

Synergism of natural antimicrobials and high pressure pasteurization for inactivation



of *Listeria monocytogenes* in a processed dairy product

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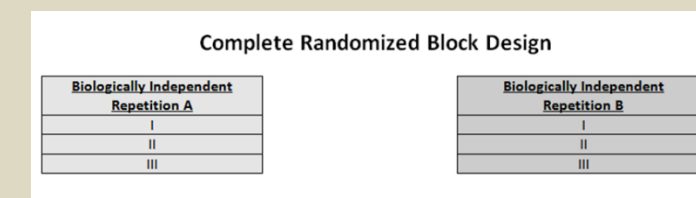


ABSTRACT

A 2010-2015 multistate outbreak associated with contaminated ice-cream with *Listeria monocytogenes* had drawn the attention of researchers and popular press to safety of ice cream products. This study investigated fate of *Listeria monocytogenes* during shelf-life of the product and utilization of an emerging interventions to eliminate the pathogen from ice-cream base. A natural antimicrobial in combination with elevated hydrostatic pressure (0 to 380 MPa) were investigated for decontamination of the inoculated product. While the bacterium did not ($P \geq 0.05$) proliferate appreciably during storage it remained detectable after two weeks of storage. A reduction of >3.0 Log CFU/ml was achievable due to synergism of heat, mild hydrostatic pressure, and natural antimicrobial that translates to $>9.99\%$ reduction of the bacterium.

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.

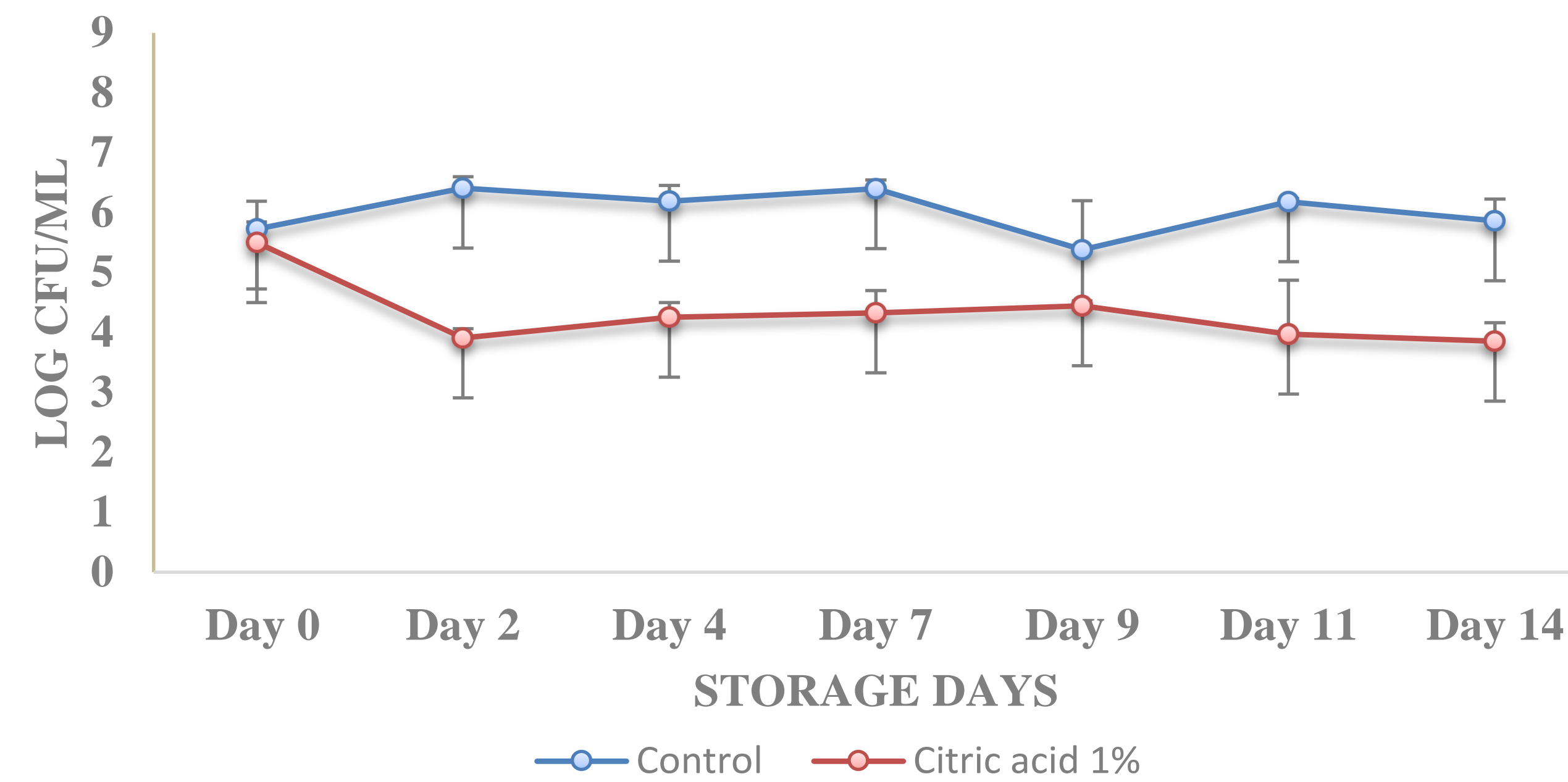


Listeria monocytogenes

- *Listeria monocytogenes* could spread through bloodstream and nervous system.
- Pregnant women are particularly susceptible to Listeria infection.
- Listeria, unlike other foodborne bacteria, tolerates salty environments and cold temperature.
- Listeria causes two (2) forms of diseases
 - In healthy people, non-invasive gastrointestinal illness
 - Invasive form, which can cause septicemia and meningitis
- In 2011 the CDC reported about 1,591 cases of listeriosis.
- It is associated with raw milk, inadequately pasteurized milk, cheese, ice cream, raw veggies, raw poultry and meats.
- **Blue Bell Outbreak:**
 - Listeria outbreak it Blue Bell occurred in April 2015
 - Only 3 states had facilities to have listeria strands found: Oklahoma, Alabama, and Texas
 - Arizona, Kansas, Texas, and Oklahoma had a total of ten (10) human illnesses.

Figure 1. Survival of *Listeria monocytogenes* and background microflora in ice-cream base with and without 1% citric acid at -20°C .

Survival of *Listeria monocytogenes* in ice-cream base at -20°C



Survival of Background Microflora in ice-cream base at -20°C

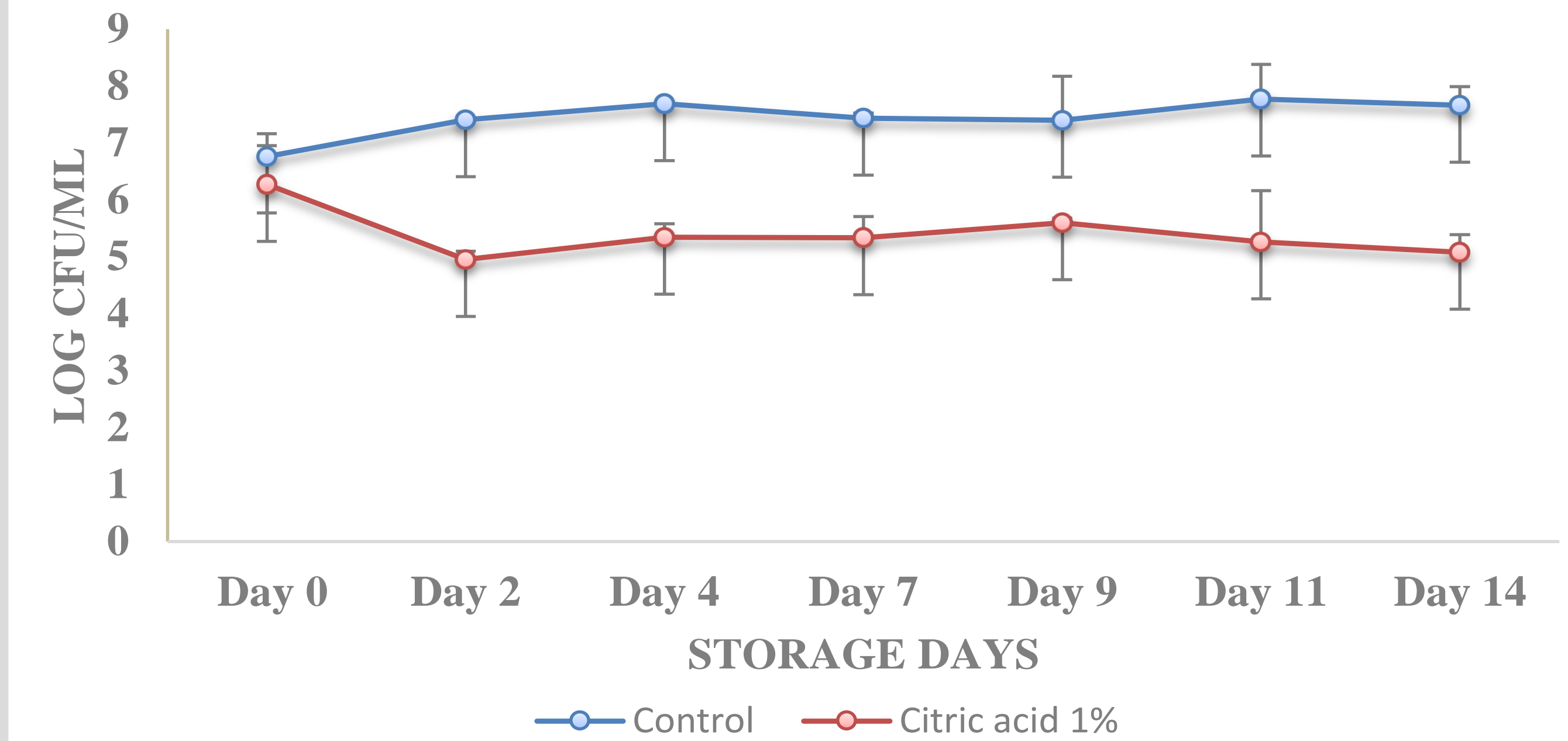
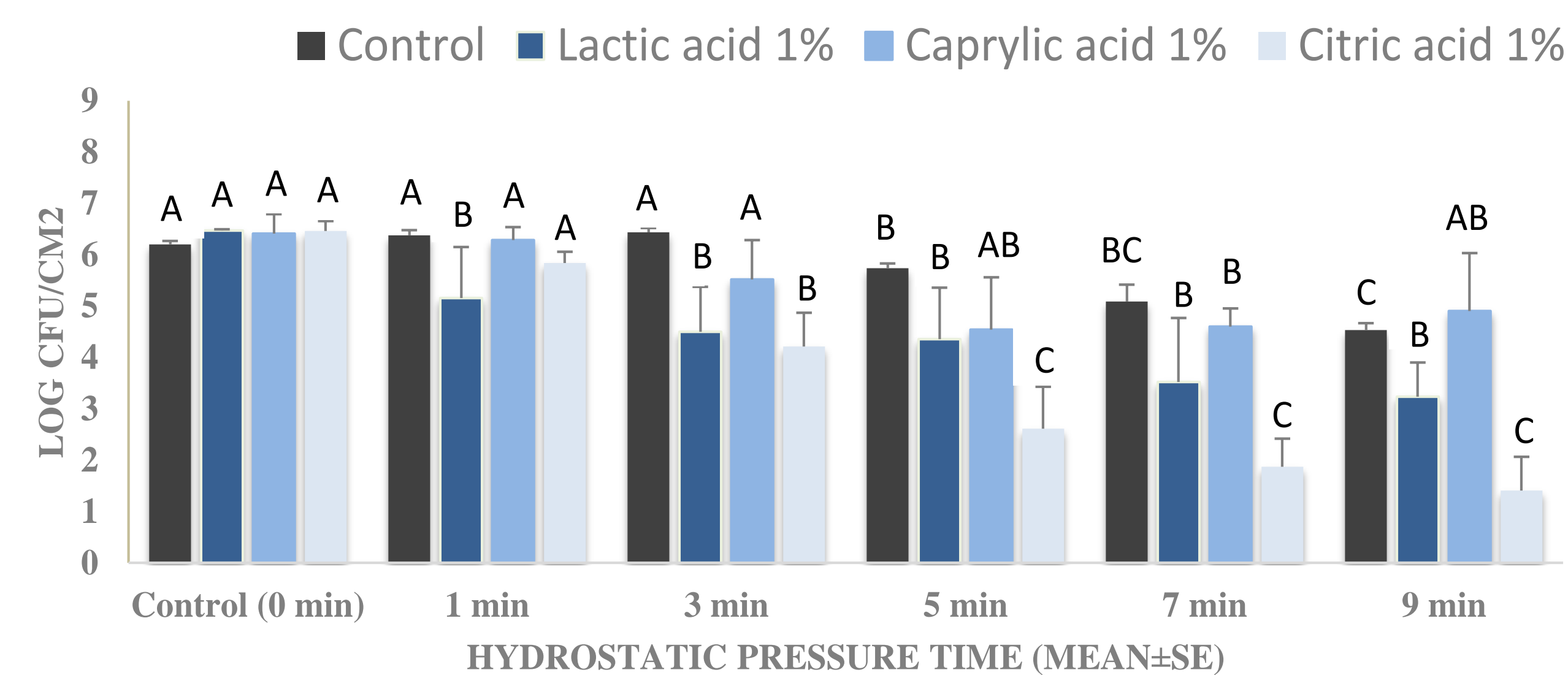
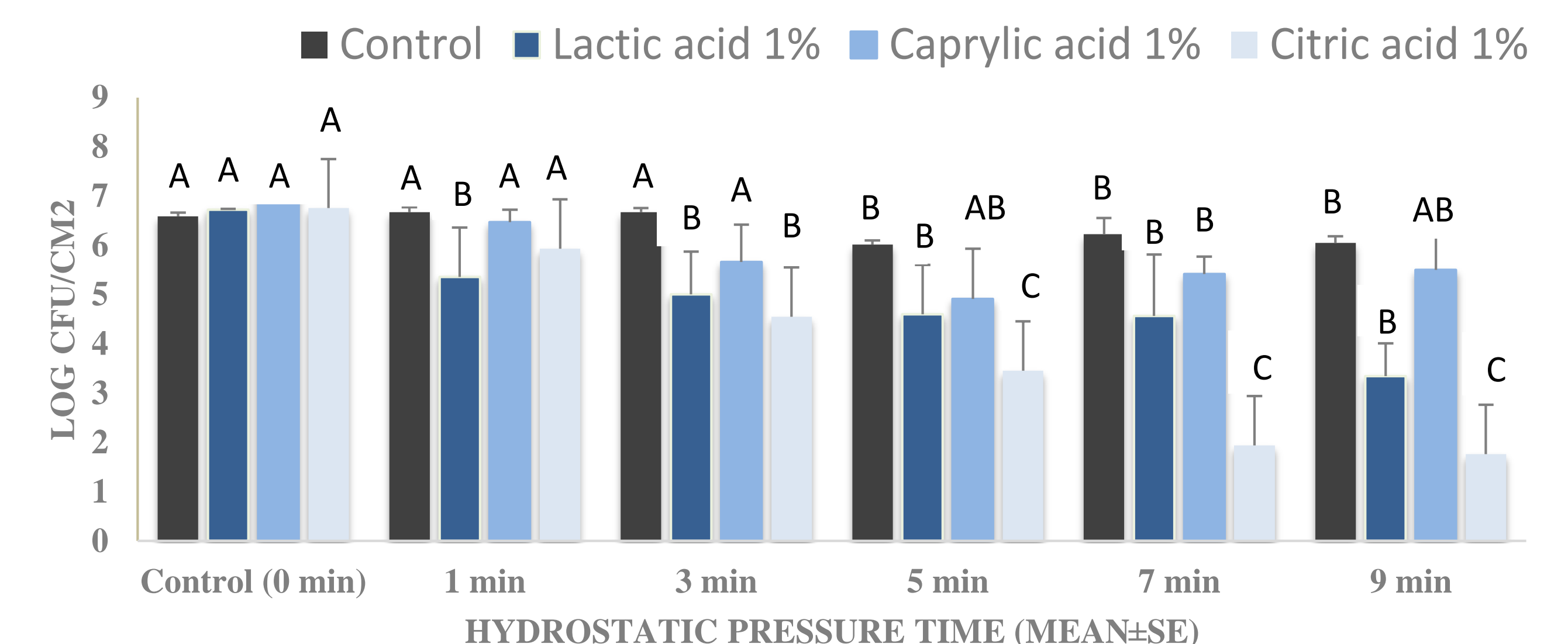


Figure 2. Inactivation of *Listeria monocytogenes* and background microflora in ice-cream base using elevated hydrostatic pressure with and with out 1% citric acid, lactic acid, and/or caprylic acid.

Inactivation of *Listeria monocytogenes* in ice-cream base at 55k PSI (380 MPa) at 4°C



Inactivation of Background Microflora in ice-cream base at 55k PSI (380 MPa) at 4°C



Study Highlights and Summary

Survival Experiment:

- During the survival experiment background microflora were 6.77 ± 0.4 and 7.66 ± 0.3 log CFU/mL on days 0 and 14, respectively, counts were similar ($P > 0.05$) after two weeks of aerobic storage.
- Similarly, *Listeria monocytogenes* counts were 5.72 ± 0.2 and 5.86 ± 0.4 log CFU/mL on days 0 and 14, respectively, these counts were also similar ($P > 0.05$) after two weeks of aerobic storage at -20°C .

High Pressure Processing Inactivation Experiment:

- A treatment at 55K PSI for 9 minutes reduced ($P < 0.05$) the pathogen counts for 1.66, 3.24, 1.51, 5.05 log CFU/ml of control samples and those with 1% lactic acid, caprylic acid, and citric acid, respectively.
- Similarly, background microflora were reduced ($P < 0.05$) microbial load for 0.54, 3.37, 1.94, 4.98 log CFU/ml for control samples and those with 1% lactic acid, caprylic acid, and citric acid, respectively.



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.

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