Effects of Lactic Acid and Elevated Hydrostatic Pressure against Wild-Type and Rifampicin-Resistant O157 and Non-O157 Shiga Toxin-Producing *Escherichia coli* in Meat Homogenate

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ABSTRACT

Introduction. Shiga toxin-producing *Escherichia coli* (STEC), is responsible for approximately 176,000 illnesses, 3,700 hospitalizations, and 30 deaths in the US annually. These pathogenic bacteria are very diverse and more than 400 STEC serovars have been isolated affecting individuals. Non-O157 Shiga toxin-producing *Escherichia coli* (serogroups other than O157:H7) are presently the cause of over 60% of STEC-induced illnesses and are prevalent in ground beef and other non-intact meat products.

Method. Hydrostatic pressure of 350 MPa (51K PSI) were applied at various time intervals (0 to 7 minutes) for inactivation of six-strain mixture of wild-type *Escherichia coli* O157 and six-strain mixture of *Escherichia coli* O26, O45, O103, O111, O121, O145 as well as their spontaneous rifampicin-resistant phenotypes. The pressure processing unit was equipped with water jacket and circulating water bath surrounding the reaction chamber for precise application of hydrostatic pressure at controlled temperature of 4 ºC. Experiments were conducted in two biologically independent repetitions, as blocking factors of a randomized complete block design and were conducted in Barocycler Reaction PULSE Tubes, with internal pressure, temperature, and compression rate monitored every 3 seconds using Barocycler HUB PBI Software. Results were analyzed using LSD-based ANOVA by OpenEpi software.

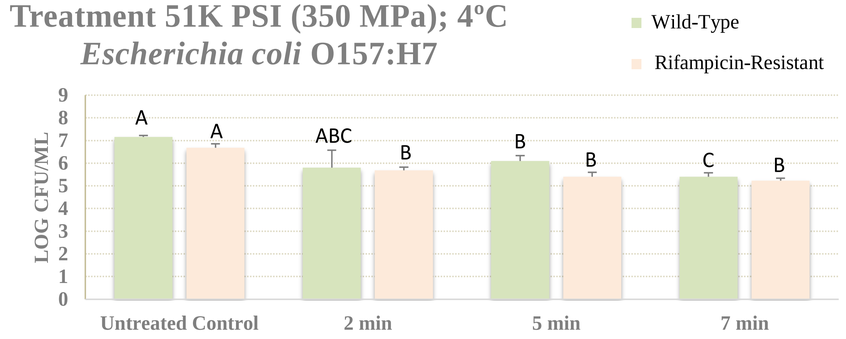
Results. Wild-type *Escherichia coli* O157 were reduced (P < 0.05) from 7.16 ± 0.1 to 5.41 ± 0.2 when exposed to treatments at 350 MPa for 7 minutes. Corresponding reductions for the same phenotype and serogroup were 6.81 ± 0.2 to <1.22 ± 0.5 for samples treated at 350 MPa for 7 minutes in presence of 1% lactic acid. The wild-type non-O157 serogroup showed similar trends, with 2.6 and 5.5 log CFU/mL reductions (P < 0.05) after treatments at 350 MPa for 7 minutes for control and lactic acid samples, respectively. Reductions of rifampicin-resistant non-O157 serogroups were similar to their spontaneous wild-type phenotype.

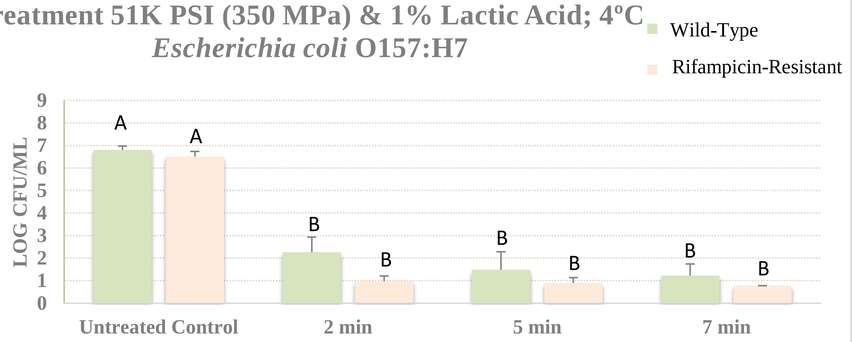
Significance. Results of this study indicate lactic acid could appreciably enhance decontamination efficacy of high pressure processing against Shiga toxin-producing *Escherichia coli*. The rifampicin-resistant and wild-type phenotypes of both O157 and non-O157 serogroups showed similar sensitivity to lactic acid and elevated hydrostatic pressure thus could be used interchangeably in microbiological challenge studies.

Design, Methods, and Analyses

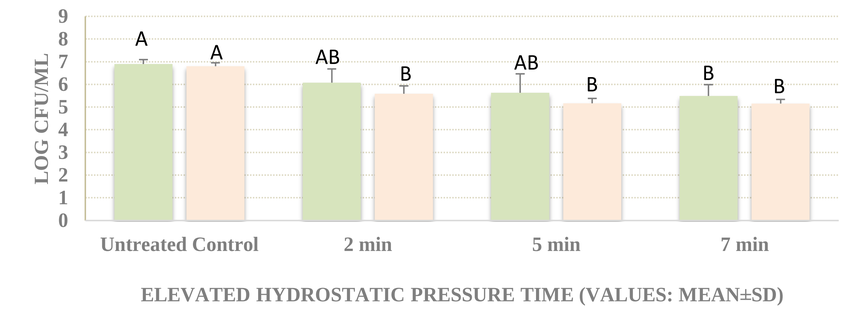
- ❑ Two biologically independent repetitions (i.e., two blocking factor).
- ❑ Each block, containing three instrumental replications.
- ❑ Each instrumental replication had two microbiological repetitions.
- ❑ Inoculation, microbiological analyses, and enumeration of the bacteria were based on Bacteriological 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 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 Open Epi Software. Values were log-transformed prior to the analysis.

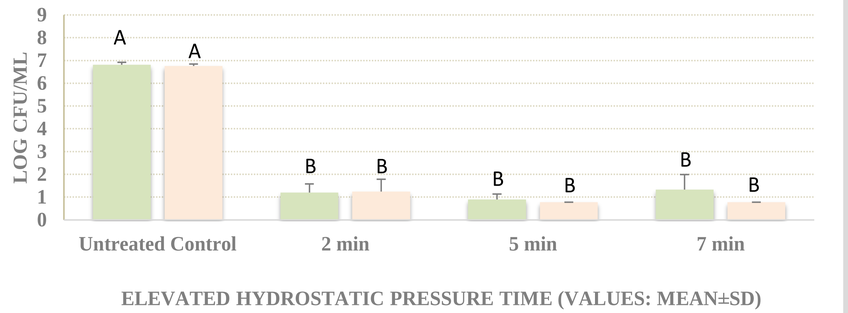
Effects of Lactic Acid and Elevated Hydrostatic Pressure against Wild-Type and Rifampicin-Resistant *Escherichia coli* O157:H7





Effects of Lactic Acid and Elevated Hydrostatic Pressure against Wild-Type and Rifampicin-Resistant Non-O157 *Escherichia coli*





Study Highlights and Summary

- ❑ Wild-type *Escherichia coli* O157 were reduced (*P* < 0.05) from 7.16 ± 0.1 to 5.41 ± 0.2 when exposed to treatments at 350 MPa for 7 minutes.
- ❑ Corresponding reductions for the same phenotype and serogroup were 6.81 ± 0.2 to <1.22 ± 0.5 for samples treated at 350 MPa for 7 minutes in presence of 1% lactic acid.
- ❑ The wild-type non-O157 serogroup showed similar trends, with 2.6 and 5.5 log CFU/mL reductions (P < 0.05) after treatments at 350 MPa for 7 minutes for control and lactic acid samples, respectively.
- ❑ Reductions of rifampicin-resistant non-O157 serogroups were similar to their spontaneous wild-type phenotype.
- ❑ Results of this study indicate lactic acid could appreciably enhance decontamination efficacy of high pressure processing against Shiga toxin-producing *Escherichia coli*.
- ❑ The rifampicin-resistant and wild-type phenotypes of both O157 and non-O157 serogroups showed similar sensitivity to lactic acid and elevated hydrostatic pressure thus could be used interchangeably in microbiological challenge studies.

High Pressure Processing Unit equipped with water jacket and circulating water bath for precise application of hydrostatic pressure at controlled temperature. Public Health Microbiology Laboratory, TSU.

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