Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

# Mathematical modeling of *Salmonella typhimurium* inactivation under high hydrostatic pressure at different temperatures

*Osman Erkmen* *

*Department of Food Engineering, Faculty of Engineering, University of Gaziantep, 27310 Gaziantep, Turkey*

ABSTRACT

Inactivation curves of *Salmonella typhimurium* under high-hydrostatic pressures (HHPs) (200, 250, 300 and 350 MPa) at different temperatures (15, 25, 35 and 45 °C) in tryptone soy broth were analyzed using the modified Gompertz model. The phase of disappearance (time for inactivation of all cells, $\lambda$) and the inactivation rate ($\mu$) of *S. typhimurium* were inversely related. Inactivation rates of *S. typhimurium* were higher ($P < 0.05$) at 45 °C than 15, 25 and 35 °C under HHPs from 200 to 350 MPa. The $\mu$ values were −2.66, −6.06, −7.67 and −7.99 $min^{-1}$ at 200, 250, 300 and 350 MPa HHP treatments, respectively, at 45 °C. A negative $\mu$ value (always negative) indicates that an increase (become more negative) in $\mu$ with increasing pressure or temperature is related to the *S. typhimurium* inactivation process. The $\mu$ values were also increased with increasing temperature from 15 to 45 °C at same treated pressures. Increased pressure and temperature had significant effects on the survival of *S. typhimurium*. The temperature dependence of the inactivation rate constant was analyzed based on the Arrhenius, linear and square-root models. The pressure sensitivity (low $E_\mu$) determined based on the Arrhenius model was lower at high pressure. $E_\mu$ (activation energy) value was 1.94 kJ/mol at 350 Mpa, and 42.88, 12.99 and 3.73 kJ/mol at 200, 250 and 300 MPa, respectively. Results of this study enable the prediction of microbial inactivation exposed to HHPs at different temperatures.



*Keywords:* Mathematical modeling; *Salmonella typhimurium*; High hydrostatic pressure

## 1. Introduction

The use of mathematical models in describing the behavior of microorganisms is a helpful tool for improving food safety (Zwietering et al., 1990; Buchanan, 1993; Bozkurt and Erkmen, 1999). At present, the Gompertz equation has become the most widely used model for describing microbial growth or inactivation (Zwietering et al., 1990; McMeekin et al., 1992; Palumbo et al., 1992; van Impe et al., 1992; Buchanan, 1993; McClure et al., 1993; Muarmans et al., 1993; Bozkurt and Erkmen, 1999; Erkmen, 2001).

There has been growing interest in using high hydrostatic pressure (HHP) processing as a non-thermal food preservation technique. High pressure has emerged as a commercial alternative to traditional thermal processing methods for foods (Garrriga et al., 2004; Fonberg-Broczek et al., 2005). A major advantage of non-thermal methods of food preservation is the inactivation of microorganisms without severe heating and therefore there is minimal damage to the flavor, color, texture and nutritional value of the food (Aleman et al., 1996; Mussa and Ramaswamy, 1997; Ponce et al., 1999). HHP technology (from 200 to 1000 MPa) permits microbial inactivation at low or moderate temperatures for the purpose of food pasteurization (Hoover et al., 1989; Knorr, 1993; Ledward, 1995; Vachon et al., 2002; Dogan and Erkmen, 2003; Erkmen and Dogan, 2004; Ritz et al., 2006). HHP is being investigated as a non-thermal processing technique to destroy foodborne microorganisms in order to enhance safety and shelf life of perishable foods (Wei et al., 1991; Chen et al., 1993; Patterson et al., 1995; Malicki et al., 2005).

The objectives of the present study were (i) to analyze the effect of HHP pressures at different temperatures on *Salmonella typhimurium* inactivation parameters obtained by fitting models to experimental counts and (ii) to evaluate the effect of

* Tel.: +90 342 3601200; fax: +90 342 3601013.
E-mail address: erkmen@gantep.edu.tr.
Received 10 February 2008; Accepted 21 May 2008
0960-3085/$ – see front matter © 2008 The Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.fbp.2008.05.002

temperature at different pressure levels on the phase of disappearance (time for inactivation, λ) and the inactivation rate (μ) parameters.

## 2. Materials and methods

### 2.1. Culture preparation

*Salmonella typhimurium* KUEN 1357 was obtained from University of İstanbul, Faculty of Medicine, Microorganism's Culture Collection Research and Applied Center, İstanbul, Turkey. The cultures were maintained on tryptone soy agar (TSA; Difco, Detroit) slants and stored at 4 °C. The cultures for experiments were subcultured twice from slant culture by inoculating in 10 ml of tryptone soy broth (TSB; Difco, Detroit), and incubated at 35 °C for 18 h.

### 2.2. Preparation of cell suspensions for pressurization

Subcultured *S. typhimurium* was inoculated at a 0.1 ml level into TSB (pH 7.0) in duplicate and incubated at 35 °C for 18 h. Cultures were diluted using sterile TSB to obtain cell number about $5.75 \times 10^7$ colony forming unit (cfu) $ml^{-1}$, which was determined by serial dilution in a diluent containing 0.1% sterile peptone water and then cultured on brain heard infusion agar (BHIA, Difco, Detroit) followed by incubation at 35 °C for 24 h (Erkmen, 2007).

### 2.3. Equipment

A hydrostatic pressure vessel (internal diameter 4 cm; length 12 cm; maximum pressure tolerance level = 1500 MPa) with internal volume of 150 $cm^3$ and a hydraulic unit (Kon hidroliksan; hydrolic pressure and manufacturing industry Inc., Konya, Turkey) were used for ultra high hydrostatic pressurization. The pressure vessel and piston were made of steel type 45WCRV7 which was processed into the required sizes at the Mechanical Engineering Department, Faculty of Engineering, University of Gaziantep, Gaziantep, Turkey.

### 2.4. Ultra high hydrostatic pressure treatment

Ten ml from each of stock culture was placed into sterile polyethylene bags (sterilized by 0.1% $H_2O_2$; 5.5 cm × 4.0 cm). The bags were sealed after eliminating air inside. In pressurization study, bags containing 18 h culture suspensions in TSB were exposed to 200, 250, 300, 350 and 400 MPa pressure from 15 to 45 °C.

The bags were placed in a hydrostatic pressure vessel in high-pressure equipment. HHP levels were generated using deionized water pressurized by a hydrostatic pump. Each treatment was a combination of a pressure level and duration. The rates of pressure increase and release times were about 25 MPa $s^{-1}$ on high-pressure apparatus. Pressurization time reported in this study did not include the pressure increase and release times. The vessel temperature has been adjusted to required experimental temperature in a water bath and held at constant temperature during pressurization. Immediately after pressurization duplicate bags were removed, cooled in an ice bath and used for enumeration (within about 3 min after pressurization) of viable cells.

Pressurized and control cell suspensions in bags were serially diluted in 0.1% sterile peptone (Difco, Detroit) water. Diluted or non-diluted samples were spread plated on BHA to enumerate viable *S. typhimurium* cells (Erkmen, 2007). Duplicate samples were used in this count. The experiments were repeated two times and the average counts were recorded as the number of cfu $ml^{-1}$.

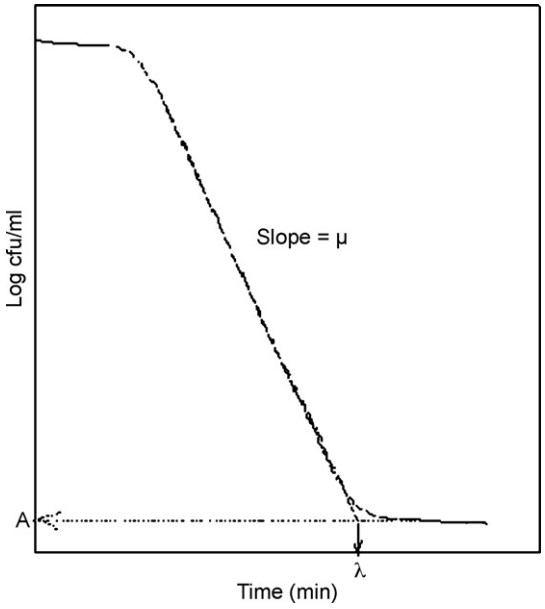


**Fig. 1 – Microbial inactivation curve with biological parameters as functions of time.**

### 2.5. Microbial growth modeling

The modified Gompertz equation was applied to experimental data counts (Zwietering et al., 1990):

$$\log N = \log N_0 + A \exp\left\{-\exp\left[\frac{\mu e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

where log *N* (cfu) is the logarithm of the number of *S. typhimurium* cells at time *t*, and log $N_0$ ($cfu_0$) the logarithm of the number of *S. typhimurium* cells at time 0. *A* (log cfu) is the lower asymptote value, μ the inactivation rate ($min^{-1}$), λ the phase of disappearance (time for inactivation, min), and *t* the time (min). Biological parameters (μ, λ and *A*), which are shown in Fig. 1, were determined using the modified-Gompertz model.

### 2.6. Fitting of the data and statistical analysis

Fitting of data for the modified Gompertz model was performed by non-linear regression analysis using SigmaPlot 8.0 (Jandel Scientific, San Francisco, USA) with the Marquardt–Levenberg algorithm; this is a search method for minimizing the sum of squares of the differences between the fitted and experimental data (Buchanan, 1993; Bozkurt and Erkmen, 1999; Bozkurt and Erkmen, 2001). The algorithm calculates the set of parameters: regression coefficient ($R^2$), residual sum of squares (RSS), μ, λ and *A*. The biological parameters (μ, λ, and *A*) of *S. typhimurium* were compared using an ANOVA module of the SPSS 15.0 software for Windows (SPSS Inc., SPSS Inc. Headquarters, 233 S. Wacker Drive, 11th floor Chicago, IL 60606) for different HHPs and temperatures to find the relationship between these parameters.