4 ºC; (**D**) After 3 day habituation, treated by caprylic acid at 4 ºC.

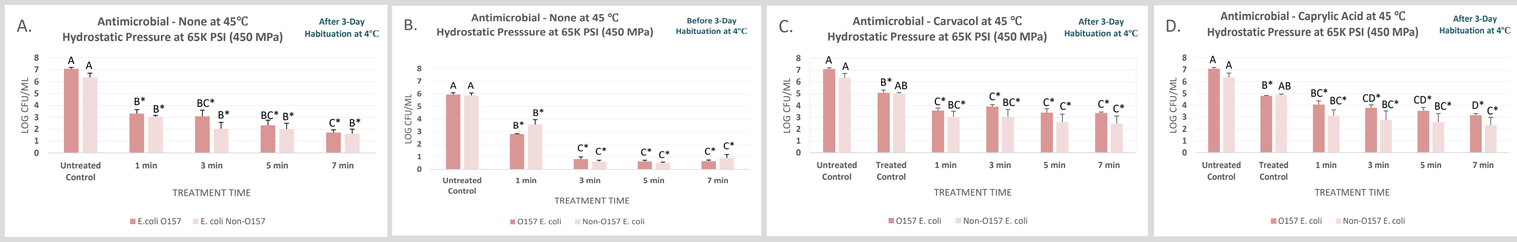

Figure 2. Inactivation of six-strain cocktail of habituated *E. coli* O157:H7 (ATCC® numbers BAA 460, 43888, 43894, 35150, 43889, 43890) and the 'Big Six' non-O157 STEC habituated strain serogroups mixtures (ATCC® numbers BAA 2196, BAA 2193, BAA 2215, BAA 2440, BAA 2219, BAA 2192) in sterilized blue berry juice, treated by carvacol (0.1%), caprylic acid (0.1%) and elevated hydrostatic pressure at 450 MPa (Barocycler Hub 880, Pressure Bioscience Inc., South Easton, MA, USA) for 0, 1, 3, 5, and 7 minutes at 45 ºC. In each graph, and for each pathogen separately, columns of each time interval followed by different uppercase letters are representing log CFU/ml values (mean ± SE) that are statistically ($p < 0.05$) different (Tukey-adjusted ANOVA). Uppercase letters followed by * sign are statistically ($p < 0.05$) different than the untreated control (not treated with antimicrobial) (Dunnett-adjusted ANOVA). (**A**) After 3 day habituation, treated by no antimicrobial at 45 ºC ; (**B**) Before 3 day habituation, treated by no antimicrobial at 45 ºC; (**C**) After 3 day habituation, treated by carvacol at 45 ºC; (**D**) After 3 day habituation, treated by caprylic acid at 45 ºC

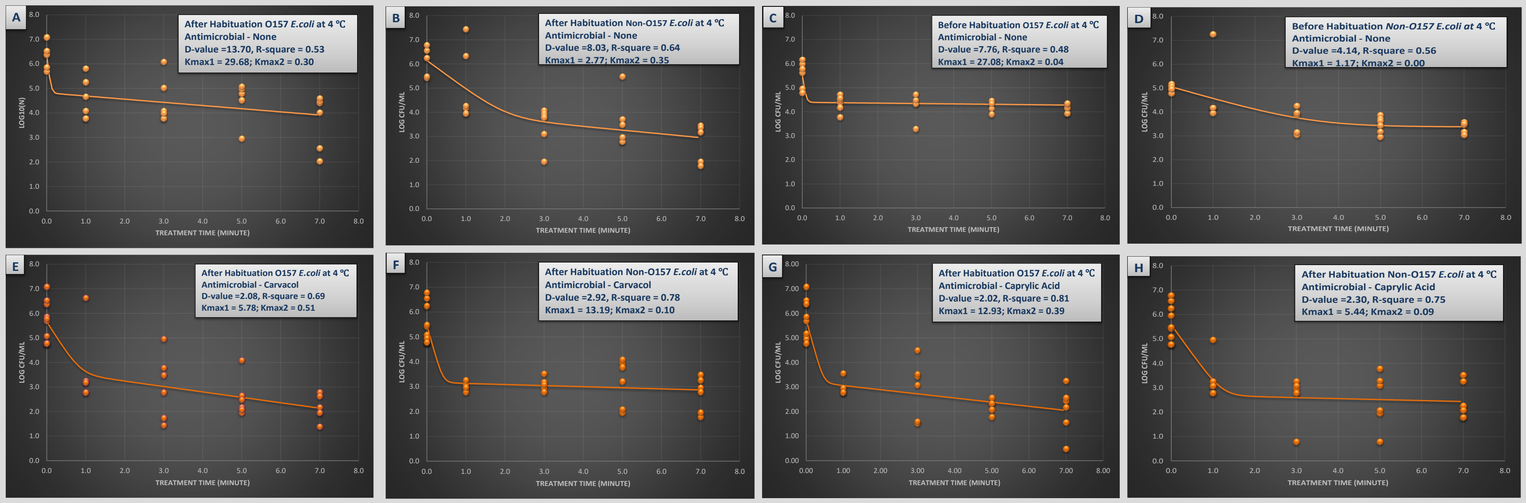

Figure 3. Inactivation rates for six-strain habituated mixture of *E. coli* O157:H7 (ATCC® numbers BAA 460, 43888, 43894, 35150, 43889, 43890) and the 'Big Six' non-O157 STEC habituated strain serogroups (ATCC® numbers BAA 2196, BAA 2193, BAA 2215, BAA 2440, BAA 2219, BAA 2192) exposed to carvacol, caprylic acid, and elevated hydrostatic pressure (Barocycler Hub 440, Pressure BioScience Inc., South Easton, MA) in sterilized blue berry juice. Using the GInaFiT software, the provided Kmax values are selected from the best-fitted model (goodness-of-fit indicator of R^2 values, $\alpha = 0.05$). Kmax values indicates the expressions of number of log cycles of reduction in 1/min unit for each pressure/temperature combination. Presented d-values are calculated based on best-fitted linear model, showing time required for one log (90%) of microbial cell reductions of the habituated microbial cell mixture. (**A**). Habituated *E. coli* O157 treated by no antimicrobial at 4 ºC with $R^2 = 0.53$; (**B**). Habituated *E. coli* Non-O157 treated by no antimicrobial at 4 ºC with $R^2 = 0.64$; (**C**). Non-habituated *E. coli* O157 treated by no antimicrobial at 4 ºC with $R^2 = 0.48$; (**D**). Non-habituated *E. coli* Non-O157 treated by no antimicrobial at 4 ºC with $R^2 = 0.56$; (**E**). Habituated *E. coli* O157 treated by carvacol (0.5%) at 4 ºC with $R^2 = 0.69$; (**F**). Habituated *E. coli* Non-O157 treated by carvacol (0.5%at 4 ºC with $R^2 = 0.78$; (**G**). Habituated *E. coli* O157 treated by caprylic acid (0.5%) at 4 ºC with $R^2 = 0.81$; (**H**). Habituated *E. coli* Non-O157 treated by caprylic acid (0.5%) at 4 ºC with $R^2 = 0.75$.