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Monte Carlo analysis of the product handling and high-pressure treatment effects on the *Vibrio vulnificus* risk to raw oysters consumers

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Short version of title: *Vibrio vulnificus* risk in untreated and high pressure processed raw oysters

ABSTRACT

A Monte Carlo procedure considering the variability in oyster handling from harvest to raw consumption estimated reductions in the number of *Vibrio vulnificus* induced septicemia cases achieved by high-pressure processing (HPP). The calculations yielded pathogen load distributions in raw oysters from harvest to consumption. In the warm season, 2-6 min treatments at 250 MPa and 1°C would lower the predicted number of septicemia cases associated with raw oyster consumption from 4,932 to less than four per 100 million consumption events (95% confidence). This study highlighted that HPP conditions should be selected according to the seasonal pathogen load and environment temperature. Finally, the procedure emphasized that the variability in the *V. vulnificus* population at harvest, before and after HPP treatments, reflecting in part the microbiological quantification methods used, significantly affected the estimated number of septicemia cases. Therefore, improving microbiological quantification should provide better predictions of the number of septicemia cases.

Keywords: oyster, high pressure processing, *Vibrio vulnificus*, Monte Carlo analysis, beta-Poisson dose response models, seafood poisoning risk

1. **Introduction**

Oysters and other bivalve mollusks are filter feeders and thus concentrate microbial pathogens and other environmental contaminants (He et al., 2002). Among pathogens found in oysters, *Vibrio vulnificus* poses a severe health threat due to its ability to cross the intestinal barrier reaching the blood stream and causing primary septicemia (up to 55% fatality rate) (Chiang and Chuang, 2003). *V. vulnificus* proliferates in warm seawater (>20°C) with moderate salinity (5-25%), and thus consumption of raw oysters is particularly unsafe during summer days (Motes et al., 1998). The Interstate Shellfish Sanitation Conference (ISSC) proposed the application of shellfish post-harvest treatments reaching a 3.52-log reduction in *V. vulnificus* counts with an endpoint of non-detectable counts at the level of <30 cfu/g (ISSC, 2011). To reach this safety standard, high pressure processing (HPP) at 200-300 MPa is likely the best alternative amidst oyster pasteurization treatments. HPP is a well-established processing technology (Mújica-Paz et al., 2011) able to reduce pathogen levels (Cook, 2003; Kural and Chen, 2008) while shucking oysters (Torres and Velazquez, 2005) and yielding products with high consumer acceptance (Cruz et al., 2011).

The efficacy of HPP treatments eliminating *V. vulnificus* in oysters harvested from different seasons can be determined by microbial risk assessment (MRA). MRA is an essential tool in the management of foodborne pathogens risks and the development of international food trade standards. MRAs, particularly quantitative MRAs, are resource-intensive tasks in which the reported incidence frequency of food-borne disease outbreaks (FBDOs), the pathogen load in the

66 foods involved, and the food amount consumed per serving are used to generate
67 a pathogen dose-illness response model for a given population group (Holcomb
68 et al., 1999; Teunis et al., 2008). The Beta-Poisson model (Eq. 1), a recurring
69 mathematical expression for pathogen dose-illness response predictions,
70 assumes that the food microbial load is known and can be described by a
71 Poisson distribution. Furthermore, a single pathogen, with a probability described
72 by a Beta distribution of surviving the patient defense mechanisms, is deemed
73 sufficient to cause the illness. The parameter α determines the shape of the
74 dose-response curve, whereas β regulates the shift along the vertical axis
75 (Holcomb et al., 1999; Strachan et al., 2005; Teunis and Haavelar, 2000).

$$P_{ill}(d;\alpha;\beta) = 1 - (1 + d/\beta)^{-\alpha} \quad (1)$$

76

77 Dose-response model development difficulties include a general lack of studies
78 using human volunteers, and when available, these studies are restricted to
79 healthy populations (Coleman and Marks, 1999; Foegeding, 1997) violating
80 essential statistical principles. Moreover, most published dose-response models
81 assume that all pathogen strains are equally virulent as in the case of FBDOs
82 involving *V. vulnificus*. Additionally, bioassays performed to determine strain
83 virulence rely primary on animal models, and thus their results may not be valid
84 for humans. Furthermore, although animal studies indicate that most strains are
85 virulent, not all appear to be so in humans. Consequently, the seasonal and
86 regional distribution of virulent *V. vulnificus* strains remains unknown (DePaola et
87 al., 2003).

The first and most important step when assessing processing technologies is to select operating conditions for specific food applications reducing known microbial risks by a specific number of decimal reductions, or by lowering the pathogen survival probability to a specified level (e.g., 1 spore in 10^9 containers). Another frequently overlooked aspect is that kinetic studies rarely discern between sub-lethal and lethal effects of microbial inactivation treatments. In addition, effects on sensory or nutritional quality caused by the entire food microflora are ignored when a single microorganism is being targeted. More recently, regulatory agencies have begun to require evidence that these process objectives are met with a high probability typically set at a 95% confidence interval (*95CI*) considering all relevant variability factors (Fernandez et al., 1999; Rieu et al., 2007; Smout et al., 2000). Monte Carlo based calculations have been used to meet this new regulatory requirement for different food products and processes (Chotyakul et al., 2011a; Chotyakul et al., 2011b; Salgado et al., 2011). In this work, the impact factors and their variability involved in the handling of raw oysters from harvest to consumption were analyzed with a Monte Carlo procedure to estimate the *V. vulnificus* load with 95CI at consumption. The risk of consuming these untreated and HPP-treated raw oysters was then determined by a dose-response analysis.

2. Materials and Methods

2.1. Parameters in the chain from harvest to oyster consumption

The following paragraphs describe the data sources and their variability for the

handling factors considered in this study and the calculations required by the Monte Carlo model predicting the *V. vulnificus* risk when consuming untreated and HPP-treated raw oysters.

2.1.1 Vibrio vulnificus counts at harvest

The information collected by Motes et al. (1998) for the 1994-95 harvest from multiple sites in the northern México Gulf coast was pooled to describe the initial *V. vulnificus* load in raw oysters used in this study (log $N_{harvest}$; Table 1). Since the load of this pathogen depends mostly on seawater temperature, microbial counts were grouped into warm (summer, Jun-Sep), transitional (spring, Apr-May; fall, Oct-Nov), and cold (winter, Dec-Mar) seasons.

INSERT TABLE 1

2.1.2 Oyster transportation from harvest site to processing facilities

The time during which oysters remain unrefrigerated after harvest ($t_{holding}$; h) varies widely depending on the fishery boat operation. Reports by the World Health Organization (WHO), and the U.S. Center for Food Safety and Applied Nutrition (CFSAN), show that a Beta-PERT distribution can describe this transportation time. The following minimum (*min*), maximum (*max*), and most likely (*mlk*) $t_{holding}$ values were used in this study: (a) spring, summer, and fall seasons: *min* = 3 h, *max* = 10 h, *mlk* = 7 h; and, (b) winter season: *min* = 2 h, *max* = 11 h, *mlk* = 8 h (FDA and CFSAN, 2005; WHO and FAO, 2005).

The growth of *V. vulnificus* during transportation was estimated by the three

phase linear model (Eq. 2, Buchanan et al., 1997; WHO and FAO, 2005) where μ_{max} is the maximum growth rate (cfu/h), and A is the highest *V. vulnificus* load found in oysters sold to consumers and defined as 10^6 cfu/g_{oyster} by WHO and FAO (2005). Moreover, the maximum growth rate (μ_{max}; Eq. 3) was assumed directly proportional (m = 0.011 cfu/h·°C) to the difference between the transportation ambient temperature (T_{air}; Table 2) and a minimum growth temperature (T_0 = 13°C) (Kaspar and Tamplin, 1993; WHO and FAO, 2005). These authors assume that when the transportation temperature is lower than the minimum growth temperature (13°C), *V. vulnificus* cells enter a viable but nonculturable (VBNC) state in which there is neither microbial growth nor death. Therefore, Eq. 3 selects the maximum value between zero and the temperature difference term ($T_{air} - T_0$) through the function "max" to distinguish the growth response below and above 13°C.

$$\log N_{transportation}(t) = \min\left[\log N_{harvest} + \mu_{\max} \cdot t_{holding}, A\right] \quad (2)$$

$$\mu_{\max}(T) = \max\left[0, m \cdot (T_{air} - T_0)\right] \quad (3)$$

INSERT TABLE 2

2.1.3 Oyster post-harvest processing and storage

In this study, harvested oysters for raw consumption were considered to be stored under the typical 5-10°C commercial temperature conditions (Cook et al., 2002; FDA and CFSAN, 2005; Su and Liu, 2007), or subjected to HPP

152 treatments and then stored at the same conditions. Under these storage
153 conditions, several reports indicate that *V. vulnificus* counts will decrease at a
154 rate of 0.041 log units per day (Cook et al., 2002; WHO and FAO, 2005). The
155 time before raw consumption ($t_{refrigeration}$; days) under 5-10°C was described by
156 the following Beta-PERT distribution parameters determined in a national survey
157 within the United States: (a) *min* = 1 day; (b) *max* = 21 days; (c) *mlk* = 7 days
158 (Cook et al., 2002; WHO and FAO, 2005). Thus, the load of *V. vulnificus* at retail
159 (N_0; cfu/g_{oyster}) was calculated as in Eq. (4):

$$\log N_0(t) = \log N_{transportation} - 0.041 \cdot t_{refrigeration} \quad (4)$$

160

161 Table 3 shows HPP treatments at 250 MPa and 1°C approaching the pressure
162 processing conditions (293 MPa, 8°C, 2 min) for which Ma and Su (2011)
163 achieved ≥ 3.52 decimal reductions of *V. parahaemolyticus* as recommended for
164 shellfish post-harvest processing (ISSC, 2011) . As in the case of untreated
165 oysters, the rate of reduction in *V. vulnificus* counts for HPP treated oysters
166 (N_{HPP}; cfu/g_{oyster}) during cold storage was calculated by considering the same
167 rate of 0.041 log units per day when stored under the typical 5-10°C commercial
168 temperature conditions (Eq. 6) (Cook et al., 2002; WHO and FAO, 2005).

$$\log N_{HPP} = \log N_{transportation} - SV_{HPP} \quad (5)$$

$$\log N_0(t) = \log N_{HPP} - 0.041 \cdot t_{refrigeration} \quad (6)$$

169 INSERT TABLE 3

2.1.4 Oyster consumption and risk assessment

WHO correlated records of septicemia incidences caused by *V. vulnificus* (no specific strain) from 1995 to 2001 with estimates of *V. vulnificus* counts at consumption to obtain Beta-Poisson model parameters (Table 4) describing the relationship between probability (P_{ill}) of developing septicemia and *V. vulnificus* load (Anonymous, 2005). In this study, the pathogen dose was calculated by multiplying the *V. vulnificus* load in untreated and HPP-treated oysters after cold storage (N_0; cfu/g_{oyster}; Eq. 4), the meat amount per oyster (Table 4; Anonymous, 2005), and the number of oysters consumed per serving obtained from a published survey given in the "Consumption vector" column of Table 4 (Degner and Petrone, 1994). The latter may be interpreted as in the following example: according to the survey, 61 persons claimed to have consumed six oysters per serving. Therefore, the number 6 appeared 61 times in the 306-element vector describing the number of oysters per serving.

INSERT TABLE 4

2.2. Monte Carlo estimations

The mean (μ_{REP}) and standard deviation (σ_{REP}) values reported for parameters following normal or log-normal distributions: *V. vulnificus* counts at harvest, ambient temperature during transportation from harvest site to processing plant, meat weight per oyster, Beta-Poisson model parameters, and HPP decimal reductions; were used with Microsoft Excel™ to generate random data vectors.

192 The Excel add-in developed by Hayes and Jacobs (2011) was used for variables
193 estimated through the Beta-PERT distribution: transportation time from harvest
194 site to processing facilities, and storage refrigeration time. The number of oysters
195 consumed per serving was the only variable not following a parametric statistical
196 distribution. Therefore, the number of oysters consumed per serving used in this
197 study was generated based on the frequency of each oyster consumption
198 number as reported in the published survey with 306 participants (Degner and
199 Petrone, 1994). The number of oysters consumed per serving was selected
200 randomly from this vector.

201

202 *2.2.1 Determination of the random probability distribution data vectors size*

203 Randomized vectors with n = 10, 50, 100, 200, 500 and 1,000 elements were
204 generated to assess the effect of the vector size on the Monte Carlo estimation
205 method as implemented in this study (Figure 1). These calculations were
206 performed with the spring season data and for SV_{HPP} = 6.3±1.5, since these
207 conditions represented the input with the highest variability. The 95% confidence
208 interval (*95CI*) of the logarithm of illness probability (log P_{ill}) after ingesting
209 untreated and HPP-treated oysters were calculated 1,000 times for each random
210 vector size. An ANOVA test was performed to detect first significant differences
211 and then mean comparisons of 95CI values were performed with a Tukey test
212 (95% confidence level) to determine which vector size was adequate to deliver
213 consistent estimations of log P_{ill}. Once the optimal vector size was determined,
214 the same calculations were repeated for warm (summer), transitional (fall/spring),

and cold (winter) seasons to ensure that log P_{ill} determinations remained consistent.

INSERT FIGURE 1

2.2.2 Effect of seawater temperature on dose response analysis

Warm (summer), transitional (fall/spring), and cold (winter) seasons calculations with the optimized random vector size were implemented to assess the effect of seawater temperature on the risk of consuming untreated and HPP-treated oysters, and to evaluate the *V. vulnificus* load for each oyster handling step from harvest to consumption.

2.2.3 Assessing the impact of reductions in the variability in parameters subject to raw oyster producer control

The reported standard deviation of several factors in oyster-handling steps can be reduced by changes in commercial oyster production practices and improvements in data collection (e.g., microbial enumeration methods). Reductions in the variability of some factors without a change in their mean values may lower significantly the estimated probability of septicemia infections. Half-reductions in the standard deviation ($0.5\sigma_{REP}$) of the following parameters were considered in this study: (a) microbial counts at harvest (Log $N_{harvest}$); (b) ambient temperature during transportation from harvesting site to processing facilities (T_{air}); (c) transportation time ($t_{holding}$); (d) storage time at 5-10°C

($t_{refrigeration}$); and, (e) *V. vulnificus* decimal reductions achieved by high pressure processing (SV_{HPP}).

3. Results

3.1 Size of the random probability distribution data vectors

Logarithmic illness probability (Log P_{ill}, 95% confidence) values for untreated oysters stabilized quickly ($n \geq 50$; Figure 2a) whereas values for HPP-treated oysters varied widely when $n < 200$ (Figure 2b). Moreover, a mean comparison analysis indicated no significant difference of Log P_{ill} for n = 100-1,000 for both untreated and HPP-treated oysters ($p_{value} > 0.05$). The variation coefficient (*VC*) remained below 5.1% for all cases when the vector size was at least n = 100. For n = 1,000, the *VC* reached values below 0.5 and 1.5% for untreated and HPP-treated oysters, respectively (Figure 2b). Furthermore, simulations with n = 1,000 for all other seasons resulted in similar consistent results for *95CI* Log P_{ill} calculations (*VC* ≈ 1.5%; data not shown). Consequently, random data vectors with 1,000 elements were sufficient for the Monte Carlo analysis used in this study.

INSERT FIGURE 2

3.2 HPP treatments at 250 MPa and 1°C

At 95% confidence, all treatments were predicted to meet the post-harvest processing objective, although the 2 min process yielded significantly higher

decimal reductions ($SV_{HPP} \geq 4.56$; $p_{value} < 0.05$) than the 4 and 6 min treatments ($SV_{HPP} \geq 4.29$ and 3.88, respectively). This apparent contradiction with the reported decimal reduction mean values (see Table 3) reflects the lower variability of the least severe treatment ($\sigma_{REP} = 0.2$) as compared to the more severe processes ($\sigma_{REP} = 0.7$ and 1.5). The large experimental data dispersion reported for the 4 and 6 min treatments may reflect experimental artifacts, or enumeration method limitations, as revealed by the Monte Carlo analysis here presented. Moreover, the Monte Carlo analysis showed that the predictions of the mildest HPP treatment analyzed in this study (250 MPa, 1°C, 2 min) is clearly sufficient to pasteurize oysters in accordance to the ISSC recommendation.

INSERT FIGURE 3

3.3 Dose exposure for untreated and HPP-treated oysters in different seasons

Seawater temperature affects significantly the pathogen loads in oysters reported for each season ($p_{value} > 0.05$) as reflected in the estimated *V. vulnificus* dose of untreated (horizontal axis) and HPP-treated oysters (vertical axis) shown in Figure 4. The pathogen level range (90% confidence) were highest for summer (log d_0 = 5.02 to 7.35 cfu/serving; Figure 4a) and considerably lower for the winter season (log d_0 = 0.80 to 2.79 cfu/serving; Figure 4b). *V. vulnificus* exposure doses for the spring and fall seasons (data not shown) displayed the broadest range, covering nearly 4 log units in the horizontal axis (log d_0 = 2.68 to 6.59 and 2.23 to 6.22 cfu/serving, respectively). The calculated arithmetic means for spring and fall were intermediate values (log d_0 = 4.65 and 4.29, respectively)

between those generated for the summer (log d_0 = 6.19) and winter (log d_0 = 1.75) seasons. The data variability difference between the predicted HPP treatments was observed clearly in the range of log d_{HPP} (90% confidence) value for the 2 min (2.2 to 2.5 log units) and the 6 min (5.5 to 5.6 log units) processes (Figure 4).

INSERT FIGURE 4

3.4 Dose response modeling for untreated and HPP-treated oysters harvested in different seasons

The estimated illness probability values did not approach log P_{ill} = 0 (P_{ill} = 1) even when the exposure dose reached extremely high values (Figure 5). The illness probability approached a maximum value log $P_{ill} \approx$ -4.31 for the summer season at dose levels approaching 10^7 cfu/serving. The reason for this data behavior is that the parameter values for the Beta Poisson model used in this study have limitations because they were determined from public health records and thus do not include extremely low dose values rarely causing an FBDO, nor extremely high doses theoretically expected to affect 100% of the population. Still, the analysis did confirm that the summer consumption of untreated oysters should be avoided since they cause an unacceptably high public health risk, estimated at 4,932 septicemia cases for every 100 million consumption events (*95CI = log* P_{ill} = -4.307, Table 5).

INSERT FIGURE 5

The estimated probability of acquiring septicemia through oyster consumption reduced significantly (p_{value} < 0.05) for all HPP treatments analyzed in Table 5. The 95% confidence intervals of septicemia cases during summer would be substantially decreased to just 2-4 cases for every 100 million consumption events (log P_{ill} = -7.34 to -7.62), whereas no cases would be expected to occur in the winter (log P_{ill} = -11.73 to -12.26). These estimated values suggest that intervention technologies reducing raw oyster consumption risk such as HPP treatments should be applied with different intensity throughout the year, i.e., the same low consumer exposure dose can be achieved throughout the year but operational costs would be reduced by eliminating over processing when the pathogen load in the untreated oyster is low. The definition by regulatory agencies of a minimum consumer risk is a necessity since complete absence of a pathogen in oysters is not possible. Low risks of foodborne illness are present even after severe food processing conditions.

INSERT TABLE 5

3.5 Effect of the standard deviation reduction in the oyster handling conditions on estimated V. vulnificus counts

At the 90% confidence interval, a slight but significant bacterial growth (≈ 0.2-1.2 log units; p_{value} >0.05) was estimated to occur during transportation from oyster harvesting sites to the process facility in all seasons except in the winter (Table 6). This modest pathogen growth is followed by a decrease in *V. vulnificus* counts during refrigerated storage (≈ 0.2-0.4 log units; Table 6).

INSERT TABLE 6

Temperature and storage time during boat fishery and retail operations are susceptible to important fluctuations as shown in Section 2.1. Additionally, environmental factors at the oyster harvest site and the microbial quantification methodologies can induce variability affecting the predicted *V. vulnificus* counts. Therefore, the effect of the statistical variability in the handling steps from harvest to consumption on the *V. vulnificus* load distribution in raw oysters was determined to identify steps in which variability could be controlled to lower P_{ill} estimations. To investigate the effect of the handling steps variability, Log P_{ill} distributions were generated with the same mean but with standard deviation reduced by 50% ($0.5\sigma_{REP}$) for the oyster handling parameters listed in section 2.2.3. The range of the estimated Log P_{ill} values narrowed significantly when lowering the reported σ_{REP} values for log $N_{harvest}$, SV_{HPP}, or by simultaneously decreasing the σ_{REP} for all variables ($p_{value} < 0.05$). Whereas the latter yields no specific recommendations on how to improve oyster, these results suggest the need to identify those factors responsible for high Log $N_{harvest}$ values, and to repeat inactivation experiments with large standard deviations in *SV* values. .

4. Conclusions

This study showed that a Monte Carlo analysis facilitates the inclusion of the statistical variability of the multiple factors affecting the selection of the operating

conditions for processing technologies reducing microbial risks such as HPP treatments of raw oysters. The analysis showed also that consumers and processors would benefit from food safety regulations enforced by requiring consumption risk reductions achieved by intervention designs considering statistical variability. This approach is a superior regulatory standard alternative to specifying a number of decimal reductions or an endpoint microbial load value.

Consumption of HPP-treated raw oysters should be encouraged as the analysis here presented predicted an important reduction in the number of septicemia cases caused by *Vibrio vulnificus*. For example, the HPP conditions evaluated in this study reduced the risk in the summer to fewer than 4 cases per 100 million raw oyster consumption events. The predicted HPP treatment effect met the shellfish pasteurization standard ($SV_{HPP} \geq 3.52$) by delivering with 95% confidence 3.88-4.56 decimal reductions in *V. vulnificus* counts. Moreover, the estimated septicemia risk would drop from an unacceptable 4,932 cases per 100 million consumption events to less than 4 incidents even for the mildest HPP treatment analyzed in this study (250 MPa, 2 min, 1°C, Table 5). In addition, over-processing could be minimized by selecting this least-severe HPP treatment at a lower processing cost and yielding a higher expected sensorial quality as compared to the 4 and 6 min treatments at the same pressure and temperature levels.

The intervention treatment severity, e.g., the selection of the HPP time and pressure level, should reflect the pathogen load in raw oysters for each season. In the case very low pathogen loads, an HPP treatment appears unnecessary.

Finally, this study showed that the natural and quantification variability of *V. vulnificus* at harvest and after HPP treatments have a significant influence on the estimation of raw oyster consumption risk. Therefore, raw oyster sampling, and *V. vulnificus* quantification methods, must be rigorously scrutinized prior to the implementation of treatment and handling recommendations based on risk analysis.

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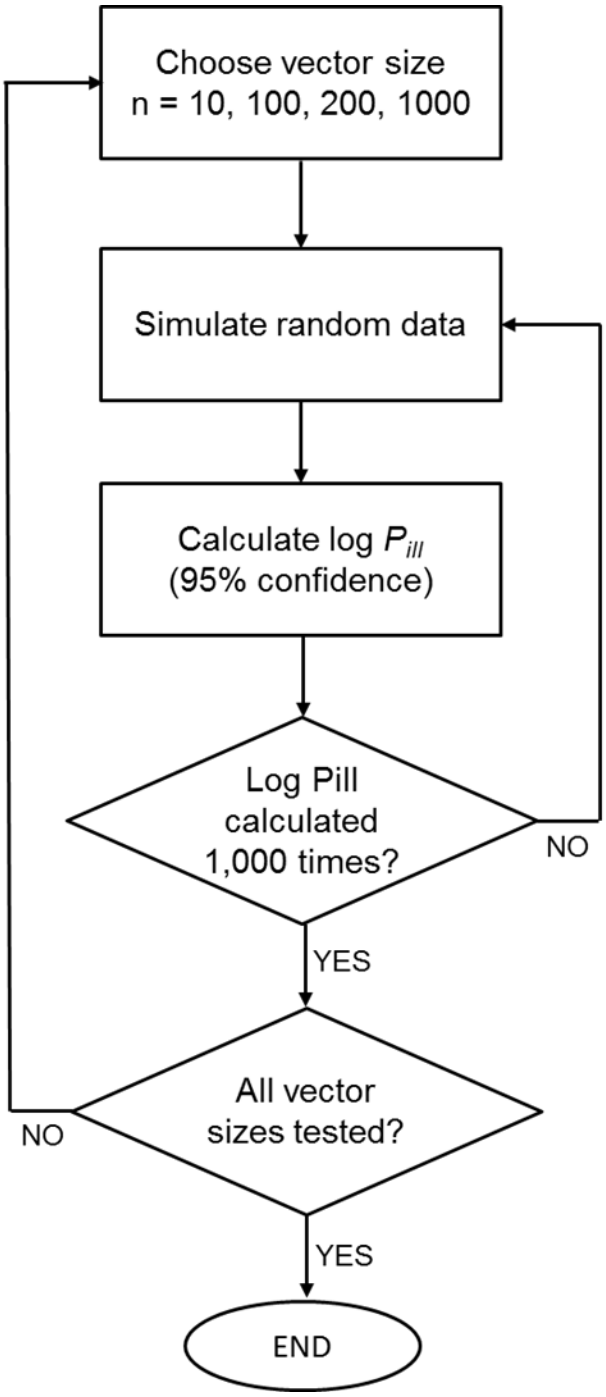
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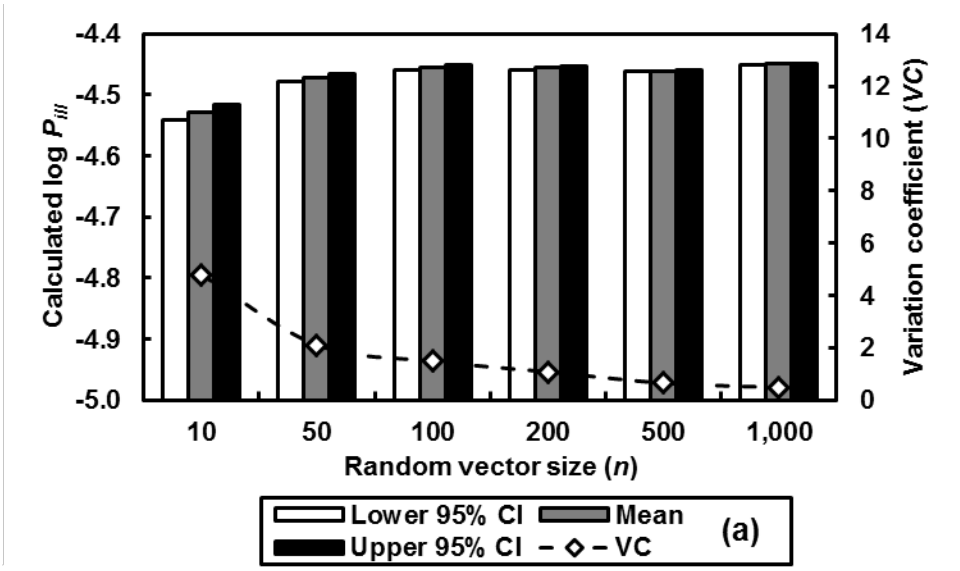
n = 10, 100, 200, 1000
Simulate random data
Calculate log P_{ill}
(95% confidence)
Log Pill
calculated
1,000 times?
NO
YES
All vector
sizes tested?
NO
YES
END

401

402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-4.5
-4.6
-4.7
-4.8
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12
10
8
6
4
2
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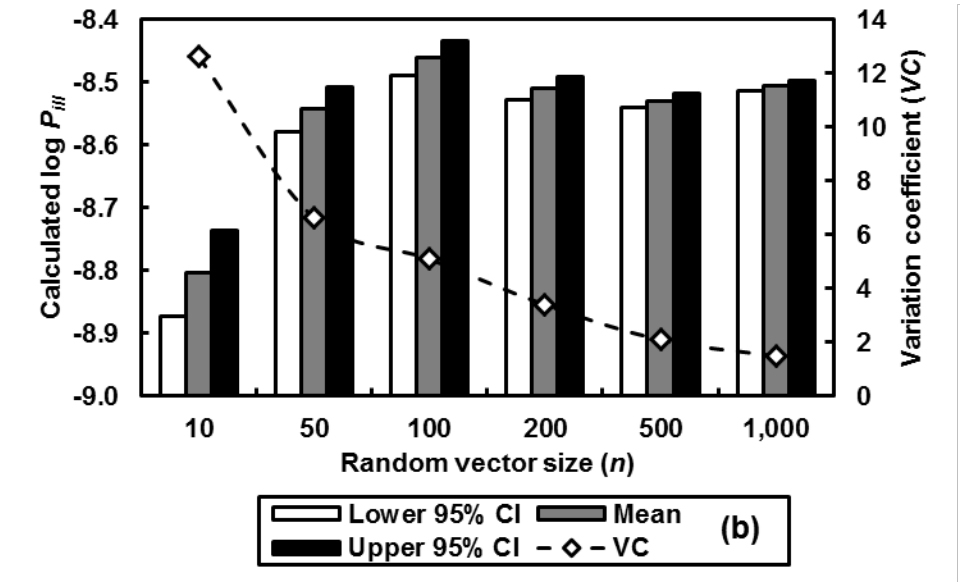
100
200
500
1,000
Random vector size (n)
Lower 95% CI
Mean
Upper 95% CI
VC
(a)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-9.0
Calculated log P_{III}
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12
10
8
6
4
2
0
Variation coefficient (VC)
10
50
100
200
500
1,000
Random vector size (n)
Lower 95% CI
Mean
Upper 95% CI
VC
(b)

403

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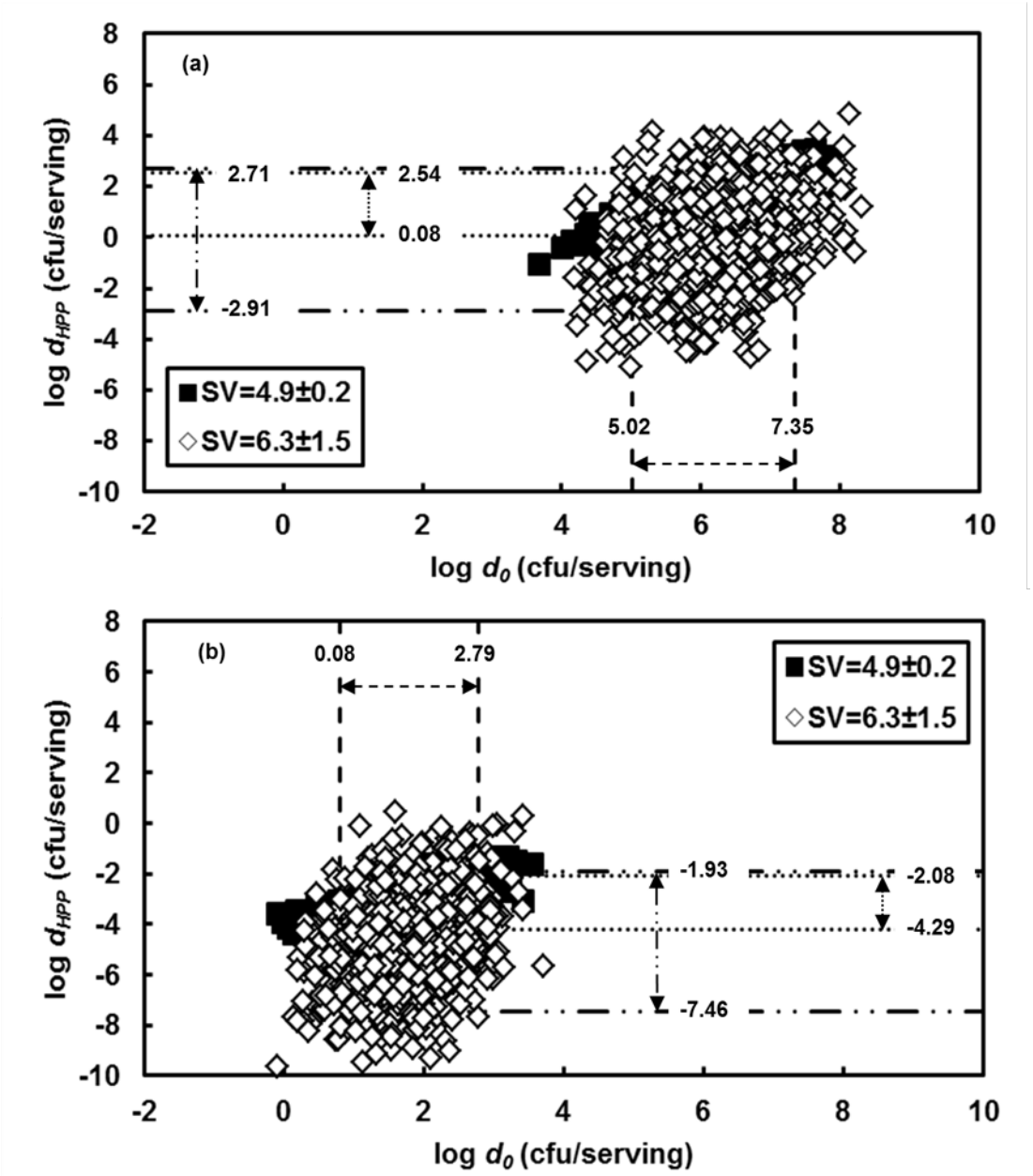
(a)
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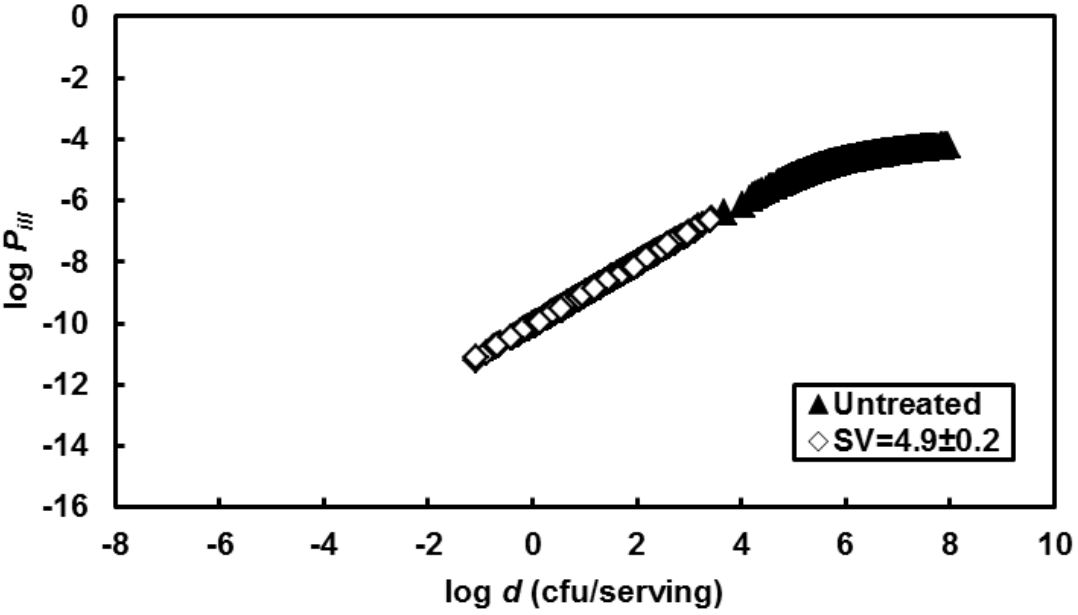
2.54
0.08
-2.91
SV=4.9±0.2
SV=6.3±1.5
5.02
7.35
log d_{HPP} (cfu/serving)
log d_0 (cfu/serving)
(b)
0.08
2.79
-1.93
-2.08
-4.29
-7.46
SV=4.9±0.2
SV=6.3±1.5
log d_{HPP} (cfu/serving)
log d_0 (cfu/serving)

406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log P_{iii}
-8
-6
-4
-2
0
2
4
6
8
10
log *d* (cfu/serving)
▲Untreated
◇SV=4.9±0.2

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Abbreviations

95CI	95% Confidence interval, i.e., 950 of 1,000 calculated values will fall in this interval
A	Maximum *V. vulnificus* concentration in oysters (10^6 cfu/g_{oyster})
d	Pathogen dose (cfu/serving)
d_0	*V. vulnificus* dose in raw unprocessed oysters (cfu/serving)
d_{HPP}	*V. vulnificus* dose in raw oysters reduced by high pressure processing (HPP) (cfu/serving)
m	*V. vulnificus* growth rate as a function of temperature (0.011 cfu/h·°C)
max	Maximum value parameter of a Beta-Pert distribution
min	Minimum value parameter of a Beta-Pert distribution
mlk	Most likely value parameter of a Beta-Pert distribution
n	Number of elements of the random data vector
N_0	*V. vulnificus* load in raw unprocessed oysters (cfu/g_{oyster})
N_{HPP}	*V. vulnificus* load remaining after HPP treatments (cfu/g_{oyster})
$N_{harvest}$	*V. vulnificus* at harvesting site (cfu/g_{oyster})
$N_{transportation}$	*V. vulnificus* load after transportation from harvesting site to processing facilities (cfu/g_{oyster})
P_{ill}	Septicemia acquisition probability as a function of an ingested pathogen dose
SV_{HPP}	Number of microbial decimal reductions achieved by HPP treatments
T_0	Minimum growth temperature of *V. vulnificus*, 13°C
T_{air}	Temperature at which oysters are transported from the harvest site to the processing facilities (°C)
t	High pressure treatment time (min)
$t_{holding}$	Time interval in which oysters remain unrefrigerated after harvesting (h)
$t_{refrigeration}$	Time interval in which oysters are stored at 5-10°C (days)
VC	Variation coefficient
α	Shape parameter in the Beta-Poisson dose response model
β	Scale parameter in the Beta-Poisson dose response model
μ_{max}	Maximum growth rate parameter in the three phase linear model (cfu/h)
μ_{GEN}	Mean value generated with Monte Carlo procedure
σ_{GEN}	Standard deviation used in the Monte Carlo procedure
μ_{REP}	Mean value reported in literature
σ_{REP}	Standard deviation reported in literature

Table 1

Vibrio vulnificus counts (MPN/g) at different sampling locations. Data from Motes et al. (1998)

	Warm (Summer)		Transitional (Spring/Fall)		Cold (Winter)	
Sampling site	μ_{REP}	σ_{REP}	μ_{REP}	σ_{REP}	μ_{REP}	σ_{REP}
Alabama	3.26	0.62	1.99	1.26	-0.52	0.29
Florida	3.03	0.67	1.72	1.18	-0.40	0.45
Louisiana	3.38	0.43	2.34	0.80	0.08	0.52
Gulf	3.20	0.59	2.00	1.12	-0.30	0.52
log $N_{harvest}$	3.22	0.58	2.01	1.09	-0.29	0.45

Table 2

Reported ambient temperature (°C) during the transportation of oysters from northern México Gulf Coast harvest sites to processing facilities (WHO and FAO, 2005)

	Spring	Summer	Fall	Winter
μ_{REP}	23.3	27.2	16.4	13.1
σ_{REP}	4.1	2.0	5.5	4.3

Table 3

Decimal reductions (SV_{HPP}) of *Vibrio vulnificus* in HPP-treated oysters at 250 MPa and 1°C. Modified from Kural and Chen (2008)

time (min)	μ_{REP}	σ_{REP}
2	4.9	0.2
4	5.4	0.7
6	6.3	1.5

446 **Table 4**
447 Meat weight, oyster consumer and Beta Poisson model data utilized for dose-response modeling

Meat weight[a]		**Consumption vector**[b]	**Beta-Poisson parameters**[c]		
Log(g_{meat}/oyster)		**Oysters per serving (Frequency)**		**Log_{10} (Parameter)**	
μ_{REP}	***σ_{REP}***		**Parameter**	***μ_{REP}***	***σ_{REP}***
1.18	0.15	1, 2, 3 (9x), 4 (10x), 5 (15x), 6 (61x), 7, 8 (11x), 10 (15x), 12 (95x), 13, 15 (5x), 17, 18 (8x), 20 (8x), 24 (37x), 25 (5x), 30 (3x), 36 (7x), 40 (3x), 45 (1x), 48 (4x), 50 (3x), 60	α	-5.0321	0.00667
			β	5.0398	0.01840

[a] FDA and CFSAN (2005); [b] Degner and Petrone (1994); [c] WHO and FAO (2005)

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Table 5

Logarithm of septicemia acquisition probability (Log P_{ill}) after raw oyster consumption during different seasons as predicted by the Monte Carlo estimation procedure

Season	HPP treatment	Mean	*95CI*	*05CI*
Spring	Untreated	-5.660	-4.479	-7.376
	2 min	-10.297	-8.269	-12.215
	4 min	-10.775	-8.498	-13.062
	6 min	-11.675	-8.643	-14.499
Summer	Untreated	-4.651	-4.307	-5.205
	2 min	-8.759	-7.527	-9.982
	4 min	-9.294	-7.624	-10.912
	6 min	-10.216	-7.340	-12.962
Fall	Untreated	-5.947	-4.574	-7.830
	2 min	-10.691	-8.678	-12.724
	4 min	-11.242	-8.830	-13.583
	6 min	-12.091	-9.200	-14.959
Winter	Untreated	-8.327	-7.290	-9.266
	2 min	-13.252	-12.145	-14.286
	4 min	-13.728	-12.264	-15.154
	6 min	-14.026	-11.734	-15.497

95CI = 95% confidence interval; ***05CI*** = 5% confidence interval

Table 6

Estimated *Vibrio vulnificus* counts (cfu/g of oyster) at different stages of the oyster handling chain

			Untreated oysters	HPP-treated oysters	
Season	**Harvest**	**Transportation**	**Retail**	**HPP[d]**	**Retail**
Spring	2.004[a]±1.099[b]	2.777±1.139	2.433±1.144	-2.114±1.165	-2.451±1.163
	(0.213, 3.865)[c]	(0.850, 4.636)	(0.534, 4.285)	(-4.047, -0.233)	(-4.437, -0.527)
Summer	3.238±0.567	4.304±0.615	3.961±0.626	-0.586±0.659	-0.931±0.684
	(2.344, 4.178)	(3.308, 5.343)	(2.964, 5.021)	(-1.658, 0.505)	(-2.020, 0.233)
Fall	2.067±1.119	2.386±1.173	2.043±1.181	-2.508±1.190	-2.844±1.204
	(0.209, 3.861)	(0.428, 4.212)	(0.057, 3.950)	(-4.480, -0.621)	(-4.296, -0.979)
Winter	-0.292±0.430	-0.144±0.494	-0.487±0.515	-5.054±0.536	-5.391±0.558
	(-1.022, 0.409)	(-0.927, 0.697)	(-1.319, 0.404)	(-5.909, -4.112)	(-6.300, -4.416)

[a]Generated mean; [b]Generated standard deviation; [c]Generated 90% confidence interval; [d]250 MPa, 1°C, 2 min

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