Use of High Hydrostatic Pressure Processing on Three Human Pathogenic Strains of *Vibrio parahaemolyticus* in Live Gulf Coast Oysters (*Cassostrea virginica)*

G.J. Flick Jr.[1*], D. M. Wall-Bourne[1], and L. Ankenman Granata[1]

[1] Food Science and Technology Department, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 (flickg@vt.edu)

Introduction

Vibrio parahaemolyticus is a ubiquitous inhabitant of coastal marine water and is part of the natural flora of bivalve shellfish (Yeung and Boor, 2004). Molluscan shellfish extract, filter, and concentrate this organism from their environment. *Vibrio parahaemolyticus* is the leading bacterial cause of seafood-associated gastroenteritis in the United States. Few studies have been performed on the sensitivity of clinical *Vibrio parahaemolyticus* to high hydrostatic pressure processing (HPP) in *Cassostrea virginica* oysters. Ten clinical strains known to have caused foodborne illness outbreaks were obtained from the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA). Phase one of the study was to determine the parameters that completely inactivated *Vibrio parahaemolyticus*, in the stationary phase, using various pressures and hold times. After growth to the stationary phase (7 $\log_{10}$) in T_1N_1 broth (Trypticase 1%, NaCl 1%), 4 ml of each strain was subjected to all combinations of the 4 pressures and 5 hold times (pressures 35,000, 40,000, 45,000, and 50,000 psi [241, 276, 310, and 345 MPa] and hold times of 1, 2, 3, 4, and 5 min). Inactivation pressure and time combinations are shown in Table 1.

The second phase involved propagating the three most resistant strains, using the strains to contaminate Gulf coast shell stock oysters, and processing the contaminated oysters to determine the efficacy of HPP to inactivate the bacteria in oysters. The three most piezotorelant strains selected for assimilation in oysters and HPP treatments were DIE 12-052499, TX 2103, and ATCC 17802 (Table 1).

	Inactivation	
Strain	**Pressure**	**Hold Time**
AQ 4037	241 MPa	1 min
ATCC 17802	**241 MPa**	**3 min**
	276 MPa	**2 min**
ATCC 17803	241 MPa	1 min
BAC 98-3547	241 MPa	1 min
CPA7-08-1699	241 MPa	3 min
DIE 12-052499	**241 MPa**	**3 min**
	276 MPa	**2 min**
SPRC 10290	241 MPa	1 min
SPRC 10293	241 MPa	2 min
	276 MPa	2 min
TX 2062	241 MPa	3 min
	276 MPa	2 min
TX 2103	**241 MPa**	**5 min (Still viable)**
	276 MPa	**5 min**

Table 1 Strains of clinical *Vibrio parahaemolyticus* bulb data for inactivation in the Stationary Phase

Materials and Methods

Following Cook's (2003) protocol for obtaining cell suspensions growth to stationary phase (7 log_{10}) in T_1N_1 broth (Trypticase 1%, NaCl 1%), pelleted cells were reconstituted in phosphate buffered saline (PBS) and 4 ml of each strain was sealed in disposable transfer pipets (SAMCO, San Fernando, CA). The pipets bulbs were subjected to HPP with all combinations of the following pressures and hold times 35,000, 40,000, 45,000, and 50,000 psi (241, 276, 310, and 345 MPa)) and 5 hold times (1, 2, 3, 4, and 5 min). Live oysters (*Crassostrea virginica*) were obtained from Cowart Seafood Corp, Lottsburg, VA and shipped overnight to the Virginia Tech Food Science Seafood Laboratory. The oysters were washed to remove any mud and placed in aerated artificial seawater tanks (20 ppt salinity in 30 liter tanks maintained at 18°C) for 4 hours before inoculation. Using Cook's (2003) protocol for obtaining cell suspensions, 20 mls of the resulting bacterial concentration of 10^8 CFU/ml was used to inoculate tanks containing oysters, resulting in a final concentration of $\pm 10^5$ cells/ml seawater. The temperature in the room was raised to 22°C (71.6°F). After 18 hour uptake, the oysters were removed and randomly placed into Cryovac® vacuum bags and sealed. The oysters were triple bagged and then subjected to the HPP parameters listed above.

After HPP, nine oysters were divided into three replicate samples. The oysters were shucked, and the FDA's Bacteriological Analytical Manual (BAM) preferred laboratory procedures for microbiological analyses of seafoods was followed for *Vibrio* isolation and identification. Equal parts oyster and PBS were homogenized for 2 min using a Seward Stomacher 400 Circulator (Tekmar Co., Cincinnati, OH). From this, serial dilutions were made in PBS and Most Probable Numbers (MPN) of *Vibrio parahaemolyticus* were determined by inoculating 3 x 1 ml portions of the 1:10, 1:100, 1:1000, and 1:10,000 dilutions into 10 ml of Alkaline Peptone Saline (APS). The tubes were incubated overnight at 35°C. From the top 1 cm of the APS tubes containing the three highest dilutions of sample showing growth, a 3-mm loopful was streaked onto TCBS (Thiosulfate Citrate Bile Salts Sucrose) for *V. parahaemolyticus.* The TCBS plates were incubated at 35°C overnight. Typical blue-green colonies on the TCBS were counted and recorded. Representative colonies were picked for confirmation using API 20E® diagnostic strips. The number of MPN tubes containing confirmed *V. parahaemolyticus* colonies was compared with the 3-tube-MPN chart and the results expressed as *V. parahaemolyticus* MPN/g of oysters.

Results

Figure 1 shows the MPN (Most Probable Number) for *Vibrio parahaemolyticus* DIE 12-052499 for each pressure and time used. HPP parameters of 345 MPa for 2 min and 310 MPa for 3 min both resulted in a 4.2 log reduction. Figure 2 shows the MPN for each pressure and time used for *V. parahaemolyticus* TX 2103. HPP parameters of 310 MPa for 2 min, and 345 MPa for 2 min resulted in a 4.2 log reduction. Figure 3 shows the MPN for *V. parahaemolyticus* ATCC type strain 17802 from each pressure and time parameter, ATCC 17802 was inactivated using 276 MPa for 2 min , 310 MPa for 2 min and 345 MPa for 1 min all resulting in a 4.0 log reduction.

Conclusion

From the three most piezotolerant strains run it appears that a pressure of 310 MPa (40,000 psi) with a 3 min hold time at room temperature does inactivate the pathogen. The level of oyster uptake of the *Vibrio* inoculum was 10^5, however we feel that the HPP parameters listed above could easily inactivate clinical *Vibrio* spp. in oysters at concentrations greater than 5.0 log. Sensory panels have evaluated oysters at both 310 and 345 MPa and the oysters were found to be acceptable.

References

Cook, D.W. 2003. Sensitivity of *Vibrio* species in phosphate-buffered saline and in oysters to High-pressure processing. *Journal of Food Protection.* 66(12): 2276-2282

Yeung, P.S.M. and K.J. Boor. 2004. Epidemiology, Pathogens, and Prevention of Foodborne *Vibrio parahaemolyticus* infections. *Foodborne Pathogens and Disease.* 1(2): 74-88.

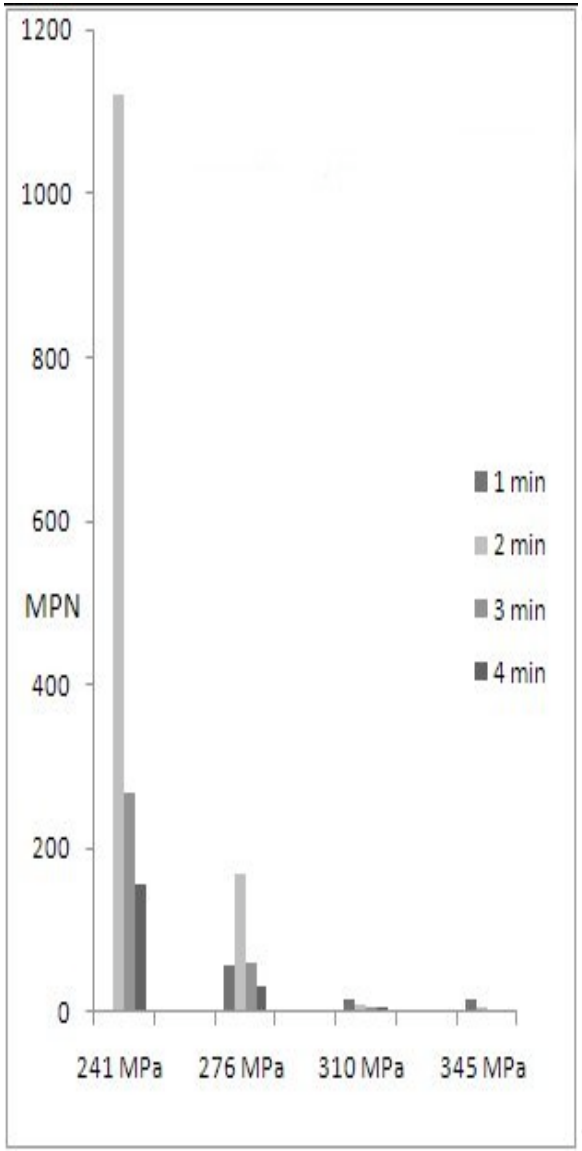

Figure 1. MPN Data for *V. p.* DIE 12-052499 using Various Pressures and Hold

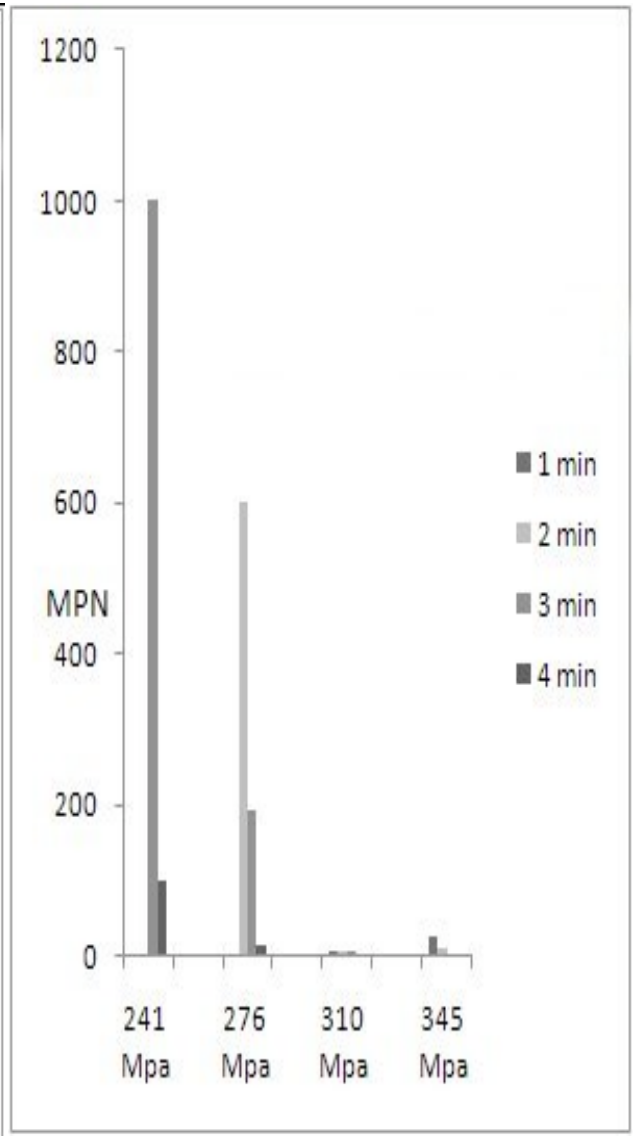

Figure 2. MPN Data for *V. p.* TX 2103 using Various Pressures and Hold Times

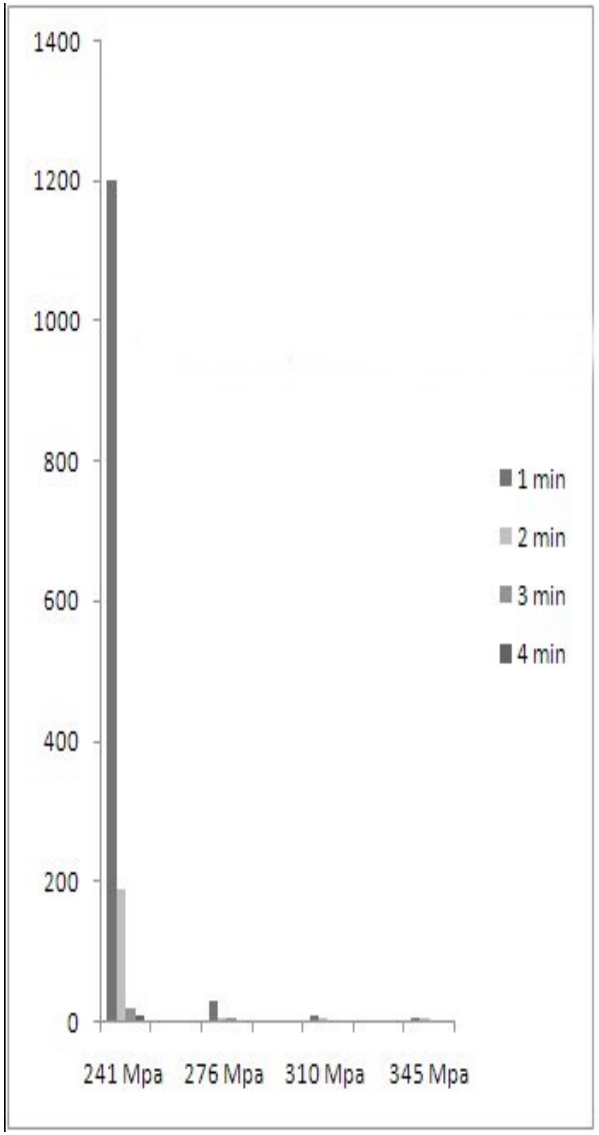

Figure 3. MPN Data for *V. p.* ATCC 17802 using Various Pressures and Hold Times