Prediction of a Required Log Reduction with Probability for *Enterobacter sakazakii* during High-Pressure Processing, Using a Survival/Death Interface Model[∇]

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A probabilistic model for predicting *Enterobacter sakazakii* inactivation in trypticase soy broth (TSB) and infant formula (IF) by high-pressure processing was developed. The modeling procedure is based on a previous model (S. Koseki and K. Yamamoto, *Int. J. Food Microbiol.* 116:136-143, 2007) that describes the probability of death of bacteria. The model developed in this study consists of a total of 300 combinations of pressure (400, 450, 500, 550, or 600 MPa), pressure-holding time (1, 3, 5, 10, or 20 min), temperature (25 or 40°C), inoculum level (3, 5, or 7 log₁₀ CFU/ml), and medium (TSB or IF), with each combination tested in triplicate. For each replicate response of *E. sakazakii*, survival and death were scored with values of 0 and 1, respectively. Data were fitted to a logistic regression model in which the medium was treated as a dummy variable. The model predicted that the required pressure-holding times at 500 MPa for a 5-log reduction in IF with 90% achievement probability were 26.3 and 7.9 min at 25 and 40°C, respectively. The probabilities of achieving 5-log reductions in TSB and IF by treatment with 400 MPa at 25°C for 10 min were 92 and 3%, respectively. The model enabled the identification of a minimum processing condition for a required log reduction, regardless of the underlying inactivation kinetics pattern. Simultaneously, the probability of an inactivation effect under the predicted processing condition was also provided by taking into account the environmental factors mentioned above.

In recent years, minimal food-processing techniques have been developed as part of a worldwide trend to produce food that retains its original flavor, taste, and texture. Of particular interest are various nonthermal processing techniques that have been studied with a view to achieving minimal food processing (23). Over the last two decades, high-hydrostatic-pressure processing (HPP) has emerged as an attractive technology for nonthermal microbial inactivation in food processing (7, 8, 16, 22). Microbial inactivation kinetics that describe changes in microbial number over time have been used to determine optimal conditions of HPP (3). In most cases, HPP-induced inactivation kinetics of microorganisms do not follow first-order kinetics on a semilog plot (log linear) but tend to follow nonlinear curves with tailing, like the inactivation kinetics for many other procedures such as thermal processing (25). Appropriate functions such as the Weibull, log-logistic, and modified Gompertz models have been applied to microbial inactivation kinetics to describe the nonlinear curves of HPP-induced microbial inactivation on a semilog plot (5, 6, 9, 10, 31).

The main concern for the food processor in ensuring microbiological safety is to set processing criteria for achieving a required log reduction of the microbial population. This point is also the focus of concepts such as the food safety objective (FSO), performance objective (PO), and performance criterion suggested by the International Commission on Microbiological Specifications for Foods and Codex Alimentarius (11, 18). The performance criterion concept signifies the change

required to reach a hazard level at each step of the food chain in order to meet a PO or FSO. The determination of the *D*-value, the time required at a specific temperature to obtain a 1-log reduction, and the *z*-value, the temperature increase required to decrease the *D*-value by 90%, has been widely applied to thermal inactivation processes to assess the inactivation effect and set a processing condition for achieving a required log reduction. These concept values are calculated for inactivated microorganisms that follow log-linear kinetics. However, these values are not applicable to HPP-inactivated microorganisms that display nonlinear kinetics. The calculation of a *D*-value from nonlinear inactivation kinetics results in an underestimation or overestimation of the log reduction, depending on the calculation method used. This is because the *D*-value for nonlinear kinetics is not constant and the total time to achieve a required log reduction is not proportional to the level of reduction (25, 29). Although some models based on the Weibull model were introduced to determine a required log reduction from nonlinear inactivation kinetics, one needs to calculate a more elaborate model and choose an appropriate equation depending on the curve shapes (24). It is not easy for a nonexpert to master the calculation procedure. In general, the evaluation of the inactivation effect on the basis of a survival curve is conducted by using the difference between the initial cell numbers (normally 6 to 8 log₁₀ CFU) and reduced cell numbers induced by some treatment. When the inactivation curve follows log-linear kinetics, the same log reduction will be obtained regardless of the inoculum level. For example, 5-log reductions from 8 log to 3 log and 6 log to 1 log would show the same treatment time with log-linear kinetics. These reductions, however, would not always appear to require the same treatment time with nonlinear kinetics (15, 21, 30). In addition, these two 5-log reductions underlie different net re-

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ductions of microbial cell numbers. Therefore, we should examine the net log reduction to obtain an accurate treatment time for a required log reduction by taking into account the inoculum level.

Recently, a survival/death interface model, which is a new predictive modeling procedure used to determine bacterial behavior after HPP inactivation as a probability of survival or death, was developed (19). In this procedure, microbial cells in a medium at an arbitrary initial inoculum level are treated by HPP and then viable cells are either detected (survival) or not detected (at <1 CFU/ml; death). The probability of death after HPP is then modeled using logistic regression. The modeling procedure is used to predict a minimal processing condition to achieve a required log reduction, which represents a net log reduction that takes into account the inoculum level, independent of the underlying inactivation kinetics. In addition, the certainty of the predicted inactivation effect under the predicted processing condition can be estimated simultaneously, because of the probability-based approach.

In the present study, we focused on *Enterobacter sakazakii* in reconstituted powdered infant formula as an application food for HPP. This study dealt with the investigation of HPP used to produce infant formula prior to spray drying. Analyses of the HPP-induced inactivation kinetics of *E. sakazakii* in reconstituted infant formula have been reported previously (14, 26). The reported *E. sakazakii* inactivation kinetics showed nonlinear curves with tailing. The objective of this study was to develop an *E. sakazakii* HPP inactivation model using the survival/death interface model. The development of this new model allows the prediction of a minimal processing condition for a required log reduction by using many parameters, such as the applied pressure, pressure-holding time, temperature, inoculum level, and type of medium. Furthermore, this new model provides a probability that the predicted processing criteria will achieve a required log reduction. The results of the present study will contribute to the setting of processing criteria for infant formula production that take into account the effects of plural environmental factors, the net log reduction, and the probability of achieving the targeted log reduction regardless of the underlying inactivation kinetics pattern.

MATERIALS AND METHODS

Cell preparation. The bacterium *E. sakazakii* ATCC 29544 was used in this study. This strain showed the greatest resistance to pressure among the four strains ATCC 12868, ATCC 29004, ATCC 29544, and ATCC 51329 (14). The strains were maintained at -85°C in Trypticase soy broth (TSB [pH 7.2]; Merck, Darmstadt, Germany) containing 10% glycerol. The TSB consisted of peptone from casein (1.5 g/liter), peptone from soy meal (5.0 g/liter), and sodium chloride (5.0 g/liter). A sterile disposable plastic loop was used to transfer the frozen bacterial cultures into 10 ml of TSB in a glass tube. The cultures were incubated without agitation at 30°C and transferred using loop inocula at three successive 24-h intervals to obtain a more homogeneous and stable inoculum. The bacterial cell suspension ($\sim 10^9$ CFU/ml) was subjected to serial 10-fold dilutions in TSB or rehydrated infant formula to produce three adjusted concentrations of approximately 3, 5, and 7 log₁₀ CFU/ml.

Commercial powdered infant formula (Sukoyaka; Bean Stalk Snow Co., Ltd., Sapporo, Japan) was used as a real food substrate in this study. The powdered infant formula consists of protein (12.3%, wt/wt), fat (27.8%, wt/wt), carbohydrate (54.9%, wt/wt), several vitamins (A, B₁, B₂, B₆, B₁₂, C, D, E, and K), and mineral salts (Ca, Fe, K, Mg, Cu, and Zn). The infant formula powder was rehydrated according to the manufacturer's instructions. The powdered infant formula (2.6 g) was mixed with 20 ml of sterile water at 70°C , and the mixture was

then shaken thoroughly in a sterile tube. After the infant formula reached ambient temperature, it was inoculated with the bacterial suspension.

HPP. Cell suspensions in TSB and reconstituted infant formula (3 ml) were put into a sterile polyethylene bag and heat sealed following the exclusion of air bubbles. The bag was placed into a hydrostatic pressurization unit, which contained a pressure chamber measuring 3 cm in diameter and 15 cm in height (HIGHPREX R7K-3-15; Yamamoto Suiatsu Kogyosho, Osaka, Japan). HPP with pressure ranging from 400 MPa to a maximum of 600 MPa in 50-MPa increments was carried out at 25 and 40°C for 1 to 20 min with water as the pressure medium. The apparatus was equipped with a temperature controller. The temperature of the medium (water) in the pressure vessel during pressurization was measured using K type thermocouples. The come-up rate was about 250 MPa/min, and the pressure release time was less than 10 s. The HPP time reported for an experiment at constant pressure does not include the come-up and pressure release times. Following treatment, samples were stored in crushed ice for up to 1 h until the commencement of bacterial analysis. A total of 300 combinations of pressure (400, 450, 500, 550, or 600 MPa), pressure-holding time (1, 3, 5, 10, or 20 min), temperature (25 or 40°C), inoculum level (3, 5, or 7 log₁₀ CFU/ml), and medium (TSB or infant formula) were examined in triplicate.

Determination of survival or death of *E. sakazakii* after HPP. After HPP, the presence or absence of viable *E. sakazakii* bacteria was determined using a direct plating method. Each undiluted pressurized sample (0.25 ml) was surface plated in quadruplicate onto nonselective agar (tryptic soy agar [TSA]; Merck). Plates were incubated at 37°C for 48 h and then were assessed for either colony formation (survival) or no colony formation (death). The detection limit was 1 CFU/ml. Moreover, each pressurized sample (1 ml) was aseptically removed from each plastic bag, added to TSB (9 ml), and incubated at 25°C for 30 days to take into account the recovery of bacterial cells from injury. Recovery from injury was found previously to occur at temperatures lower than the optimum growth temperature (2, 4, 20). The turbidities of cultures were observed, and samples were taken every 5 days for up to 30 days with a sterile loop and streaked onto duplicate TSA plates. Plates were then incubated at 37°C for 48 h. The survival of *E. sakazakii* after HPP was indicated by the presence of colonies on TSA plates.

Model development. For each replicate response of *E. sakazakii*, survival and death were scored with values of 0 and 1, respectively. Data were fitted to a logistic regression model by using R statistical software (28) based on the approach described by Ratkowsky and Ross (27) and a modified model that we developed previously (19). The three models proposed in this study were of the form shown below:

$$\text{Logit}(P) = a_0 + a_1\text{press} + a_2 \ln(\text{time}) + a_3\text{temp} + a_4\text{IC} + a_5\text{medium} \quad (1)$$

$$\begin{aligned} \text{Logit}(P) = a_0 + a_1\text{press} + a_2 \ln(\text{time}) + a_3\text{temp} + a_4\text{IC} + a_5\text{medium} \\ + a_6(\text{temp} \times \text{press}) \end{aligned} \quad (2)$$

$$\begin{aligned} \text{Logit}(P) = a_0 + a_1\text{press} + a_2\text{time} + a_3\text{temp} + a_4\text{IC} + a_5\text{medium} + a_6(\text{press} \\ \times \text{time}) + a_7(\text{press} \times \text{temp}) + a_8(\text{press} \times \text{IC}) + a_9(\text{press} \times \text{medium}) \\ + a_{10}(\text{time} \times \text{temp}) + a_{11}(\text{time} \times \text{IC}) + a_{12}(\text{time} \times \text{medium}) \\ + a_{13}(\text{temp} \times \text{IC}) + a_{14}(\text{temp} \times \text{medium}) + a_{15}(\text{IC} \times \text{medium}) \\ + a_{16}(\text{press} \times \text{press}) + a_{17}(\text{time} \times \text{time}) + a_{18}(\text{temp} \times \text{temp}) \\ + a_{19}(\text{IC} \times \text{IC}) \end{aligned} \quad (3)$$

where logit(P) is an abbreviation for $\ln[P/(1 - P)]$. $\ln$ is the natural logarithm, P is the probability of survival (range, 0 to 1), a_i values are coefficients to be estimated, press is the pressure applied (in megapascals), time is the pressure-holding time (in minutes), temp is the temperature (in degrees Celsius) at which the treatment was conducted, IC is the inoculum concentration of *E. sakazakii* bacteria (in log₁₀ CFU per milliliter) in the tested solution, and medium is the type of medium (TSB or infant formula). Since the medium type is not a quantitative variable in this analysis, the variables of TSB and infant formula were coded as dummy variables with values of 0 and 1, respectively (1). A logarithm of time was applied since the relationship between the effect of microbial inactivation and the pressure-holding time is not linear (5, 6, 9, 10, 31). The parameters were estimated using the glm() function in the R software program. Significant parameters were selected to minimize Akaike's information criterion (AIC) (13) by using the stepAIC() function in the MASS package of the R program. AIC is a measure of the goodness of fit of an estimated statistical model. The predicted survival/death interfaces for P

values of 0.1, 0.5, and 0.9 were calculated using KaleidaGraph, version 4.0 (Synergy Software, Reading, PA).

Model validation. The developed model was validated by cross validation (CV) using the leave-one-out method (13). Leave-one-out CV involves using a single observation from the original data set as the validation datum and the remaining observations as the training data. This process was repeated so that each observation in the data set was used once as the validation datum. This calculation was conducted using the `cv.glm()` function in the `boot` package of the R program.

RESULTS

Model development. The survival or death response of *E. sakazakii* to 300 combinations of factors was monitored, with triplicates tested for each set of conditions. The initial inoculum levels were 3.0 ± 0.3 , 5.0 ± 0.2 , and 7.0 ± 0.2 log CFU/ml. Therefore, we used initial inoculum levels of 3, 5, and 7 log₁₀ CFU/ml in the presented models. The inoculum levels of 3, 5, and 7 log₁₀ CFU/ml represented low, medium, and high contamination levels, respectively.

For all experimental conditions, there was no recovery of HPP-treated *E. sakazakii* cells that were incubated in TSB or reconstituted infant formula at 25°C for 30 days. The results pertaining to the survival or death of *E. sakazakii* obtained immediately after HPP treatment were consistent with the assessment taken after 30 days of storage at 25°C. Although most of the conditions (90.3%) consistently resulted in either all survival responses or all death responses in all three replicate trials, some conditions (9.7%) produced responses that differed among trials. The 900 data points (300 combinations $\times$ 3 replicates) generated by the trials were analyzed using the three logistic regression models. Estimated parameters that were selected to minimize the AIC values and standard errors of the three models are shown in Table 1. The performances of the models were evaluated using various statistical indices (Table 2). AIC is a balanced index of both the goodness of fit and the number of parameters. A smaller AIC indicates a better model to describe the nature of the data sets. Max-rescaled R^2 values and Hosmer-Lemeshow goodness-of-fit statistics for P values represent the goodness of fit. If these values are close to 1, there is a good fit to the data (17). A higher percentage of concordance indicates a better fit to the data. The CV prediction error is an index of model validation using the leave-one-out CV method. A smaller CV value indicates a better model. All of the indices of model 3 showed better performance than those of the other two models. However, model 3 comprised 10 parameters, and most of these were interaction factors. The employment of this model, being more complex than the other two models, made it especially difficult to delineate the effect of each interaction parameter on model prediction. The use of a simpler model yielded a better understanding of the effects of parameters. Therefore, the simpler models 1 and 2 yielded a better understanding than model 3. Although the AIC of model 1 was slightly larger than that of model 2, the Hosmer-Lemeshow goodness-of-fit statistics for P values and CV prediction errors for model 1 were slightly better than those for model 2. Thus, model 1 was selected as the best model in this study and subsequently employed for further analysis.

Model prediction. Comparative results of the predicted survival/death interfaces derived from model 1 and the observed responses of *E. sakazakii* are shown in Fig. 1. Figure 1 shows

TABLE 1. Parameters used in the logistic regression models for survival and death of *E. sakazakii* in infant formula or TSB after HPP

Model	Parameter ^a	Estimate	SE	Probability
1	Intercept	-27.76	2.77	<2e-16
	Press	0.05	0.00	<2e-16
	ln(time)	3.51	0.34	<2e-16
	Temp	0.27	0.03	<2e-16
	IC	-2.03	0.20	<2e-16
	Medium	8.23	0.77	<2e-16
2	Intercept	-19.21	2.05	<2e-16
	Press	0.03	0.00	0.00
	ln(time)	3.53	0.34	<2e-16
	Press $\times$ temp	0.001	0.00	<2e-16
	IC	-2.04	0.20	<2e-16
	Medium	8.24	0.77	<2e-16
3	Intercept	-1.97	2.18	0.37
	IC	-6.34	1.21	0.00
	Medium	20.54	3.83	0.00
	Press $\times$ time	0.00	0.00	0.00
	Press $\times$ IC	0.01	0.00	<2e-16
	Press $\times$ medium	-0.02	0.01	0.00
	Time $\times$ IC	0.08	0.02	0.00
	Temp $\times$ IC	0.07	0.01	<2e-16
	Time $\times$ temp	-0.03	0.01	0.00
	IC $\times$ IC	-0.35	0.10	0.00

^a Press, applied pressure (in megapascals); time, pressure-holding time (in minutes); IC, inoculum concentration (in log₁₀ CFU per milliliter); temp, processing temperature (in degrees Celsius); medium, TSB or infant formula.

the effects of the applied pressure and pressure-holding time at different inoculum levels (3, 5, and 7 log₁₀ CFU/ml) in different media (TSB and infant formula) at different temperatures (25 and 40°C). Overall, the survival/death interfaces are consistent with observed data. All variable factors mentioned above greatly influenced the pressure-holding time required for *E. sakazakii* inactivation induced by HPP. *E. sakazakii* cells in infant formula showed higher resistance to pressure than those in TSB, demonstrating that the medium type significantly affects bacterial inactivation. This result is consistent with the findings in previous reports (14, 26). As the inoculum level increased, the pressure-holding time required for bacterial inactivation was extended. In order to eliminate the larger inoculum of *E. sakazakii*, a longer pressure-holding time and higher pressure level were required. However, an increased temperature was shown to enhance the effect of bacterial inactivation by HPP. A representation of the significant effect of increased temperature is shown in Fig. 2. The representation of this

TABLE 2. Comparison of model performances for survival and death of *E. sakazakii* in infant formula or TSB after HPP

Model	AIC	HL statistic ^a (df; P value)	Max-rescaled R^2	% Concordance	CV prediction error ^b
1	280.82	4.60 (8; 0.80)	0.86	98.4	0.07
2	279.05	8.12 (8; 0.42)	0.86	98.4	0.08
3	243.13	3.18 (8; 0.92)	0.89	98.9	0.05

^a HL statistic, Hosmer-Lemeshow statistic.

^b The cross-validation (CV) prediction error was determined by the leave-one-out CV method.

FIG. 1. Survival/death interface for *E. sakazakii* after HPP. The black and white circles represent survival and death, respectively, in all three replicate experiments. The black and white squares represent responses that differed among the three replicate trials. Model predictions from model 1 were compared with data used to generate the model for the effects of the pressure and pressure-holding time with different inoculum levels (3, 5, and 7 log₁₀ CFU/ml) at 25 and 40°C on the potential for the death of *E. sakazakii* after HPP in TSB or infant formula (IF) at three levels of probability, as follows: *P* values of 0.1 (bottom curves), 0.5 (middle curves), and 0.9 (top curves). The interface with *P* of 0.5 (50% probability of death) is indicated by the solid lines; dotted and dashed lines represent predictions at *P* of 0.1 (less conservative) and 0.9 (more conservative), respectively.

model prediction (Fig. 2) permits visual determination of a processing time for a required arbitrary log reduction with arbitrary probability. These survival/death interface models enable the identification of minimum processing criteria, along with the probability for achieving a required bacterial log reduction.

Probabilistic assessment of survival. Since model 1 expresses the odds ratio of the survival of *E. sakazakii*, the probability of survival can be calculated with respect to the pressure-holding time under different pressure conditions and at different inoculum levels, as shown in Fig. 3. The results show that as the pressure-holding time is increased, the probability of inactivation increases. Figure 3a and b illustrate the effects of the temperature and medium type, respectively, on the probability of inactivation. Selecting a higher temperature or suspending bacteria in the simpler medium, TSB (2% [wt/vol] protein solution; see “Cell preparation” in Materials and Methods), enhanced the probability of *E. sakazakii* inactivation by HPP.

Another aspect of the survival/death interface model is

shown in Fig. 4 as a change in the probability of inactivation for achieving a required log reduction. Figure 4 shows that as the required log reduction (inoculum level) increases, the probability of inactivation under any arbitrary processing condition decreases. Figure 4a and b illustrate the effects of the temperature and medium type, respectively, on the probability of achieving a required log reduction. Even at an inoculum level greater than 5 log₁₀ CFU/ml, a higher temperature or simpler medium (TSB) resulted in a higher probability of inactivation.

DISCUSSION

In this study, a probabilistic model was developed for determining a processing condition to achieve a required log reduction that took into account the effects of various parameters on *E. sakazakii* inactivation induced by HPP. The model is not based on the kinetics of inactivation but provides a method for predicting the inactivation effect and determining the probability of log reduction. The modeling of bacterial survival and death after HPP took into account many factors, such as the

FIG. 2. Representation of the survival/death interface with respect to the pressure-holding time at 500 MPa with the inoculum level predicted by model 1 for *E. sakazakii* in infant formula (IF) at 25°C (a) and 40°C (b). The black and white circles represent survival and death, respectively, in all three replicate experiments. The black and white squares represent responses that differed among the three replicate trials. The interface with P of 0.5 (50% probability of growth) is indicated by the solid lines. The dashed and dotted lines represent predictions at P of 0.1 (more conservative) and 0.9 (less conservative), respectively.

applied pressure, pressure-holding time, temperature, and medium type, in order to determine a minimum processing condition for achieving a required log reduction (Fig. 1). In addition to enabling the identification of a minimum processing condition, this new model provides the probability of the inactivation effect under the predicted processing condition. Furthermore, the influences of the process magnitude, inoculum level, temperature, and medium type on the probability of the inactivation effect were determined stochastically (Fig. 2 and 3).

The modeling of microbial inactivation induced by HPP has been investigated to develop and fit an appropriate function to

describe nonlinear kinetics. However, the reductions would not always correspond to the same treatment times on nonlinear inactivation curves (15, 21, 30). In addition, the conventional concept of log reduction from a high inoculum level based on a semilog plot may lead to the inappropriate interpretation of the results because the net reductions of microbial cell numbers inevitably differ depending on the inoculum level. In contrast, the newly developed modeling procedure does not depend on inactivation kinetics for estimating a log reduction. The model is used to determine whether all viable bacteria, with arbitrary initial cell numbers, are inactivated (death) or not (survival) after treatment under an arbitrary HPP condi-

FIG. 3. Representation of the changes in the probability of inactivation with respect to the pressure-holding time under different pressure conditions and at different inoculum levels. (a) Effect of temperature on the HPP-induced *E. sakazakii* inactivation with an inoculum level of 7 log₁₀ CFU/ml in TSB treated at 400 MPa. (b) Effect of the medium type on HPP-induced *E. sakazakii* inactivation with an inoculum level of 5 log₁₀ CFU/ml in TSB and infant formula (IF) treated at 500 MPa and 25°C.

FIG. 4. Representation of changes in the probability of inactivation with respect to the inoculum level under different pressure conditions. (a) Effect of temperature on HPP-induced *E. sakazakii* inactivation in infant formula (IF) treated at 500 MPa for 10 min. (b) Effect of the medium type on the HPP-induced *E. sakazakii* inactivation by treatment at 400 MPa and 25°C.

tion. This procedure evaluates the net microbial reduction number. The probability of death is then modeled using logistic regression. This modeling procedure has been applied to the prediction of the HPP-induced inactivation effect on *Listeria monocytogenes* in a buffer system (19). However, the effects of the temperature and medium type on HPP-induced inactivation of *E. sakazakii* were not examined in the previous study. The predictive model developed in the present study allowed us to quantitatively identify the effects of the temperature and the medium type as a dummy variable on HPP-induced *E. sakazakii* inactivation.

Since the modeling procedure employed in this study was that of a generalized linear model, the categorical data were treated as quantitative variables by coding various numbers (dummy variables) (1). The estimated coefficient of the medium type, for example, 8.23 for model 1 (Table 1), represented a value by which the experimental data set used in this study could be explained. Thus, the estimates are expected to change according to the experimental data set. Since bacterial inactivation behavior changes according to food type, the estimated coefficient will also be expected to change. However, since plural categorical variables can be applied to the generalized linear model, an integrated predictive model could be developed and analyzed to predict the inactivation effect for several food types by using one model. Further studies would lead to the development of an integrated model.

In this study, we considered that the use of HPP to produce infant formula is prior to spray drying (12). During processing, one of the options is to blend all of the predried ingredients, such as milk, milk derivatives, soy protein, carbohydrates, fats, minerals, vitamins, and some food additives, together in water to form a liquid mix, which is then spray dried into a powder. HPP, instead of heat treatment, could be applied to the pasteurization processing of the liquid mixture prior to spray drying. Some heat-sensitive ingredients such as protein and vitamins would be retained with the use of HPP, thereby facilitating the production of high-quality and safe products.

Furthermore, employment of the new model described in this study would be useful for food processing in terms of microbiological food safety requirements based on the concepts of FSO and PO, since microbial inactivation conditions that cause the required log reduction can be determined, along with the achievement probability. The new model can also be employed to determine processing criteria that meet FSO or PO requirements.

Data concerning pressure-holding time used for model development in this study did not include the pressure come-up time. The pressure come-up times generally differ depending on the scale and/or performance of the pressurization apparatus. Since the microbial inactivation effect may be affected by the pressure come-up rate, especially with a short treatment time, the model developed in this study cannot be applied to all HPP apparatus. Obviously, the effect of the pressure come-up rate on the microbial inactivation effect should be investigated in the future. However, the modeling procedure presented in this study is capable of incorporating the effect of the pressure come-up rate as one of the parameters.

In conclusion, the survival/death interface model enables the prediction and evaluation of HPP-induced *E. sakazakii* inactivation in reconstituted infant formula with a probability of achieving a required log reduction that takes into account the effect of plural factors. The model will contribute to setting processing criteria corresponding to FSO and/or PO guidelines. Furthermore, the modeling procedure would contribute to progress regarding predictive microbiology as a new and different trend for a microbial inactivation model.

APPENDIX

The source codes of the R program for the estimation of parameters in the proposed three models are as follows.

```
# Set data sets
d<- read.csv("ESdata_all.csv")
library(MASS)
```

```

library(boot)

#Model 1 log-time simple model
fit1<- glm (DS~Press+log(Time)+Temp+IC+type, fami-
ly=binomial(link="logit"), data=d)
fit1<- stepAIC(fit1)
summary(fit1)
# Leave-one-out cross validation for binomial responses
cost<- function(r, pi=0) mean(abs(r-pi)>0.5)
cv.fit1<- cv.glm(d, fit1, cost, K=nrow(d))$delta

#Model 2 log-time Temp*Press cross term model
fit2<- glm (DS~Press+log(Time)+Temp+I(Press*Temp)+
IC+type, family=binomial(link="logit"), data=d)
fit2B<- stepAIC(fit2)
#Leave-one-out cross validation for binomial responses
cost<- function(r, pi=0) mean(abs(r-pi)>0.5)
cv.fit2B<- cv.glm(d, fit2B, cost, K=nrow(d))$delta

#Model 3 all parameters and all cross terms model
fit3<- glm (DS~Press+Time+Temp+IC+type+I(Press*
Time)+I(Press*Temp)+I(Press*IC)+I(Press*type)+I(Time*
Temp)+I(Time*IC)+I(Time*type)+I(Temp*IC)+I(Temp*
type)+I(IC*type)+I(Press*Press)+I(Time*Time)+I(Temp*
Temp)+I(IC*IC), family=binomial(link="logit"), data=d)
fit3B<- stepAIC(fit3)
# Leave-one-out cross validation for binomial responses
cost<- function(r, pi=0) mean(abs(r-pi)>0.5)
cv.fit3B<- cv.glm(d, fit3B, cost, K=nrow(d))$delta

```

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