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Microbial Modeling in Quantitative Risk Assessment for the Hazard Analysis and Critical Control Point (HACCP) System: A Review

Sea Cheol Min[1,2]* and Young Jin Choi[3]

[1]*Division of Food Science, Seoul Women's University, Seoul 139-774, Korea*
[2]*Department of Food Science and Toxicology, University of Idaho, Agricultural Science Building, Moscow, ID 83844, USA*
[3]*Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea*

Abstract Quantitative risk assessments are related to implementing hazard analysis and critical control points (HACCP) by its potential involvement in identifying critical control points (CCPs), validating critical limits at a CCP, enabling rational designs of new processes, and products to meet required level of safety, and evaluating processing operations for verification procedures. The quantitative risk assessment is becoming a standard research tool which provides useful predictions and analyses on microbial risks and, thus, a valuable aid in implementing a HACCP system. This paper provides a review of microbial modeling in quantitative risk assessments, which can be applied to HACCP systems.

Keywords: risk assessment, hazard analysis and critical control points (HACCP), microbial model, probability model, sensitivity analysis

Introduction

Many mathematical models describing growth and inhibition of microorganisms have been developed in past years. By a quantitative approach using the models, either a worst-case, what-if, or statistical approach can be made in risk assessment (1).

The quantitative risk assessment (QRA) is a useful tool that has emerged for assessing risk of foodborne illness (2). The risk assessment is typically done in a computer environment using alternate assumptions and situations and consists of 4 main steps: hazard identification, hazard characterization, exposure assessment, and risk characterization (3,4).

1. Hazard identification: The presence of a pathogen in a food is associated in this step. The hazard identification involves identification of any microbiological agents in foods capable of causing adverse health effects (3).
2. Hazard characterization: Hazard characterization is the qualitative and quantitative evaluation of the nature of the adverse health effects associated with microbiological agents in food.
3. Exposure assessment: Exposure assessment is the qualitative and quantitative evaluation of the intake of microbiological agents via food. It estimates pathogen population and the likelihood of its being ingested by consumers.
4. Risk characterization: Risk characterization involves qualitative and quantitative estimation of the probability of occurrence and severity of known or potential adverse health effects in a given population. Results from hazard identification, hazard characterization, and exposure assessment are incorporated into the risk characterization.

*Corresponding author: Tel: +82-2-970-5635; Fax: +82-2-970-5631
E-mail: smin@swu.ac.kr
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Microbial modeling in quantitative risk assessment has been rapidly developed, resulting in many publications regarding their applications, including hazard analysis and critical control point (HACCP) implementations. Thus, there's a need to review the modeling critically. The objectives of this paper are to (i) describe fundamental information about QRA, (ii) review the use of QRA for the HACCP systems, (iii) provide mathematical models available for QRA, and (iv) discuss about usages of the models to conduct QRA.

Quantitative Risk Assessment (QRA)

QRA for the HACCP The objectives of risk assessment are as follows (1,3,5):

1. To answer 3 risk questions about (i) what can go wrong, (ii) how likely is it, and (iii) what would be the consequences if it goes wrong.
2. To identify the critical control point (CCP) for a HACCP system, which is defined as any step where control can be applied that is essential to prevent or eliminate a hazard or reduce it to an acceptable level (6).
3. To validate the critical limit at CCPs, which is defined as the maximum or minimum value to which a microbiological hazard must be controlled at a CCP to prevent, eliminate, or reduce the identified hazard to an acceptable level (6).
4. To predict consequences of an insufficient control of a critical control point.
5. To provide information, which is used to identify interventions that can prevent relevant risks.
6. To enable the rational design of new processes and products to meet required levels of safety and shelf life.
7. To evaluate processing operations.
8. To be used as an educational tool, particularly for non-technical people, by generating graphs.
9. To save resources, time, labor, and costs using models.

Food safety regulatory agencies are taking a new approach to ensuring the safety of food supply based on the HACCP system (3). The objectives 1 through 7 are related to planning and implementation of HACCP systems. The hazard analysis, as a part of the implementation of a HACCP system, can be transformed into a more meaningful managerial tool by using elements of QRA. QRA can be used to determine which hazards should be essential to control, reduce, or eliminate (3). The QRA is also a tool to derive or validate control measures and critical limits at CCPs. The effect of control measures can be quantified, which enables occurrence of contaminants in the end-products estimated. Thus, risk assessment can help in developing more effective HACCP plans. Obvious users for QRA are agencies and regulators responsible for food inspection, food standards, and disease surveillance.

Microbial modeling Modeling quantifies the effects of the interactions between two or more factors and allows interpolation of combinations of factors not explicitly tested. A practical control of microorganisms depends on a combination of preservative factors, with none of the factors at levels capable of inhibiting the microorganisms by themselves. Mathematical models are the best way to make predictions in these circumstances (7).

Models can provide useful information for making decisions in safety-related situations. For example, a time-to-growth can estimate whether there is likely to be a risk in a particular food after a specified time-temperature storage. Models also can show which factor has the major influence (7). The consequences of an alteration in process events, such as changes in preservative formulations or thermal-treatment conditions, can also be immediately determined using models.

Stepwise procedure for QRA A procedure for stepwise QRA has been developed (8). In level 1 of the procedure, rough risk assessments are performed in which orders of magnitude for microbial processes are estimated by the use of simple models. Second, the main determinants of risk are studied more accurately and quantitatively. This method identifies the scope of the most important hazards, the risk-determining process steps, and risks. The results of level 1 are used in level 2. In level 2, specific models are generally used to describe the risk-determining phenomena quantitatively. The results of the models can then be compared, to estimate risk on a broad basis. Also in level 2, effects of possible changes in process or product parameters can be estimated. The results of level 2 can be used in level 3, which is the most detailed level, conducting calculations and simulations using detailed-specific models (e.g., stochastic models) (8).

Mathematical Models

Mathematical models for QRA have been considered under 2 main headings: Growth-inhibition models and probability models. The growth-inhibition models describe the growth rate of microorganisms of concern. The probability models predict the likelihood of a event (e.g., spore germination) within a given time period (9). Mathematical models are recommended to be validated by experiments before use (10).

The hypotheses underlying the modeling approach are that nutrients will not limit growth until spoilage has occurred or infectious dose levels are exceeded and environmental factors (e.g., temperature, pH, and water activity (A_w), gaseous atmosphere) rule the rate and extent of microbial proliferation. A detailed knowledge of the growth responses of microorganisms to those environmental factors enables prediction of the extent of microbial proliferation in foods during processing, distribution, and storage by monitoring the environment presented to the microorganism (9).

Growth-inhibition models can be divided by primary, secondary, and tertiary models (11). Primary models describe the growth or inactivation curve or probability of growth; secondary models describe the kinetic parameters of primary models in terms of environmental conditions; tertiary models integrate data for all aspects of responses of microbes to their environment into expert systems or decision support systems (8).

Primary growth models Most of the models shown in Table 1 are empirically-used equations or analytical solutions of differential equations, describing the number of microorganisms at a time under constant environmental conditions.

The simplest way to describe growth is by assuming first-order kinetics. Growth can then be described by an exponential function (Table 1). Little attention has been paid to modeling the duration of the stationary phase or the decline phase because food is usually overtly spoiled and can contain a high level of pathogens by the time this phase begins. However, fermented foods and some vacuum packed foods are important exceptions (12).

Orders of magnitude for growth can easily be estimated by using the exponential growth function, neglecting lag time (l) and stationary growth (Table 1). The assumption l=0 results in fail-safe predictions. On the basis of the estimated order of magnitude, it can be decided whether growth is one of the main determinants of risk (8).

Bacterial growth is also often described by sigmoidal curves. Several sigmoidal functions used to describe the growth curve empirically are the logistic, Gompertz, Richards, Schnute, and Stannard models (13). The Gompertz model, introduced by Gibson *et al.* (14), has become the most widely used primary model for describing microbial growth (7) (Table 1). Zwietering *et al.* (15) statistically compared the sigmoidal functions for describing the growth of *Lactobacillus plantarum* and concluded that the Gompertz function was statistically sufficient to describe the growth and was the easiest to use.

The Baranyi (16) model also has an important practical advantage over most other sigmoidal models and probability models (13,17). However, the Baranyi model is less empirical than the Gompertz. An important disadvantage of the Gompertz model is that it does not give exactly $n=n_0$ at $t=0$. For relatively short processes the lack of this information may have significant effects on predicted growth (8).

Secondary growth models Secondary models describe the influence of environmental factors (e.g., temperature, pH, and water activity) on the parameters of primary model

(12). Secondary kinetic models can be divided into 4 main model types: (i) Belehradek or square root type models, (ii) Arrhenius type models, (iii) modified Arrhenius or Davey models, and (iv) polynomial or response surface models (7,8). Examples of the secondary growth models are shown in Table 2.

The gamma model is a square root type of model and uses dimensionless growth factors to calculate the relative effects of environmental variables on the specific growth rate (Table 2). The growth factors are defined for pH, water activity, and temperature. The gamma model determines a specific growth rate and provides quantitative insight into the relevance of several environmental conditions for growth (8). The gamma model is often applied since the parameters can be found in literature for many pathogens. The gamma model is simple in structure, easy to interpret, and has few parameters. Moreover, new variables can easily be included in the model.

Polynomial models are a group of secondary growth models (Table 2). Multiple linear regression is often used to determine the best fit values for the parameters in polynomial models. Due to the fact that the parameters are determined only to obtain best fits to data, the parameters are not biologically meaningful.

Primary inactivation models Some examples of inactivation models are shown in Table 3. For many years, thermal inactivation has been described by first-order kinetics. In past years, other models have been developed, which found significant deviations from log linear inactivation (8). The main practical problem of the present thermal inactivation models is that they are still hard to be used for general predictive purposes because of the lack of parameter values for tailing and shoulders phenomena (8).

Secondary inactivation models Several secondary inactivation models have been developed relating inactivation parameters to environmental factors. The model types resemble secondary growth model types. The model types shown in Table 3 are linear Arrhenius-Davey and polynomial models. The use of secondary models is generally largely restricted because the parameters of the models are often very specific (8).

Probability models Currently, most of the models predicting the microbial load are deterministic, predicting a single value for an output. However, the microbial quality and safety need to be characterized by a certain level of variation. Typical examples of variation are: variation in growth conditions (e.g., temperature, pH, and water activity), measurement uncertainty, and variation among strains (18). Variability and uncertainty in models describing pathogen populations in food are accommodated through the use of probability models. The QRA offers a way to organize and combine published data from different laboratories into probability distribution functions (PDFs), which can be more reflective of the survival of target microorganisms than data from any single article (2). Stochastic prediction using probability models is a key element for performing QRA (18).

Monte Carlo simulation is a general method to deal with stochastic models. The simulation has been applied for QRA (19-22). To obtain the Monte Carlo simulation, a deterministic mathematical model fit on the experimental data is assumed to generate a perfect dataset. The model prediction at each point of independent variable is considered as the mean value of a normal distribution. This results in a dataset, which can include a perturbation of the perfect dataset and thus a realistic representation of a dataset. This dataset is subsequently fitted with a growth model or inhibition model. The procedure of fitting of data is repeated many times (more than 100 times) with different values selected from the probability distributions of parameters. The Monte Carlo simulation, thus, results in distributions of parameters of the used model (18).

Sensitive analysis The difference in the model output due to the change in the input variable is referred to as the sensitivity. Sensitivity analysis of risk models is used to identify the most significant risk factors (3). The characteristics and usages of the most common methods for sensitivity analysis are summarized in Table 5.

The response surface model is a regression equation that is fitted using standard regression techniques. The model may contain linear quadratic, cubic, or reciprocal terms and include interaction or cross product terms (3). Methods such as regression analysis and response surface method (RSM) may not be able to provide robust insights either because they assume linearity or because they require specification of a functional form. Analysis of variance (ANOVA) and scatter plots are model independent. ANOVA is used for a probabilistic analysis, but does not provide insight into the relationship between the output and the most sensitive inputs directly.

Different sensitivity method can lead to different rank ordering of risk factors because each sensitivity analysis method is typically based on a different assumption regarding appropriate ways of measuring sensitivity. Thus, applying two or more methods, preferably with dissimilar foundations, is generally recommended to increase confidence on the identification of risk factors (3). The comparison can provide insight regarding whether the methods perform similarly in practice despite different theoretical foundations (3,8).

Tertiary models Tertiary levels are computer software routines that turn the primary and secondary level models into user-friendly programs (7,12).

The US Department of Agriculture (USDA)'s Pathogen Modeling Program (PMP) is computer-based software, which estimates the growth or inhibition of foodborne microorganisms in food counting such factors as growth, lethality, and survival in culture broth and food products (23). Growth models based on the primary Gompertz function and secondary-response surface equations were combined into the PMP. The program has a series of menu screens asking for input on the desired models, interesting microorganisms (e.g., *Bacillus cereus*, *Clostridium botulinum*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, *Shigella flexneri*, *Staphylococcus aureus*, and *Yersinia enterocolitica*) and environmental conditions (e.g., pH, sodium chloride level, temperature, and nitrite concentration). The PMP is available without charge from http://ars.usda.gov/Services/docs.htm?docid=6786. The

Table 1. Primary growth models for quantitative risk analyses

Model	Variables and parameters	Characteristics	References
$Ln(n)=\ln(n_0)+\mu \cdot t$	n: number of microbial cells n_0: initial number of the cells μ: specific growth rate t: time	Simplest model. No exponential nor sigmoidal curves.	(7)
Baranyi model $Ln(n)=\ln(n_0)+\mu_{max}A_n(t)-$ $Ln(1+(\exp(\mu_{max}A_n(t))-1)/(\exp(A))$	μ_{max}: maximum specific growth rate, A: maximum level of increase ($\ln(n_\infty/n_0)$)	Models have an important practical advantage over most other sigmoidal models and probability models. This model is less empirical than the Gompertz function.	(16)
Gompertz model $n_t=n_0+a_1\exp(-\exp(-a_2(t\text{-}\tau)))$	a_1: difference in $\log_{10}$ counts between the inoculum and the stationary phase a_2: a slope term τ: time at the inflection point	The model was used for parameterizing the growth of *C. botulium*.	(7, 12)
Gompertz model $Ln(M_t/M_0)=a_2\exp\{-\exp[(\mu e/a_2)(t_1-t)+1]\}$	a_2: log difference in cell numbers from inoculum to stationary phase μ: maximum specific growth rate t_1: lag time	The parameters in the Gompertz model were reformed to have the parameters directly represent the growth rate and the lag phase.	(7, 15)
$M_t=a_5+[a_6/(1+\exp(\tau-t/g))]$	M_t: population at time t measured in optical density units a_5: value of the lower asymptote (^{a} M_0) a_6: maximum population t: time at the inflection point g: generation time	The logistic function is similar in shape to the Gompertz model.	(29)
$dM/dt=\alpha_{(t)}\mu_{(N)}M$	$\alpha_{(t)}$: adjustment function whose value depended on the environmental change $\mu_{(N)}$: potential specific growth rate $\alpha_{(t)}\mu_{(N)}$: actual specific growth rate	This model was developed with an emphasis on lag phase reflecting the time necessary for a cell to adjust to the new environment. Generally, the smaller the initial value of α, the longer the lag phase and the slower initial growth rate.	(7, 30)

Table 1. Continued

Model	Variables and parameters	Characteristics	References
$n_t = n_{max} - \ln(1+(\exp(n_{max}-n_0)-1)\exp(-\mu_{max}A(t))$	n_t: logarithm of population n_0: logarithm of initial population n_{max}: logarithm of the maximum population μ_{max}: maximum specific growth rate A(t): definite integral of the adjustment function	This model describes a lag and exponential growth phase. The fits of a set of *Listeria* growth data by this function estimated the growth rate to be about 10% slower than those determined using the Gompertz function. This discrepancy was attributed to the Gompertz slope being too steep at the inflection point relative to the entire exponential phase slope. The 4-parameter model fitted growth data better than the Gompertz function as judged by goodness-of-fit and standard errors of the estimates.	(7)
$M_B = M_A \exp(k_1 \cdot t)$	M_A and M_B: populations for the initial and activated cells k_1: rate parameter The exponential growth rate was $M_B = M_C/2^{(t/g)}$, where M_C was the population of actively growing cells and g was designated as the generation time. $g = a_1 + a_2 \sum (\text{population} \times \text{time})$, where a_1 was the basic generation time and a_2 was the sum-growth parameter.	Whiting and Cygnarowicz-Provost (31) constructed the growth and decline model by assuming that spore germination, lag phase, or recovery from injury was a first-order process or that the apparent rate was controlled by a single first order step.	(7, 31)
$M_t = M_0 2^{(Y_{1t}-Y_{2t})}$ With $Y_{1t} = a_1[1-(1+(t/a_2)+(t/a_3)2+(t/a_4)3/6)\exp(-t/a_5)]$, representing a growth function and $Y_{2t} = \exp((t-a_6)/a_5) - \exp(-(t-a_4)/a_5) - \exp(-a_4/a_5) + \exp(a_4/a_5)$, representing a death function	a_1-a_6: model parameters M_t: population M_0: initial population	The model accurately fitted the growth and decline of *Yersinia enterocolitica* in broths of varying pH and sodium chloride concentrations stored at different temperatures.	(7, 32)

Table 2. Secondary growth models for quantitative risk analyses

Model	Variables and parameters other than time (t)	Characteristics	References
Square root models $\mu = c \times (\text{temperature} - T_{\min})^2$ $\sqrt{k} = a_1(T - T_{\min})\{1 - \exp[a_2(T - T_{\min})]\}$ $\sqrt{k} = b[(A_w - A_{w\min})(pH - pH_{\min})]^{1/2}(T - T_{\min})$	T_{min}: theoretical temperature at which the growth rate of the modeled organism is predicted to be zero based on the extrapolation of the regression line fitted to the data. Consider two temperatures, T_{ref}, a temperature at which the growth rate (μ_{ref}) of the organism is known, and T_{test} at which we want to estimate the growth rate, μ_{ref}, then: $\mu_{test} = \mu_{ref} \times \left[\frac{c \times (T_{test} - T_{\min})^2}{c \times (T_{ref} - T_{\min})^2}\right]$ $\mu_{test} = \mu_{ref} \times \left[\frac{(T_{test} - T_{\min})}{(T_{ref} - T_{\min})}\right]^2$ The term $\left[\frac{(T_{test} - T_{\min})}{(T_{ref} - T_{\min})}\right]^2$ derives the growth rate at one condition from that measured at some other condition a_1: slope for the increasing rate T_{min}: extrapolated temperature at k=0 for the increasing rate (notional minimum temperature for growth) a_2: slope for the decreasing rate T_{max}: extrapolated temperature at k=0 for the decreasing rate k: rate of growth b: coefficient to be estimated A_w: water activity A_{wmin}: a notional minimum water activity for growth pH_{min} is a notional minimum pH for growth	Easy to interpret. Parameters can be found in literature. Nonlinear regression if pH and/or A_w are included. No theoretical foundation.	(12, 33-35)
Square root: gamma model $\mu = \mu_{opt} \cdot \gamma(T) \cdot \gamma(pH) \cdot \gamma(A_w)$	$\gamma(T) = \left(\frac{(T - T_{\min})}{(T_{opt} - T_{\min})}\right)^2$ $\gamma(pH) = \frac{(pH - pH_{\min})(pH_{\max} - pH)}{(pH_{opt} - pH_{\min})(pH_{\max} - pH_{opt})}$ $\gamma(A_w) = \frac{A_w - A_{w\min}}{1 - A_{w\min}}$	Parameters found in literature. For every variable relative effect can be calculated. Nonlinear regression if pH and/or A_w are included.	(8, 36)

Table 2. Continued

Model	Variables and parameters other than time (t)	Characteristics	References
Gamma concept $\mu = f(temperature) \times f(a_w) \times f(pH) \times$ $f(acid) \times f(other_1) \times f(other_2) \times \ldots f(other_n)$	μ: rate of growth	The model relies on the observation that many factors that affect microbial growth rate act independently, and each measurable factor can be represented by a discrete term that is multiplied by each other term. The cumulative effect of many factors at suboptimal levels can be estimated by multiplying the relative inhibitory effect of each factor.	(9, 36)
Arrhenius-Eyring $\mu = \dfrac{\rho_{25}\dfrac{T}{298}\exp\left\{\dfrac{H_A}{R}\left(\dfrac{1}{298}-\dfrac{1}{T}\right)\right\}}{\left(1+\exp\left\{\dfrac{H_L}{R}\left(\dfrac{1}{T_{1/2L}}-\dfrac{1}{T}\right)\right\}+\exp\left\{\dfrac{H_H}{R}\left(\dfrac{1}{T_{1/2H}}-\dfrac{1}{T}\right)\right\}\right)}$	R and T: universal gas constant and absolute temperature, respectively ρ(25): scaling factor equal to the response rate (1/K) at 25°C H_A: activation energy of the rate-controlling reaction H_L: activation energy of denaturation of the growth-rate-controlling enzyme at low temperatures H_H: activation energy of denaturation of the growth-rate-controlling enzyme at high temperatures $T_{1/2L}$: lower temperature at which half of the growth-rate-controlling enzyme is denatured $T_{1/2H}$: higher temperature at which half of the growth-rate-controlling enzyme is denatured	Parameters often used as fit parameters, instead of estimates of biologically relevant parameters.	(9, 37, 38)
Linear Arrhenius-Davey $\mathrm{Ln}(\mu) = a + \dfrac{b}{T} + \dfrac{c}{T^2} + dA_w + eA_w^2$ $\mathrm{Ln}(k) = -E/(RT) + a_1(pH)^2 + a_2(pH) + a_3$ $\mathrm{Ln}(k) = a_0 + a_1/T + a_1/T^2 + a_3A_w + a_4A_w^2$	a, b, c, d, and e: fit parameters E: enthalpy R: gas constant T: temperature in Kelvin a_n: model parameters k: rate of growth	A linear Arrhenius model for the effect of temperature and A_w was determined in foods by Davey to satisfactorily predict growth rates. Parameters are not biologically meaningful.	(7, 8, 39-41)
$\mathrm{Log}(M/M_0) = \log[F_1(1+\exp(-k_1t_1))/(1+\exp(k_1(t-t_1)))]$ $+\log[(1-F_1)(1+\exp(-k_2t_1))/(1+\exp(k_2(t-t_1)))]$	F_1: fraction of population in the major group k_1: inactivation rate parameter for the major population k_2: inactivation rate parameter for the subpopulation t=time t_1: lag period	A logistic model was proposed by for enhanced thermal destruction of *L. monocytogenes* and *S. aureus* by a lactoperoxidase system. The model was expanded to include a shoulder and two slopes	(7, 42)
Polynomial model $\mathrm{Log}(\mu) = a + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n}\sum_{j=1}^{n} b_{ij} x_i x_j$ $Y = a + b_1X_1 + b_2X_2 + \ldots + b_iX_i + \ldots b_nX_1^2$ $+ \ldots b_iX_i^2 + \ldots + b_vX_1X_2 + \ldots + b_ZX_iX_j$	a, $b_{1,2,\ldots z}$: model parameters $X_{1,2,\ldots,i,j}$: variables	Only applicable to the situation for which it was developed. Extrapolation is not allowed. It does not have theoretical foundation. Parameters are not biologically meaningful. The model uses many parameters, which can lead to description of errors.	(8, 9)

Table 3. Inactivation models for quantitative risk analyses

Type	Model	Variables and parameters	Characteristics	References
Primary	$\mathrm{Ln}(n)=\ln(n_0)-kt$	k=2.303/D t=time	Exponential	(8)
Primary	$n=(n_{0a}+n_{0d})\exp\left[\frac{-t}{\theta_i}\right]-n_{od}\exp\left[\frac{-t}{\theta_{ai}}\right]$	θ: position of maximum slope θ_i: time constant for inactivation θ_{ai}: combined time constant for inaction and activation n_{0a}, n_{0d}: initial population sizes of activated spores and dormant spores respectively	Mainly focused on description of shoulder. Used for activated and dormant spores	(43)
Primary	$\mathrm{Log}(n)=\log(n_0)+\log\left(\frac{2F_1}{1+\exp[k_1 t]}+\frac{2(1-F_1)}{1+\exp[k_2 t]}\right)$	F_1, 1-F_1: two fractions of bacteria k_1, k_2: specific inactivation rates for the two fractions	Mainly focusing on tailing.	(42)
Primary	Gompertz model $\mathrm{Log}(n)=\log(n_0)+a\cdot\exp[-\exp(b+ct)]-a\cdot\exp[-\exp(b)]$	a, b, c: fit parameters	Empirical	(44)
Secondary	$\mathrm{Ln}(k)=a+\frac{b}{T}+\frac{c}{pH}+\frac{d}{pH^2}$	a, b, c, d: fit parameters T: temperature in Kelvin	Linear Arrhenius-Davey	(45)
Secondary	$\mathrm{Log}\left(\frac{2.303}{k}\right)=\log(D)=a+b_1T+b_2pH\ldots b_ZT^2$	a, b_1, b_2, …, b_z: fit parameters T: temperature in Kelvin	Polynomial	(46)

Table 4. Probability models for quantitative risk analyses

Model	Characteristics	References
$\text{Log}(R_I/R_G)=a+b_1(\%NaCl)+b_2(pH)+b_3(\%NaCl)^2+b_4(pH)^2$ $+b_5(\%NaCl)(pH)$, where R_I is the number of cells inoculated into the system and R_G is the number having initiated growth, a, $b_1,\ldots,b_5$ are coefficients to be determined	The effect of environmental conditions on the probability (P) of a single cell initiating growth was modeled by the polynomial expression.	(9)
$\text{Log}_{10}P(\%)=5\left(\frac{e^y}{1+e^y}\right)-3$, where the effect of environmental variables is expressed in 'y' by the expression: $y=b_1+b_2\times(T)+b_3\times(S_t-LP)+b_4\times T\times(S_t-LP)$, where $b_1,\ldots,b_4$ are coefficients to be determined, T is temperature, S_t the elapsed time, and where LP, the time to toxigenesis, was modeled by: $LP=a+b_5T+b_6(1/T)+b_7(I)$, where *I* is the inoculum concentration, and a, $b_5,\ldots\ b_7$ are values to be determined.	Lindroth and Benigeorgis (47) recognized that the probability of growth detection within a given time was dependent upon the lag time and initial inoculum density.	(9, 47)
$P(t)=\frac{P_{\max}}{(1+\exp[k(\theta-t)])}$, where P(t) is probability of growth at time t, P_{max} is maximum probability, k is a rate constant, and θ is time to the midpoint of the function	The model was developed to describe the probability of one spore or vegetative cell initiating growth and toxigenesis.	(48)

Table 5. Sensitivity analysis methods

Method	Characteristics	Use	References
Nominal range sensitivity analysis (Local sensitivity analysis or threshold analysis)	<Advantage> Relatively simple method that is easily applied. Easy to understand. <Disadvantage> The model does not include effect of interactions or correlated inputs. The results can be used to rank order risk factors only if there are no significant interactions among the inputs, and if ranges are properly specified for each input. Analysis can be repeated for any number of individual model inputs. It needs nominal range for each input.	The method evaluates the effect of a model input on outputs by individually varying only the model inputs across its entire range of plausible values, while holding all other inputs at their nominal values. The results of nominal range sensitivity are most valid when applied to a linear model. The results are potentially misleading for nonlinear models.	(3, 49)
Regression analysis	If the coefficient is statistically significant, then there is strong evidence of sensitivity. The magnitude of statistically significant regression coefficients can be used to help determine the ranking of the inputs according to their sensitivity if the inputs or the coefficients are normalized (between -1 and 1 by correlation transformation) to remove dimensional effects. By normalization, the round-off errors can be minimized and all regression coefficients have the same unit; hence, regression coefficients can be compared on an equal basis. <Advantage> Generalized linear models (GLM) (e.g., Logistic regression and Poisson regression) provide flexibility to use correlated input data and non-normal error distributions <Disadvantage> It works best only if each input is statistically independent of every other input. The residuals of a least squares regression analysis must be normally distributed and independent.	Most properly performed on an independent random sample of data.	(3, 49, 50)
Analysis of variance (ANOVA)	The output is assumed to be normally distributed. <Advantage> No assumption is needed regarding the type of underlying model and both continuous and discrete inputs can be analyzed using ANOVA. <Disadvantage> If the inputs are correlated, then the effect of each individual input on the response variable can be difficult to assess. Time consuming for a large number of inputs with interactions.	ANOVA determines whether there is a statistical association between an output and one or more inputs.	(3, 50, 51)

Table 5. Continued

Method	Characteristics	Use	References
Response surface method (RSM)	Monte Carlo simulation methods are typically used to generate multiple values of each model input and to calculate corresponding values of the model output. <Advantage> It is easy to apply iterative numerical procedures to the response surface, such as optimization or Monte Carlo simulation, compared to the original model. The values of its coefficients may provide a useful indication of sensitivities. <Disadvantage> Most response surface studies are based on fewer inputs than the original model. Thus, the effect of all original inputs on the sensitivities cannot be evaluated in the response surface method.	The method used to represent the relation between a response variable and one or more explanatory inputs. Sensitivity of the model output to one or more of the selected input can be determined by inspection of the functional form of the response surface. It is employed for an optimization of processes. Graphical.	(3, 50, 52)
Scatter plot	A graphical sensitivity analysis method. <Advantage> Graphical The method is easy and often recommended as a first step in sensitivity analysis. <Disadvantage> No quantitative sensitivity	An input value and the corresponding output value are plotted as points on a scatter plot. It allows for the identification of potentially complex dependencies between inputs and an output. An understanding of the nature of the dependencies can guide the selection of other appropriate analysis methods. Visual assessment of the influence of individual inputs on an output.	(3, 54)

PMP would be a useful tool for (23):

1. Estimating bacterial growth or decline in a particular food at specific time and temperatures.
2. Identifying potential critical control points where the model indicates that at a certain level controllable factors will either permit or suppress microbial growth.
3. Reformulating product based on conditions that influence microbial growth.
4. Providing graphical modeling tools that can be used as instructional aids for demonstrating to employees the impact of HACCP implementation.

Food micromodel was developed by a consortium of industry and government researchers (12). It has predictive equations for growth, survival, and death of pathogens. Growth models for *L. monocytogenes*, *Y. enterocolitica*, *B. cereus*, *Campylobacter jejuni*, psychrotrophic *C. botulinum*, *Salmonella*, and *S. aureus* include the factors of temperature, pH, and water activity (7). The program is available from http://www.lfra.co.uk/micromodel/index.html.

Food spoilage predictor (http://www.hdl.com.au/html/products.htm) is commercial software that models the effect of water activity and fluctuating temperature on the growth of *psychrotolerant pseudomonds* (12). The model has been extensively validated in milk, meat products, and seafood (12).

Seafood spoilage predictor was developed to predict shelf life of seafood at different storage temperature. The software can evaluate the effect of fluctuating temperatures on shelf life of seafood determined by specific microorganisms (12). It is available without charge from http://www.dfu.min.dk/micro/ssp/.

A proposed international web-based compendium of models and growth data, termed 'ComBase' is also well advanced (http://wyndmoor.arserrc.gov/combase/) (12).

Variability

Variability in virulence and the growth responses of different strains of the same species exists (24). Specifying the magnitude of this variability is important in QRA to indicate the confidence of predicted parameters (12). The nature and magnitude of variability and uncertainty associated with predictive models is not completely understood. In some models, the upper and lower confidence intervals are missing (23).

The magnitude of the variability in response times of microorganisms is usually highly skewed, necessitating model fitting with some mathematical transformation of the measured response (12). A logarithmic transformation of values for time parameters are frequently closer to being normally distributed than untransformed values (7).

Models have been used on representative strains or mixtures of representative strains to characterize the range of growth responses that correspond to the environment. A worst-case situation that all unfavorable events occur at the same time is inherent in many predictive models that may lead to conservative control measures or an overestimation of the risk (1,25).

Model Selection

Models using a large number of parameters (e.g., higher-order polynomials) were more prone to unreliability because the predictions of such models often changed dramatically near the limits of the interpolation region (12, 26). Comparison of results from different models does not always substantially contribute to a broad view on risk if process variations are more significant than model variables, which rules out differences between models. In this case, the accuracy of the model predictions does not justify the use of more complex models and it would be efficient to use the simplest model available (8).

The exponential model is the simplest dose-response model used in microbial risk assessment and predicts a direct proportionality between dose and risk of illness below the asymptotic dose. The Beta-Poisson and hypergeometric models also predict a direct proportionality between dose and risk of infection in the low-dose region (12).

Even though the use of stochastic variables may not change the conclusions from non-stochastic models, the variations of predicted parameters and risk factors are recommended to be presented stochastically to determine the significance of the variations.

Considerations Prior to Applications

It is not yet possible to rely solely upon any predictive modeling programs to determine the safety of foods and processing systems. They usually cannot include all influences on bacterial growth and survival and do not consider the protective buffering effects of various food components when converting predictions from experimental broth cultures to different food matrices.

The models in the programs do not include the inhibitory or supporting effect that endogenous bacteria and their population may have on pathogens. These effects will vary in different seasons, locales, climates, and other unforeseen events in an establishment.

The models do not usually account for increased resistance of bacteria to certain treatments, induced by prior conditioning of cells, such as heat shock (23).

Predictive modeling programs must not simply replace microbial validation, experimental challenge studies, or the judgment of a trained and experienced microbiologist in hazard analysis (23). Predictive modeling programs need to be used as support tools. They should be used in a conservative manner and other factors should play a role in making critical decisions about a process or deviation (23). The Food Safety and Inspection Service (FSIS) does not approve nor advise industry on the proper use of specific modeling programs (23).

Developing reliable QRA will require the skills of both microbial ecologists and mathematical modelers (12). Model developers must specify assumptions and limitations of the models. The type of microorganisms and the ranges of factors need to be clearly described to validate the models.

Directions for Microbial Modeling

Additional factors (e.g., anion effects from acidulants) need to be continuously investigated and considered for microbial modeling (7).

The models also need to be developed to account effects

of the physiological state and culture history of the test cells and to simultaneously estimate growth and inactivation by integrating growth and inactivation data (7,27).

The growth of food pathogens can be significantly affected by that of spoilage flora in foods and the populations of both pathogenic and spoilage organisms can reject food from consumption. Thus, models that can simulate comparative growths of both organisms are sought (7).

Statistical criteria for determining error or confidence intervals of predicted parameters need to be agreed on and applied in the modeling. To conduct this, the nature and magnitude of variability and uncertainty associated with predictive models need to be further studied.

Case Study

Background and objective Outbreaks of salmonellosis associated with the consumption of raw almonds were reported in 2001/2002 and 2003/2004. Raw almonds are now known as a vector for salmonellosis (22). There is an interest in investigating effects of various interventions on the reduction in the number of cases of salmonellosis, caused by the consumption of raw almonds. In this case study, an edible film incorporating an antimicrobial lactoperoxidase system (LPOS) was assumed to be applied to reduce the salmonellosis. Thus, the objective of the study was to use a previously developed QRA using Monte Carlo simulation to predict the risk associated with consumption of raw almonds and almonds coated with the LPOS-antimicrobial edible film. The QRA was conducted to predict how much the antimicrobial coating reduces salmonellosis risk and which variables have the greatest effect on the predicted annual number of cases of the salmonellosis. This prediction is practical for the HCCP system for almond processes in determining and validating any related CCPs and critical limits at the CCPs.

Materials and methods The QRA developed by Danyluk *et al.* (22) describing the risk associated with consumption of raw almonds was used. All the variables used in the study of Danyluk *et al.* (22) was used without modification; level of contamination, handler storage time, pre-process storage time, pre-process reduction, post-process storage time, post-process reduction, retail storage time, retail reduction, consumer storage time, consumer storage temperature, consumer reduction, *Salmonella* contamination/serving (CFU/28 g), probability of illness/serving, U.S. consumption of raw almonds, *Salmonella* prevalence (% positive 100 g samples), *Salmonella*-positive 100 g samples consumed, contaminated servings in positive 100 g sample, servings consumed containing *Salmonella*, and simulated log reduction. All the values for the variables in this study were identical to those of Danyluk *et al.* (22) except for the value for the simulated log reduction. The log-reduction of *Salmonella* (4.0±0.5 log) achieved by the antimicrobial coating incorporating LPOS (28) was used as the value for the simulated log reduction variable. Computer software (@RISK, Palisade) was used to perform Monte Carlo simulations of 10,000 iterations.

Results Figure 1 illustrates probability distributions of salmonellosis from consumption of raw almonds and the antimicrobial-coated almonds. The value ‘0’ on the horizontal axis on log predicted illness (cases/year) stands for the probability that one case of salmonellosis occurs per year. Similarly, the value ‘2’ indicates probability that 100 cases of salmonellosis occur per year. Thus, the sum of probability values on 0 indicates probability that more than 1 case of salmonellosis occurs annually. The results from the analysis are summarized in Table 5. The results suggest that the antimicrobial coating significantly reduces the probability of salmonellosis (Fig. 1 and Table 5).

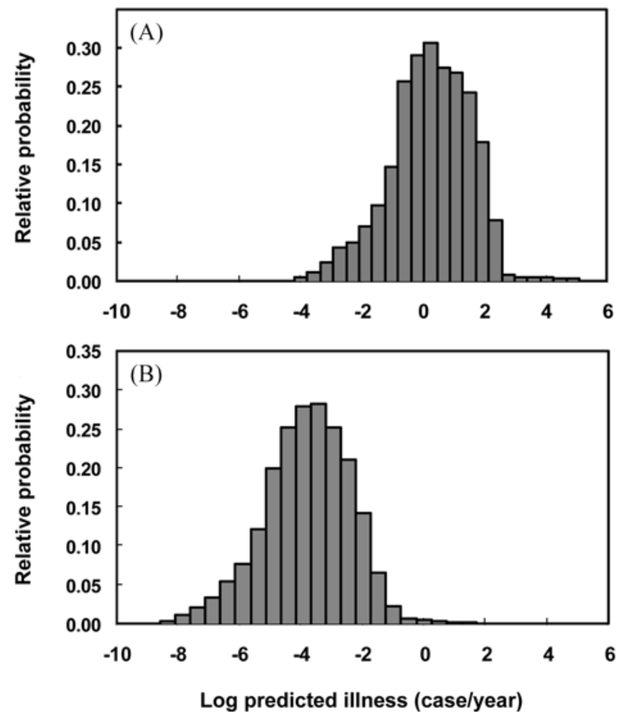

Fig. 1. Probability distributions of salmonellosis from consumption of raw almonds (A) and almonds coated with the edible film incorporating the lactoperoxidase system (B).

Table 6. Summary of results from Monte Carlo simulations predicting the risk of salmonellosis from consumption almonds

Almonds	Probability (%)		
	≥1 case/year	≥10 case/year	≥100 case/year
Raw almonds	78	48	21
Coated almonds[1)]	0.39	0.08	0.01

[1)]Almonds coated with the edible film incorporating a lactoperoxidase system.

A sensitivity analysis was performed to evaluate which variables have the greatest effect on the predicted annual number of cases of salmonellosis from consumption of almonds. The results are demonstrated in Fig. 2. Higher the absolute value of the correlation coefficient, greater effect on the predicted illness/year. The results imply that the primary factors influencing the estimate of number of salmonellosis cases per year from consumption of raw almonds were total handler storage time, reduction during consumer storage, level of contamination, and number of contaminated servings while those from the consumption of the antimicrobial-coated almonds include the simulated

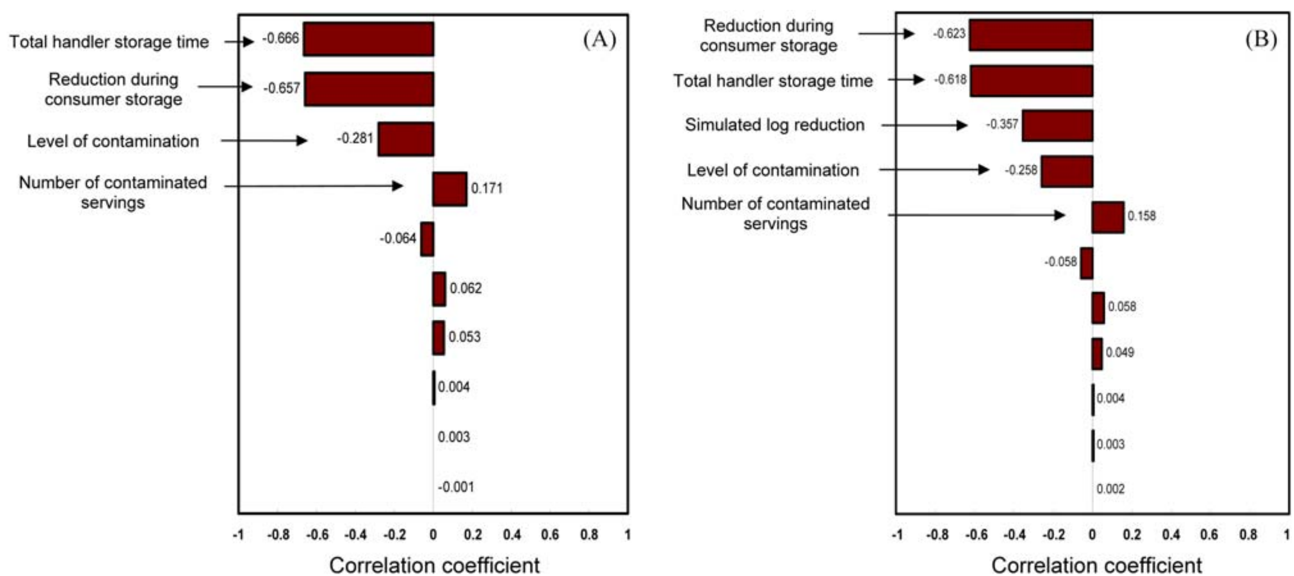

Fig. 2. Sensitivity analysis results predicting relative significance of the variables for Salmonellosis outbreak from consumption of raw almonds (A) and almonds coated with the edible film incorporating the lactoperoxidase system (B).

log reduction, the log reduction in the number of *Salmonella* achieved by applying the antimicrobial coating to contaminated raw almonds. This indicates that the antimicrobial coating can effectively reduce Salmonellosis outbreaks from almond consumption.

In summary, the variables used in this study can be set with the value of interest in the QRA to investigate the effect of the variable on the annual number of cases of the salmonellosis, which will be used to identify CCP as well as the critical limits at each CCP. The use of this QRA is anticipated not only for determination of critical limits, but also their validation. For example, the 4-log reduction was used as the value for the simulated log reduction variable in the case study and it was found from the sensitivity analysis that the simulated log reduction is one of the primary variables affecting the predicted salmonellosis cases. The antimicrobial coating process would be considered as a CCP and the concentration of LPOS, which results in the 4-log reduction of *Salmonella*, will be one of the critical limits in the coating process if the antimicrobial coating with the 4-log reduction satisfies the degree of reducing the Salmonellosis outbreaks. If the 4-log reduction is admitted as a critical value, any antimicrobial coatings can be validated based on the criteria that the antimicrobial coating to be applied reduce the number of *Salmonella* cells by 4 logs. The QRA has the potential to be used to manage almonds safety issues as implication of the HACCP system for almond processes.

Conclusion

The use of mathematical models is becoming a standard research tool and a valuable aid in evaluating and designing food processes. The predictions made by microbial modeling are serviceable for planning and validating HACCP plans. Model-based quantitative risk assessment, a powerful combination of food microbiology, modeling, and applied statistics, can provide useful insights for agencies and regulators responsible for food inspection, food standards, and disease surveillance. The use of microbial modeling on a commercial basis will be fully realized by continuously-obtaining reliable data and models with the involvement of the food industry.

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