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Alternative approaches to predicting microbial behaviour: A probabilistic modelling approach for microbial inactivation and a revised web-tool, the Microbial Responses Viewer

Shige Koseki*

National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

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ABSTRACT

An alternative predictive model for microbial inactivation and a novel web-based tool for the application of predictive microbiology are reviewed in this paper. The developed model, based on probabilistic concepts, enabled the identification of minimum processing conditions necessary to obtain a required log reduction, regardless of the underlying inactivation kinetics. The model also provides the probability distribution of the inactivation effect. The revised web-tool, the MRV (Microbial Responses Viewer), provides information concerning growth/no growth boundary conditions and the specific growth rates of queried microorganisms. The MRV enables users to retrieve microbial growth/no growth information intuitively. Using the MRV, food processors can easily identify appropriate food design and processing conditions.

1. Introduction

Predictive microbiology is a well-established and well-recognised scientific discipline with a burgeoning body of literature (Brul, van Gerwen, & Zwietering, 2007; McKellar & Lu, 2004; McMeekin, Olley, Ross, & Ratkowsky, 1993). The quantitative evaluation of microbial responses in food environments allows us to define appropriate processing conditions and formulations for processed foods. However, it seems that most of the outcomes of predictive microbiology studies conducted to date are not necessarily translated into practical uses in real food processing situations. For example, although extremely complex mathematical models might be worth using to gain a basic understanding of microbial responses, the application of such complex models would not be easy in practice. An alternative approach to microbial inactivation modelling for the practical use will be needed for appropriate evaluation. Several web-based prediction and/or retrieving system for microbial responses in food environments, such as the Pathogen Modeling Program (Buchanan, 1993) and ComBase (Baranyi & Tamplin, 2004), have been developed to facilitate the practical use of the outcomes of predictive microbiology studies. However, the existing tools do not necessarily meet the demands of the food industry. Our research group has recently developed a novel web-based data-retrieving tool that provides microbial growth/no growth conditions and is intended for practical use in the food processing setting. This paper describes alternative approaches to evaluate microbial responses in foods that were presented during a keynote lecture at the 7th International Conference on Predictive Modelling of Foods (Koseki, 2011).

2. An alternative approach to evaluate the effects of microbial inactivation

2.1. Background and objectives

The primary concern for food processors with respect to ensuring microbial safety is determining the process criteria necessary to achieve a required bacterial log reduction. Setting such criteria is also the focus of concepts such as the Food Safety Objectives (FSOs), Performance Objectives (POs), and Performance Criterion (PC) as suggested by the International Commission on Microbiological Specifications for Foods (ICMSF) and Codex Alimentarius (Codex Alimentarius Commissions, 2005; ICMSF, 2002). To meet the POs, food processors need to determine appropriate process conditions, such as the combination of process magnitude and time, for a required log reduction. The decimal reduction time (*D*-value) has been widely applied to determine the thermal

* Tel./fax: +81 29 838 8064.
E-mail address: koseki@affrc.go.jp.

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inactivation conditions for required log reductions. The *D*-value concept is based on microbial inactivation kinetics that follow log-linear kinetics. Hence, the *D*-values are not applicable to microbial inactivation that displays nonlinear kinetics, as is the case for high pressure processing (HPP). The *D*-value for nonlinear kinetics is not constant, and the total time necessary to achieve a required log reduction is not proportional to the number of decimal reductions (Cebrián, Michiels, Mañas, & Condón, 2010; Chen & Campanella, 2012; Gounadaki, Skandamis, Drosinos, & Nychas, 2007; Ma et al., 2009; Molinari, Pilosof, & Jagus, 2004; Peleg & Cole, 1998; Tassou, Panagou, Samaras, Galiatsatou, & Mallidis, 2008; Uesugi, Woodling, & Moraru, 2007). The calculation of a *D*-value from nonlinear inactivation kinetics results in the underestimation or overestimation of the log reduction depending on the calculation method used. Although some models based on the Weibull model have been introduced to determine the required log reduction from nonlinear inactivation kinetics, one needs to use a more elaborate model and to choose the appropriate equation depending on the curve shape (Chen & Campanella, 2012; Peleg, 1999). It is not easy for a non-expert to master the calculation procedure. Thus, a simple and easy-to-use calculation procedure to determine the treatment time necessary to achieve the required log reduction, one that does not depend on the inactivation curve shape, is needed. Furthermore, conventional deterministic kinetics-based evaluations of microbial inactivation cannot take into account variability and/or uncertainty. Probabilistic approaches to the evaluation of microbial inactivation are needed to describe the variability and/or uncertainty of appropriate microbial risk assessments.

A survival/death interface model has been developed in which a new predictive modelling procedure is used to determine bacterial behaviour after HPP inactivation as a probability of survival or death (Koseki & Yamamoto, 2007). Recently, Valdramidis et al. (2009) investigated the optimisation and design of HPP for apple juice production. In those procedures, the probability of death after processing is modelled using a logistic regression. The modelling procedure is used to predict the minimal processing conditions necessary to achieve a required log reduction independent of the underlying inactivation kinetics. In addition, the certainty of the predicted inactivation effect under the predicted processing conditions can be estimated simultaneously. Herein, the probabilistic aspects of the modelling procedure will be discussed using the previously published model for predicting the level of *Cronobacter sakazakii* inactivation in reconstituted infant formula (IF) by HPP (Koseki, Matsubara, & Yamamoto, 2009).

2.2. Modelling procedure

As described in the previous report (Koseki et al., 2009), IF inoculated with *C. sakazakii* at different inoculum levels (3, 5, and 7 log CFU/ml) was treated by HPP ranging from 400 to 600 MPa at 50 MPa increments at different temperatures (25 and 40 °C). For each replicate response of *C. sakazakii*, survival and death were scored with values 0 and 1, respectively. The data were fitted to the following simple logistic regression model using R statistical software (R Development Core Team, 2012).

$$Logit(P) = a_0 + a_1 Press + a_2\ ln(Time) + a_3 Temp + a_4 IC \qquad (1)$$

where $Logit(P)$ represents ln $[P/(1-P)]$, ln is the natural logarithm, P is the probability of survival (range: 0–1), a_i are coefficients to be estimated, *Press* is the applied pressure (MPa), *Time* is the amount of time that the pressure is held (min), *Temp* is the temperature at which the treatment was conducted (°C), and *IC* is the inoculum level ($\log_{10}$ CFU/ml) of *C. sakazakii* in the reconstituted IF. The parameters were estimated using the glm() function in the R software program (Ver. 2.14.1). Because there are some weak points in more complex models including interaction and/or quadratic parameters (Koseki & Yamamoto, 2007; Ratkowsky, 2002), the simple model was employed.

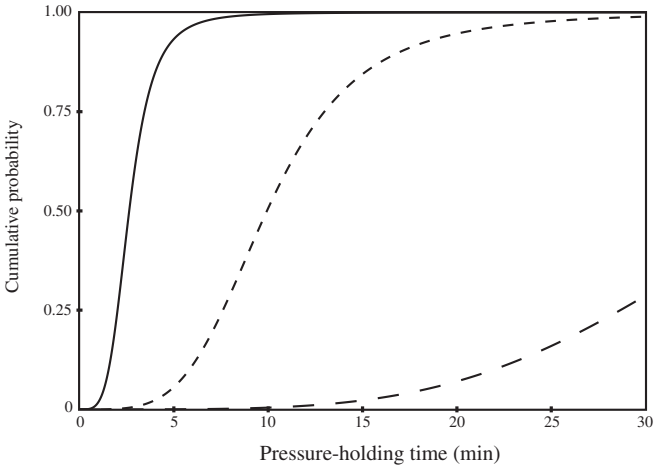

Fig. 1. Changes in the cumulative probability for the inactivation of *C. sakazakii* corresponding to a 5-log reduction in reconstituted infant formula under different pressures at 25 °C. Solid line: 600 MPa, dotted line: 500 MPa, dashed line: 400 MPa.

2.3. Determination of the treatment time for a required log reduction

The resulting model was obtained:

$$Logit(P) = -34.20 + 0.05 \times Press + 4.10 \times ln(Time) + 0.28 \times Temp - 1.91 \times IC \qquad (2)$$

The accuracy of the estimated model (Eq. (2)) was sufficient as demonstrated by goodness-of-fit indices such as the percent concordance (99.2%), maximum-rescaled R^2 (0.92), and low cross-validation error of prediction (0.03) was observed. Because the developed model expresses the natural logarithm of the odds $(P/(1-P))$ of the survival of *C. sakazakii*, the probability of survival can be described as following transformation of Eq. (1):

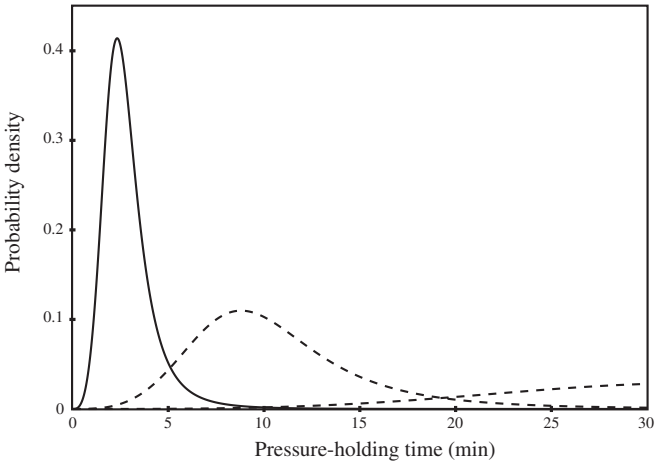

Fig. 2. Probability density distribution for the inactivation of *C. sakazakii* corresponding to a 5-log reduction in reconstituted infant formula under different pressures at 25 °C. Solid line: 600 MPa, dotted line: 500 MPa, dashed line: 400 MPa.