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Comparison of Kinetic Models To Describe High Pressure and Gamma-Irradiation Used To Inactivate *Vibrio vulnificus* and *Vibrio parahaemolyticus* Prepared in Buffer Solution and in Whole Oysters

XIAOPEI HU,[1] PARAMESWARAKUMAR MALLIKARJUNAN,[1]* JAHEON KOO,[2] LINDA S. ANDREWS,[3] AND MICHAEL L. JAHNCKE[2]

[1]*Biological Systems Engineering Department, Virginia Polytechnic Institute & State University, 200 Seitz Hall, Blacksburg, Virginia 24060;* [2]*Virginia Seafood Agricultural Research and Extension Center, Virginia Polytechnic Institute & State University, Virginia Sea Grant Program, 102 South King Street, Hampton, Virginia 23693; and* [3]*Experimental Seafood Processing Laboratory, Coastal Research & Extension Center, Mississippi State University, 2710 Beach Boulevard, Biloxi, Mississippi 39531, USA*

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ABSTRACT

Comparisons of different models in inactivation kinetics were conducted on data obtained from high-pressure and gamma-irradiation processing. *Vibrio vulnificus* (MO-624) and *Vibrio parahaemolyticus* (03:K6 TX-2103) suspended in phosphate-buffered saline (pH 7.4, 10^7 CFU/ml) were exposed to pressures from 207 to 379 MPa for 1 to 20 min. Inoculated whole oysters (10^6 CFU/g) were exposed to pressure from 276 to 379 MPa for 1 to 15 min. Pure cultures and inoculated oysters (10^6 CFU/g) also were irradiated (gamma-irradiation) at doses of less than 3 kGy. Four mathematical models, the Bigelow model, Arrhenius equation, Fermi equation, and Weibull frequency distributions, were applied to microbial survival data, and performances of the different kinetic models were compared. Weibull frequency distributions can predict the high-pressure inactivation of *Vibrio* spp. with more accuracy in both pure cultures and inoculated oyster samples. The Fermi model provided a better description of gamma-irradiation inactivation kinetics compared with the traditional Bigelow model.

Nonthermal processing of foods using high hydrostatic pressure or irradiation helps retain nutritional and sensory properties, destroys pathogenic microorganisms, and extends product shelf life. The mechanism underlying the process and kinetic parameters associated with inactivation of bacteria, spores, and enzymes has been described by Tewari and Jayas *(15)*, Farkas and Hoover *(7)*, and Sendra et al. *(14)*. In most nonthermal inactivation studies, *D*- and *z*-values are still routinely used based on the assumptions that microbial destruction is exponential and that the logarithmic relationship between microbial inactivation and processing time is linear; however, recent reviews of nonlinearity of semilogarithmic survivor curves indicate that first order kinetics, which dominate current microbial kinetics modeling, may not be appropriate for many nonthermal processes.

Peleg and Cole *(12)* challenged the assumption of first order kinetics by questioning the insensitivity of linear regression data fitting and the temperature dependence of the generated models *(5)*. They proposed that the survival curve is a cumulative form of the resistance distribution of the exposed population. Thus, the semilogarithmic survival curve can be of any shape, and the linear curve is a special case of the Weibull distribution of resistances with a shape factor of 1 *(11)*. For a microbial resistance curve that has a sigmoid shape, with respect to the forcing agent a Fermi's equation, which is used to describe mechanical changes of biomaterials at and around their glass transition temperature, may be a better model to quantify the relationship *(10)*. This model has been successfully applied to microorganisms exposed to pulsed electric fields. Several other important implications of this approach have been demonstrated *(10)*; however, this method has not been applied to other nonthermal processing techniques.

The main objective of this study was to compare different inactivation kinetics models applied to microbial survivor curves for high-pressure and gamma-irradiation treatments to eliminate *Vibrio vulnificus* and *V. parahaemolyticus* in pure cultures and in inoculated oysters.

MATERIALS AND METHODS

Clinical strains of *V. parahaemolyticus* (03:K6 TX-2103) and *V. vulnificus* (MO-624) were used in this study. Pure cultures of both strains were incubated overnight in tryptic soy agar (TSA) with a final concentration of 1% NaCl and suspended in phosphate-buffered saline (PBS, pH 7.4) *(4)* to obtain initial concentrations of approximately 10^8 CFU/ml. Approximately, 2 ml of the pure cultures were placed in duplicate into heat-sealed 5.1- by 10.2-cm Kapak pouches (Kapak Corporation, Minneapolis, Minn.), which are made of laminated films of polyethylene terephthalate and linear low-density polyethylene. These pouches were then placed inside a second 16.5- by 20.3-cm Kapak pouch for pressure treatment.

Whole eastern oysters (*Crassostrea virginica*) were also in-

* Author for correspondence. Tel: 540-231-7937; Fax: 540-231-3199; E-mail: kumar@vt.edu.

TABLE 1. *Kinetics parameters for high-pressure treatment of* Vibrio parahaemolyticus *O3:K6, TX-2103) in PBS (pH 7.4)*[a]

Pressure (MPa)	Bigelow			Arrhenius			Weibull			
	D	R^2	A_f	*D*	R^2	A_f	*a*	*b*	R^2	A_f
207	3.28	0.94	1.11	4.03	0.93	1.31	0.95	0.87	0.91	1.05
241	1.82	0.89	1.92	2.43	0.93	2.13	0.36	0.77	0.88	1.04
276	1.26	0.95	1.12	1.47	0.93	1.23	0.58	0.98	0.72	1.09
310	1.04	0.88	1.27	0.89	0.93	1.28	1.96	2.49	0.90	1.06

[a] *D,* decimal reduction time (min); R^2, correlation coefficient; A_f, accuracy factor; *a* and *b,* scale and shape factors of Weibull distribution.

oculated with *V. parahaemolyticus* O3:K6 TX-2103 to initial concentrations of 10^6 CFU/g. Fifteen to 20 live oysters were cultured in 30.3 liters of seawater in an aquarium tank. To obtain high concentrations of bacteria in each oyster, 100 ml of freshly cultured *V. parahaemolyticus* and *V. vulnificus* (TSA broth with 1% final NaCl concentration) was poured into the tank and mixed. An initial microbial concentration of 10^6 CFU/ml in the oyster tissues was obtained after overnight exposure. The oysters were refrigerated, and then three or four whole oysters were put into heavy duty (4.5 mm thickness) 20.3- by 30.5-cm Kapak pouches for treatment. Duplicate pouches were heat-sealed into another pouch with 10 ml of disinfectant. The pouches were not vacuum packed, and there were small air bubbles present inside the pouches. High-pressure treatments were conducted in duplicate. The samples were treated in a high-hydrostatic-pressure vessel (EPSInc, Engineered Pressure Systems, National Forge, Colo.). This equipment has a chamber size of 22 liters. The come-up time for the pressure unit varies according to the applied pressure. The recorded come-up time was 5.7 to 8 min as pressure increased from 275 to 550 KPa. A 50:50 mixture of water and Houghto-safe 620 (water-glycol) was used as the pressurizing medium. The pure cultures of *V. parahaemolyticus* and *V. vulnificus* and inoculated shelled oysters were treated with pressures of 207, 241, 276, 310, 345, and 379 MPa (30,000 to 55,000 psi) for 0 to 20 min.

In the irradiation study, oysters were treated using a research-size irradiator (Shepherd Model 484, J. L. Shepherd and Associates, San Fernando, Calif.) with a radioactive source of cobalt-60 at a dose rate of 900 rads/min. A dosimetry study was performed to determine the best location in the irradiation chamber for oysters in a 4-liter container to achieve the most consistent dose. Both pure culture and oysters received a dose of 0 to 3 kGy. Irradiation processing was performed in four replicates for broth cultures and six replicates for oyster samples.

Pure culture of *V. parahaemolyticus* and *V. vulnificus* and oyster homogenate were serially diluted in PBS. Approximately 5 ml of pure *Vibrio* culture was transferred into a sample cup and plated onto TSA (with 1% NaCl final concentration) using a spiral plater (Microbiology International, Frederick, Md.). Plates were incubated at 35°C for 18 to 20 h. Viable plate counts of *V. parahaemolyticus* and *V. vulnificus* in oyster homogenate were determined using thiosulfate–citrate–bile salts–sucrose *(6)* and modified cellobiose–polymyxin B–colistin *(6)* plates and the hydrophobic membrane filtration method *(4).* In the irradiation study, for *V. parahaemolyticus* and *V. vulnificus* determinations, instead of the hydrophobic membrane filtration method, a three-tube most probable number method was used *(6).* Additional identification of *V. vulnificus* and *V. parahaemolyticus* was conducted using appropriate biochemical tests, i.e., TSA with 8% NaCl and API-20E test strips (bioMérieux, Inc., Hazelwood, Mo.).

The death rate constant (*k*) and decimal reduction time (*D*) were calculated using a differential first order kinetics equation and the microbial survival curve including the cumulative pressure come-up time. Four models, Bigelow, Arrhenius, Fermi, and Weibull distribution, were applied to data on bacterial numbers, and the performances of different kinetics models were compared. The accuracy factor (A_f), a measure of the precision of a developed model, was used to evaluate the different predictive models, as proposed by Ross *(13)* and expressed as

$$A_f = 10^{[\Sigma \log(\text{predicted}/\text{observed})]/n}$$

where *n* is the number of observations used in the calculation.

RESULTS AND DISCUSSION

The microbial inactivation data for *Vibrio* spp. in pure culture and in oysters after high-pressure processing were previously published by Koo et al. *(9).* The *D*-values for high-pressure treatment of *V. vulnificus* and *V. parahaemolyticus* are presented in Tables 1, 2, and 3. For *V. parahaemolyticus* pure culture in PBS buffer, as the pressure increased from 207 to 310 MPa, *D*-values decreased from 3.28 to 1.04 min. In comparison, *D*-values calculated using the differential form of the first order kinetics equation and the Arrhenius equation were higher at lower pressures, but the discrepancy was reduced as the pressure increased. At 310 MPa, the *D*-value obtained by the Arrhenius equation was smaller than that obtained from a traditional linear regression method. For *V. vulnificus* in pure culture, *D*-values decreased from 1.58 to 1.04 min as pressure increased from 207 to 276 MPa. Compared with *D*-values obtained by the Arrhenius equation, similar phenomena were observed for

TABLE 2. *Kinetics parameters for high-pressure treatment of* Vibrio vulnificus *(MO-624) in PBS (pH 7.4)*[a]

Pressure (MPa)	Bigelow			Arrhenius			Weibull			
	D	R^2	A_f	*D*	R^2	A_f	*a*	*b*	R^2	A_f
207	1.58	0.93	1.28	1.93	0.91	1.36	2.07	1.92	0.95	1.11
241	1.13	0.85	1.40	1.23	0.91	1.47	1.94	2.56	0.93	1.11
276	1.04	0.79	1.45	0.87	0.91	1.45	2.73	4.35	0.99	1.00

[a] *D,* decimal reduction time (min); R^2, correlation coefficient; A_f, accuracy factor; *a* and *b,* scale and shape factors of Weibull distribution.

TABLE 3. *Kinetics parameters for high-pressure treatment of* Vibrio parahaemolyticus *(O3:K6 TX-2103) in raw oysters*[a]

Pressure (MPa)	Bigelow			Arrhenius			Weibull			
	D	R^2	A_f	*D*	R^2	A_f	*a*	*b*	R^2	A_f
276	3.27	0.94	1.09	4.19	0.78	1.23	1.36	0.98	0.87	1.05
310	2.68	0.86	1.11	2.96	0.78	1.12	0.95	0.91	0.83	1.05
345	2.02	0.94	1.13	2.09	0.78	1.11	1.11	1.11	0.78	1.05

[a] *D*, decimal reduction time (min); R^2, correlation coefficient; A_f, accuracy factor; *a* and *b*, scale and shape factors of Weibull distribution.

the *V. parahaemolyticus* strain in pure cultures; however, *D*-values calculated using a traditional linear regression model for *V. parahaemolyticus* concentrations in raw oysters were lower than those from the Arrhenius equation at all measured pressures. Theoretically, the differential form of the first order kinetics using the Arrhenius equation should provide a more accurate description of bacterial inactivation kinetics because it considers the effect of the pressure come-up as gradual instead of instantaneous. Therefore, *D*-values calculated by including the entire pressure come-up time into the calculation should be higher; however, because of the lack of data on the pressure come-up profile, especially at higher pressures, which have longer buildup times, the expected results were not obtained.

V. vulnificus (MO-624) was more sensitive to pressure treatments than was *V. parahaemolyticus* (TX-2163), as indicated by the lower *D*-values at the same pressure level. This finding agrees with results obtained by Berlin et al. *(3).* These data suggest that oyster tissues may provide protection to *V. vulnificus* and *V. parahaemolyticus*, as indicated by the increased *D*-values under the same pressure.

The Weibull frequency distribution provided the best fit in all instances, although parameters *a* and mean t_c (data not shown) did not indicate any trends to allow comparisons among various treatments. The Bigelow model provided the next best fit. The results disagreed with those of Fernández et al. *(8),* who stated that parameters *a* and mean t_c can be considered the characteristic time required for inactivation and can be used to compare the degree of resistance correlated with different microorganisms. The application of the Weibull distribution function in high-pressure inactivation requires additional examination for the physical meaning underlying these parameters.

The deviation of the experimental data from the predicted values using the Arrhenius approach was probably due to the lack of additional information on the pressure buildup profile in the beginning of the experimental time period.

The accuracy factor was a good index for indicating the precision of the predictive models. Although the correlation coefficient (R^2) was generally used to compare the performance of model fitting, it cannot be considered an appropriate index in this study because data collected at different pressures were not the same. Processing times were reduced at higher pressures, resulting in fewer data points and therefore relatively higher R^2 values.

The microbial inactivation data of *Vibrio* spp. by high-pressure processing in pure culture and in oysters was reported by Andrews et al. *(2).* The kinetics parameters for the irradiation treatment and the performance evaluation of various models are presented in Table 4. *V. parahaemolyticus* (TX-2103) was more resistant to irradiation than was *V. vulnificus* (MO-624), results consistent with those for heat resistance and pressure resistance studies for *V. parahaemolyticus* and *V. vulnificus (1, 3).* However, the *D*-values for *V. parahaemolyticus* in irradiated oyster tissues were lower than those for *V. parahaemolyticus* in pure cultures. The reason for this difference is not evident, and additional investigations are needed.

Fermi's equation produced slightly better predictions of the experimental data as indicated by the A_f value closer to 1. Correspondingly, the R^2 value demonstrated the same trend. D_c, which is defined as the critical value of irradiation dosage when the survival level is 50%, was 0.60 kGy for *V. parahaemolyticus* and 0.50 kGy for *V. vulnificus* in PBS and 0.57 kGy for *V. parahaemolyticus* in oysters. This finding agreed with *D*-values obtained from the Bigelow model. Therefore, D_c can be considered a characteristic constant for comparing resistance of microorganisms. The kinetics parameter *a* describes the steepness of the survival curve around the critical dosage. This analogy was assumed to hold for this system based on the research of Peleg *(10),*

TABLE 4. *Kinetics parameters for irradiation of* Vibrio parahaemolyticus (O3:K6 TX-2103) and Vibrio vulnificus *(MO-624) in PBS (pH 7.4) and in raw oysters*[a]

Inoculant	Bigelow			Fermi			
	D	R^2	A_f	*a*	D_c	R^2	A_f
V. parahaemolyticus in buffer	0.24	0.91	1.18	0.11	0.60	0.93	1.13
V. vulnificus in buffer	0.19	0.84	1.26	0.09	0.50	0.88	1.24
V. parahaemolyticus in oysters	0.22	0.81	1.23	0.10	0.57	0.87	1.26

[a] *D*, decimal reduction time (min); R^2, correlation coefficient; A_f, accuracy factor; *a*, kinetics parameter of Fermi equation, which describes the steepness of the survival curve around the critical dosage; D_c, critical value of the irradiation dosage where the survival level is 50%.

who applied this approach to microbial survival in pulsed electric fields. The assumption of this analogy was that nearly all microorganisms are not affected by radiation until a certain dosage is achieved, and the results were similar to the data indicating that microorganisms are little affected by electric fields of less than 4 to 8 kV/cm.

Weibull frequency distributions can predict the high-pressure inactivation of *Vibrio* spp. with more accuracy in both pure cultures and inoculated oyster samples. The Fermi model provided a better description of irradiation inactivation kinetics compared with the traditional Bigelow model. Critical dosages obtained from each study showed close correlations with microbial resistance. However, the characteristic parameter from the Weibull distribution did not allow for resistance comparison among treatments.

Thus, the nonlinear relationship of microbial survivor curves should be critically evaluated in nonthermal processing studies. Verification of characteristic parameters requires additional application of different models to determine the inactivation kinetics for different microorganisms so that a database can be established for parameter comparisons.

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