

# The use of matrix-adapted bacterial isolates of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* for the validation of high pressure treated juices

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FDA

## Abstract

**Background:** Manufacturers of high pressure-processed (HPP) juices are required to demonstrate efficacy as part of process control to comply with FDA's Juice HACCP regulation i.e., a 5-log reduction of the pertinent microorganism. However, there is no consensus on validation study approaches for bacterial strain selection or preparation.

**Purpose:** To compare HPP inactivation of matrix-adapted *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* isolates in buffer and apple juice and their appropriateness in process validation.

**Methodology:** Ten strains each of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* were grown individually in TSBYE using three different growth conditions: neutral, cold-adapted, and acid-adapted. Cold-adapted cells were prepared at 17°C. Acid-adapted cells were prepared in intermediate pH 5.0 TSBYE (HCl adjusted). Approximately 6 log CFU/mL of bacterial strains were inoculated into buffered peptone water (BPW) at pH 3.50±0.10 (HCl adjusted) and apple juice at pH 3.50±0.10 (store purchased) and treated at sublethal pressures of 500 MPa for *E. coli* O157:H7 and 200 MPa for *Salmonella* and *L. monocytogenes* (180s, 4°C initial). Analyses were conducted 0h, 24h and 48h (4°C storage) post-HPP on non-selective media.

**Results:** *E. coli* O157:H7 exhibited greater barotolerance than *Salmonella* spp. and *L. monocytogenes*. Following same-day as treatment analyses of cells in BPW, *E. coli* O157:H7 strain TW14359 demonstrated resistance and *E. coli* O157:H7 strain SEA13B88 was significantly more sensitive to HPP ( $p < 0.05$ ). *Salmonella* strains from neutral and acid-adapted growth conditions behaved similarly (<1.5 log reduction); however, cold-adapted preparations were barosensitive (>3 log reduction), with *S. Cubana* and *S. Montevideo* showing greater resistance compared to other *Salmonella* strains. Multiple *L. monocytogenes* strains demonstrated greater resistance to HPP (<2 log reduction) under cold-adapted and acid-adapted conditions, while *L. monocytogenes* strains CDC and ScottA were significantly more barosensitive (approximately 4 log reduction). Following same-day as treatment analyses of cells in apple juice, increases in barotolerance were observed from all microorganisms under all growth conditions compared to BPW. Time-dependent loss in viability occurred in all post-HPP storage samples.

**Conclusion:** These results suggest under the conditions tested, bacterial strain and preparation methods, as well as matrix composition, influence HPP efficacy and should be considered when conducting validation studies.

## Introduction

The fruit and vegetable juice industry has shown a growing trend in minimally processed juices. A frequent technology used for functional juices is cold pressure, which refers to the application of high pressure processing (HPP) at low temperatures for a mild treatment to inactivate foodborne pathogens.

During HPP treatment of juice products, a pathogen may be capable of developing resistance to not only to pressure, but may be able to adapt to acidic conditions within the juice matrix or withstand refrigerated temperatures during shelf-life storage.

The objective of this study was to compare HPP inactivation of matrix-adapted *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* isolates within an acidic buffer and their appropriateness in process validation.

## Materials and Methods

### Bacterial Isolates

**Table 1. Bacterial isolates associated with outbreaks.**

<i>E. coli</i> O157:H7	<i>L. monocytogenes</i>	<i>Salmonella</i> spp.
960212 (sprouts)	7-16-3 (dairy isolate)	Anatum (sprouts)
960218 (sprouts)	573-035 (taffy apple outbreak)	Cubana (alfalfa sprouts)
F4546 (alfalfa sprouts)	1838 (cabbage)	Hartford (orange juice)
F4637 (sprouts)	California (U of Georgia)	Montevideo (tomato)
F5853 (sprouts)	CDC (coleslaw)	Muenchen (sprouts)
H3482 (sprouts)	F2363 (human epidemic LA1985)	Newport J1894 (tomato)
M-11-0450i-1 (hazelnut)	LIS0827 (avocado)	Saintpaul (alfalfa sprouts)
Sakai (radish sprout)	MAD328 (environmental isolate)	Tennessee (alfalfa sprouts)
SEA13B88 (apple juice)	ScottA (human isolate)	Thompson RM1987 (cilantro)
TW14359 (spinach)	V37 (bulk milk)	Typhimurium (orange juice)

### Adaptive Growth Conditions

Stationary phase cells were grown in:

- Neutral conditions → TSBYE at standard formula pH levels (pH 7.30 ± 0.2) and grown overnight at 37°C.
- Cold-adapted conditions → neutral TSBYE and grown for 48 h in a circulating water bath at 17°C
- Acid-adapted conditions → pH intermediate TSBYE at pH 5.0 ± 0.2 adjusted with 10N hydrochloric acid (HCl). *E. coli* O157:H7 and *Salmonella* spp. cultures were grown overnight at 37°C. *L. monocytogenes* cultures required 48 h at 37°C to reach stationary phase.

### Sample Preparation

Individual strains were centrifuged at 4,000 rpm for 5 min at 4°C and triple washed in Butterfield's phosphate buffer (BPB) followed by resuspension in BPB.

Buffered peptone water (BPW) was prepared at pH 3.5 adjusted with HCl. Apple juice (pH 3.50±0.10) was purchased from local grocery store. Inoculation at 1.0% (approx. 6 log CFU/mL) into 150 mL of acid-adjusted BPW or apple juice was done for each strain under each growth condition. Samples were prepared the day of HPP treatment and kept at 4°C before and after treatments.

Samples were packaged in 3-mil co-extruded film vacuum pouches and double heat-sealed. Pouches were then double packaged with an outer 2.5-mil PET/PE pouch and vacuum-sealed.

### HPP Treatment & Analysis

Samples were pressurized by an Avure 24L HPP unit.\* Pressure treatment was targeted at sublethal levels. During preliminary trials, sublethal pressure levels were found to be:

- 500 MPa for *E. coli* O157:H7 (180 s, 4°C initial temp.)
- 200 MPa for *L. monocytogenes* and *Salmonella* (180 s, 4°C initial temp.)

\*Inoculated control samples were prepared and kept at 4°C without pressure application

Pouches were analyzed same-day as HPP treatment, 24 h post-HPP cold storage (4°C), and 48 h post-HPP cold storage (4°C). [24 h and 48 h data not shown.]

The surviving populations were serially diluted using BPW and were enumerated by spread plating on TSAYE plates. Samples were conducted in triplicate with three repetitions for each experiment.



Figure 1. Avure 24L unit within IFSH pilot plant.

## Results and Discussion

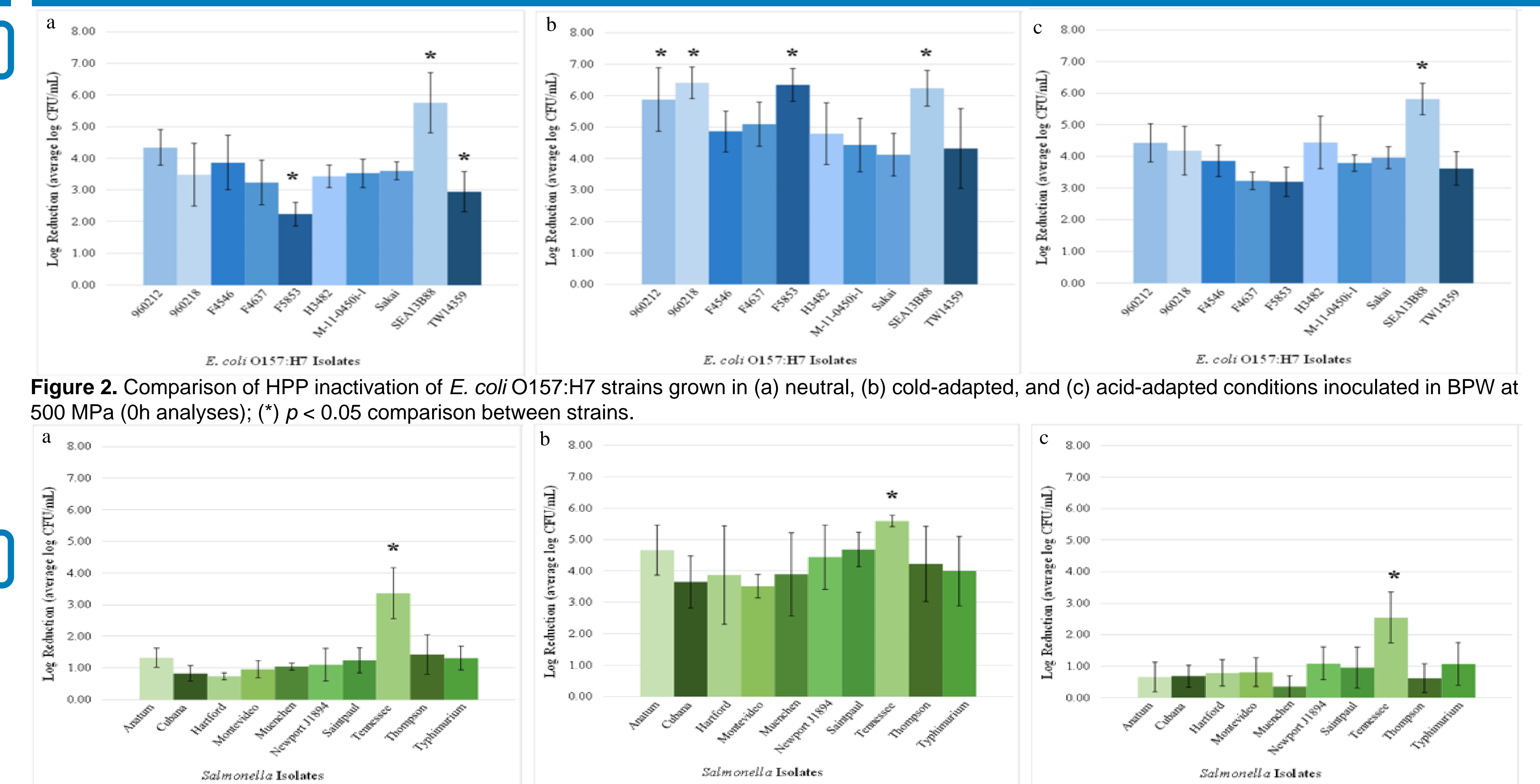


Figure 2. Comparison of HPP inactivation of *E. coli* O157:H7 strains grown in (a) neutral, (b) cold-adapted, and (c) acid-adapted conditions inoculated in BPW at 500 MPa (0h analyses); (\*)  $p < 0.05$  comparison between strains.

Figure 3. Comparison of HPP inactivation of *Salmonella* strains grown in (a) neutral, (b) cold-adapted, and (c) acid-adapted conditions inoculated in BPW at 200 MPa (0h analyses); (\*)  $p < 0.05$  comparison between strains.

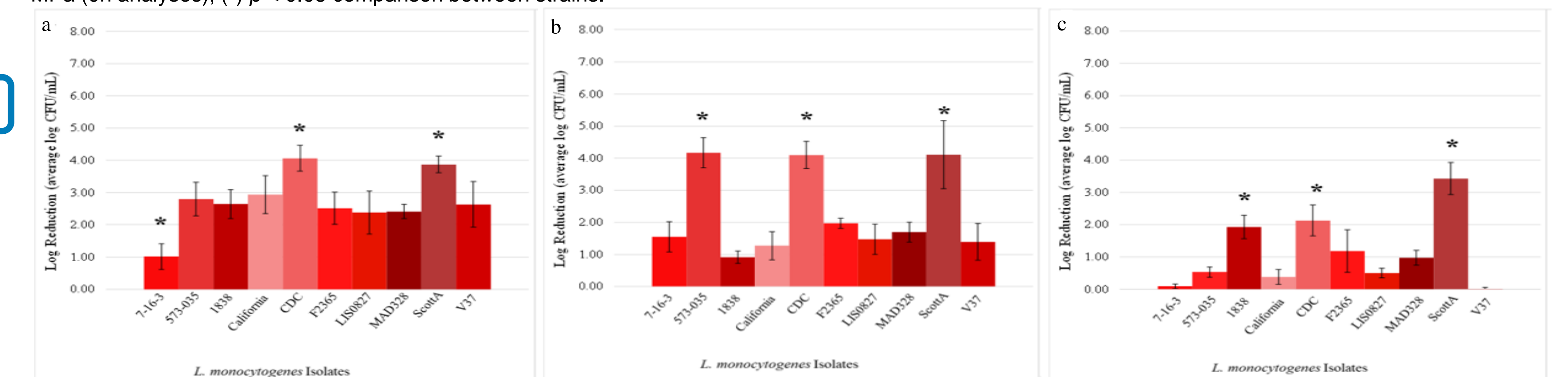


Figure 4. Comparison of HPP inactivation of *L. monocytogenes* strains grown in (a) neutral, (b) cold-adapted, and (c) acid-adapted conditions inoculated in BPW at 200 MPa (0h analyses); (\*)  $p < 0.05$  comparison between strains.

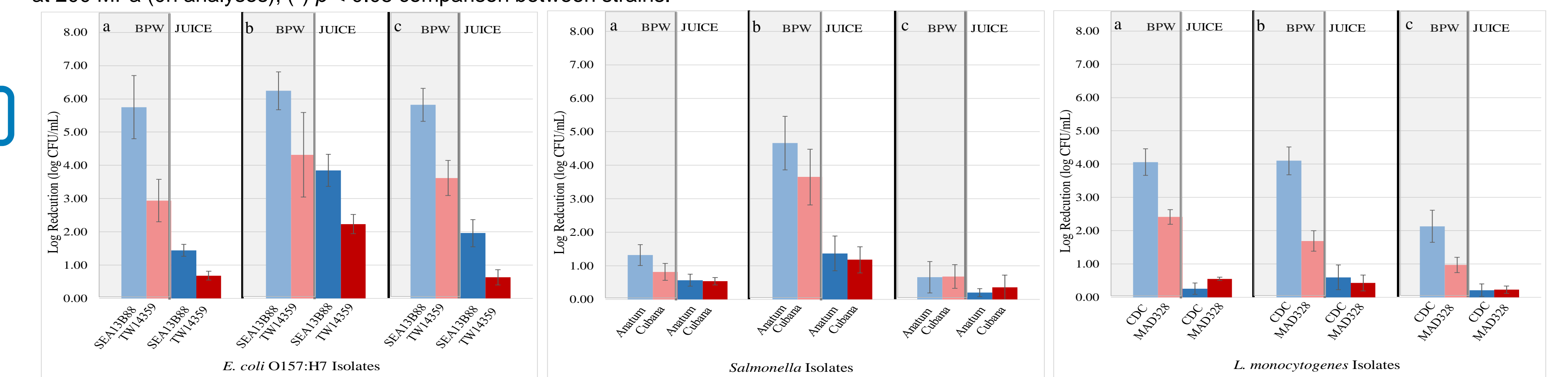


Figure 5. Comparison of HPP inactivation of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* strains grown in (a) neutral, (b) cold-adapted, and (c) acid-adapted conditions inoculated in BPW and apple juice at 500 MPa for *E. coli* O157:H7 and 200 MPa for *Salmonella* and *L. monocytogenes* (0h analyses).

## Conclusion

- E. coli* O157:H7 exhibited greater barotolerance than *Salmonella* spp. and *L. monocytogenes*, loss in viability was seen for all pathogens in all growth conditions during cold storage.
- In BPW, *E. coli* O157:H7 strains F5853 and TW14359 demonstrated resistance and *E. coli* O157:H7 strain SEA13B88 was most sensitive in all growth conditions
- Salmonella* isolates grown in neutral and acid-adapted conditions had similar results (<1.5 log reduction), except for *S. Tennessee*. Cold-adapted preparations were highly sensitive.
- Multiple cold- and acid-adapted *L. monocytogenes* strains had <2 log reduction while *L. monocytogenes* strains CDC and ScottA were significantly more sensitive in all growth conditions.
- Greater barotolerance was observed in apple juice from all microorganisms under all growth conditions compared to BPW.