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Modeling and predicting inactivation of *Escherichia coli* under isobaric and dynamic high hydrostatic pressure

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ABSTRACT

Published experimental isobaric inactivation curves of *Escherichia coli* cells in peptone solution and raw milk cheese were fitted with the Weibull model (with a fixed shape parameter) and the pressure dependent rate parameter of the Weibull model by log-logistic equation. These were used to predict these microorganisms' nonisobaric survival curves under different pressure profiles. The assumption has been that the inactivation rate in nonisobaric processes is a function of the instantaneous pressure and the corresponding survival ratio. This could be expressed by a difference equation and can be solved incrementally by a software like Microsoft Excel® which is freely available in the web. Although the predictions were not perfectly matched with the data, reasonable estimates were obtained under different pressure profiles. In principle, the same methodology can be used to predict nonisobaric survival curves during pulsed high pressure treatments if the temperature effects are negligible.

Industrial relevance: There is now enough evidence that a log-linear isothermal survival curve of bacterial cells is an exception rather than a rule and the same can be said about semilogarithmic survival curves of organisms exposed to high hydrostatic pressure (HHP). Therefore accurate description of survival curves with other modeling techniques is essential. Moreover, userfriendly software like Microsoft Excel® is more familiar than the other softwares and could help the people in the food industry to predict the nonisobaric inactivation of microorganisms.

1. Introduction

High hydrostatic pressure (HHP) is considered to be a promising alternative to thermal treatments during the last 20 years (Jordan, Pascual, Bracey, & Mackey, 2001). The application of HHP treatment ranging from 100 to 1000 MPa could successfully inactivate the microorganisms and hence allows the preservation of foods (Buzrul, Alpas, Largeteau, & Demazeau, 2007). The number of studies examining the inactivation kinetics of microorganisms by HHP has enormously increased during the last few years (Chen & Hoover, 2003; Buzrul & Alpas, 2004; Buzrul, Alpas, & Bozoglu, 2005). All these studies indicated that first-order kinetics is an exception rather than the rule for HHP inactivation of microorganisms just like the thermal inactivation (Peleg & Cole, 1998; van Boekel, 2002; Buzrul & Alpas, 2007).

HHP treatment can consist of a single exposure period (one pressure cycle; i.e., long holding time) or the application of multi-pulsed pressure in shorter periods (has an equal total duration for holding period but shorter holding times for each pulse). Pulsed HHP treatment can effectively be used to inactivate microorganisms (Alemán et al., 1998); moreover, it is a convenient way to reduce the pressure level needed to inactivate the microorganisms (Donsì, Ferrari, & Maresca, 2007). However, it is also important to predict the inactivation of microorganisms during nonisobaric pressure treatment such as pulsed HHP processes. The main objectives of this communication were to present a method of predicting nonisobaric inactivation of *Escherichia coli* whose isothermal survival curves have upward concavity (i.e., non-linear survival curves or tailing) and to demonstrate that the generation of the predicted curves can be done with a general purpose software like Microsoft Excel®, which can be downloaded freely from the web.

2. The method

2.1. Primary, secondary and tertiary models

Let us assume that the effect of temperature during HHP processing is minimized i.e., experiments are done in a temperature range so that temperature does not contribute to the microbial inactivation. Let us also assume that the isobaric survival curves of the treated microorganisms, in the pertinent medium and pressure range, all obey the power law or Weibullian model (Peleg & Cole, 1998):

$$\log_{10} S(t) = -b(P)t^{n(P)} \quad (1)$$

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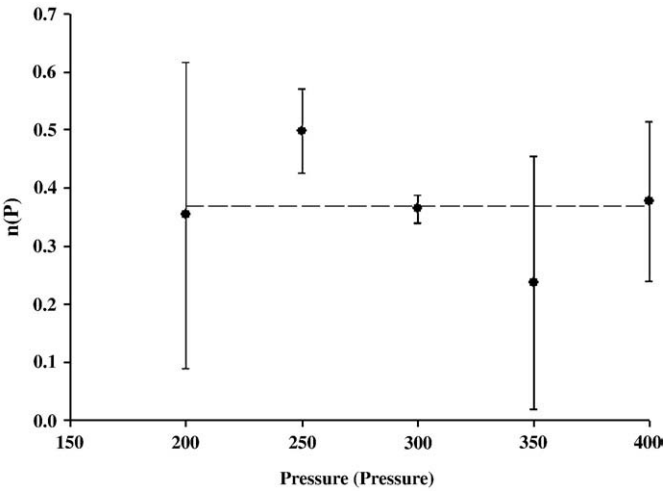

Fig. 1. Shape parameters for the survival curves of *E. coli* ATCC 25922 [$n(P)$] ± 95% confidence intervals of the Weibull model Eq. (1). Dashed line indicates fixed n value ($n = 0.37$) of the reduced Weibull model Eq. (3).

where $S(t)$ is the survival ratio i.e., $S(t) = N(t) / N_0$, $N(t)$ and N_0 are the number of survivors after an exposure time t and initial number of microorganisms (CFU mL^{-1} or g^{-1}), respectively. The model has two parameters; b is pressure dependent rate parameter and n is the pressure dependent shape parameter. Such a model presents the main advantage of being very simple and sufficiently robust to describe both monotonic downward concave (shoulder) survival curves ($n>1$) and monotonic upward concave (tailing) survival curves ($n<1$). Traditional first-order model (Eq. (1)) is then a special case ($n = 1$) of the Weibull model [in fact, different expressions of the Weibull model exist in literature (Mafart, Couvert, Gaillard, & Leguerinel, 2002; van Boekel, 2002). They all result with the same fitting when they are applied to the data (Buzrul, 2007). Why we select Eq. (1) as a model will be explained below]. Moreover, if the distribution of the lethal events (heat, pressure, disinfectant, etc.) is indeed Weibullian, then $n(P)$ is expected to be either pressure independent or only a weak function of pressure (Peleg, Normand, & Corradini, 2005).

It is well known that when a microbial population is pressurized, substantial mortality occurs when the pressure reaches a certain level. In this lethal range, the inactivation rate increases as the pressure is raised. A convenient model that captures these features is the log-logistic equation (Campanella & Peleg, 2001):

$$b(P) = In\{1 + \exp[k(P - P_c)]\} \quad (2)$$

where In is the natural logarithm and k and P_c are the parameters of Eq. (2). According to this model, P_c marks the pressure region, where intensive inactivation starts and k is the rate at which $b(P)$ climbs as the pressure increases.

When the power $n(P)$ is fixed, i.e., $n(P) = n$, the isobaric survival curves equation becomes:

$$\log_{10} S(t) = -b(P)t^n \quad (3)$$

where $b(P)$ is defined by Eq. (2).

Peleg and Penchina (2000) assumed that the inactivation rate in non-isothermal processes is a function of the momentary temperature and the corresponding survival ratio. This procedure explained in great detail in several studies (for example Peleg, Penchina, & Cole, 2001; Corradini, Normand, & Peleg, 2005). This assumption can also be applied for the nonisobaric pressure treatments such as pulsed HHP processes. Furthermore, the rate model obtained from the above assumption could be expressed by a difference equation in which case it can be solved incrementally with a software like Microsoft Excel®. The derivation of difference equation was given by Peleg et al. (2005) in great detail and this incremental method was posted on the web as freeware [Microsoft Excel® (RealTimePasteurizationData.xls) program is available at http://www-unix.oit.umass.edu/~aew2000/SalmSurvival.html].

2.2. Procedure

2.2.1. Isobaric survival curves

Two recent studies were selected as the database: Koseki and Yamamoto (2007) used *E. coli* ATCC in 0.1% peptone solution and Shao, Ramaswamy, and Zhu (2007) used *E. coli* O157:H7 in raw milk cheese for HHP inactivation experiments. The data reported in their figures were scanned and digitized by using a software program (WinDIG 2.5; written by Mr. Dominique Lovy, Geneve, Switzerland). The reported data as $\log_{10}N(t)$ versus time were converted into $\log_{10}S(t)$ versus time. Eq. (1) was fitted to these data as the primary model. From Eq. (1) $n(P)$ values were obtained and these values were used to propose a fixed n value for the overall data. Then, Eq. (3) was fitted to the data to obtain new $b(P)$ values. These $b(P)$ values versus pressure were fitted to Eq. (2) as a secondary model and k and P_c were estimated.

2.2.2. Nonisobaric survival curves

The nonisobaric inactivation data were also obtained from the same studies; Koseki and Yamamoto (2007) also reported pressure profiles in figures; however, Shao et al. (2007) only mentioned about their compression and decompression rates. Therefore pressure-time data were simulated for the latter. Pressure profiles of Koseki and Yamamoto (2007) were scanned and digitized by the same method described above and then these (pressure and time) values were written in a Microsoft Excel® sheet. From the web page http://www-unix.oit.umass.edu/~aew2000/SalmSurvival.html, a Microsoft Excel® program (RealTimePasteurizationData.xls; model by Micha Peleg and program by Mark D. Normand) was downloaded and digitized pressure and time values (written in the Excel® sheet) were pasted to this program. In fact, this program was written for thermal inactivation of microorganisms. Nevertheless, as mentioned above it can also be applied for the nonisobaric HHP treatments. Values of k and P_c obtained from Eq. (2) and fixed n value of Eq. (3) were entered. Then, by clicking the solve button the nonisobaric survival curve was obtained. Since in this program parameter $b(P)$ was defined by k and P_c among the alternatives of different expressions of the Weibull

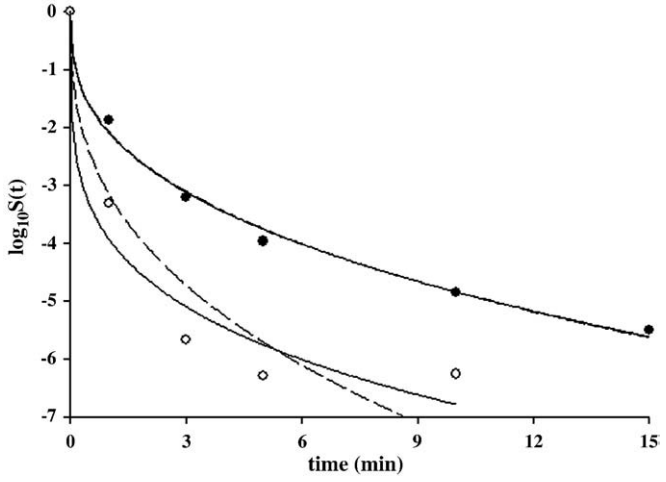

Fig. 2. Survival curves of *E. coli* ATCC 25922 in 0.1% peptone water at 300 (closed circles) and 350 (open circles) MPa. Solid lines indicate that data were fitted with the Weibull model Eq. (1) and dashed lines indicate that data were fitted with the reduced Weibull model Eq. (3) with $n = 0.37$. The experimental data are from Koseki and Yamamoto (2007).