

Fate and Decontamination of O157 and Non-O157 Serogroups of Shiga Toxin-Producing *Escherichia coli*, including ATCC 43895, as Affected by Elevated Hydrostatic Pressure



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ABSTRACT

Various serogroups of Shiga toxin-producing *Escherichia coli* including O157, O26, O45, O103, O111, O121 and O145 had been involved in an array of outbreaks associated with meat products. Statistics derived from CDC foodborne outbreak database confirms this concern, delineating 423 outbreaks associated with ground beef from 1998 to 2015. This study investigates the effects of elevated hydrostatic pressure on reduction of wild-type and rifampicin-resistant variants of the above-mentioned seven serogroups of Shiga toxin-producing *Escherichia coli*. Vast majority of treatments below two minutes showed low efficacy ($P \geq 0.05$) in reducing the serogroups. Wild-type and rifampicin-resistant variants of the pathogen showed comparable sensitivity ($P \geq 0.05$) for vast majority of tested strains.

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.
- Inoculation, microbiological analyses, and enumeration of the bacteria were based on Bacteriological Analytical Methods (BAM) of the U.S. Food and Drug Administration (FDA).
- Hydrostatic pressure (Barocycler Hub440, Pressure BioScience Inc., South Easton, MA) of 55,000 PSI (379 MPa) were applied at various time interval 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 Open Epi Software. Values were log-transformed prior to the analysis.



O157 and Non-O157 *Escherichia coli*

- Escherichia coli* is one of the predominant enteric species in the human gut (Fouladkhah et al., 2013).
- Results from several studies suggest control measures for O157 may be effective for non-O157 STEC. More research is needed to uncover unique characteristics and resistances of non-O157 STEC.
- Most *E. coli* bacteria are harmless, but some produce a toxin (Shiga-toxin) that can cause serious illness, including bloody diarrhea, blood-clotting problems, kidney failure, and death.
- Like generic *E. coli*, toxin-producing Shiga-toxigenic *Escherichia coli* (STEC) are Gram-negative, rod-shaped bacteria, but are characterized by the production of Shiga toxins (Stx). A subset of STEC called enterohemorrhagic *Escherichia coli* (EHEC) includes only those that could cause serious illness. Serotype O157:H7 is the prototypic EHEC strain (Fouladkhah et al., 2012).
- EHEC O157:H7 was first identified in an outbreak, in 1982, and further gained recognition in 1992-1993 outbreak in which hamburgers from a fast-food restaurant were the vehicle.
- Raw or undercooked ground beef products are the vehicles most often implicated in O157:H7 outbreaks (CDC Food Tools).
- All people are believed to be susceptible to hemorrhagic colitis, but young children and the elderly are more susceptible and at higher risk for the illness to progress to more severe complications.

Figure 1. Inactivation of O157 and Non-O157 Shiga Toxin-producing *Escherichia coli* and a 1992-1993 pacific northwest outbreak strain.

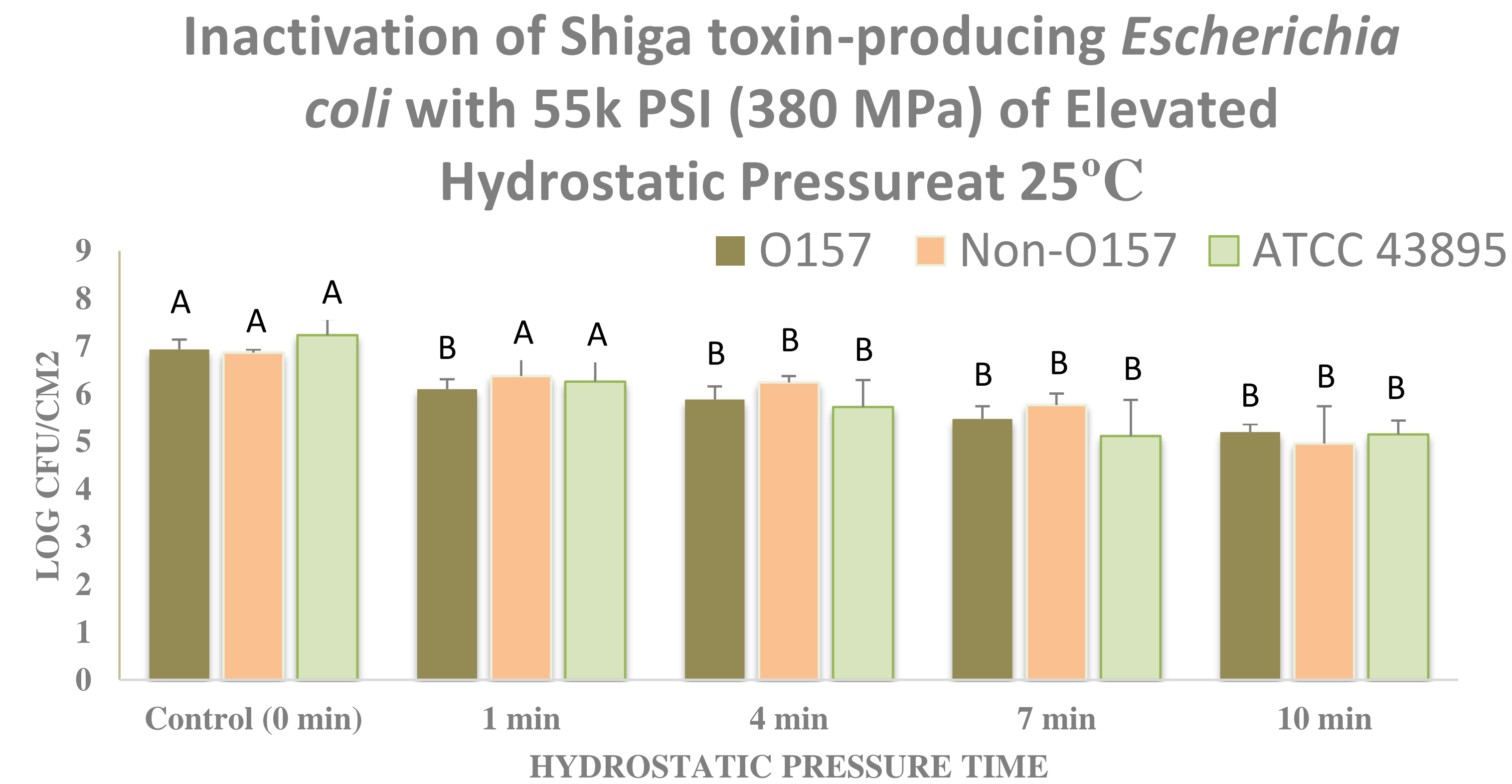


Figure 2. Survival of O157 and Non-O157 Shiga Toxin-producing *Escherichia coli* in meat homogenate.

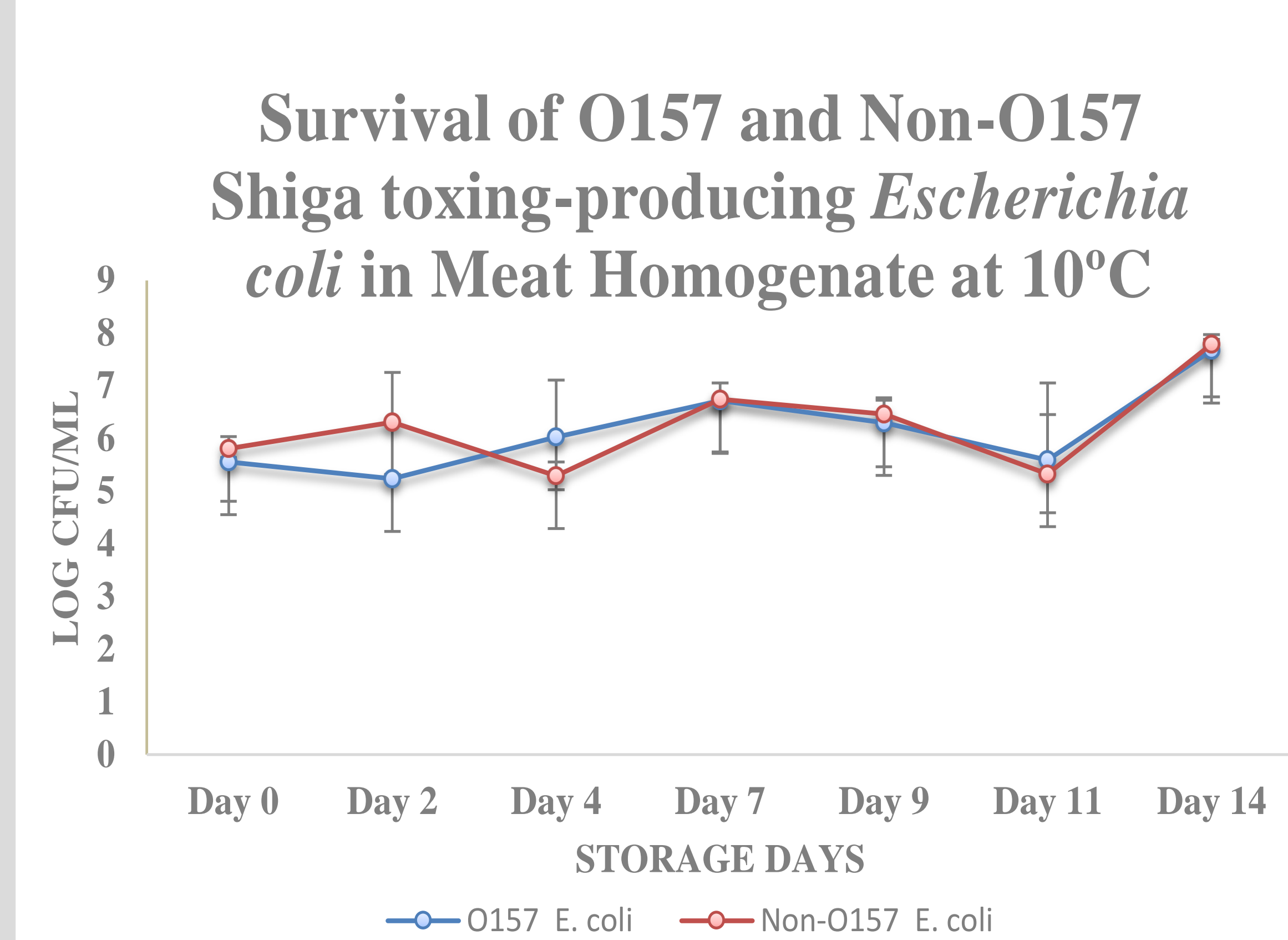


Table 11. Temperature recordings before and after high pressure treatments and pH recordings after neutralization with D/E broth.

		pH	Temperature	
			Before Treatment	After Treatment
<i>E. coli</i> O157:H7	Control	7.22 ± 0.6	24.47 ± 0.4	24.12 ± 0.5
	10 minutes	6.96 ± 0.5	24.20 ± 0.4	24.02 ± 0.2
	7 minutes	7.39 ± 0.2	24.32 ± 0.7	24.08 ± 0.9
	4 minutes	7.34 ± 0.2	24.25 ± 0.5	24.62 ± 0.6
	1 minutes	7.18 ± 0.5	25.00 ± 0.0	25.00 ± 0.0
Non-O157 <i>E. coli</i>	Control	7.03 ± 0.4	24.13 ± 0.4	24.12 ± 0.2
	10 minutes	7.32 ± 0.3	24.30 ± 0.3	24.30 ± 0.5
	7 minutes	7.39 ± 0.2	24.08 ± 0.9	24.07 ± 1.0
	4 minutes	7.52 ± 0.0	24.40 ± 0.7	24.40 ± 0.6
	1 minutes	7.47 ± 0.3	25.00 ± 0.0	25.00 ± 0.0
ATCC 43895 strain	Control	7.08 ± 0.6	23.97 ± 0.4	23.92 ± 0.5
	10 minutes	7.30 ± 0.4	24.15 ± 0.3	24.18 ± 0.5
	7 minutes	7.37 ± 0.2	24.02 ± 0.7	23.88 ± 0.7
	4 minutes	7.45 ± 0.2	24.15 ± 0.8	24.28 ± 0.6
	1 minutes	7.32 ± 0.3	25.00 ± 0.0	25.00 ± 0.0

Study Highlights and Summary

Survival Experiment:

- During the aerobic storage at 10°C, O157 *E. coli* strains were increase ($P > 0.05$) from 5.55 ± 0.1 to 7.67 ± 0.2 log CFU/mL of inoculated meat homogenate on days 0 and 14, respectively.
- Similarly, Non-O157 *E. coli* strains were increase ($P > 0.05$) from 5.81 ± 0.2 to 7.79 ± 0.2 log CFU/mL of inoculated meat homogenate on days 0 and 14, respectively.
- The tested O157 and Non-O157 serogroups showed similar ($P > 0.05$) survival pattern during the aerobic storage.

High Pressure Processing Inactivation Experiment:

- A treatment at 55K PSI for 10 minutes reduced ($P < 0.05$) the pathogen counts for 1.74, 1.91, 2.08 log CFU/ml for O157, Non-O157, and 1992-1993 outbreak strain samples, respectively. This translates to 90 to 99% reductions.
- Overall, in vast majority of tested time, and hydrostatic pressure, O157 and Non-O157 *E. coli* showed similar ($P > 0.05$) sensitivity and reduction patterns. The 1992-1993 *E. coli* O157:H7 outbreak strain also showed similar sensitivity ($P > 0.05$) to treatments of hydrostatic pressure at control temperatures.

References

- Fouladkhah, A., Geornaras, I., Sofos, J. Biofilm formation of O157 and non-O157 Shiga toxin-producing *Escherichia coli* and multidrug-resistant and susceptible *Salmonella* Typhimurium and Newport and their inactivation by sanitizers. *Journal of Food Science* 2013. 78(6): M880-M886.
- Fouladkhah, A., Geornaras, I., Yang, H., and Sofos, J. Lactic Acid Resistance of Shiga Toxin-Producing *Escherichia coli* and Multidrug-resistant and Susceptible *Salmonella* Typhimurium and *Salmonella* Newport in Meat Homogenate. *Food Microbiology* 2013. 36(2): 260-266
- CDC Foodborne Outbreak Online Database (FOOD Tool): <https://www.cdc.gov/foodborneoutbreaks/>

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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, TSU.