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Inactivation of *Salmonella enteritidis* during boiling of eggs

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Abstract

A series of inactivation curves for *Salmonella enteritidis* were determined for boiling eggs using different conditions of time and temperature. No significant influence of egg weight could be found on the temperature evolution in the yolk. The inactivation curves consistently showed an initial slow decline in bacterial number at lower temperatures, after which a very rapid inactivation took place. It was not possible to reproduce this behavior using a traditional inactivation model. A pragmatic model existing in two parts was therefore constructed. When the temperature is below a certain threshold, the inactivation follows a second order temperature dependence. Above the temperature threshold, standard Bigelow inactivation kinetics are assumed.

This model could describe the data reasonably well, provided that the decimal reduction time in the Bigelow model was assumed to be different for a fast or slow heating process, respectively. The results suggest that the bacteria are more resistant towards a slower heating process, which is confirmed by analyzing the raw data. A fail-safe model can be obtained by using the parameters associated with the slow heating process. The statistical properties of the calibrated model are satisfactory, and a cross-validation shows that it can be used for egg boiling conditions outside its calibration range.

Keywords: *Salmonella enteritidis*; Thermal inactivation; Eggs; Modeling

1. Introduction

It is well known that *Salmonella* infections are responsible for a high number of bacterial foodborne diseases (Mead et al., 1999). *Salmonella enteritidis* is reported as one of the most frequently isolated serotypes and is closely associated with eggs and egg products (McKellar and Knight, 2000). It is therefore important to assess the behavior of *S. enteritidis* in eggs and egg dishes (Baker et al., 1998). The percentage of eggs contaminated in the retail market is estimated as 0.01%, and 0.5–8.3% of the eggs produced by an infected flock may be contaminated (Chantarapanont et al., 2000). The number of *S. enteritidis* cells is generally quite low (<20/egg) (Humphrey et al., 1991), but temperature abuse can lead to a very high number of *Salmonella*, especially in the yolk (Schoeni et al., 1995). Adding to th erisk is the fact that *Salmonella* bacteria appear to have a

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higher heat resistance in liquid eggs and liquid egg yolks than in pure albumen (Muriana et al., 1996; Doyle and Mazzotta, 2000).

Boiled eggs are a very common type of egg dish in Belgium (Grijspeerdt et al., 1999). Three types of boiled eggs are generally distinguished: soft-, medium- and hard-boiled. The risk of microbial contamination is minimal for hard-boiled eggs (Baker et al., 1983; Humphrey et al., 1989; Chantarapanont et al., 2000), but medium- and certainly soft-boiled eggs are a potential risk factor for salmonellosis. According to Chantarapanont et al. (2000), the inactivation rate is dependent on the weight and the initial temperature of the egg. The required time to inactivate *S. enteritidis* is longer for larger and colder eggs. They also found that the American Egg Board boiling method (emerging the eggs in water of ± 23 °C, bringing the water to boiling with subsequent cooling) was more efficient than boiling the eggs in water at 100 °C for a certain time, as described by Humphrey et al. (1989). Baker et al. (1983) studied the survival of *S. typhimurium* in inoculated eggs during cooking and found that boiling for 7 min was necessary to kill all bacteria, for initial levels of contamination ranging between 10^4 and 10^7 CFU/ml. Hechelmann and Rödel (1993) came to the same conclusion.

To quantify in more detail the inactivation of *S. enteritidis* during the egg boiling process, the time–temperature trajectories together with the *Salmonella* bacterial count evolution were measured. A mathematical model for bacterial inactivation was then fitted to this data. Thermal inactivation of microorganisms is generally described by the thermal death time, or *D*-value, and the *z*-value that characterizes the temperature dependence of *D*. Both parameters have been used extensively to characterize thermal resistance of bacteria. Their use for dynamic conditions seems rather limited, however (Ross et al., 1998). The standard procedure for modeling bacterial inactivation or growth as function of temperature is essentially the same: several inactivation (or growth) curves are determined at constant temperature, after which, the temperature-dependence of the kinetic parameters is established. Due to the nature of the experimental conditions, a truly dynamic model is needed to model the *Salmonella* inactivation. It can be debated that parameter estimation could be more accurate when based on stationary data. It is, however, not the aim of this paper to take a position in this discussion; the fact is that it would be very difficult to reproduce the bacterial inactivation in constant temperature experiments that are representative for the conditions occurring during boiling of eggs due to the coagulation of egg yolk. The model describing the inactivation process at constant temperature is generally referred to as a primary model, the temperature functional dependence of the kinetic parameters as a secondary model (Whiting, 1995). Combining both types can lead to a truly dynamic model. Geeraerd (1999) gives an elaborate overview of primary and secondary bacterial inactivation models.

2. Experimental data

2.1. Time–temperature profile

A series of 12 weighed eggs were boiled in water. Each egg was brought in 1 l of boiling water and taken out after a progressively increasing time. Immediately thereafter, the temperature was measured in the yolk by putting the probe of a digital fast responding thermometer (Testo 926) through the eggshell. Trials were conducted with large (63–73 g) and medium (53–63 g) sized eggs. This measuring process was done starting with eggs at room temperature (slightly varying around 22 °C) and at refrigeration temperature (6 °C). This measurement series was done both for water maintained at boiling temperature and water starting at room temperature. The resulting temperature profiles are shown in Fig. 1. For all data series, each point measurement was repeated at least four times, except for the case of boiling eggs at room temperature starting from water at room temperature, where only two replications were used. For the second method of boiling (water starting from room temperature), the water temperature is plotted as well.

A simple heat transfer model (Eq. (1)) could very well describe the time–temperature profiles, as is evident by the high R^2 values (Table 1):

$$\frac{dT}{dt} = -h(T - T_{water})$$

$$T(0) = T_0 \qquad (1)$$

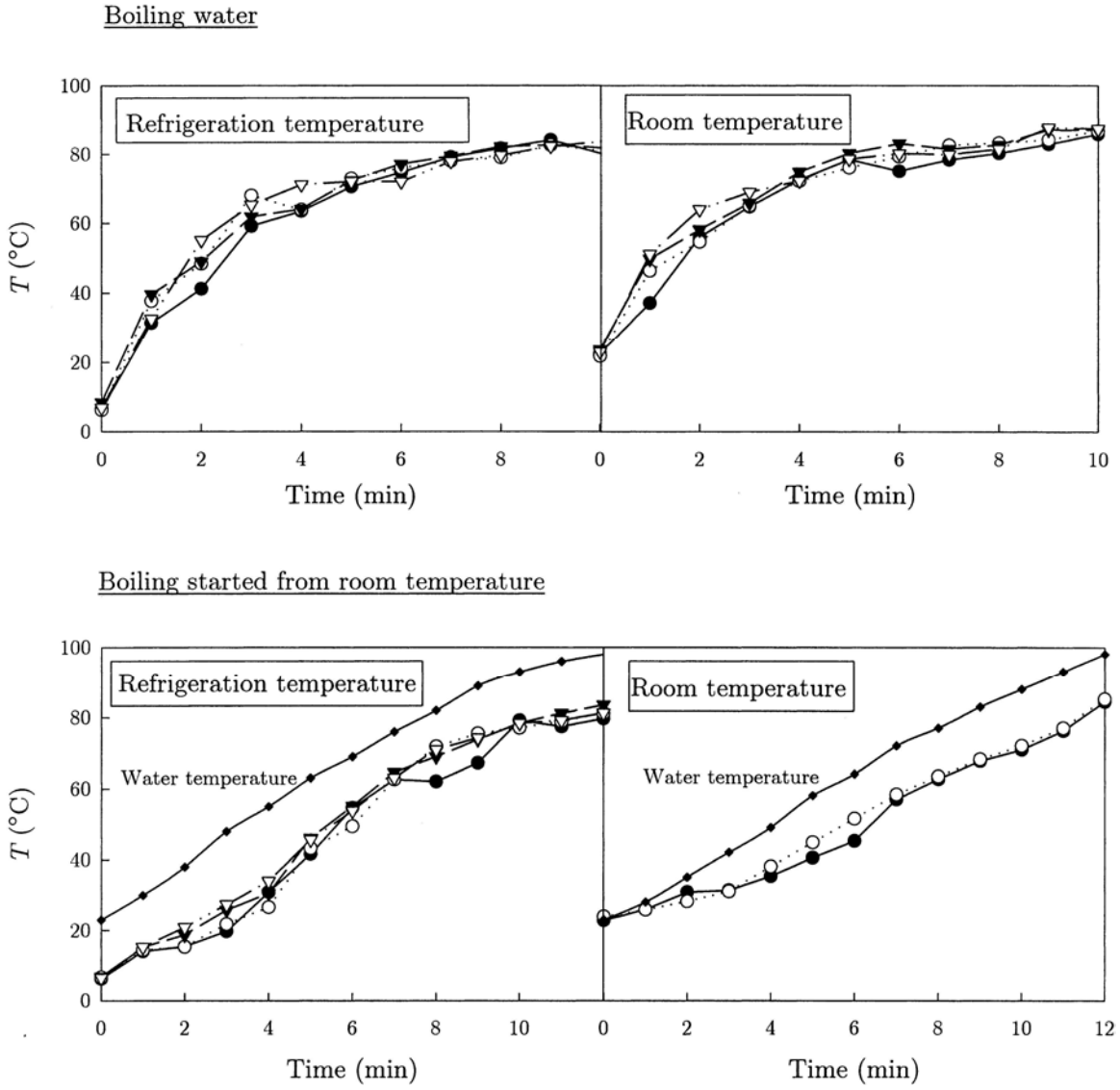

Fig. 1. The time–temperature profiles in the egg yolk during boiling for the different boiling methods. The different symbols denote replications. For the experiments started with water at room temperature, the water temperature is plotted as well.

With h as the overall heat transfer coefficient (min^{-1}). For constant water temperature T_{water}, Eq. (1) reduces to:

$$T = T_{\mathrm{water}} + (T_0 - T_{\mathrm{water}})\mathrm{e}^{-ht} \quad (2)$$

For the experiments using boiling water, Eq. (2) was used to fit the temperature curves, while Eq. (1) was used to fit the time–temperature profiles of the experiments starting from water at room temperature. Only one parameter, i.e. the estimated heat transfer coefficient, had to be estimated (Table 1). The confidence limits on these estimated coefficients overlapped for the replicated experiments, so it is safe to conclude that there is no significant difference between the replications. This implies that egg weight

Table 1
Estimations and confidence limits for the heat transfer coefficient

Starting temperature		Optimal value	95% lower limit	95% upper limit	R^2
Egg	Water				
Refrigeration	boiling	0.22	0.20	0.25	0.97
		0.24	0.20	0.29	0.95
		0.24	0.20	0.28	0.96
		0.25	0.20	0.29	0.95
Room	boiling	0.21	0.18	0.25	0.98
		0.23	0.20	0.26	0.98
		0.25	0.21	0.29	0.97
		0.25	0.20	0.30	0.97
Refrigeration	room	0.39	0.32	0.46	1.00
		0.42	0.33	0.51	0.99
		0.48	0.40	0.56	1.00
		0.49	0.40	0.57	0.98
Room	room	0.33	0.30	0.37	0.99
		0.37	0.34	0.40	1.00

is not a determining factor for its heating and that the starting temperature of the egg has no effect on the intervariability. Therefore, it seems justified to use the average temperature of the replicates for the model fitting. It must be remarked that the not-significant difference is only verified for the measuring frequency of 1 min. A shorter measuring interval could reveal differences, although this seems unlikely.

2.2. *Salmonella* concentration

A series of 15 medium-sized eggs, without visible shell defects, were inoculated with *S. enteritidis* MB1328 (DVK-CLO, Belgium), isolated from infected eggs. The yolk was injected with 100 μl of an overnight culture in Buffered Peptone Water (BPW, Oxoid, UK) using a sterile needle. The opening of the eggshell was then sealed with a drop of glue. The eggs were placed at 37 °C overnight to allow *Salmonella* to reach its asymptotic concentration of about 10^8 CFU/ml. The yolk would then be completely colonized by *Salmonella*, which guarantees a reasonably constant starting concentration in all the eggs (Hechelmann and Rödel, 1993). Due to inter-egg variability, it is very difficult to obtain a stable concentration when starting with intermediate *Salmonella* concentration. To verify that there is no concentration decline, five saturated eggs were measured over a time range of 8 h, with a frequency of 2 h. There was absolutely no indication of bacterial deterioration over this time span; the *S. enteritidis* concentration remained constant with a log mean value of 8.21 CFU/ml and a standard deviation of 0.22. The inoculated eggs were then stored at room temperature (± 22 °C) and in the refrigerator (6 °C), respectively. Analogously to the temperature measurements, a series of 12 eggs were boiled in water. With a measuring frequency of 1 min, the eggs were broken and the yolk was separated from the white. The yolk was homogenized in 10 ml Ringer (Oxoid) and plated out on xylose desoxycholate agar (XLD, Oxoid) and tryptic soy agar (TSA, Oxoid). After incubation at 37 °C during 24 h, the colonies on the XLD plates were counted. The TSA plates served as a control for detecting non-*Salmonella* contamination. Eggs contaminated with non-*Salmonella* bacteria were excluded from the study. The initial concentration of *Salmonella* in the yolk was determined by measurements on three non-boiled inoculated eggs. Each measurement series was done in duplicate.

The inactivation data are shown in Fig. 2, in a similar manner as the time–temperature profiles in Fig. 1. The scatter in the curves results from normal measurement error and variation due to the use of a different egg for each point. Using the outlined experimental procedure, it is not possible to distinguish between these two sources of error, so they will be considered as one. However, the trends in the curves are obviously the same; after an initial slow decline, the concentration drops very sharply. The concentration after this inactivation front was below the detection limit, so it was assumed that there was one cell left to allow logarithmic transformation of the values (Jarvis, 1989). Comparing Fig. 2 with Fig. 1 clearly indicates that this inactivation front seems to coincide with a yolk temperature between 75 and 80 °C, independent from the starting temperature.

3. Mathematical model

It was attempted to describe the data using the model of Geeraerd (1999), developed for accurately modeling the shoulder, linear and tailing parts that are often observed in microbial isothermal inactivation curves. The basic reasoning behind the model is comparable to that of the microbial growth model of Baranyi et al. (1993). The reader is referred to the

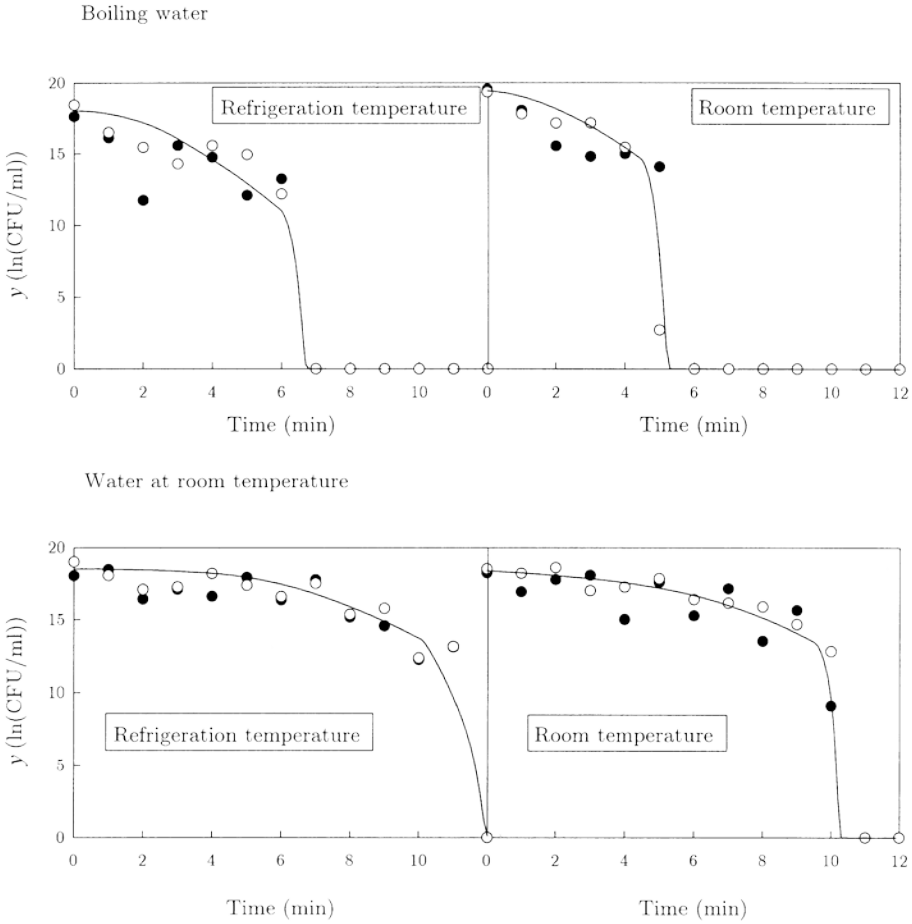

Fig. 2. The *S. enteritidis* inactivation curves and the model fit. The different symbols denote replications.

original paper for the mechanistic background of the model.

$$\frac{dy}{dt} = -k(T)\frac{1}{1+e^{q_p}}(1-e^{y_{res}-y})$$

$$\frac{dq_p}{dt} = -k(T) \quad (3)$$

With $y \triangleq \ln(x)$, $q_p \triangleq \ln(Q_p)$ and $y_{res} \triangleq \ln(x_{res})$. x is the bacterial concentration (CFU/ml) and x_{res} is the residual bacterial concentration (CFU/ml), which is connected with the tailing region of the isothermal inactivation curve. Q_p is a dimensionless parameter representative for the physiological state of the microbial population and is related to the shape of the shoulder. The specific inactivation rate k (min^{-1}) is dependent on temperature via the Bigelow model (Fujikawa and Itoh, 1998; Versyck et al., 1999):

$$k(T) = \frac{2.303}{D_{ref}} e^{\frac{2.303}{z}(T-T_{ref})} \quad (4)$$

D_{ref} (min) is the decimal reduction time, corresponding to the reference temperature T_{ref} (°C). z (°C) is the number of degrees change of temperature required to effect a tenfold change in D_{ref}.

A new model was constructed to allow fitting the observed inactivation profiles. The model is basically the traditionally used first order inactivation model,

while the temperature dependence of k is empirically adapted so that the model response can follow the slow initial inactivation front. Examining the derivatives of the inactivation curves revealed a sort of second order temperature dependence at lower temperatures.

$$\frac{dy(t)}{dt} = -k(T)$$

$$k(T) = \begin{cases} aT^2, & T < T_s \\ -\frac{2.303}{D_{ref}} e^{\frac{2.303}{z}(T(t)-T_{ref})}, & T \geq T_s \end{cases} \quad (5)$$

$$y(0) = y_0$$

There are four model parameters: a switching temperature T_s (°C), a proportionality constant a ($°C^{-2}$ min^{-1}) describing the inactivation behavior at low overall temperature, and D_{ref} and z, the well-known Bigelow parameters. The aim of this modeling approach was not to describe the observed behavior mechanistically, as this is outside the scope of this paper, but to minimize the influence of the initial slow inactivation on the model description of the sharp inactivation front. The latter falls within the area of interest from the point of view of microbiological food safety.

4. Model fitting

The Geeraerd model as well as the alternative model were simultaneously fitted to all available data sets using a non-weighted least-squares approach. All code was implemented in Mathematica 4.1 (Wolfram Research, Illinois). The minimization of the sum of squares was accomplished using the direction set method of Brent (1973). The integration of the differential equations was done using the built-in solver of Mathematica. For both models, the initial concentration y_0 was not estimated but the measured values were used. This is justified because the measured y_0 is the average of three independent measurements.

4.1. Geeraerd model

The model fit obtained with the Geeraerd model was not satisfactory, and the typical problem that occurred is illustrated with the fitting example shown in Fig. 3. The initial slow inactivation phase proved to be impossible to reproduce, causing the simulated inactivation front to be deformed. This model was developed for mild heat treatment and seems unable to cope with the steep temperature profile. Alternative sigmoidal inactivation models (Xiong et al., 1999) will give the same problem, as long as the temperature dependence model is not adapted. Therefore, another approach was followed in order to try to depict this phenomenon.

4.2. Alternative model

Fitting the alternative model as such to the data resulted in a non-optimal fit. The individual inactivation curves could be reproduced very well, but fitting the model simultaneously to all the data resulted in problems reproducing the inactivation front for the measurement starting from boiling water on the one hand, and the one starting with water at room temperature on the other. The shape of the fitted model appeared to be quite accurate, but the inactivation front fell somewhere in between the two extremes. Splitting up D_{ref} into a different D_{ref_1} and D_{ref_2} for both cases resulted in a good fit (Fig. 2). The model shows some systematic overestimation in the initial inactivation zone, but the inactivation front is very well simulated. The regression was highly

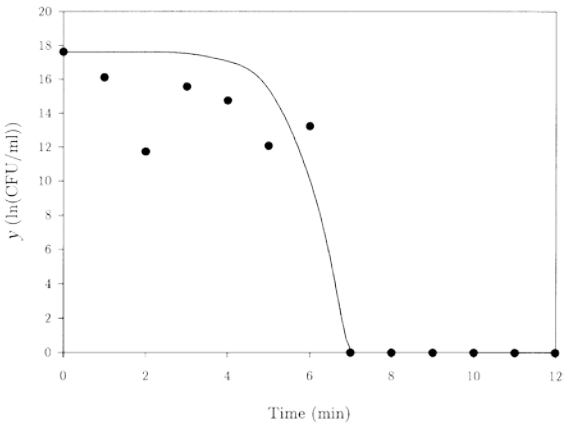

Fig. 3. Typical model fit of the Geeraerd model. For this example, the *Salmonella* inactivation data in eggs stored at refrigeration temperature and boiled in water of 100 °C was used.

significant, as was verified by an F-test (significance level $\alpha < 10^{-10}$) (Froment and Bischoff, 1990). The model was fitted to both measurements for each of the four experimental conditions. In Fig. 2, the simulated curve was only plotted for the average initial *Salmonella* concentration of the two replications, in order not to overload the figure. The optimal parameter values are indicated in Table 2.

In the case of experiments starting with boiling water, the bacteria are killed at a slightly higher yolk temperature than starting from water at room temperature, as indicated by the lower D_{ref} value in the former case. This could indicate that the bacteria are more heat resistant when they are subjected to a more gradually increasing temperature. This different heat resistance as a function of heating rate is already apparent after a closer examination of Figs. 1 and 2. As an objective measure of the heat treatment intensity, similar to the F_0-value for sterilization processes, the pasteurizing effect PE introduced by McKellar et al. (1994) can be used. It was calculated for each of the four experimental setups up to t_p, the time at which the inactivation front starts (Eq. (6)). t_p was calculated as the inflection point of an interpolating polynomial.

$$\mathrm{PE} = \frac{1}{t_0}\int_0^{t_p} e^{-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right)}\,dt \qquad (6)$$

where E_a = activation energy (394.470 kJ/mol, from Palumbo et al. (1995)), $T_0 = 345$ K and $t_0 = 15$ s. Table 3 shows the resulting PE values. The required PE is clearly considerably larger for the cases of gradual heating, in line with the model fitting results.

Adapting the model parameters depending on the environmental conditions is not the best of practices, certainly when considering the predictive capabilities of a model. When the most accurate predictions are necessary, one should use D_{ref_1} and D_{ref_2} according to the heating regime. To obtain fail-safe predictions, D_{ref_2} should be used.

Another possibility of handling this phenomenon would be to make D_{ref} an explicit function of the temperature history. Establishing and calibrating this functional relationship would require a considerable effort by doing dedicated experiments.

Table 2
Optimal parameter values and confidence ranges of the 95% joint confidence region for the new inactivation model

Parameter	Optimal value	Confidence range
T_s (°C)	66.74	{63.92–72.19}
a (°C^{-2} min^{-1})	2.8×10^{-4}	$\{2.29 \times 10^{-4}$–$3.57 \times 10^{-4}\}$
D_{ref_1} (65 °C)	555.32 min	{161.11–833.01}
D_{ref_2} (65 °C)	5496.34 min	{1455.56–8910.97}
z (°C)	3.44 °C	{3.33–4.01}

Table 3
PE values for the different experimental set-ups

Water	Eggs	t_p (s)	PE
Boiling	refrigeration temperature	6.5	0.33
	room temperature	5.3	0.68
Starting at room temperature	refrigeration temperature	11.5	2.12
	room temperature	10.4	2.52

4.2.1. Parameter significance and correlation

One of the most important questions in any regression exercise is whether the model parameters can be estimated significantly from the available data. Instead of determining the individual confidence intervals, as is done most often, the complete joint confidence region was calculated (Rosso et al., 1995). It is well known that individual confidence intervals, where all other parameters are kept at their optimum value, yield overly optimistic answers in the case of non-linear regression. The joint confidence region defines the region of joint parameter uncertainty, and it is determined when all the parameters vary simultaneously (Froment and Bischoff, 1990). It is constructed using the following expression:

$$\mathrm{SSE} \leq \mathrm{SSE}_{opt}\left(1 + \frac{p}{n-p}F(1-\alpha, p, n-p)\right) \qquad (7)$$

SSE is the residual sum of squares at a specific parameter combination, SSE_{opt} is the SSE at optimal parameter values, n is the number of experiments, p is the number of parameters and $F(1-\alpha,p,n-p)$, the classical F-distribution at confidence level $(1-\alpha)$% with $(p, n-p)$ degrees of freedom. To construct the joint confidence region, the parameter space has to be divided in a grid. In each grid node, the SSE has to be evaluated. When Eq. (7) is fulfilled, the parameter combination at the grid point falls within the confidence region.

The 95% confidence limits derived from the joint confidence region are summarized in Table 2. Although the estimation error for D_{ref_1} and D_{ref_2} are quite large, every parameter could be significantly estimated. Note that the intervals are not symmetric, which is a consequence of the nonlinearity of the model.

It is not only important to know whether the parameters can be significantly estimated, but also to know to what extent the estimates are intercorrelated. These correlations can be obtained by calculating the correlation matrix (Seber and Wild, 1989), which indicates a relative high correlation only between D_{ref_2} and z. It is not uncommon that the parameters of an Arrhenius-like model tend to be correlated (Nash and Walker-Smith, 1987). Optimal experiments can help to maximize parameter decorrelation (Bernaerts et al., 2000), but are not easy to implement in this real-life scenario. Note that the correlation between D_{ref_1} and D_{ref_2} is very close to zero, which was expected as they do not appear in the same experimental context.

	D_{ref_1}	D_{ref_2}	z	a	T_s
D_{ref_2}	1	-4.7×10^{-10}	0.65	−0.55	−0.22
D_{ref_2}	-4.7×10^{-10}	1	0.76	−0.21	−0.46
z	0.65	0.76	1	−0.50	−0.50
a	−0.55	−0.21	−0.50	1	0.86
T_s	−0.22	−0.46	−0.50	0.86	1

4.2.2. Model adequacy

Because there were replicated experiments available, the statistical adequacy of the model can be determined using the following F-value (Bates and Watts, 1988):

$$F_a = \frac{\dfrac{\mathrm{SSE} - \sum_{i=1}^{n_e}(n_i - \bar{n})^2}{n - p - n_e + 1}}{\dfrac{\sum_{i=1}^{n_e}(n_i - \bar{n})^2}{n_e - 1}} < F(1-\alpha, n-p-n_e+1, n_e-1) \quad (8)$$

with n_i as the individual experimental points and $\bar{n}$ the arithmetic mean of the n_e replicated observations. If F_a does not exceed $F(1-\alpha, n-p-n_e+1, n_e-1)$ then the model is statistically adequate at the $(1-\alpha)$% level.

For the case under study here, the total number of experimental points $n = 104$ and the number of model parameters $p = 5$. For each data set, all data points are replicated ($n_e = 52$). The estimation of the pure error variance is determined by determining the weighted arithmetic mean of the estimates at every individual replicate. Using this procedure, the model was shown to be statistically adequate ($F_c = 1.43 < F(0.95, 48,51) = 1.6$), meaning that the lack of fit is mainly due to the natural experimental variance and not due to the model structure itself.

5. Cross-validation

The model is statistically adequate and all regression coefficients are significant, but the true value of a model can only be tested when applying it to data not used to calibrate it. There were analogous time–temperature and *Salmonella* inactivation data available as the data used for the model fitting, but they were measured a year before by another analyst. The inactivation of *S. enteritidis* in egg was determined in duplicate, starting from boiling water. The time–temperature curve was measured as well. This time–temperature data was fed to the model and the inactivation curve was simulated. As can be seen

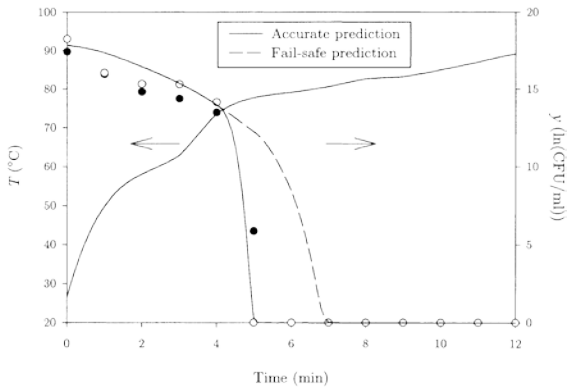

Fig. 4. Time–temperature profile and bacterial concentration measurements for the cross-validation data. Both the accurate and fail-safe model predictions are shown. The different symbols denote replications.

in Fig. 4, the model output describes the data quite well. The initial decline phase shows some systematic overestimation but the sharp inactivation front is captured very accurately. This illustrates the good predictive capacities of the model, at least for *Salmonella* inactivation in boiling eggs. The application in other egg dishes can be expected to lead to reasonable results, but should be interpreted with some caution, in view of the dependence of D_{ref} on the time–temperature trajectory. For fail-safe predictions, D_{ref_2} should be used. Such a fail-safe simulation is also indicated in Fig. 4.

6. Discussion

Although the exact boiling conditions were sometimes difficult to extract from the papers, the time–temperature profiles measured in the egg yolk during boiling in this study agreed quite well with the data from Baker et al. (1983) and Hechelmann and Rödel (1993), but not with those obtained by Chantarapanont et al. (2000). The temperature rise is considerably faster in our experiments. In these papers, the temperature in the yolk was measured in situ using a thermocouple. Chantarapanont et al. (2000) mention that the thermocouple was positioned as close as possible in the center of the yolk, while this is not clear in the paper of Baker et al. (1983). The temperature rise in the center will obviously be slower than at the border of the yolk. Our method introduces some inter-egg variability and there is a minor time-delay (in the order of 10 s) between the time sampling and the actual temperature measurement. The replications show that this variability is only minimal, and the cross-validation data show this to be true even between analysts. By putting the temperature probe through the shell in the yolk, the exact position of the temperature measurement is more diffuse, although it was tried to position the probe as close to the center of the yolk as possible. Taking the average of the replications, it can be expected that our measurement is more representative of the center temperature of the yolk as a whole, as opposed to a fixed probe position in the exact center of the yolk, of which it is not certain that it will be the cold spot since the yolk is not symmetrically oriented in the egg. Since we incubated the inoculated eggs, *Salmonella* will be present uniformly throughout the whole yolk, making it the logical choice to measure a more averaged temperature. The sharp inactivation front occurring during approximately the same measured temperature indicates that this is a justified approach. Chantarapanont et al. (2000) cooked the eggs only 30 min after inoculation, so that *S. enteritidis* would not grow and spread around the entire egg yolk, making it more logical to measure the exact spot of inoculation. From the modeling point of view, the assumption of a uniform temperature profile in the yolk is an approximation of reality, but could be dealt with by using multidimensional modeling. However, the required experimental and computational efforts would be considerable.

The heat source used by Chantarapanont et al. (2000) to cook the eggs appears to be less powerful than the one used in this paper, which is evident from the water temperature profile during the American Egg Board boiling process, comparable with the experiments starting from water at room temperature. This difference can certainly explain the slower temperature rise in the yolk in their study. But even considering the differences in the measuring locations in the yolk, the temperature differences seem still far off for the experiments starting with boiling water. We cannot really find a reasonable explanation for the differences between our results and those from Baker et al. (1983) on the one hand, and those from Chantarapanont et al. (2000) on the other. It is peculiar

that the *Salmonella* inactivation data presented by Chantarapanont et al. (2000) are in the same range as ours. To pick a specific example: a strain of *S. enteritidis* with a decimal reduction time of 6.4 min in yolk of 56 °C was completely inactivated in the yolk of a medium egg at starting temperature of 10 °C between 3 and 6 min in boiling water. The temperature at the measurement position in the yolk was 23.1 °C at 3 min and 50.7 °C at 6 min, respectively. It seems unlikely that all *S. enteritidis* would be killed by such a mild temperature treatment (corresponding with a PE of $\pm 2 \times 10^{-6}$), and it is even contradictory with the decimal reduction time that was determined independently in the same paper.

We could not find any influence of egg weight on the temperature profile, also in contradiction with Chantarapanont et al. (2000). Again, the location of temperature measurement could partly explain this observed difference. It has also to be remarked that Chantarapanont et al. (2000) did not do a statistical comparison between the temperature progresses in the two types of eggs.

An intriguing aspect of the inactivation curves presented here is the initial slow inactivation phase, where the bacterial concentration diminishes even at relative low yolk temperatures. This phenomenon is not expected and cannot be explained as a broad initial shoulder, because it could not be reproduced with the Geeraerd model. The fact that the *S. enteritidis* concentration did not drop in a saturated egg during 24 h storage at room temperature indicates that the high bacterial concentration is not responsible for this low-temperature inactivation. A similar slow initial inactivation at lower temperature was also described by Hou et al. (1996) who studied the pasteurization of intact shell eggs. This inactivation is probably due to temperature gradients in the egg yolk. The outer layer of the yolk will heat more rapidly than the center, so the bacteria in these outer layers will be killed off more rapidly. They represent only a fraction of the total bacterial concentration. Whatever the reason of this initial inactivation, it is not contributing significantly to the microbiological safety of the product, which is why it is accounted for empirically in the model used to fit the inactivation profiles.

The experimental results indicate that inactivation of *S. enteritidis* is complete after 7 min when boiling eggs at 6 °C in boiling water and after 5 min when the eggs were previously stored at room temperature (± 22 °C). This coincides roughly with a yolk temperature of ± 80 °C, slightly depending on the specific heating regime. Chantarapanont et al. (2000) found similar results, although their measuring frequency of the *Salmonella* inactivation was too crude to make a true comparison. Humphrey et al. (1989) came to different conclusions; according to their measurements, eggs should be boiled for at least 10 min to kill 10^8 CFU/ml *S. enteriditis*. In so far it is justified to make a comparison with the inactivation rate of *S. typhimurium*, our results are very consistent with Baker et al. (1983). When starting with water at room temperature, the inactivation time is between 10 and 12 min depending on the starting temperature of the egg. This is also in agreement with the results from Chantarapanont et al. (2000).

The modeling efforts presented in this paper illustrate the difficulty of extrapolating modeling results obtained using experiments with bacteria in ideal conditions to real-life situations. Existing inactivation models could not reproduce the slow bacterial inactivation at lower temperatures, which is consistently present in our experiment. The alternative model, using an empirical temperature dependence introduced here, was able to fit the data very well, and was demonstrated to be statistically sound. The fact that D_{ref} must be split up as a function of the heating rate in order to obtain an accurate model fit shows a shortcoming of the model. Probably, a more sophisticated temperature dependence model could help resolve this problem. The use of novel techniques such as neural networks could be of interest here (Hajmeer et al., 1997; Geeraerd et al., 1998), although the risk of overfitting is larger with these complex models.

The cross-validation example illustrates further that this calibrated model could be useful to predict *S. enteritidis* inactivation in boiling eggs. The extension to other egg dishes is straightforward, although it seems the best practice is to apply the fail-safe approach. The model could also be very beneficial to use in quantitative risk analysis studies (Whiting and Buchanan, 1997; Baker et al., 1998), where it could enhance the reliability of the final results.

Finally, it must be remarked that the conditions of the inactivation experiments presume a worst-case

scenario. The egg yolk is saturated with *S. enteritidis*, a condition that will occur in practice only after significant temperature abuse of the eggs. Assuming a deterministic model, model prediction validity should not be influenced by the initial concentration.

Notation

a	Proportionality constant ($^\circ C^{-2}$ min^{-1})
D_{ref}	Decimal reduction time corresponding to a reference temperature T_{ref} (min)
k	Specific inactivation rate (min^{-1})
n	Number of observations
n_e	Number of replications
x	Microbial population density (CFU/ml)
x_{res}	Residual microbial population density (CFU/ml)
p	Number of model parameters
PE	Pasteurizing effect
q_p	Natural logarithm of Q_p
Q_p	Dimensionless parameter representative for the physiological state of the microbial population
SSE	Sum of squared errors
t_p	Estimated time of inactivation front (min)
T	Temperature (°C)
T_s	Switching temperature (°C)
T_{ref}	Reference temperature (°C)
T_{water}	Water temperature (°C)
T_0	Initial yolk temperature (°C)
y	Natural logarithm of the microbial population density [ln(CFU/ml)]
y_{res}	Natural logarithm of the residual bacterial concentration [ln(CFU/ml)]
y_0	Natural logarithm of the initial bacterial concentration [ln(CFU/ml)]
z	The number of degrees change required for a tenfold change in D_{ref} (°C)

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