Modelling of inactivation of *Geobacillus stearothermophilus* spores exposed to superheated steam

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Head, D.S. and S. Cenkowski. 2010. **Modelling of inactivation of *Geobacillus stearothermophilus* spores exposed to superheated steam**. Canadian Biosystems Engineering/Le génie des biosystèmes au Canada. 52: 3.1–3.10. Spores of *Geobacillus stearothermophilus* at two inoculum levels [3 and 6 log colony forming units per gram (cfu/g)] were challenged with superheated steam (SS) at a velocity of 0.35 m/s and temperatures of 130, 145, 160, and 175°C. A tendency of tailing was observed in the survivor curves [plots of survival ratio, log (N/N_o), against processing time] as a monotonic upward concavity. Therefore, non-linear regression models, the Weibull and Kamau models, instead of the traditional first-order kinetics model were used to describe the survivor curves. The Weibull model was successfully fitted to all survivor curves obtained in this study, whereas the Kamau model agreed only for some. A high correlation between the predicted and observed values of spore survival ratio were obtained for the SS treatment in the range of 145–175°C at both inoculum levels, and for SS treatment at 130°C at low inoculum level. The Weibull model was expanded by describing its parameters (α, β) as a function of SS temperature for the range of 130–175°C. This allowed accurate predictions of the spore survival ratio based on the SS temperature and processing time only (with the exception of predictions for spores at high inoculum level challenged with SS at 130°C). Hence, the Weibull model is recommended for describing and modelling of inactivation of *G. stearothermophilus* spores in SS at higher temperatures (145–175°C). **Keywords:** modelling, spores, inactivation, *Geobacillus stearothermophilus*.

Les spores de *Geobacillus stearothermophilus* à deux niveaux d'inoculation [3 et 6 log unités de formation de colonies par gramme (cfu/g)] ont été exposés à de la vapeur surchauffée (SS) à une vitesse de 0,35 m/s et à des températures de 130, 145, 160 et 175°C. Une tendance de conservation a été observée dans les courbes de survie (graphiques du ratio de survie, log (N/N_o), en fonction du temps de traitement) selon un modèle à concavité monotonique ascendante. En conséquence, des modèles de régression non-linéaire, les modèles Weibull et Kamau, plutôt que le modèle cinétique traditionnel de premier ordre ont été utilisés pour décrire les courbes de survie. Le modèle Weibull a représenté de manière efficace toutes les courbes de survie obtenues dans cette étude, alors que le modèle Kamau ne s'est avéré valide que pour quelques courbes seulement. Une corrélation élevée entre les valeurs prédites et les valeurs observées du ratio de survie des spores était obtenue pour le traitement SS pour des températures variant de 145–175°C aux deux niveaux d'inoculation, et pour le traitement SS à 130°C au niveau d'inoculation le plus faible. Le modèle Weibull a été ajusté en adaptant ses paramètres (α, β) en fonction de la température SS pour la plage de 130–175°C. Ceci a permis des prédictions précises du ratio de survie des spores basées sur la température SS et le temps de traitement seulement (à l'exception des prédictions pour les spores traitées à un haut taux d'inoculation à 130°C). Par conséquent, le modèle Weibull est recommandé pour décrire et modéliser l'inactivation des spores *G. stearothermophilus* en SS à des températures élevées (145–175°C). **Mots clés:** modélisation, spores, inactivation, *Geobacillus stearothermophilus*.

INTRODUCTION

Thermal inactivation of bacterial vegetative cells and spores has been traditionally presumed to follow the first-order kinetics model:

$$\log\left(\frac{N}{N_o}\right) = -kt \tag{1}$$

where N_o is the initial number of microorganisms (cfu/g), N is the number of microorganisms at time t (cfu/g), t is the treatment or processing time (s or min), and k is a rate constant (1/s or 1/min) (Peleg 2002; van Boekel 2002). From this equation the classic D-value (time necessary for one log reduction in the number of microorganisms) can be obtained by calculating the reciprocal of k ($1/k = D$). This simple first-order model is widely accepted within food industry because of its simplicity and usefulness for calculation of processing times needed to achieve commercial sterility (van Boekel 2002). Some researchers urge to re-examine the use of first-order kinetics model and the D-value for calculation of food sterility on industrial scale (Couvert et al. 2005; Peleg 2006).

According to the first-order kinetics model presented in a semi-log plot, the ratio of microorganisms surviving a heat treatment is expected to depend linearly on the duration of the treatment. However, it has been shown that in reality the ratio of surviving microorganisms is not always linearly correlated with treatment duration, and deviations such as shoulders, tails (biphasic curves), upward or downward concavity occur in survivor curves (Shull et al. 1963; Juneja and Marks 2005; Bialka et al. 2008). Heat destruction of bacterial spores is now believed to be dependent on the strain, treatment duration and temperature (thermal history) (Tremoulet et al. 2002), as well as on the presence of sub-populations of spores with different heat resistance arising from the spore physiological state (i.e., activated, dormant, or so-called ‘‘super dormant’’ spores) (Sapru et al. 1993; Iciek et al. 2006). In spore inactivation, the shoulders occurring in survivor curves have been mainly associated with the activation phenomenon, whereas tails have been considered to be the indication of resistance heterogeneity within the population of spores (Xiong et al. 1999; Manas and Pagan 2005). Simultaneously occurring phenomena of spore activation and inactivation may result in non-linearity of survivor curves obtained for spores (van Boekel 2002). A number of models have been proposed to describe the non-linearity in survivor curves; logistic (i.e., the Kamau model), vitalistic, as well as the Weibull distribution models (Kamau et al. 1990; Xiong et al. 1999; Guan et al. 2006). The latter models are simple (only two parameters determine the curve path) and versatile as they can accurately fit either straight, concave downwards or upwards survivor curves (Manas and Pagan 2005).

Superheated steam (SS), also known as dry steam, is generated when additional sensible heat is delivered to saturated (wet) steam, raising the steam temperature above the corresponding boiling point at a given pressure. Superheated steam, due to its high heat intensity and high processing temperatures (usually between 100 and 200°C), has a potential to largely reduce microbial load.

Geobacillus (formerly *Bacillus*) *stearothermophilus* is a Gram-positive, thermophilic, spore-forming bacterium often associated with plant food products. The spores of *G. stearothermophilus* are very heat resistant to moist heat, and may survive canning and sterilization operations. Flat-sour spoilage of properly processed low acid canned foods may occur if the products are stored at elevated temperatures (Ng and Schaffner 1997).

In our previous work (Head et al. 2008) the survivor curves obtained for *G. stearothermophilus* spores challenged with SS in the range of 130–175°C and a velocity of 0.35 m/s showed non-linear dependence on the treatment duration. Therefore, the objectives of this work were: (i) to apply the Weibull and Kamau models to the experimental data obtained from the aforementioned study; (ii) to assess the adequacy of fit of these models and recommend the best model describing inactivation of *G. stearothermophilus* spores exposed to SS.

MATERIALS and METHODS

Microorganism and maintenance

Geobacillus stearothermophilus ATCC 10149 (Charm Sciences Inc., Malden, MA, USA) was maintained on tryptic soy agar (TSA) slants (Difco Laboratories, Detroit, MI, USA) following growth on TSA (24–36 h; 55°C). Slants were maintained at 4°C for subsequent use.

Spore production

G. stearothermophilus spores were produced according to the method of Kim and Naylor (1966) with an addition of a freeze-drying step. The resultant spore crop was frozen in liquid nitrogen and freeze-dried using a Viritis 10–146MP-BA vacuum freezer (Viritis Inc., Gardiner, NY, USA). The dried spore material was lightly ground into a powder using a sterile mortar and pestle, and mixed with sterilized sand (commercially available decorative white sand). Spore concentration per gram of the mix was targeted so that 3 or 6 log cfu/g could be inoculated for treatment with SS. Spore concentration was estimated by serial dilution in 0.1% peptone (Difco Laboratories, Detroit) followed by direct plate counting on TSA (55°C for 24–36 h) (Swanson et al. 1992). The spore-sand mix was kept at 4°C before treatment with SS.

Superheated steam processing system

A superheated steam processing system developed in the Department of Biosystems Engineering at the University of Manitoba was used for challenging of the spores. The main components of the system were: a 9 kW electric steam generator (model MB9L; Sussman Electric Boilers, New York, NY, USA), steam conveying pipelines, a pressure reducing regulator (type 95L NPT; Fisher Controls International Inc., Marshalltown, IA, USA), an electric superheater (2 kW single phase heating element located in the pipe conveying steam), a processing chamber, a hot-air supply system to the jacket of the chamber, and a data acquisition and control system.

Briefly, pressurized saturated steam was produced by the steam generator. Superheated steam was generated by reducing the pressure of saturated steam to near 1 atmosphere. The steam was then conveyed via pipelines through the steam superheater, where its temperature was adjusted to the desired level. Superheated steam was then directed to the processing chamber (containing spore-sand mix) and subsequently condensed. During processing, hot air was forced through the air jacket surrounding the processing chamber to maintain adiabatic conditions in the chamber.

The sample tray consisted of two squares of Spectra nylon mesh (20 μm mesh opening, 14% open area, and 55 μm thickness; Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) housed between two tight-fitting aluminum rings (inner diameter 6.3 cm and outer diameter 7.5 cm). A layer of spore-sand mix (1 g) was placed between the two mesh squares in the sample tray. The sample tray was then placed in the processing chamber and exposed to SS for up to 30 s come-up time (depending on the operating temperature) before actual timing of the SS treatment commenced.

Treatment conditions and enumeration of surviving spores

Spore-sand mix was exposed to SS at temperatures of 130, 145, 160, and 175±2°C and a velocity of 0.35±0.02 m/s for different durations of time (0.5–100 min depending on temperature). Three trials were performed at each processing time. At each SS temperature, the experiments were performed long enough to achieve at least one log reduction in the number of spores, and to allow calculation of the classical *D*-value. The *D*-values were obtained from the slope of the regression line fitting the entire survivor curve at each temperature with one exception. When spores at the high inoculum were treated with SS at 130°C for up to 480 min, a one log reduction in spore survivors was not achieved. Therefore, the *D*-value for that temperature was estimated from the data obtained for the first 60 min of processing.

Surviving spores following exposure to SS were determined using serial dilution in 0.1% peptone (Difco Laboratories, Detroit, MI, USA) followed by duplicated spread plating on TSA medium, incubation at 55°C for 24–36 h, and enumeration. Results (reported as log cfu/g of the spore-sand mix) were used to calculate survival ratio, $\log(N/N_o)$, of spores at each processing time for construction of survivor curves.

Data analysis and model evaluation

Weibull model

The Weibull model takes into account the biological variation within a population of microorganisms with respect to thermal inactivation. As a result, the model is a statistical model of distribution of different inactivation times (van Boekel 2002) and has a cumulative form of:

$$\log\left(\frac{N}{N_o}\right) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta} \quad (2)$$

where N_o is the initial number of microorganisms (cfu/g), N is the number of microorganisms at time t (cfu/g), t is the treatment or processing time (s or min), α is the characteristic time or scale parameter (s or min), and β is the shape parameter (non-dimensional). In a semi-log plot, the Weibull distribution corresponds to a concave upward survivor curve when $\beta < 1$, concave downward curve if $\beta > 1$, and is linear if $\beta = 1$. The first-order kinetics approach (straight line in a semi-log plot) is therefore a special case of the Weibull model. Although the Weibull model is empirical, a link can be made with the physiological effects due to inactivating agents (i.e., heat) acting on microorganisms. For example, when $\beta < 1$, the remaining organisms have the ability to adapt to the applied stress and it is more difficult to inactivate them, whereas when $\beta > 1$, the remaining organisms become increasingly more damaged.

Historically, the Weibull model has been used in failure engineering to describe time to failure in electronic and mechanical systems (van Boekel 2002). The 90% percentile of the failure time distribution – called reliable life, t_R, can be calculated from the parameters α and β in the Weibull model as follows:

$$t_R = \alpha(2.303)^{\frac{1}{\beta}} \quad (3)$$

where α and β are the scale and shape parameters, respectively (van Boekel 2002). The t_R is analogous to the classic *D*-value when the first log reduction is considered (Manas and Pagan 2005; Buzrul 2007).

Kamau model

The Kamau model is a logistic model that can describe non-linear survivor curves and has a general form of:

$$\log\left(\frac{N}{N_o}\right) = \log 2 - \log[1 + e^{kt}] \quad (4)$$

where, N_o is the initial number of microorganisms (cfu/g), N is the number of microorganisms at time t (cfu/g), t is the treatment or processing time (s or min), e is the base of the natural logarithm ($e \approx 2.71$), and

$$k = 4[(dN/dt)_{max}]/N_o \quad (5)$$

The differential $(dN/dt)_{max}$ is the slope of survivor curve at the point of inflection (maximum killing rate), and $k/4$ is the maximum specific killing rate (Kamau et al. 1990).

Survivor curves having two distinct killing phases (biphasic curves) can be analyzed with the Kamau model in a two-term exponential form of Eq. 4:

$$\log\left(\frac{N}{N_o}\right) = \log\left[\frac{2n}{1 + e^{k_1 t}} + \frac{2(1-n)}{1 + e^{k_2 t}}\right] \quad (6)$$

where n and $(1-n)$ represent two fractions (sub-populations) of organisms differing in heat resistance, and k_1 and k_2 are the specific killing rates for the two sub-populations, respectively (Kamau et al. 1990). The above form of the model assumes exponential killing of both sub-populations, but at different, independent rates.

Model evaluation

Sigma Stat (version 3.5, Systat Software Inc., Point Richmond, CA, USA) was used for non-linear regression analysis and for determination of the parameters of the Weibull and Kamau models. The models were constructed using averages from two experimental data sets, and the third data set was used for model validation. Adequacy of the goodness of fit of each model to the experimental data was determined by the adjusted correlation coefficient (R^2_{adj}) and standard error of estimate (root mean square error, RMSE). The one with the highest value of R^2_{adj} and the lowest value of RMSE was selected as the best model describing *G. stearothermophilus* spore inactivation in SS. Additionally, the pattern of residuals [the difference between observed and predicted values of $\log(\frac{N}{N_o})$] calculated for predictions of each model was examined. Models were validated against a third experimental data set of $\log(\frac{N}{N_o})$. High values of the correlation coefficient (R^2) of this linear regression indicated high accuracy of the model.

RESULTS and DISCUSSIONS

Figures 1–3 show the fit of the Weibull and Kamau models (Eqs. 2 and 6, respectively) to the survivor curves obtained for *G. stearothermophilus* spores, at two inoculum levels, challenged with SS of different temperatures. The Weibull model fitted all survivor curves obtained for the spores at both inoculum levels (Figs. 1 and 2), whereas the Kamau model fitted only the survivor curves obtained for 130, 145, and 160°C at high inoculum, and for 160°C at low inoculum (Fig. 3). It is possible that the

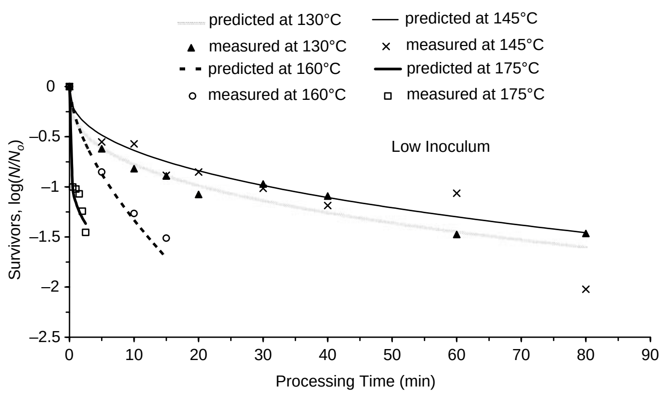

Fig. 1. Fit of the Weibull model (Eq. 2) to survivor curves obtained for *G. stearothermophilus* spores (at 3 log cfu/g, i.e., low inoculum level) challenged with superheated steam at 130–175°C.

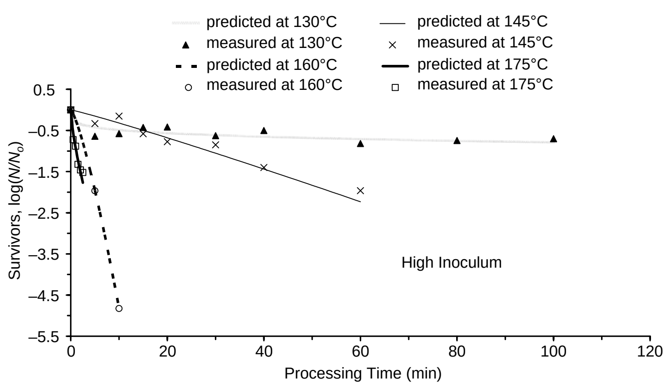

Fig. 2. Fit of the Weibull model (Eq. 2) to survivor curves obtained for *G. stearothermophilus* spores (at 6 log cfu/g, i.e. high inoculum level) challenged with superheated steam at 130–175°C.

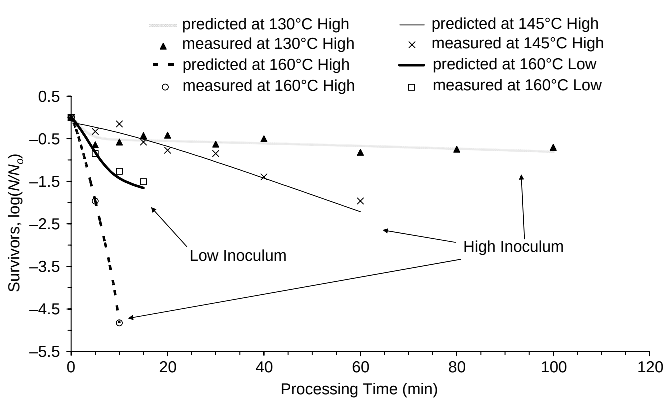

Fig. 3. Fit of the Kamau model (Eq. 6) to survivor curves obtained for *G. stearothermophilus* spores (at 3 and 6 log cfu/g, i.e. low and high inoculum levels, respectively) challenged with superheated steam at 130–160°C.

lack of success in fitting the Kamau model to all experimental data was caused by the fact that this model was proposed for describing inactivation of bacterial vegetative cells (*Listeria monocytogenes* and *Staphylococcus aureus*) and may not entirely apply to spore inactivation kinetics.

For each model, the residuals (difference between the observed and predicted values) of the survival ratio, $\log(N/N_o)$, of spores were calculated for each SS temperature and plotted against processing time (Figs. 4–6). There were no noticeable patterns of the residuals, and generally the spread of the residuals was random (especially for SS at 160 and 175°C), indicating a good fit to the experimental data. The parameters of goodness of fit (RMSE and R^2_{adj}) of the Weibull and Kamau models are summarized in Table 1. The RMSE measures the average deviation between the observed and fitted (predicted) values; thus smaller values of RMSE indicate a better fit of a model (Buzrul and Alpas 2004, 2007). Generally, smaller values of RMSE were obtained for the values of survival ratio at low inoculum level than those at high inoculum level. Values of the adjusted coefficient of correlation (R^2_{adj}) approaching 1 indicate a good fit of a model to the experimental data. Very high values of the R^2_{adj} were obtained for the Weibull model applied to the inactivation data obtained at low inoculum level at all SS temperatures (R^2_{adj} of 0.93–0.99). At high inoculum level, the R^2_{adj} of the Weibull model applied to experimental data for SS at 145–175°C were also very high (0.95–0.99), but for SS at 130°C the R^2_{adj} value decreased to 0.70, indicating a poorer fit of the Weibull model. The poorer fit of the Weibull model applied to data obtained for the SS treatment at 130°C (high inoculum level) may have been caused by a complex, irregular shape (concave upwards and concave downwards and substantial tailing) of the survivor curve.

For the models validation purposes, the correlation coefficient (R^2) and a slope of the regression line of the observed and predicted values of survival ratio were determined (Table 2). When data for spore inactivation in the SS treatment at 130°C was analyzed, the Weibull model provided more accurate predictions (indicated by R^2 and slope approaching 1) of survival ratio at low inoculum level than at high inoculum level, whereas

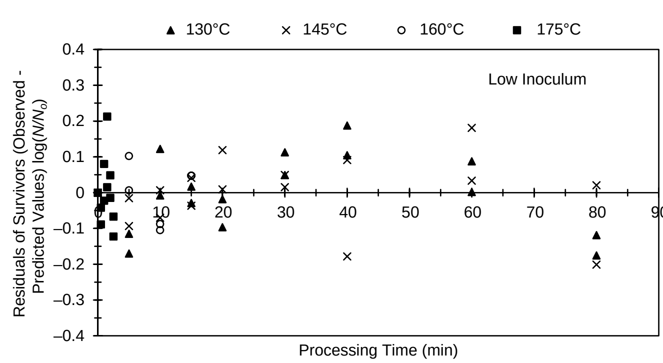

Fig. 4. Residuals of survival ratio obtained for the Weibull model (Eq. 2) predictions at low inoculum level (3 log cfu/g).

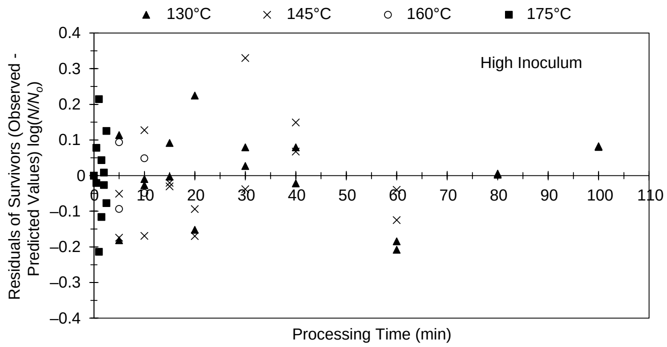

Fig. 5. Residuals of survival ratio obtained for the Weibull model (Eq. 2) predictions at high inoculum level (6 log cfu/g).

a reversed trend was noted for predictions at 145°C (Table 2). For higher SS temperatures (160 and 175°C) similar predictions of survival ratio were observed at both inoculum levels. Also, similar predictions of survival ratio were obtained from the Kamau and Weibull models fitted to the survivor curves for the same SS temperature (at the same inoculum level). Rajan and others (2006) tested two non-linear models (the Weibull and log-logistic models) for their applicability to describe inactivation of *Bacillus stearothermophilus* spores in egg patties exposed to pressure-assisted thermal processing. They reported that the Weibull model fitted more accurately to survivor curves of *Bacillus stearothermophilus* than did the log-logistic model.

Parameters of the Weibull and Kamau models obtained for spore survivor curves at each SS temperature and inoculum level are summarized in Tables 3 and 4, respectively. In most instances, the value of the shape parameter (β) in the Weibull model was less than 1, indicating concave upward shape of the survivor curves. The concave upward shape of survivor curves could indicate that spores became increasingly adapted to the heat as SS treatment progressed. Another explanation of the concave upward shape of curves could be phenomena of activation and inactivation of spores, which occurred simultaneously but at different rates during SS treatment. More research is needed to elucidate the physiological changes occurring in the spores exposed to SS. Fernandez et al. (2002) reported a mean value of 0.88 for the β parameter when the Weibull model was used to describe joint effect of temperature (80–95°C) and pH (4.7–6.2) on thermal inactivation of *Bacillus cereus* spores. In a study by Buzrul and Alpas (2007), the Weibull model was used to describe survivor curves of food-borne pathogens (*Listeria monocytogenes, Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus*) exposed to mild heat (60°C). Values of the shape parameter obtained for survivor curves of those pathogens were less than 1. The authors interpreted this as an evidence of the remaining cells adapting to stress, or perhaps being the sturdy ones. Couvert and others (2005), reported values of the β parameter in the range of 0.62 to 2.92, when spores of *Bacillus pumilus* were exposed to different heating temperatures (88–104°C), heating medium pH (4.7–7.0), and recovery medium pH (5.27–7.0). Conflicting reports on the value of the β parameter ($\beta < 1$ or $\beta > 1$) for the same microorganism, can be found in the literature. This contradiction may be explained by the fact that conditions of the cell (i.e., coming from the stationary or the exponential growth phase), the age of vegetative cell or spore, the presence of compounds in the heating medium, heating conditions (i.e., pH, ionic strength, etc.), and choice of recovery media and conditions, strongly influence thermal death kinetics (van Boekel 2002).

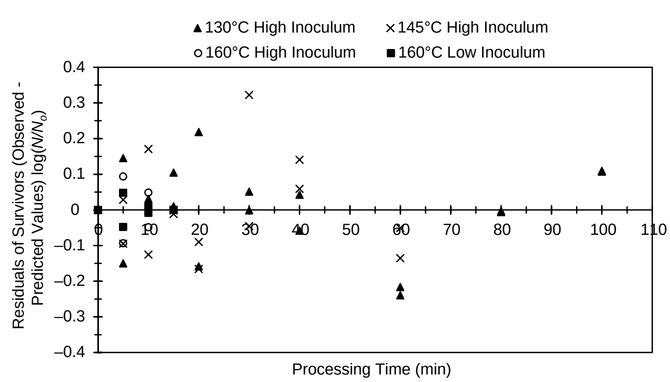

Fig. 6. Residuals of survival ratio obtained for the Kamau model (Eq. 6) predictions at low and high inoculum levels (3 and 6 log cfu/g, respectively).

Table 1. Goodness of fit parameters of the Weibull and Kamau models (Eqs. 2 and 6, respectively) predicting survival ratio of *G. stearothermophilus* spores challenged with superheated steam.

SS* temperature (°C)	Inoculum level (log cfu/g)	Model	RMSE**	$R^{2\dagger}_{adj}$
130	3	Weibull	0.11	0.93
		Kamau	–[§]	–
	6	Weibull	0.12	0.70
		Kamau	0.13	0.64
145	3	Weibull	0.10	0.94
		Kamau	–	–
	6	Weibull	0.14	0.96
		Kamau	0.14	0.96
160	3	Weibull	0.08	0.98
		Kamau	0.03	0.99
	6	Weibull	0.09	0.99
		Kamau	0.11	0.99
175	3	Weibull	0.10	0.94
		Kamau	–	–
	6	Weibull	0.12	0.95
		Kamau	–	–

*SS, superheated steam.
**RMSE, root mean square error (standard error of estimation).
† R^2_{adj}, adjusted coefficient of correlation.
§ Model did not fit the experimental results.

Table 2. Validation of the Weibull and Kamau models (Eqs. 2 and 6, respectively) – results of linear regression of the observed and predicted values of survival ratio of *G. stearothermophilus* spores challenged with superheated steam.

SS* Temperature (°C)	Inoculum Level (log cfu/g)	Model	Slope	R^2**
130	3	Weibull	1.05	0.96
		Kamau	–†	–
	6	Weibull	0.84	0.74
		Kamau	0.82	0.76
145	3	Weibull	0.75	0.86
		Kamau	–	–
	6	Weibull	1.12	0.97
		Kamau	1.09	0.97
160	3	Weibull	1.10	≈1.00
		Kamau	1.11	0.99
	6	Weibull	0.99	1.00
		Kamau	1.00	1.00
175	3	Weibull	1.00	0.96
		Kamau	–	–
	6	Weibull	1.10	0.96
		Kamau	–	–

*SS, superheated steam.
**R^2, correlation coefficient of the regression line.
†Model did not fit the experimental results.

The α and β parameters in the Weibull model can be used to calculate the reliable life (t_R), the time in which one log reduction in the number of surviving organisms will occur. The reliable life calculated for the first log reduction of an organism is analogous to the classic *D*-value often used in food industry for calculations of processing time, even though the two values have a different meaning (t_R has a probabilistic interpretation while *D*-value is the reciprocal of a first-order rate constant) (Buzrul 2007). The values of t_R obtained from the parameters of the Weibull model fitted to *G. stearothermophilus* spore inactivation data, as well as the *D*-values obtained from the first-order kinetics model applied to the same inactivation data [reported by Head et al. (2008)] are listed in Table 3. For spores at high inoculum level, the values of the t_R and the *D*-values were similar (except for the SS treatment at 130°C). However, the t_R values obtained for spores at low inoculum level were much lower than the classic *D*-values obtained by following the first-order kinetics. For spores at low inoculum level, over-processing could be avoided if the Weibull model was used rather than the classical first-order kinetics model to predict processing time needed for one log reduction of spores. Similar observation was made by Buzrul (2007), who applied the first-order kinetics model and the Weibull model to inactivation data of lactic acid bacteria (*Lactobacillus frigidus*) in beer. The author reported over-processing (when the target was one log reduction) of beer during pasteurization when the classical *D*-value obtained by following the first-order kinetics model was used instead of the t_R value obtained following the Weibull model.

From the regression results for the Weibull model it was noted that generally the α parameter had a decreasing trend, and the β parameter had a tendency to increase in their value with an increase of SS temperature (Table 3). The k_1 and n parameters in the Kamau model also appeared to depend on the SS temperature (Table 4). However in the case of the Kamau model, the regression analysis was based only on the three available points obtained for the data at one inoculum level, which may not be reliable enough to ensure whether the observed trend was factual or accidental. Dependence of the α and β parameters in the Weibull model on treatment temperature was studied by van Boekel (2002), who applied the Weibull model to inactivation data (taken from the literature) of microbial vegetative cells. Out of fifty-five case studies analyzed, seven case studies confirmed a linear dependence of the β parameter on the treatment temperature. However, the α parameter in its

Table 3. The parameters* of the Weibull model (Eq. 2) used to obtain values of survival ratio of *G. stearothermophilus* spores challenged with superheated steam.

SS** temperature (°C)	Inoculum Level (log cfu/g)	α	β	t_R†	*D*-value§
130	3	1.90	0.35	20.78	65.9
130	6	5.88	0.21	316.53	101.7
145	3	3.82	0.39	31.05	63.0
145	6	13.25	1.08	28.60	29.0
160	3	1.59	0.61	6.25	9.3
160	6	1.56	1.29	2.97	2.1
175	3	0.005	0.18	0.44	2.1
175	6	0.35	0.72	1.13	1.5

*α, scale parameter (min); β, shape parameter (non-dimensional).
**SS, superheated steam.
†t, reliable life (min) calculated from $t_R = \alpha(2.303)^{1/\beta}$, and analogous to the *D*-value for the first log reduction.
§*D*-values (min) were obtained by fitting the first-order kinetics model to the inactivation data (values reported by Head et al. 2008).

Table 4. The parameters* of the Kamau model (Eq. 6) used to obtain values of survival ratio of *G. stearothermophilus* spores challenged with superheated steam.

SS** Temperature (°C)	Inoculum Level (log cfu/g)	k_1	k_2	n
130	3	–[§]	–	–
130	6	0.011	0.660	0.314
145	3	–	–	–
145	6	0.092	4.605	0.761
160	3	0.088	0.554	0.051
160	6	0.986	0.805	1.168
175	3	–	–	–
175	6	–	–	–

*k_1, specific killing rate for organisms in n sub-population; k_2, specific killing rate for organisms in $1-n$ sub-population; n, fraction (sub-population) of organisms having heat resistance different from that of sub-population $1-n$.
**SS, superheated steam.
[§]Model did not fit the experimental results.

logarithm form depended linearly on temperature in fifty-two case studies.

The Weibull model fitted all survivor curves for both inoculum levels of *G. stearothermophilus* spores treated with SS and was effective at predicting the spore survival ratio for each SS temperature. Thus, the Weibull model was considered for investigation if survival of spores exposed to SS could be modelled when only SS temperature and processing time were known. Different values were obtained for the parameters of the Weibull model for each SS temperature when the model was applied to the experimental data. Therefore, a unifying equation for the Weibull model based on SS temperature was made. The α and β parameters were expressed as a linear function of SS temperature:

$$\alpha = a_2 - a_1 T \tag{7}$$

$$\beta = b_2 - b_1 T \tag{8}$$

where, a_1, a_2, b_1, and b_2 are coefficients, and T is SS temperature (°C). These relationships (Eqs. 7–8) of the model parameters were, therefore, inserted into the original equation of the Weibull model (Eq. 2) to obtain an expanded Weibull model equation:

$$\log\left(\frac{N}{N_o}\right) = -\frac{1}{2.303}\left(\frac{t}{(a_2 - a_1 T)}\right)^{(b_2 - b_1 T)} \tag{9}$$

Non-linear regression of the expanded Weibull model (Eq. 9) was performed (Table 5) and the values of survival ratio of *G. stearothermophilus* spores at each inoculum level were predicted based solely on SS temperature and processing time (Figs. 7–8). The residuals of the $\log(N/N_o)$ predictions (using the expanded Weibull model) were plotted against processing time for all SS temperatures at each inoculum level (Figs. 9–10). An examination of those plots indicated a good fit of the expanded Weibull model (Eq. 9) to the experimental data for spores at low inoculum level treated with SS at 130 and 175°C and a slightly poorer fit to the data for spores treated with SS at 145°C (over-prediction at processing time <60 min) and 160°C (under-prediction at processing time >5 min) (Figs. 7 and 9). The inactivation of spores at high inoculum level treated with SS at 160 and 175°C was described by the expanded Weibull model fairly well (Figs. 8 and 10). However, an over-prediction of the $\log(N/N_o)$ was seen for spores treated with SS at 145°C (at processing time <60 min) and under-prediction of the survival ratio was seen for spores treated with SS at 130°C (at processing time >5 min).

The results of the validation of the expanded Weibull model (Eq. 9) obtained for all SS treatments are summarized in Table 6. In general, the expanded Weibull model provided more accurate predictions of survival ratio for *G. stearothermophilus* spores at low inoculum level than at high inoculum level. Specifically, high values of the R^2 (0.82–0.97) obtained for all SS treatments of spores at low inoculum level indicated accurate predictions of the survival ratio. The inactivation of spores at high inoculum level was accurately predicted for the SS treatments at 145 and 160°C (R^2 of 0.82 and 0.99, respectively), less accurately predicted for the SS treatment at 175°C (R^2 of 0.70) and inaccurate predictions were obtained for the SS treatment at 130°C (R^2 of 0.17)

Table 5. Regression results for the expanded Weibull model (Eq. 9).

Inoculum level (log cfu/g)	Parameter	Coefficient	Regression value	RMSE*	R_{adj}^2**
3	α	a_1	0.0334	0.214	0.58
		a_2	5.8816		
	β	b_1	0.00002		
		b_2	0.3027		
6	α	a_1	0.0189	0.473	0.76
		a_2	4.358		
	β	b_1	−0.0501		
		b_2	−6.853		

*RMSE, root mean square error (standard error of estimation).
**R_{adj}^2, adjusted coefficient of correlation.

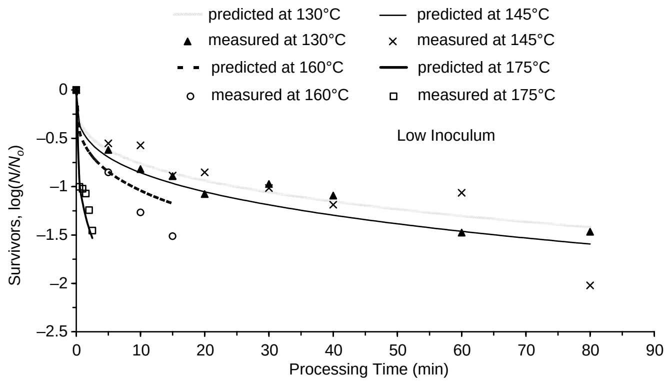

Fig. 7. Comparison of predicted results based on the expanded Weibull model (Eq. 9) and measured values of survival ratio for *G. stearothermophilus* spores at low inoculum level (3 log cfu/g) challenged with superheated steam at 130–175°C.

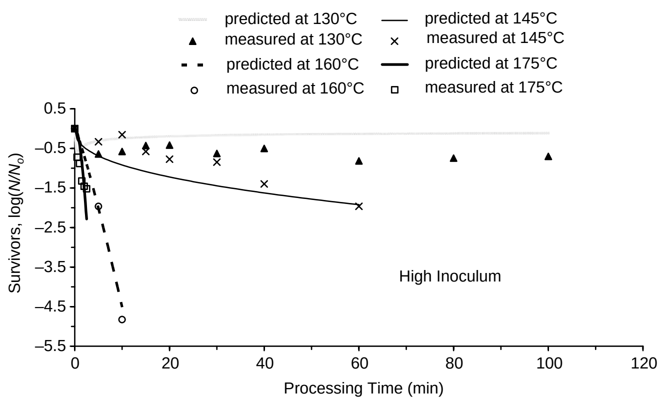

Fig. 8. Comparison of predicted results based on the expanded Weibull model (Eq. 9) and measured values of survival ratio for *G. stearothermophilus* spores at high inoculum level (6 log cfu/g) challenged with superheated steam at 130–175°C.

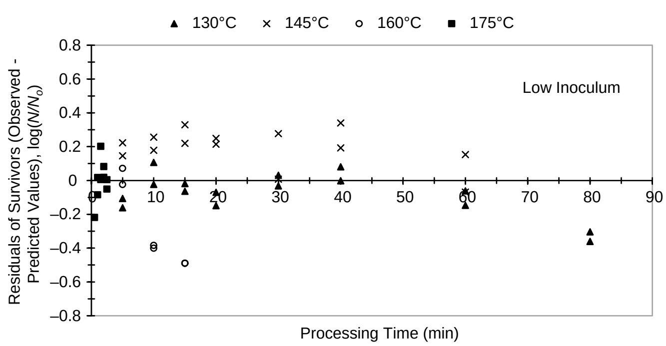

Fig. 9. Residuals of survival ratio obtained for the expanded Weibull model (Eq. 9) predictions at low inoculum level (3 log cfu/g).

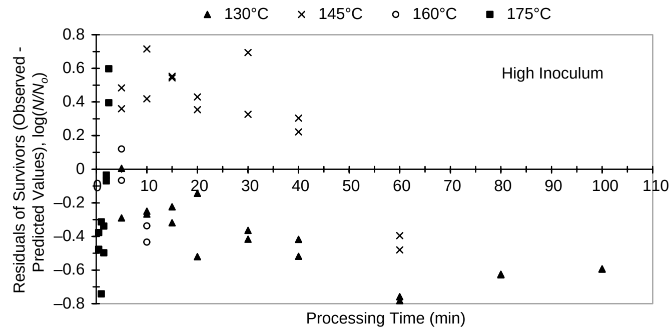

Fig. 10. Residuals of survival ratio obtained for the expanded Weibull model (Eq. 9) predictions at high inoculum level (6 log cfu/g).

(Table 6). It was noted earlier that the Weibull model did not fit well to the experimental data obtained for the SS treatment at 130°C (at high inoculum level). This was most probably caused by very irregular shape (concave upwards and concave downwards and substantial tailing) of that survivor curve. That initial poor fit of the Weibull model applied to experimental data at 130°C caused an even poorer fit, once the Weibull model was expanded to include mathematical relationships of SS temperature and processing time. Therefore, it was concluded that the Weibull model can be used for describing and modelling of inactivation of *G. stearothermophilus* spores with SS at higher temperatures (145–175°C). Use of such processing temperatures, however, may cause partial or total degradation of labile biological components in some foods, as well as shorten their storage stability.

CONCLUSIONS

The Weibull model successfully fitted all survivor curves obtained for *Geobacillus stearothermophilus* spores treated with SS, whereas the Kamau model did not fit the curves obtained for the SS treatment at 175°C at high inoculum

Table 6. Validation of the expanded Weibull model (Eq. 9) – results of linear regression of the observed and predicted values of survival ratio of *G. stearothermophilus* spores challenged with superheated steam.

SS* temperature (°C)	Inoculum level (log cfu/g)	Slope	R^2**
130	3	0.93	0.97
	6	0.15	0.17
145	3	0.78	0.82
	6	0.82	0.82
160	3	0.78	0.97
	6	0.93	≈1.00
175	3	1.09	0.97
	6	1.28	0.70

*SS, superheated steam.
**R^2, correlation coefficient of the regression line.

level and the curves obtained for the SS treatment at 130, 145, and 175°C at low inoculum level. A good fit of the Weibull model to survivor curves and a high correlation between the predicted and the observed values of survival ratio were obtained at all SS temperatures and for both inoculum levels (with the exception of data for spores at high inoculum level challenged with SS at 130°C).

The Weibull model was expanded by describing its parameters (α, β) as a function of SS temperature for the range of 130–175°C. This allowed accurate predictions of the spore survival ratio based on the SS temperature and processing time only (with the exception of predictions for spores at high inoculum level challenged with SS at 130°C). A further improvement of the Weibull model could be attempted by including the effects of SS velocity on the model parameters.

The Weibull model is recommended for describing and modelling of inactivation of *Geobacillus stearothermophilus* spores with SS at higher temperatures (145–175°C). The use of high SS temperatures, however, may be limited to products that are not thermally sensitive as their properties and storage stability may be compromised.

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