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Modeling the synergistic effect of high pressure and heat on inactivation kinetics of *Listeria innocua*: a preliminary study

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Abstract

The survival curves of *Listeria innocua* CDW47 by high hydrostatic pressure were obtained at four pressure levels (138, 207, 276, 345 MPa) and four temperatures (25, 35, 45, 50 °C) in peptone solution. Tailing was observed in the survival curves. Elevated temperatures and pressures substantially promoted the inactivation of *L. innocua*. A linear and two non-linear (Weibull and log–logistic) models were fitted to these data and the goodness of fit of these models were compared. Regression coefficients (R^2), root mean square (RMSE), accuracy factor (A_f) values and residual plots suggested that linear model, although it produced good fits for some pressure–temperature combinations, was not as appropriate as non-linear models to represent the data. The residual and correlation plots strongly suggested that among the non linear models studied the log–logistic model produced better fit to the data than the Weibull model. Such pressure–temperature inactivation models form the engineering basis for design, evaluation and optimization of high hydrostatic pressure processes as a new preservation technique.

Keywords: High hydrostatic pressure; *Listeria innocua*; Weibull; Log–logistic

1. Introduction

Heat treatment has been long regarded as one of the most widely used and most effective means for destruction of spoilage and pathogenic microorganisms in foods. Although it is an efficient process to inactivate microorganisms, it cannot be used to treat heat-labile compounds. Therefore new processes tend to use non-thermal preservation treatments such as electrical fields, ionization, light pulses, high hydrostatic pressure (HHP) and ultrasounds. Some of these systems already have regulatory approval and are commonly used in the industry, while others continue to be developed and evaluated for potential commercial application [1,2]. Over the past 15 years, high pressure has emerged as a commercial alternative to traditional thermal processing methods for some foods such as fruit juices, jams and guacamole. As compared to thermal processing HHP processing can inactivate microorganisms at lower treatment temperatures, therefore treated foods possess the same or nearly same sensory and nutritional qualities as the untreated product [3]. HHP is a three-variable process consisting of pressure, time and temperature. For effective use of this method in food preservation it is necessary to study the interaction of these factors and determine the minimum conditions to obtain desirable levels of microbial destruction while maintaining a maximum degree of sensory and nutritional quality [4].

The inactivation kinetics of microorganisms using heat has been studied extensively and traditionally it has been assumed to follow first-order kinetics. It assumes that all the cells or spores in a population have

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equal resistance to lethal treatments, which results in a linear relationship between the logarithm of the number of survivors and treatment time. However when the linearity of the data is started to be questioned [5,6] and significant deviations from linearity have been reported frequently [2,7,8]; several non-linear models were also used to predict the survival curves of microorganisms which were inactivated by heat treatment.

Although the inactivation kinetics of microorganisms using heat has been extensively studied, information on the inactivation kinetics of microorganisms under high pressure, especially under simultaneous application of pressure and other processing techniques, is still limited. Accurate prediction of the effectiveness of HHP processing against foodborne pathogens based on inactivation kinetics is essential to permit production of safe products [3]. The patterns of inactivation kinetics of microorganisms using HHP are quite variable. Some investigators indicated first-order kinetics. For instance, Erkmen et al. [9] studied inactivation kinetics of *Escherichia coli* by HHP in broth, milk, peach and orange juice. *E. coli* seemed to obey first-order kinetics in the range of 300–700 MPa. However, various other authors observed curvature and tailing which indicates linear model is not appropriate in describing data [10,11]. Different kinds of deviations from linearity have been observed such as sigmoid curves, curves with a shoulder and especially with a tailing and correspondingly various models have been proposed to describe these non-linear curves such as Baranyi, Buchanan, Membre, Log–logistic, Gompertz and Weibull [6,12–18]. Among these, log–logistic [15] and Weibull [6] models have been successfully used in describing the non-linear inactivation of different microorganisms under various experimental conditions [3,11]. Therefore these two non-linear models were selected and compared with traditional linear model in this study.

Listeria innocua is a non-pathogenic microorganism but for many characteristics (such as growth and biochemical characteristics) it is similar to pathogenic *Listeria monocytogenes* and therefore a useful surrogate for the foodborne pathogen *L. monocytogenes*. It fills this role because in addition to its very similar physiology and metabolism with*L. monocytogenes*, the non-pathogen is equally resistant to low pH, drying, heating and salt. Such hardiness makes *L. innocua* an excellent indicator in inoculated pack studies at food processing plants. Therefore it has been used as a model organism in various studies [19].

The objectives of this study were to model the survival curves of *Listeria innocua* under combined effect of HHP and heat in peptone solution using linear and two non-linear models (Weibull and log–logistic) to investigate and compare the goodness of fit of these models.

2. Materials and methods

2.1. Bacterial growth and cell suspension

Listeria innocua CDW47 (obtained from Dr. John Sofos, Colorado State University) was grown in Trypticase soy broth (Difco) for 16 h at 37 °C. The cells were harvested by centrifugation and resuspended in sterile 0.1% peptone solution to a final concentration of about 10^8–10^9 cells/ml. The cell suspensions were dispensed in 2 ml portions in sterile plastic vials (Simport Plastic). The vials were placed individually in plastic bags (Nasco Whirl-Pak), vacuum sealed and used for pressurization.

2.2. Hydrostatic pressurization

The hydrostatic pressure unit used for this study has a 25×35 cm pressure chamber filled with a mixture of water and oil (Engineered Pressure System). The temperature of the liquid in the pressure chamber can be adjusted to between 25 and 90 °C by a built-in heating system. The pressure level and time of pressurization are controlled automatically. The rate of pressure increase was about 300 MPa/min and pressure release time was less than 1 min. Before each study, the temperature of the liquid was adjusted to the desired level and the plastic vials containing cell suspensions, in duplicate, were placed in the liquid for 2–3 min. The vials were then subjected to pressurization at the desired pressure (138, 207, 276 and 345 MPa) and temperature (25, 35, 45 and 50 °C) for 5, 10, 15, 20, 25 and 30 min. Immediately after pressurization, the vials were transferred to ice-water and used for the enumeration of colony forming units (c.f.u.).

2.3. Enumeration of survivors

Cell suspensions from each vial was serially diluted in 0.1% peptone solution and each dilution (from each sample) were surface plated in duplicate giving four plates per dilution on prepoured trypticase soy agar (Difco) supplemented with 0.6% yeast extract. The plates were incubated at 37 °C for 48 h and used for the enumeration of c.f.u. The plates were also kept for an additional 48 h at 37 °C to allow injured cells to form visible colonies. Two replicates were performed on separate days and average of eight counts (two vials × two plates × two times) were used to calculate the results. The unpressurized cell suspensions were used to determine initial c.f.u./ml.

2.4. Modeling of survival curves

2.4.1. First-order kinetics

Chick [20] proposed the following differential equation to apply the theory of first-order chemical reaction to the thermal destruction of microorganisms:

$$\begin{cases} \frac{dN(t)}{dt} = -kN(t) & (0 \leqslant t < +\infty) \\ N(0) = N_0 & (N_0 > 0; t = 0), \end{cases} \quad (1)$$

where $N(t)$ and N_0 are the concentrations (c.f.u./ml) present at time t and zero, respectively; k is the death rate constant (min^{-1}); $dN(t)/dt$ (c.f.u./ml/min) is the death (inactivation) rate; $[dN(t)/dt]/N(t)$ is the relative or specific death (inactivation) rate in min^{-1}.

In terms of the base ten logarithm, the solution of Eq. (1) can be written as

$$\log \frac{N(t)}{N_0} = -\frac{t}{D} \quad (t \geqslant 0), \quad (2)$$

where D, is the D-value or the decimal reduction time in minutes (time required for one log reduction in the number of cells), namely $D = \ln(10)/k = 2.303/k$. From Eq. (2), $\log[N(t)/N_0]$ versus time t is a straight line on a semi-log plot, so Eq. (2) can be used to fit linear survival curves.

2.4.2. Weibull model

While conventional first-order model implicitly assumes that the microbial populations are homogenous in terms of their heat or pressure resistance, some researchers [6,18] assumed that at a given temperature or pressure, the time of heat or pressure exposure, which mainly causes the death of a microbial cell or a bacterial spore is variable from one individual to the other, and the dispersion of individual heat or pressure resistance was governed by a Weibull distribution, the cumulative form of which yields:

$$N = N_0 e^{-kt^r}. \quad (3)$$

Peleg et al. [6] wrote out this model in the following decimal logarithmic form:

$$\log \frac{N}{N_0} = -bt^r, \quad (4)$$

where b and r are the scale and shape factors, respectively.

Such a model presents the main advantage of remaining very simple and being sufficiently robust to describe both downward concave survival curves ($r > 1$) and upward concave curves ($r < 1$). Obviously, the model includes the traditional case where the survival curve, originated from a first order, is linear ($r = 1$) [21].

2.4.3. Log–logistic equation

In order to describe the inactivation kinetics of microorganisms, the following equation was proposed by Cole et al. [15]:

$$\log N = \alpha + \frac{\omega - \alpha}{1 + e^{4\sigma(\tau - \log t)/(\omega - \alpha)}}, \quad (5)$$

where α = upper asymptote (log c.f.u./ml); ω = lower asymptote (log c.f.u./ml); σ = the maximum rate of inactivation (log (c.f.u./ml)/log min); τ = the log time to the maximum rate of inactivation (log min).

At $t = 0$ $\log t$ is not defined, therefore a small t ($t = 10^{-6}$) was used to approximate $t = 0$. $\log N_0$ was calculated from Eq. (5) and substituted back into Eq. (5) [3,11] to give

$$\log \frac{N}{N_0} = \log N - \log N_0 = \frac{\omega - \alpha}{1 + e^{4\sigma(\tau - \log t)/(\omega - \alpha)}} - \frac{\omega - \alpha}{1 + e^{4\sigma(\tau + 6)/(\omega - \alpha)}}. \quad (6)$$

The number of parameters of log–logistic model could be reduced from 4 to 3 if we let $A = \omega - \alpha$ [3,11]. Therefore

$$\log \frac{N}{N_0} = \frac{A}{1 + e^{4\sigma(\tau - \log t)/A}} - \frac{A}{1 + e^{4\sigma(\tau + 6)/A}}. \quad (7)$$

2.4.4. Data analysis and model evaluation

Curve Expert Version 1.37 (Curve fitting system for Windows) was used for linear and non-linear regression analysis and to determine the parameters of non-linear models. The goodness of the fit of the models was assessed using regression coefficient (R^2), root mean square (RMSE) and accuracy factor (A_f). For plotting the residual graphs Microsoft® Excel 2000 was used.

R^2 measures how well a linear or a non-linear model fit the data and higher the R^2 value, the better is the adequacy of the model to describe the data [22]. RMSE measures the average deviation between the observed and fitted values. Small RMSE value of a model indicates a better fit of data for that model.

$$\mathrm{RMSE} = \sqrt{\frac{\sum (\text{fitted} - \text{observed})^2}{n - p}}, \quad (8)$$

where n is the number of observations and p is the number of parameters to be estimated.

The accuracy factor (A_f) was proposed by Ross [23] and it was used in several studies to evaluate the performance of predictive models [3,11]. A_f provides a measure of the average difference between observed and predicted values and is defined as

$$A_f = 10^{\frac{\sum |\log(\text{predicted}/\text{observed})|}{n}}. \quad (9)$$

The larger the A_f value, the less accurate is the average estimate, while a value of 1 indicates that the model produces a perfect fit to these data.

3. Results and discussion

The three models for inactivation curves of *L. innocua* by combined pressure and heat (276 and 345 MPa at 45 °C) is represented in Fig. 1. Observed values were shown as black dots and the first value (not shown on the figures) is obviously zero (when $t = 0$, N becomes N_0 therefore $\log(N/N_0)$ is 0). Considering the latter argument the

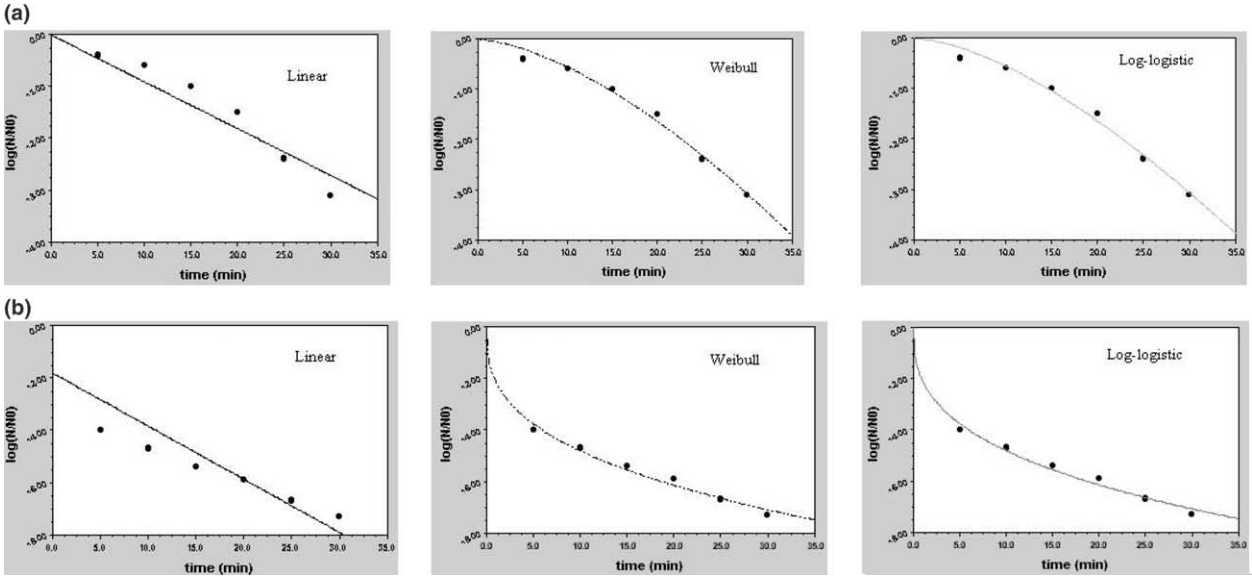

Fig. 1. Comparison of fitting of three models for the survival curves of *Listeria innocua* at 276 (a) and 345 (b) MPa at 45° C. (The Weibull and log–logistic models produced almost identical curves). Data points (observed values) were shown as black dots and the first data point (not shown on the graphs) is the zero point. Data are the average of eight counts.

Table 1
Comparison of the linear, Weibull and log–logistic models for the survival curves of *Listeria innocua* CDW47 in peptone solution

	R^2			RMSE			A_f		
	Linear	Weibull	Logistic	Linear	Weibull	Logistic	Linear	Weibull	Logistic
138 Mpa									
25 °C	0.916	0.980	0.979	0.079	0.039	0.049	1.133	1.090	1.091
35 °C	0.947	0.963	0.962	0.082	0.052	0.080	1.196	1.128	1.169
45 °C	0.868	0.974	0.984	0.152	0.068	0.062	1.750	1.288	1.306
50 °C	0.960	0.961	0.992	0.117	0.116	0.062	1.373	1.347	1.103
Mean	0.923	0.969	0.979	0.108	0.069	0.063	1.363	1.213	1.167
207 MPa									
25 °C	0.883	0.972	0.970	0.140	0.069	0.079	1.178	1.093	1.093
35 °C	0.935	0.954	0.952	0.133	0.112	0.132	1.139	1.152	1.156
45 °C	0.929	0.950	0.948	0.199	0.168	0.198	1.158	1.147	1.150
50 °C	0.942	0.947	0.944	0.202	0.195	0.231	1.219	1.258	1.267
Mean	0.922	0.956	0.953	0.169	0.136	0.160	1.174	1.162	1.167
276 MPa									
25 °C	0.990	0.997	0.998	0.065	0.036	0.035	1.053	1.044	1.027
35 °C	0.961	0.972	0.980	0.186	0.159	0.152	1.550	1.435	1.253
45 °C	0.936	0.990	0.990	0.349	0.140	0.163	1.276	1.231	1.235
50 °C	0.960	0.960	0.959	0.449	0.400	0.522	1.159	1.158	1.153
Mean	0.962	0.980	0.982	0.262	0.184	0.218	1.260	1.217	1.167
345 MPa									
25 °C	0.983	0.988	0.987	0.123	0.101	0.125	1.088	1.087	1.093
35 °C	0.943	0.961	0.974	0.449	0.371	0.352	1.093	1.116	1.138
45 °C	0.827	0.994	0.994	1.234	0.221	0.263	1.167	1.038	1.040
50 °C	0.641	0.968	0.999	2.473	0.735	0.175	1.238	1.089	1.014
Mean	0.849	0.978	0.988	1.070	0.357	0.229	1.146	1.083	1.071

linear model (just by visual inspection) was not fully appropriate in describing the data with tailing, however the non-linear models produced almost identical curves (again by visual inspection) indicating better fit at this pressure and temperature. Similar results were also observed for inactivation curves at other pressure–temperature combinations (data not shown).

The goodness of fit of the linear and two non-linear models were compared by computing the R^2, RMSE and A_f values (Table 1). Although the linear model was not appropriate with a treatment of 276 and 345 MPa at 45° C, better fits were obtained at other pressure–temperature combinations. For instance; at 138 MPa–35 °C ($R^2 = 0.947$, RMSE = 0.082, $A_f = 1.196$) and at 276 MPa–25 °C ($R^2 = 0.990$, RMSE = 0.065, $A_f = 1.053$) linear model seemed appropriate to describe the data. Nevertheless, at these pressure–temperature combinations two non-linear models produced even better fit than the linear model did as indicated by higher R^2 and lower RMSE and A_f values. Comparing the mean values in Table 1, it could be postulated that, the linear regression model produced the poorest fit to the data as indicated by its lowest R^2 and highest RMSE and A_f values. Among the non-linear models studied log–logistic model seemed to produce the better fit, however there were some exceptional cases where the Weibull model produced as good as or better fit than the log–logistic model. For example; at 276 MPa–45 °C both models have the same R^2 values (0.99), on the other hand, based on RMSE (0.140 for the Weibull, 0.163 for log–logistic)

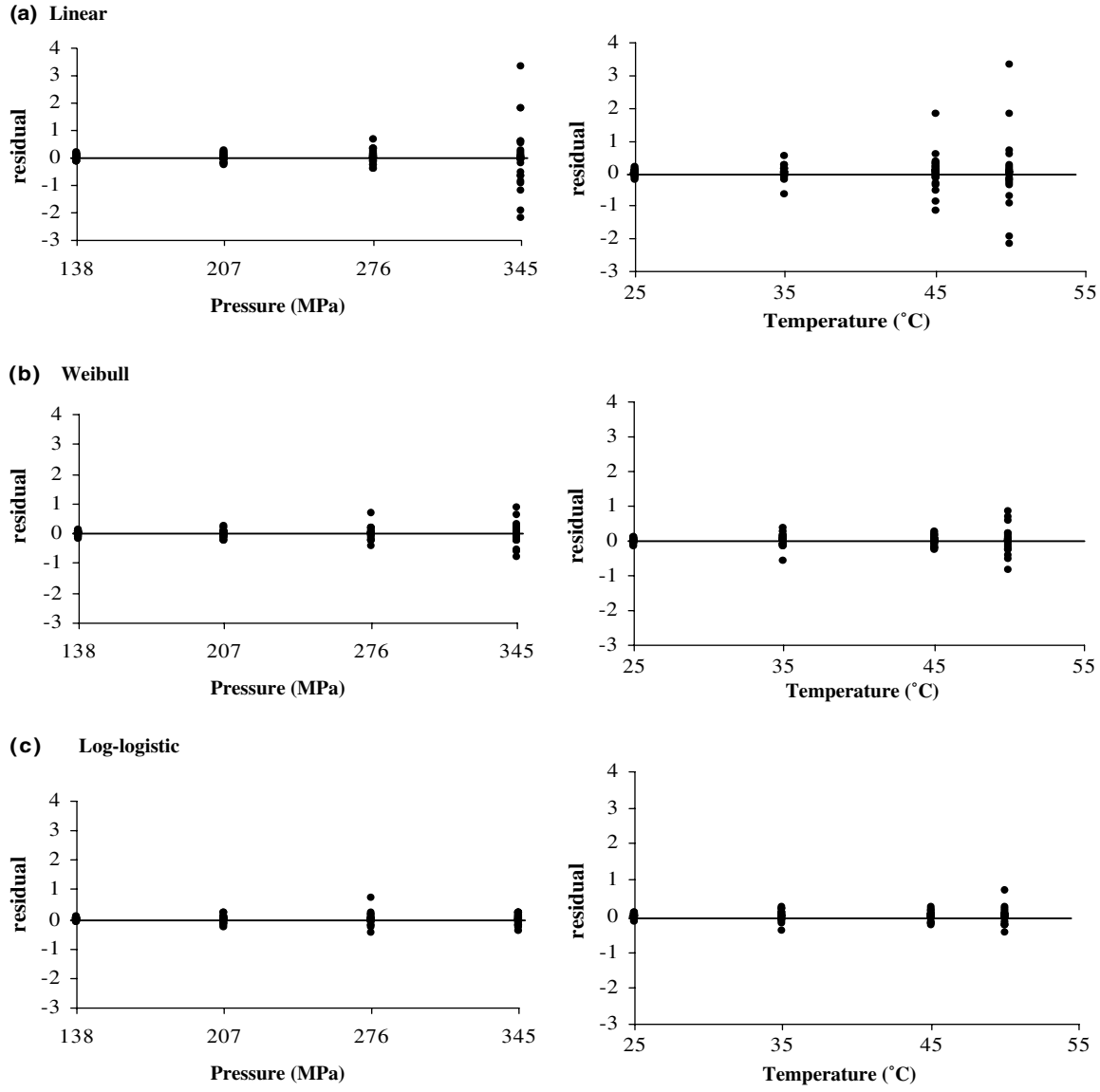

Fig. 2. Residual analysis of the inactivation of *L. innocua* at different pressures and pressurization temperatures according to linear, Weibull and log–logistic models.

and A_f values (1.231 for the Weibull, 1.235 for log–logistic) the fit is better for the Weibull model. Hence, although in general the Weibull model was slightly worse than the log–logistic model it is not easy to distinguish the goodness of fit of these two models just by investigating the mean values given in Table 1 and further analysis tool is necessary to make the differentiation. For this purpose, the residual plots and correlation between experimental and fitted values for linear and non-linear models are shown in Figs. 2–4, respectively. By investigating the residual plots one can get an idea whether a model is fully appropriate for the data being analyzed or not. Residuals are distributed randomly and they fall within a horizontal band centered around 0, displaying no systematic tendencies to be positive or negative [3,22]. Based on visual inspection of the residual plots (Fig. 2) it can be concluded that at low pressures (⩽276 MPa) and temperatures (⩽35 °C) all of the examined models seemed to be appropriate, however as pressure and pressurization temperature

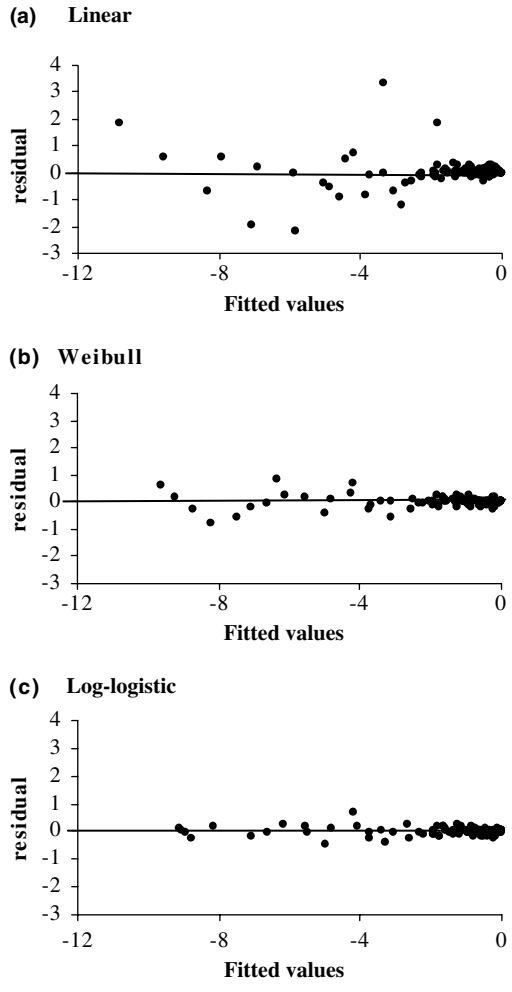

Fig. 3. Residual analysis of the inactivation of *L. innocua* according to linear, Weibull and log–logistic models.

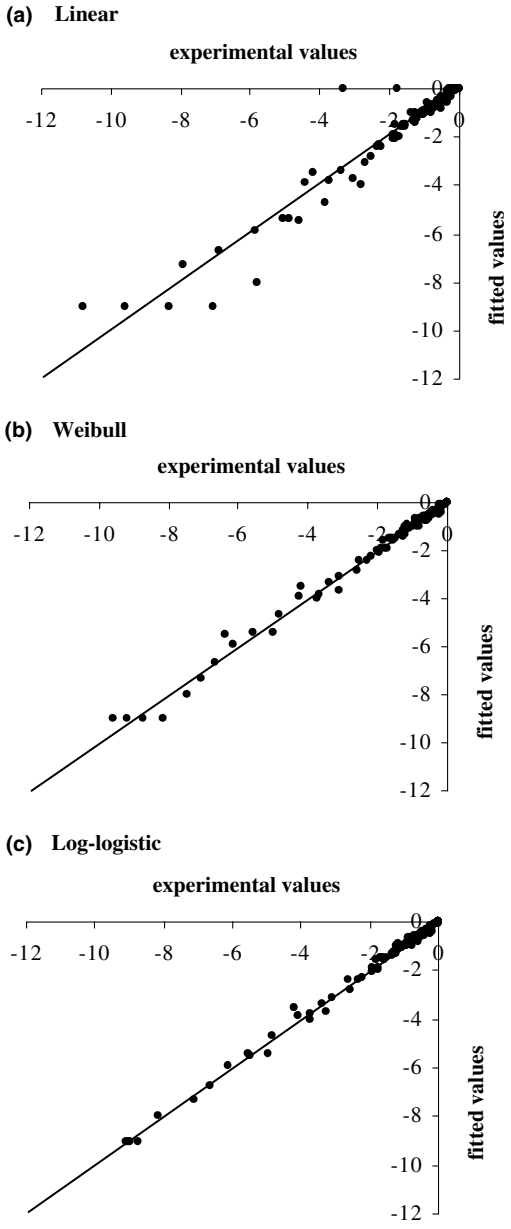

Fig. 4. Correlation between experimentally determined and calculated values according to linear, Weibull and log–logistic models. The center line is the 'line of equivalence'.

increased non-linear models were more appropriate than linear model to represent these data.

When residual versus fitted values plots (Fig. 3) were examined, it seems that the non-linear models studied were better in terms of describing the data being analyzed. Fitted versus experimental values plots (Fig. 4) proved the accuracy of the non-linear models studied. These plots (Fig. 4) indicated that there is a close relationship between observed values and predicted values for the non-linear models. Residual plot examination also states that log–logistic model is better than the Weibull model. Therefore when residual and correlation plots (Figs. 3 and 4) are used in combination with

Table 1, the difficulty in differentiating the goodness of fit of these two non-linear models could be greatly eliminated.

The effect of pressure and pressurization temperature on the values of parameters of the non-linear models were presented in Tables 2 and 3. Shape factors of Weibull model in Table 2 indicates that survival curves of *L. innocua* fitted with this model were both concave downward ($r > 1$) and concave upward ($r < 1$). This can also be seen from Fig. 1; at 276 MPa and 45° C the curve fitted with the Weibull model is concave downward, on the other hand same model produced concave upward fit at 345 MPa and 45 ° C. Almost linear survival curves were also fitted with the Weibull model (data not shown) at 50° C except for 345 MPa (see *r* values in Table 2). Although some authors [24] reported that the shape factor (*r* values) was independent of external factors such as temperature and pH, others demonstrated that shape factors were dependent on pressure at certain pressure ranges [3]. Chen et al. [3] also reported a linear relationship between scale factors (*b* values) of Weibull model and pressure. However, in this study we did not find any dependence of *r* and *b* values on pressure and temperature, therefore our results on the parameters of the Weibull model are more likely in agreement with those of Fernandez et al. [24].

When the parameters of log–logistic model are examined it is seen that *A* values varied from −56.2 to −1.3 (Table 3). Chen et al. [3,11] reduced the number of parameters of log–logistic model by setting a new *A* value and called this new model reduced log–logistic model.

Table 2
Effect of temperature and pressure on the values of parameters of the Weibull model

Temperature (°C)	25	35	45	50
b (MPa)				
138	0.11	0.011	0.0016	0.037
207	0.21	0.14	0.21	0.041
276	0.088	0.035	0.016	0.19
345	0.15	0.32	2.12	4.38
r (MPa)				
138	0.53	1.26	1.87	1.04
207	0.45	0.64	0.63	1.14
276	0.84	1.20	1.56	0.96
345	0.79	0.76	0.35	0.23

Table 3
Effect of temperature and pressure on the *A* values of log–logistic model

Temperature (°C)	25	35	45	50
A (MPa)				
138	−17.02	−8.48	−1.26	−1.37
207	−8.83	−15.08	−21.39	−26.82
276	−4.92	−2.89	−56.24	−39.71
345	−23.39	−5.72	−46.25	−9.18

This reduced model still produced a better fit than the other non-linear models studied and was comparable to the original full log–logistic model. Reducing the number of parameters of a model may serve to simplify that model. Although a model with more parameters can be expected to show a better fit to data, Baranyi et al. [25] pointed out the dangers of over-parameterization as it can result with equations that describe not only the underlying response, but also the errors specific to observed data as well. Therefore, in this study although further reduction of number of parameters at a constant pressure for varying temperature or vice versa (reduction from three to two) was possible, we did not pay much attention to select a new set of *A* values (Table 3).

The most serious deficiency in pressure process kinetics is that most parameters have been measured at a single pressure [7] and accordingly most of the authors proposed first-order or non-linear inactivation kinetics under a set of limited number of pressure and temperature values [3,11]. However, it has been strongly recommended to investigate the influence of HHP on reduction of microbial populations by collecting data at different pressures and temperatures in a statistically valid experimental design [7]. Therefore, considering the nature of inactivation kinetics, we have used four different pressure (138, 207, 276 and 345 MPa) and four different temperature (25, 35, 45 and 50 °C) values. Increasing the number of pressure and temperature parameters for studying the inactivation kinetics of microorganisms in a model system would result in a more accurate model and facilitate to select the most appropriate pressure and temperature combination to achieve the desired inactivation of *L. innocua*. This in return would help the food industry to optimize its process conditions and construct appropriate HACCP programs for food safety.

4. Conclusion

Mathematical models can be used to extrapolate dose-response information and can be an important tool to optimize production and distribution chains. In addition a statistical model of best fit would support the food technologists to optimize the economy of the inactivation approach in reality. Therefore, our primary aim was to compare the performance of three mathematical models (one linear and two non-linear) for the inactivation kinetics of *L. innocua* in peptone solution. Non-linear models produced better fit than the linear model in the pressure and temperature range studied where log–logistic model gave more accurate predictions of inactivation of *L. innocua* at different pressure and temperature levels than the Weibull model. Use of pressurization temperatures of 45 °C and higher would allow using lower pressures or shorter treatment times

for the inactivation. However, it is also known that models developed for one kind of bacteria should not be used to predict the inactivation kinetics of microorganisms in other foods, so it will be interesting to see whether these non-linear models proposed (for *L. innocua*) is also valid for other bacteria or especially for different foods as key stage in model development is validation or usage.

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