1 **Application of Grape Seed Extract in Depuration for Decontaminating *Vibrio***
2 ***parahaemolyticus* in Pacific Oysters (*Crassostrea gigas*)**

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Abstract

This study investigated the potential application of grape seed extract (GSE) in depuration to increase its efficacy in reducing *Vibrio parahaemolyticus* populations in Pacific oysters (*Crassostrea gigas*). Pacific oysters were inoculated with five clinical strains of *V. parahaemolyticus* to 10^{4-5} MPN/g and depurated in UV-irradiated artificial seawater (ASW) containing GSE at a level of 1.0% (1.8 mg/mL total phenolic contents as gallic acid equivalents) or 1.5% (3.1 mg/mL total phenolic contents as gallic acid equivalents) at 12.5 °C for up to 5 days. Changes of *V. parahaemolyticus* populations in oysters during depuration were analyzed every 24 h using the three-tube most probable number (MPN) method. The populations of *V. parahaemolyticus* in inoculated oysters decreased by 3.06 and 3.71 log MPN/g, respectively, after 4 and 5 days of depuration in ASW at 12.5°C. However, populations of *V. parahaemolyticus* in oysters were reduced by 3.01 and 4.18 log MPN/g after 2 days of depuration at 12.5°C in ASW containing 1.0 and 1.5% GSE, respectively. Further studies confirmed that depuration using ASW containing 1.5% GSE with 3.1 mg/mL total phenolic contents as gallic acid equivalents at 12.5 °C for two days was capable of achieving >3.52 log MPN/g reductions of *V. parahaemolyticus* in Pacific oysters.

Key words: *Vibrio parahaemolyticus*, oyster, depuration, grape seed extract, seafood safety

1. Introduction

Oyster contain unsaturated fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), dietary-essential amino acids (arginine, cysteine, and proline), minerals (irons, zinc, and cooper) as well as vitamin B₁₂ and is a good source of nutrients to human diet (Dong, 2009; USDA, 2016). A 100-gram serving of oyster provides more than the Recommended Dietary Allowances (RDAs) of copper, zinc and Vitamin B-12 for both men and women (Otten, Hellwig, & Meyers, 2006).

The United States produces more than 27 million pounds of oysters each year with most of them (70%) being sold for raw consumption without process (Hardesty, 2001). *Vibrio parahaemolyticus* is a foodborne pathogen naturally distributed in the marine environments and can be accumulated in shellfish digestive gland through the water-feeding activity. Illnesses caused by *V. parahaemolyticus* typically with symptoms of nausea, vomiting, abdominal cramping and watery diarrhea occurring 24 hours of infection (CDC, 2016). The United States Centers for Disease Control and Prevention (CDC) estimate that 45,000 cases of *V. parahaemolyticus* occur annually in the U.S. (FDA, 2012).

Several post-harvest processes, such as mild heat treatment (Andrews, Park, & Chen, 2000), flash freezing followed by frozen storage (Liu, Lu, & Su, 2009), irradiation (Andrews, Jahncke, & Mallikarjunan, 2003), and high pressure processing (Ma, & Su, 2011), have been developed to decrease *V. parahaemolyticus* in oysters to <30 MPN/g with a minimum 3.52-log reduction according to the National Shellfish Sanitation Program (ISSC, 2016; NSSP, 2015). However, oysters are often killed after these processes, except by low-dose irradiation. There is a need to develop a process for reducing *V. parahaemolyticus* contamination in oysters without significant adverse effects.

Depuration is a process which holds shellfish in clean seawater to allow them to purge contaminants, including sand, bacteria, toxins and trace metals. It has been utilized as a post-harvest treatment with a long history for reducing total bacteria levels in shellfish (Blogoslawski and Stewart, 1983). However, studies have reported that depuration at ambient temperature was not effective in reducing some persistent bacteria, such as *V. parahaemolyticus*, in oysters (Tamplin and Capers, 1992). Recently, we developed a refrigerated seawater (7 to 15 °C) depuration capable of decreasing *V. parahaemolyticus* populations in oysters by >3.0 log MPN/g after 5 days of the process with no adverse effect on oysters (Phuvasate, Chen, & Su, 2012). There is a need to increase the efficacy of the depuration process to deliver >3.52-log reductions of *V. parahaemolyticus* in contaminated oysters in a shorter time period.

Grape seed extract contains polyphenol compounds, such as proanthocyanidins (Jayaprakasha, Selvi, & Sakariah, 2003; Rababah, Hettiarachchy, & Horax, 2004), that has antimicrobial activity against both Gram-positive and Gram-negative bacteria including *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enterica*, and *Vibrio parahaemolyticus* (Mahmoud, 2014; Perumalla & Herriarachchy, 2011). Xi, Liu, & Su (2012) studied the bactericidal activity of green tea extract against *V. parahaemolyticus* and reported that tea extract containing equal or higher than 4.6 mg/mL total phenolic contents as gallic acid equivalents reduced a mixture of five clinical *V. parahaemolyticus* strains in a growth medium (tryptic soy broth plus 1.5% NaCl) from 4.5 log CFU/mL to a non-detectable level (<10 CFU/mL) in 8 h. A recent study reported that grape seed extract (1%, v/v), when combined with carboxymethyl cellulose (CMC) and *Zataria multiflora* Boiss essential oil (ZMEO), reduced *Pseudomonas* spp. and lactic acid bacteria by 2.8 and 2.6 log CFU/g, respectively, in rainbow trout fillets stored at 4°C for 20 days (Raeisia *et al.*, 2015). These observations suggest that grape

seed extract might be utilized as a natural antimicrobial agent in depuration process to inactivate *V. parahaemolyticus* in oysters. This study was conducted to evaluate the efficacy of refrigerated depuration using artificial seawater containing grape seed extract in reducing *V. parahaemolyticus* in Pacific oysters (*Crassostrea gigas*).

2. Material and methods

2.1. Bacterial culture preparation

Five clinical strains of *Vibrio parahaemolyticus* (10290, 10292, 10293, BE98-2029, O27-1c1) were individually grown in 10 ml tryptic soy broth (Difco, Becton, Dickinson, Sparks, MD, USA) supplemented with 1.5% NaCl (TSB-Salt) for 16-18 h at 37 °C. Each enriched culture was streaked onto a thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Difco) plate and incubated for 18-24 h at 37 °C. A single colony from each TCBS plate was picked and incubated in TSB-Salt (10 ml) for 4 h at 37 °C. The enriched TSB-Salt cultures were pooled into a 50-mL sterile centrifuge tube and harvested by centrifugation at $3000 \times g$ (Sorvall RC-5B, Kendro Laboratory Products, Newtown, CT, USA) at 5 ± 1 °C for 15 min. Pellet cells were collected and resuspended in 50 ml sterile 2% NaCl to obtain a mixed culture suspension of 10^{8-9} CFU/mL.

2.2. Antimicrobial activity of grape seed extract against *V. parahaemolyticus*

Twenty grams of grape seed extract (Bulk Supplements, Henderson, NV, USA) were added to 380 mL of deionized water (DI) and mixed by a blender (Waring Laboratory, Torrington, CT, USA) for 20 s. The blended solution (5%, w/v) was held at room temperature for 24 h and then filtrated through a 2V filter paper (Whatman, Kent, England, UK). The filtered solution was allowed to pass through a Discovery DPA-6S SPE tube (Sigma-Aldrich, St. Louis, Missouri, USA) to reduce color of the extract solution. A preliminary study using well diffusion inhibition tests revealed that grape seed extract at 1.0% concentration inhibited growth of *V.*

parahaemolyticus on tryptic soy agar supplemented with 1.5% NaCl (data not shown). Antimicrobial activity of grape seed extract against *V. parahaemolyticus* was determined by inoculating the 5-strain mixture of *V. parahaemolyticus* to TSB-Salt containing 1.0% grape seed extract at a level of 10^{5-6} CFU/mL and then incubated at 37 °C for 8 h. Changes of *V. parahaemolyticus* populations in TSB-Salt during incubation were determined at 0, 2, 4, 6, 8 h with the plate count method using tryptic soy agar (Difco, Becton, Dickinson, Sparks, MD, USA) supplemented with 1.5% NaCl (TSA-Salt) and incubation at 37 °C for 24 h. Results were reported as means of two determinations.

2.3. Determination of total phenolic contents in grape seed extract

Total phenolic contents in grape seed extract were determined according to the Folin-Ciocalteu method (Waterhouse, 2003). Grape seed extract solution (200 µL) was mixed with 15.8 ml of DI water and 1 ml of Folin-Ciocalteu Phenol reagent (MP Biomedicals, Solon, OH, USA). The sample mixture was allowed to settle at room temperature for 5 min. Three milliliters of sodium carbonate solution were then added to the mixture and incubated for 2 h at 20 °C. The solution was then measured by a UV-VIS Spectrophotometer (UV-2401 PC, Shimadzu, Kyoto, Japan) at 765 nm. Total phenolic contents (mg/mL) in the solution were reported as gallic acid equivalents according to a gallic acid standard curve established with the absorbance values of gallic acid at 0, 50, 100, 150, 250 and 500 mg/mL. Results were reported as means of three determinations.

2.4. Analysis of turbidity of artificial seawater containing grape seed extract

Turbidity of artificial seawater containing grape seed extract was analyzed with the Sper Scientific LUTU-2016 Lutron Turbidity meter (Sper Scientific, Scottsdale, AZ, USA) using the nephelometric method according to manufacturer's instructions. The turbidity meter was

calibrated with standard solutions of 0 and 100 Nephelometric Turbidity Units (NTU) before sample analysis.

2.5. Oyster preparation

Fresh Pacific oysters (*Crassostrea gigas*) with an average length of 8.0 ± 0.7 cm and weight of 29.7 ± 5.2 g were obtained from Oregon Oyster Farms (Yaquina Bay, Newport, Oregon, USA) and transported to laboratory in a cooler with ice gels on the day of harvest. Oysters were briefly washed under tap water to remove mud on the shell and then placed in a rectangular high-density polyethylene (HDPE) tank (45 by 30 by 30 cm; Nalgene, Rochester, NY, USA) containing artificial seawater (ASW) with a salinity of 30 parts per thousands (ppt) prepared from Instant Ocean Salt (Aquarium systems Inc., Mentor, OH, USA). Oysters were held in ASW at room temperature overnight with algae (Shellfish Diet 1800, Reed Mariculture Inc., Campbell, CA, USA) being added to the water and air being pumped into the tank to maintain oxygen level favorable to resume their biological activities.

2.6. Inoculation of oysters with *V. parahaemolyticus*

Oysters were transferred to a tank containing 20L ASW containing mixed culture of five clinical *V. parahaemolyticus* strains at a level of about 10^5 CFU/mL. Accumulation of *V. parahaemolyticus* in oysters was performed at room temperature for 16-18 h with water being circulated at 15 L/h to achieve a contamination level of *V. parahaemolyticus* in oysters at 10^{4-5} CFU/mL.

2.7. Depuration

Inoculated oysters were transferred to a laboratory recirculating depuration system equipped with a 15 W Gamma UV sterilizer (Current-USA Inc., Vista, CA, USA) and a water chiller (Delta star, Aqua Logic, Inc., San Diego, CA, USA). Depuration of oysters was conducted using

40 oysters in 80 L of ASW containing 1.0 % or 1.5 % grape seed extract at a water flow rate of 1500 L/h. The process was conducted at 12.5°C for up to 5 days with water being replaced with fresh ASW containing grape seed extract every 24 h. Populations of *V. parahaemolyticus* in oysters were analyzed every 24 h. A depuration condition yielded a mean reduction of *V. parahaemolyticus* in oysters by >3.52 log (MPN/g) was further studied 4 times to validate the process's capability of delivering >3.52 log (MPN/g) reduction of *V. parahaemolyticus* in individual oysters with a final level of <30 MPN/g in each oyster.

2.8. Microbiological analysis

Populations of *V. parahaemolyticus* in oysters before and after depuration were determined with the three-tube most-probable-number (MPN) method (FDA, 2016). Five oysters were randomly picked from the depuration tank at each test time and shucked with a sterile shucking knife in a stainless tray. Each oyster meat was blended in a sterile stainless blender jar with an equal volume of phosphate-buffered saline (PBS) for 1 min at high speed by a two speed laboratory blender (Waring Laboratory, Torrington, CT, USA). Twenty grams of the oyster homogenate were transferred into a sterile sampling bag and mixed with 80 g of PBS to prepare a 1:10 sample dilution with additional 10-fold dilutions being prepared with PBS. Each sample dilution was inoculated into 3 tubes of alkaline peptone water (APW) and the tubes were incubated at 37 °C for 16-18 h. After the incubation, one loopful (3 mm) of APW from each turbid tube was streaked onto individual TCBS agar plates and incubated at 35-37 °C for 18-24 h. Colonies formed on TCBS plates that were green or bluish with round (2-3 mm) shape were considered as *V. parahaemolyticus*. Total populations of *V. parahaemolyticus* were determined by converting APW tubes that were positive for *V. parahaemolyticus* to MPN/g using a MPN table. The efficacy of the UV sterilizer in disinfecting *V. parahaemolyticus* in artificial seawater

during depuration was determined daily by spreading 1 ml of ASW onto TCBS plates and incubated at 37 °C for 24 h. Results were reported as means of five determinations.

2.9. Statistical analysis

Results of the bacterial populations were transformed to log values and statistically analyzed with one-way Analysis of Variance (ANOVA) and Tukey-Kramer multiple-comparison Test using IBM SPSS 19.0 (Chicago, IL, USA). Significant differences between reductions of *V. parahaemolyticus* at various times during depuration were established at $P < 0.05$.

3. Results and Discussion

3.1. Antimicrobial activity of grape seed extract

Populations of a mixed culture of five strains of *V. parahaemolyticus* in tryptic soy broth supplemented with 1.5% NaCl (TSB-salt) was increased gradually from an initial level of 5.23 to 8.43 log CFU/mL after 8 h of incubation at 37°C (Table 1). However, populations of *V. parahaemolyticus* in TSB-Salt containing 1% grape seed extract (1.8 mg/mL total phenolic compounds as gallic acid equivalents) decreased significantly ($P < 0.05$) from 5.19 to 3.45 CFU/mL after 2 h of incubation at 37 °C and further decreased to a non-detectable level (< 10 CFU/mL) after 4 h of incubation (Table 1). These results revealed that grape seed extract exhibited strong bactericidal activity against *V. parahaemolyticus*.

The bactericidal activity of grape seed extract against foodborne pathogens has been reported in several studies. One study reported that the minimum inhibitory concentration (MIC) of grape seed extract for inhibiting growth of *E. coli* O157: H7, *L. monocytogenes* and *Salmonella* Typhimurium at levels around 4.43 log CFU spread onto a plate were 4.0, 4.0 and 8.0 mg/mL, respectively (Ahn, Grun, & Mustapha, 2004). Mahmoud (2014) found that the MIC of grape seed extract against *V. parahaemolyticus* at a level of 10^8 CFU/mL in tryptic soy broth

supplemented with 1% NaCl was 10.0 mg/mL. In this study, we observed that grape seed extract with total phenolic compounds of 1.8 mg/mL as gallic acid equivalents decreased *V. parahaemolyticus* populations in TSB-salt from 5.19 CFU/mL to non-detectable levels (<10 CFU/mL) within 4 h at 37 °C. The differences between these results are likely due to different strains of *V. parahaemolyticus* as well as grape seed extracts containing differing amounts of total phenolic compounds used in the studies.

3.2. The application of grape seed extract in depuration for reducing *V. parahaemolyticus* populations in Pacific oysters

Addition of grape seed extract (GSE) to ASW at a concentration of 1.0% increased the efficacy of depuration in reducing *V. parahaemolyticus* populations in the Pacific oysters. The populations of *V. parahaemolyticus* in laboratory inoculated oysters decreased by 3.61 log MPN/g (from 5.23 to 1.62 log MPN/g) after being depurated in ASW containing 1% GSE at 12.5 °C for 3 days (Table 2). However, a smaller reduction (3.12 log MPN/g) of *V. parahaemolyticus* in the oysters was obtained after 3 days of depuration at 12.5 °C using ASW without GSE. These results indicated that depuration with ASW containing 1% GSE at 12.5 °C for 3 days could result in >3.52 log reductions of *V. parahaemolyticus* populations in the Pacific oysters.

To verify the efficacy of depuration with ASW containing 1% GSE at 12.5 °C for reducing *V. parahaemolyticus* in oysters, an additional study was conducted to determine reductions of *V. parahaemolyticus* in individual oysters by the depuration process. A mean reduction of 3.51 log MPN/g (from five oysters) of *V. parahaemolyticus* populations in oysters was observed after 3 days of depuration at 12.5 °C with reductions in each oyster ranging from 3.12 to 3.77 log MPN/g (Table 3). These results signify that depuration with ASW containing 1% GSE (1.8

216 mg/mL total phenolic contents as gallic acid equivalents) may not always yield >3.52 log
217 reductions of *V. parahaemolyticus* in oysters.

218 Increasing the concentration of grape seed extract (GSE) in ASW from 1.0 to 1.5% greatly
219 enhanced the efficacy of the depuration in decontaminating *V. parahaemolyticus* in oysters.
220 Populations of *V. parahaemolyticus* in the Pacific oysters decreased by 3.77 and 4.18 log MPN/g
221 after one and two days of depuration in ASW containing 1.5% GSE (3.1 mg/mL total phenolic
222 contents as gallic acid equivalents), respectively (Table 4). However, it required 5 days of
223 depuration in ASW at 12.5 °C to decrease *V. parahaemolyticus* populations in oysters by 3.71
224 log MPN/g.

225 To confirm the efficacy of depuration using ASW containing 1.5% GSE (3.1 mg/mL total
226 phenolic contents as gallic acid equivalents) in decontaminating *V. parahaemolyticus* in oysters,
227 further studies were conducted to determine reductions of *V. parahaemolyticus* in individual
228 oysters by the process. Results confirmed that depuration with ASW containing 1.5% GSE was
229 able to decrease *V. parahaemolyticus* populations in the Pacific oysters by 3.55 - 4.41 log MPN/g
230 with final *V. parahaemolyticus* levels in all the oysters being reduced to <30 MPN/g (<1.48 log
231 MPN/g) after 2 days of process (Table 5). These results indicate that the efficacy of depuration
232 with ASW containing GSE appears to be associated with the amounts of total phenolic
233 compounds in the water, which was evidenced by greater reductions of *V. parahaemolyticus*
234 levels in oysters by depuration with ASW containing 1.5% GSE (3.1 mg/mL total phenolic
235 contents as gallic acid equivalents) than those with ASW containing 1.0% GSE (1.8 mg/mL total
236 phenolic contents as gallic acid equivalents). Finally, depuration with ASW containing GSE did
237 not result in mortality of the Pacific oysters. All oysters remained alive after the depuration
238 process (data not shown).

3.3. Effects of color of grape seed extract on turbidity of artificial seawater for depuration

Grape seed extract is derived from red grapes with polyphenolic compound proanthocyanidins being the major contributor to its dark color (Lea, 1992; Weber *et al.*, 2007). Addition of GSE to ASW, even at a concentration of 1%, significantly changed the color of water and increased the turbidity of water to 54 NTU. To avoid the impact of water turbidity on depuration for reducing populations of *V. parahaemolyticus* in oysters, GSE solutions were filtered through a Discovery DPA-6S SPE tube (Sigma-Aldrich, St. Louis, Missouri, USA) to reduce color of the solutions, which also reduced the turbidity of ASW containing 1.5% GSE to 2.9 NTU. While the filtration process reduced the color of the GSE solutions, it also reduced the total phenolic contents in 5% GSE solution from 38.5 to 14.6 mg/mL most likely due to removal of polyphenolic compounds (proanthocyanidins) from the grape seed extract solution. However, this study demonstrated that addition of GSE to ASW at a concentration of 1.5% (3.1 mg/mL total phenolic contents as gallic acid equivalents) for depuration at 12.5 °C greatly reduced the processing time needed to achieve >3.52-log reductions of *V. parahaemolyticus* populations in the Pacific oysters from 5 to 2 days due to the antimicrobial activity of grape seed extract against *V. parahaemolyticus*.

4. Conclusions

Addition of grape seed extract to artificial seawater for oyster depuration increased the efficacy of the process in reducing *Vibrio parahaemolyticus* populations in laboratory contaminated oysters. This study developed a depuration process at 12.5 °C using artificial seawater containing 1.5% grape seed extract (3.1 mg/mL total phenolic contents as gallic acid equivalents) to significantly reduce the depuration time from 5 to 2 days for achieving >3.52-log reductions of *V. parahaemolyticus* in the Pacific oysters. Further studies need to be conducted

262 using oysters with naturally accumulated *V. parahaemolyticus* to validate the efficacy of this
263 depuration process for decontaminating *V. parahaemolyticus* in oysters.

264

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Table 1

Antimicrobial activity of grape seed extract (GSE) against growth of *Vibrio parahaemolyticus* in tryptic soy broth plus 1.5% NaCl (TSB-Salt) with 1% GSE at 37 °C.

Incubation time (h)	Control	TSB-Salt + GSE (1%) ^a
0 ^b	5.23 ± 0.01 ^c A	5.19 ± 0.01 A
2	5.66 ± 0.03 B	3.45 ± 0.01 C
4	8.07 ± 0.01 C	ND ^d
6	8.41 ± 0.05 D	ND
8	8.43 ± 0.05 D	ND

^a Total phenolic compounds (1.8 mg/mL) as gallic acid equivalents.

^b Bacterial counts determined after 30 s of mixing bacteria culture with the medium.

^c Data were reported as means (log CFU/mL) of two determinations ± standard deviation. Data with different letters in the same column are significantly different ($P < 0.05$).

^d Non-detectable with a detection limit of <10 CFU/mL.

Table 2

Changes of *V. parahaemolyticus* populations (log MPN/g) in laboratory inoculated oysters depurated with artificial seawater (ASW) containing 1.0% grape seed extract (GSE) at 12.5 °C.

Time (days)	ASW with 1.0% GSE ^a	Control
0	5.23 ± 0.30 ^b A	5.35 ± 0.25 A
1	2.77 ± 0.26 (2.46) ^c B	3.77 ± 0.18 (1.58) B
2	2.22 ± 0.23 (3.01) C	2.86 ± 0.56 (2.49) BC
3	1.62 ± 0.45 (3.61) D	2.23 ± 0.30 (3.12) C

^a Total phenolic contents of 1.8 mg/mL as gallic acid equivalents.

^b Values were reported as means of five determinations ± standard deviation. Data with different letters in the same column are significantly different ($P < 0.05$).

^c Reduction (MPN/g) of *V. parahaemolyticus* after treatments.

Table 3

Changes of *Vibrio parahaemolyticus* populations (log MPN/g) in laboratory inoculated oysters depurated with artificial seawater containing 1.0% grape seed extract (1.8 mg/mL total phenolic contents as gallic acid equivalents) at 12.5 °C for 3 days.

Oysters	<i>V. parahaemolyticus</i> populations ^a
1	1.63 ^b (3.46) ^c
2	1.36 (3.73)
3	1.63 (3.46)
4	1.97 (3.12)
5	1.32 (3.77)
Average value	1.58 ± 0.26 (3.51)

^a Initial mean populations in oysters were 5.09 ± 0.28 (log MPN/g) determined with 5 oyster samples.

^b *V. parahaemolyticus* (log MPN/g) in oysters after treatments.

^c Reduction of *V. parahaemolyticus* (log MPN/g) from initial mean populations after treatments.

Table 4

Changes of *V. parahaemolyticus* (MPN/g) populations in laboratory inoculated oysters depurated with ASW containing 1.5% grape seed extract (GSE, 3.1 mg/mL total phenolic contents as gallic acid equivalents) at 12.5 °C.

Time (days)	ASW with 1.5% GSE	Control
0	5.17 ± 0.21 ^a A	5.17 ± 0.34 A
1	1.40 ± 0.31 (3.77) ^b B	2.97 ± 0.42 (2.20) B
2	0.99 ± 0.27 (4.18) B	2.16 ± 0.19 (3.01) C
3	1.11 ± 0.38 (4.06) B	2.13 ± 0.17 (3.04) C
4	0.94 ± 0.40 (4.23) B	2.11 ± 0.25 (3.06) C
5	0.99 ± 0.42 (4.18) B	1.46 ± 0.14 (3.71) D

^a Values were reported as means of five determinations ± standard deviation. Data with different letters in the same column are significantly different ($P < 0.05$).

^b Reduction of *V. parahaemolyticus* (log MPN/g) after treatments.

Table 5

Changes of *Vibrio parahaemolyticus* populations (log MPN/g) in individual oysters depurated with ASW containing 1.5% grape seed extract (3.1 mg/mL total phenolic contents as gallic acid equivalents) at 12.5 °C for 2 days.

Oysters	Trial 1 ^a	Trial 2 ^b	Trial 3 ^c	Trial 4 ^d
1	1.18 ^e (3.79) ^f	1.04 (3.92)	1.32 (3.86)	0.96 (3.97)
2	0.56 (4.41)	0.87 (4.09)	1.18 (4.00)	1.18 (3.75)
3	1.32 (3.65)	1.18 (3.78)	1.04 (4.14)	1.38 (3.55)
4	1.36 (3.61)	0.56 (4.40)	1.04 (4.14)	1.04 (3.89)
5	1.18 (3.79)	1.04 (3.92)	0.96 (4.22)	1.18 (3.75)

^a Initial populations in oysters ranged from 4.97 to 5.38 log MPN/g determined with 5 oyster samples.

^b Initial populations in oysters ranged from 4.96 to 5.66 log MPN/g determined with 5 oyster samples.

^c Initial populations in oysters ranged from 5.18 to 5.66 log MPN/g determined with 5 oyster samples.

^d Initial populations in oysters ranged from 4.93 to 5.66 log MPN/g determined with 5 oyster samples.

^e *V. parahaemolyticus* (log MPN/g) in oysters after treatments.

^f Reductions of *V. parahaemolyticus* (log MPN/g) from the lowest initial populations after treatments.