Modeling non-linear survival curves to calculate thermal inactivation of *Salmonella* in poultry of different fat levels

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Abstract

Survival curves of a cocktail of eight serotypes of *Salmonella* in ground poultry of different fat levels (1–12%), when heated rapidly to specified temperatures (58–65°C), were examined. Because many of the survival curves were concave, values for two parameters: the asymptotic *D*-value and the "lag" times were estimated and used to develop secondary models for estimating the time needed to obtain a 7 $\log_{10}$ relative reduction as a function of fat level and temperature. To compute the necessary time, at a given temperature and fat level, the estimated lag time should be added to the product of 7 and the estimated asymptotic *D*-value. A model was also developed for estimating the standard error of the estimated times, so that upper confidence bounds for the necessary times can be computed. It was found that lag times increase with higher fat levels. The effect of fat on *D*-values depended on the species; it is estimated that, for a given increase of fat level, the increase of the *D*-value would be greater for ground chicken than that for ground turkey. In addition, there was a statistically significant species effect on *D*-values, with higher *D*-values for ground turkey than for ground chicken at the higher temperatures studied. The thermal death curves displayed a non-linear tendency, however, for estimation purposes, a linear curve was assumed. There was not a statistically significant interaction effect of fat levels and temperatures on *D*-values, thus, for modeling, it was assumed that *z*-values were not dependent on the fat levels. The *z*-values for ground chicken and turkey were estimated to be 5.5°C and 6.1°C, respectively, and are statistically significantly different. These findings should have substantial practical importance to food processors of cooked poultry, allowing them to vary their thermal treatment of ready-to-eat poultry products in a safe manner. Published by Elsevier Science B.V.

Keywords: *D*-value; Lag times; Poultry; Concave survival curves; Spline regression; Non-linear regression

1. Introduction

Salmonella is a leading cause of bacterial food-borne disease outbreaks in the United States and continues to be a concern of public health significance. The disease caused by this organism, salmonellosis, is characterized by infection of the colon, leading to diarrhea, fever and abdominal pain, and in severe cases, dehydration and electrolyte imbalance (Zwadyk, 1992). The annual incidence in the US is 1.4 million cases, causing as many as 550 deaths (Mead et al., 1999). The cost per year, based

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on medical expenses, loss of income due to inability to work, and long-term effects of the infection, has been estimated at 1 million dollars (Snyder and Poland, 1990).

It was estimated that about 20% of raw broiler carcass samples and 45–50% of 25-g ground poultry samples in the US are contaminated with *Salmonella* (USDA-FSIS, 1996a,b,c). Most of the reported outbreaks (67%) are attributed to consumption of inadequately cooked contaminated animal products (Bean and Griffin, 1990; Tauxe, 1991). In an effort to guard against this pathogen, the United States Department of Agriculture-Food Safety and Inspection Services has implemented a 7 $\log_{10}$ relative reduction in population counts of *Salmonella* for fully and partially cooked poultry products (USDA-FSIS, 1999). The thermal-processing schedule to meet this lethality performance standard was not specified.

Cooking remains a primary means of eliminating pathogens from ground muscle foods and therefore, serves to protect against foodborne disease. Accordingly, teams of investigators have conducted thermal inactivation studies of different *Salmonella* serotypes in aqueous media and foods (D'Aoust, 1989). Various factors affecting the heat resistance have been documented, including growth temperature, stage of growth, initial population, bacterial strains, composition and pH of the heating menstruum, heat shock, and methodology used for detection of survivors (Tomlins and Ordal, 1976). Among these factors, nature of the suspending medium, particularly the fat level in meat, is very important. Fat level in meat have been documented to influence the bacterial heat resistance; *D*-value increases with the increase in fat content (Hansen and Riemann, 1963; Stumbo, 1973; Ahmed et al., 1995). However, no information is available in the published literature on the heat resistance of different *Salmonella* serotypes in poultry meat, as affected by fat levels.

The objective of this study was to quantify the heat resistance of a cocktail of eight serotypes of *Salmonella* in ground chicken and turkey containing 1–12% fat at temperatures between 58°C and 65°C. Secondary models to describe the lethality kinetics, as a function of fixed temperature and fat levels, are derived and used to estimate the time needed to obtain a 7 $\log_{10}$ relative reduction of *Salmonella*, extrapolating to temperatures greater than 65°C. In order to derive length of cooking time at a given temperature that would assure that the target lethality is obtained, estimates of standard errors of estimated times are derived and are used to construct upper 95% confidence bounds for them. The heat treatments required for a specified lethality, i.e., 7 $\log_{10}$ relative reduction of *Salmonella* in poultry, detailed in this study, could be used to support cooking times and temperatures that would inactivate salmonellae in the poultry meat, thereby minimizing the potential danger of foodborne infections from processed poultry meat.

Many of the survival curves were concave. In such situations, numerous researchers have stated that the *D*-values were measured from the "linear" portions of the curves. However, we have not seen a discussion of procedures used for identifying "linear" portions of survival curves. Clearly, some judgement is used in assigning the data points to be included in the "linear" portion of the survival curves. In this paper, spline and non-linear regressions are used to identify "linear" portions of concave survival curves.

2. Materials and methods

2.1. Organisms

A cocktail consisting of eight serotypes of *Salmonella* representing isolates from beef, pork, chicken, turkey or human clinical cases, was used in this study. The information about these strains is given in Table 1. These strains were preserved by freezing the cultures at −70°C in vials containing tryptic soy broth (TSB; Difco, Detroit, MI) supplemented with 10% (v/v) glycerol (Sigma, St. Louis, MO).

2.2. Products

Ground chicken and turkey were purchased from a local supermarket. To obtain meat with desired fat levels (low, medium and high), chicken or turkey was aseptically transferred to a sterile Waring Blender and mixed with the appropriate amount of same species fat. The proximate analysis of meats performed by Lancaster Laboratories (Lancaster, PA) indicated that the chicken contained: (i) 2% fat, 75%

Table 1
Salmonella serotypes cocktail sources

Cocktail strains	Strain designation	Source	Isolate
Salmonella Thompson	FSIS 120	FSIS	Chicken
S. enteritidis Phage type 13A	H3527	CDC	Clinical
S. enteritidis Phage type 4	H3502	CDC	Clinical
S. typhimurium Phage type DT 104	H3380	CDC	Clinical
Salmonella Hadar	MF 60404	FSIS	Turkey
Salmonella Copenhagen	8457	FSIS	Pork
Salmonella Montevideo	FSIS 051	FSIS	Beef
Salmonella Heildelberg	F5038BG1	CDC	Environmental

moisture, 1% ash and 21% protein: (ii) 6.3% fat, 72% moisture, 1.2% ash and 20% protein; (iii) 9% fat, 68% moisture, 0.89% ash and 19% protein; (iv) 12% fat, 69% moisture, 1.0% ash and 18% protein. The turkey contained: (i) 1% fat, 72% moisture, 1.0% ash and 24% protein; (ii) 7% fat, 72% moisture, 0.9% ash and 19% protein; (iii) 10% fat, 67% moisture, 1.0% ash and 17% protein; (iv) 12% fat, 68% moisture, 1.0% ash and 17% protein. The pH of the meats tested were determined using a combination electrode (Sensorex, semi-micro, A.H. Thomas, Philadelphia, PA) attached to an Orion model 601A pH meter. The meat was placed into stomacher 400 polyethylene bags (100 g/bag) and vacuum-sealed. Thereafter, five of these bags were vacuum-sealed in barrier pouches (Bell Fibre Products, Columbus, GA), frozen at −40°C and irradiated (42 kGy) to eliminate indigenous microflora. Random samples were tested to verify elimination of microflora by diluting in 0.1% (wt/vol) peptone water (PW), spiral plating (Spiral Biotech, Bethesda, MD; Model D) on tryptic soy agar (TSA; Difco) and incubating aerobically at 30°C for 48 h.

2.3. Culture preparation

To propagate the cultures, vials were partially thawed at room temperature, and 1.0 ml of the thawed culture was transferred to 10 ml of brain heart infusion broth (BHI; Difco) in 50-ml tubes and incubated for 24 h at 37°C. The inocula for use in heating tests were prepared by transferring 0.1 ml of each culture to 10-ml tubes of BHI and incubating aerobically for 24 h at 37°C. These cultures were maintained in BHI for 2 weeks at 4°C. A new series of cultures was initiated from the frozen stock on a biweekly basis.

A day before the experiment, the inocula for conducting the heating studies were prepared by transferring 0.1 ml of each culture to 10-ml tubes of BHI, and incubating aerobically for 18 h at 37°C to provide late stationary phase cells. On the day of the experiment, each culture was centrifuged ($5000 \times g$, 15 min, 4°C), the pellet was washed twice in 0.1% peptone water (wt/vol) and finally suspended in peptone water to a target level of 8–9 $\log_{10}$ cfu/ml. The population densities in each cell suspension were enumerated by spiral plating (Model D; Spiral Biotech) appropriate dilutions (in 0.1% peptone water), in duplicate, on tryptic soy agar (TSA; Difco) plates. Equal volumes of each culture were combined in a sterile test tube to obtain an eight-strain mixture of *Salmonella* (8 $\log_{10}$ cfu/ml) prior to inoculation of meat.

2.4. Sample preparation and inoculation

The cocktail of eight serotypes of *Salmonella* was added (10 ml) to 100 g of thawed, irradiated ground chicken or turkey. The inoculated meat was blended with a Seward laboratory stomacher 400 for 5 min to ensure even distribution of the organisms in the meat sample. Duplicate 5-g ground meat samples were then weighed aseptically into 30 × 19-cm sterile filtered stomacher bags (Spiral Biotech). Negative controls included bags containing meat samples inoculated with 0.1 ml of 0.1% (w/v) peptone water with no bacterial cells. Thereafter, the bags were compressed into a thin layer (approximately 1–2 mm thick) by pressing them against a flat surface, excluding most of the air, and then heat-sealed.

2.5. Thermal inactivation and bacterial enumeration

The bags containing the meat samples were incubated for 90 min at 4°C to achieve temperature equilibrium. Thereafter, the thermal inactivation

studies were carried out in a temperature-controlled water bath (Techne, ESRB, Cambridge, UK) stabilized at 58°C, 60°C, 62.5°C or 65°C, according to the procedure as described by Juneja et al. (1997). Bags for each replicate were then removed at predetermined time intervals and placed into an ice-water bath till analyses (approximately within 30 min). Surviving bacteria were enumerated by surface plating appropriate dilutions, in duplicate, on TSA supplemented with 0.6% yeast extract and 1% sodium pyruvate, using a spiral plater.

Samples not inoculated with *Salmonella* cocktail were plated as controls. Also, 0.1 and 1.0 ml of undiluted suspension were surface plated, where necessary. All plates were incubated at 28°C for at least 48 h prior to counting colonies. Incubation temperature of 28°C was used because researchers have reported that temperatures below the optimum for growth may enhance repair of heat-damaged cells (Katsui et al., 1982). Two independent experiments were done for each temperature–species combination. For each replicate experiment, an average cfu/g of two platings of each sampling point were used to determine estimates of the lethality kinetics.

3. Statistical methods

3.1. Primary inactivation model

The first step toward the goal of developing a predictive model of inactivation of *Salmonella* in poultry products was to examine, graphically, the observed survival data to determine the type of curve that needed to be modeled. The survival data cover between 5 and 6 $\log_{10}$ reductions of *Salmonella*, and appear, for the most part, to form curves that are either linear or concave followed by an asymptotic line. The negative inverse of the slope of the asymptotic line is called the asymptotic D-value, asym D, which, in this paper, will often be referred to as the D-value, D, for ease of notation. The projection of the asymptotic line on the time axis is called the lag time, Lag. For linear survival curves, Lag is 0. Thus, for a given temperature and fat level, the time needed to obtain an x $\log_{10}$ relative reduction, $t(x)$, can be approximated by: $t(x) = \text{Lag} + x$ asym D, for sufficiently large x.

A common and simple procedure for estimating these parameters is to perform the linear regression on the "linear" portion of the survival curve (eliminating the observations that do not appear to lie on a linear line). The estimate of the asymptotic D-value is the negative inverse of the estimated slope of the linear regression line, and the estimate of the lag time is the difference between the intercept of the linear regression line and the log of the observed level at time 0, divided by the negative of the slope. The problem with this procedure is that often, it is not clear the best way of identifying the linear portion of the curve. The consequences of incorrect identification of the linear portion of the curve could be either biases, in the case when not enough data points are deleted, or large variances, in the case when too many data points are deleted. Hopefully, by the careful examination of the fitted non-linear curves, significant bias is eliminated. Using estimates of non-linear survival curves involves estimating more parameter values and assuming specific assumptions (Bazin et al., 1988) for the shape for survival curves. However, it is not clear which set of curves to use, and that the use of any one set of them would not necessarily eliminate biases. The resulting estimates would be unstable, in part, because the numbers of observations at the beginning of the curves were small; per survival curve, there were only between six and eight measurements for times greater than 0. One advantage of using the "linear" portions of the curves for deriving estimates is that the estimation procedure can be replicated, simply, from the information provided in this paper, and thereby can easily be used by other researchers to develop more general models for prediction. When concave curves with asymptotic linear lines are assumed, selecting "appropriate" linear portions of the curves and then projecting outside the range of the data would provide reasonable estimates of the times needed to specified relative reductions of the cell population. As a consequence, for these reasons of stability, consistency, and ease of calculations, secondary models of asymptotic D-values and lag times were developed using the estimates from linear regressions from all the curves.

To help identify the "linear" portions of the curve and evaluate the estimates of the parameters values, non-linear curves were fit to the data, from which the

asymptotic D-values and lag times were also estimated. From the shape of the estimated non-linear curves, linear portions of the survival curve were assigned. Linear regressions were performed on the linear portions of the curves to derive estimates of the asymptotic D-values and lag times. These estimates were compared to the ones derived using the non-linear regressions. When a large discrepancy was noted, the curves were re-examined in an attempt to "explain" the discrepancy.

Different functional forms have been proposed for describing non-linear, concave survival curves (van Gerwen and Zwietering, 1998). Two forms are examined here. The first one is a spline function:

$$\ln\left(\frac{N(t)}{N_0}\right) = -bt - c(t-t_0)^+, \qquad (1)$$

where b, c and t_0 are non-negative valued parameters. The spline curve does not model the curvature that is expected in the shoulder of the concave survival curve, thus, it may not provide a good fit. When the fitted spline function did not provide a good fit, a second function that allows more curvature in the shoulder was used. In an attempt to model this curvature in a simple manner, the non-linear concave survival curves were estimated using the equation,

$$\ln\left(\frac{N(t)}{N_0}\right) = \ln\left(\frac{p(t,a,b)}{p(0,a,b)}\right) - ct, \qquad (2)$$

where $p(t, a, b)$ is a decreasing function with values between 0 and 1 and with first derivative, $p'(t, a, b)$, such that, as $t \to \infty$, $p(t, a, b) \to 0$, and a, b, and c are unknown parameters. A common and mathematical convenient function used in analyzing mortality data is the logistic (Thatcher, 1999),

$$p(t,a,b) = \frac{\exp(-b(t-a))}{1+\exp(-b(t-a))}, \qquad (3)$$

where a and b are parameters, $b > 0$. Substituting Eq. (2) in Eq. (3) derives

$$\ln\left(\frac{N(t)}{N_0}\right) = \ln\left(\frac{1+\exp(ba)}{1+\exp(-b(t-a))}\right) - (b+c)t. \qquad (4)$$

Finally, some of the observed survival data, at the lower temperatures, seemed to form convex shaped curves. Cerf (1977) offered various reasons for convexity or "tailing" of the survival curve, one of which assumed the existence of a vitalistic mechanism that would result in a variability of heat resistances within a population of apparently identical cells. The heat resistance is a genetic trait for an individual cell, and thus heat resistance is presumably a random variable with a distribution among the cells of a population. It is assumed that the probability of a cell, identified by the index j, surviving at time t, $S_j(t)$, can be written as: $S_j(t) = A(t)e^{-k(j)t}$, where $k(j)$ is a constant, referred to as the specific lethal hazard rate for the jth cell, and $A(t)$ is a function, here not specific to the cell, and such that derivative, with respect to t, of $\ln(A(t))$ approaches zero, as t approaches infinity. If there is no shoulder, so that for each cell of the population, simple first-order kinetics are assumed, then $A(t) = 1$. To describe the tailing effect, accordingly, it is assumed that $k(j)$ has distribution, F, over the population of cells, so that for the population, the proportion of the cells surviving at t would be

$$S(t) = A(t)\int e^{-kt} dF(k) = A(t)\phi_F(t), \qquad (5)$$

where $\phi_F(\cdot)$ is the Laplace transform of F. Thus, the survival curve can be described as

$$\ln\left(\frac{N(t)}{N_0}\right) = \ln(A(t)) + \ln(\phi_F(t)). \qquad (6)$$

Various distributions have been suggested (Bazin et al., 1988). Here, we consider two distributions: a normal distribution, so that $\ln(\phi_F(t)) = -\mu t + (vt)^2/2$, where μ is the mean and v is the standard deviation of F; and a gamma distribution, so that $\ln(\phi_F(t)) = -(\mu/v)^2 \ln(1 + v^2 t/\mu)$, where again μ is the mean and v is the standard deviation of F. For small v, this last expression can be approximated as $-\mu t + (vt)^2/2$. Thus, statistical evidence of tailing would exist if an estimate of v were statistically, significantly, different from 0.

The examination of these fitted curves was used to help determine the general shape of the survival curves, as explained in Results, below. All regression analyses used the logarithm of the observed

relative reduction of the measured levels as the dependent variable. For determining the values for the parameters in these models, SAS®-PC release 6.12, PROC MODEL was used.

3.2. Secondary models

To derive predictions for asymptotic D and lag time values, regression equations are developed. The common logarithm of the D-values, $\log_{10}(D)$, and the natural logarithms of 1 + the maximum of 0 and the lag times are the dependent variables. Temperature, the square of temperature, inverse of the temperature in the Kelvin scale, fat, the square and the square root of fat, and the products of these variables with the temperature variables were considered as independent variables in the regression. To account for the addition of 10-ml inoculum solution to the 100-g food matrix, the actual fat levels used in constructing the secondary models, described below, were the measured fat levels divided by 1.10.

Data analysis, described in the results section, was used to develop a model, $\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{E}$, where $\mathbf{Y}$ is the $n \times 1$ column vector of the independent variable, $\mathbf{X}$ is $n \times p$ matrix of dependent variables of temperature, fat and species, $\boldsymbol{\beta}$ is the $p \times 1$ matrix of parameter values, $\mathbf{E}$ is the $n \times 1$ matrix of error terms with covariance matrix, $\mathbf{V}$, and n is the number of observations. Since there are four temperatures and an equal number of observations per temperature, the value of n is a multiple of 4, $n = 4m$.

For the dependent variable, $\log_{10}(D)$, an analysis of variance on the residuals indicated that the variance component associated with temperature was a high percentage of the total variance. The signs positive or negative) of the residuals were highly clustered with temperature. Such a pattern could arise because there is a positive correlation of obtained results at the same temperature. Another possibility is that the model does not fit the data and here are systematic biases that are a function of temperature. There are four temperatures used in this study so that only simple, and at most quadratic, functions involving temperature can be used if estimates of the magnitude of the errors are desired. For the purposes of determining the precision of the predictions of asymptotic D-values for given values of the independent variables, the observations at a given temperature were considered as a random "block" and the covariance matrix of $\mathbf{E}$, $\mathbf{V}$, is assumed to take the form,

$$\mathbf{V} = \begin{matrix} \mathbf{C} & 0 & 0 & 0 \\ 0 & \mathbf{C} & 0 & 0 \\ 0 & 0 & \mathbf{C} & 0 \\ 0 & 0 & 0 & \mathbf{C} \end{matrix}, \tag{7}$$

where $\mathbf{C} = (\sigma_{ij})$ is an $m \times m$ intraclass covariance matrix, $\sigma_{ii} = \sigma^2$, and $\sigma_{ij} = \rho\sigma^2$, for $i \neq j$, σ is the standard deviation of individual results and ρ is the intrablock correlation, $0 < \rho < 1$. The variance, σ^2, can be written as $\sigma^2 = \sigma_b^2 + \sigma_w^2$, where σ_b^2 is the between-block variance, and σ_w^2 is the within-block variance, so that $\rho = \sigma_b^2/(\sigma_b^2 + \sigma_w^2)$. The minimum variance, unbiased, estimate of $\boldsymbol{\beta}$ is the same as the unweighted regression estimate of $\boldsymbol{\beta}$, and the covariance matrix of the estimate of $\boldsymbol{\beta}$, $\hat{\boldsymbol{\beta}}$, is

$$\mathrm{var}(\hat{\boldsymbol{\beta}}) = (\mathbf{X}^t\hat{\mathbf{V}}^{-1}\mathbf{X})^{-1}, \tag{8}$$

where $\mathbf{X}^t$ represents the transpose of the matrix $\mathbf{X}$, and $\hat{\mathbf{\beta}}$ is an estimate of $\mathbf{V}$. The estimate of $\mathbf{V}$ is made by using the PROC MIXED procedure of SAS®-PC version 6.12, with the REML option, which provides estimates of variances that are adjusted for the degrees of freedom. The degrees of freedom associated with the standard errors of predictions depend upon the values of the independent variables and are obtained by approximation procedures (SAS Institute, 1996). A heuristic explanation of the degrees of freedom is as follows: since the four blocks are defined by temperature and temperature is used as an independent variable in the regression, say as a polynomial of degree q, the degrees of freedom associated with the between-block variance component is assumed to be $df_b = 4 - (q + 1)$, and the degrees of freedom associated with the within-block variance component is assumed to be $df_w = 4(m - 1) - p + q + 1$. The degrees of freedom associated with the standard error of a prediction are estimated as an approximation (SAS Institute, 1996) and depend upon the degrees of freedom associated with the variance components. For example, if $q = 1$, $p = 6$ and $m = 8$ (as will turn out to be the case), then $df_b = 2$, and $df_w = 24$. The degrees of freedom that are approximated by the SAS® PROC MIXED

procedure associated with the estimates of the standard errors of predictions considered here range from about 2.1 to 2.5.

4. Results

Fig. 1a–h presents graphs of the observed survival data points and fitted survival curves using the non-linear models, described above in Eq. (1) using a spline regression, and Eq. (4) assuming a curved shoulder. Examination of the data and graphs revealed an apparent "outlier" observation at temperature = 62.5°C, time = 5 min, for the turkey matrix with 7% fat (Fig. 1d). The observed value of 5.57 $\log_{10}$ is just slightly lower than the observed value of 5.68 at 3 min and 1.4 $\log_{10}$ higher than the observed value of 4.205 $\log_{10}$ at 6 min. This pattern is inconsistent with the patterns for the other turkey matrices. Thus, the 5.57 $\log_{10}$ observed value was deleted from the analysis.

The curves for temperatures 62.5°C and 65°C appear, generally, to be concave with initial shoulders. For the data for the chicken matrices at 58°C and turkey matrices at 60°C, the observed curves often did not have "nice-curved" shoulders, and in some cases appear to have a convex shape. These latter set of curves were examined using Eq. (6) with $A(t) = 1$, $A(t)$ equal to a constant, assuming normal and gamma distributions for the specific lethal hazard rates for the cells in the population, with and without deleting the results at times equal to 0, and deleting the results at time equal to 15 min for the chicken matrices at 58°C. Generally, these fitted curves appear only slightly convex; the exception being the fitted curve for the two lowest fat turkey matrices at 60°C and the two highest fat chicken matrices at 58°C. For these four cases, one sided p-values were less than 0.10 for testing the standard deviation, $v > 0$. For these two chicken matrices at 58°C, when deleting the three observations at times less than 20 min, the measured D-values increased by about 10–15%, or about 0.05 $\log_{10}$ units, over those obtained using all the points; for these two turkey matrices at 60°C, when deleting the two observations at times less than 10 min, the measured D-values increased by about 25%, or 0.10 $\log_{10}$ units, over those obtained using all the points. Thus, from the 16 curves at temperatures 58°C and 60°C, only four seem to have substantial statistical evidence of a convex shape, and there does not appear to be a consistent pattern of occurrences. Consequently, we conclude that there is not sufficient evidence for assuming convex survival curves. For these curves, it is thus assumed that the linear model is true and the lag time is 0.

As stated above, the assignment of the "linear" portions of the observed survival curves was aided by results obtained from spline and non-linear regressions. When convergent and unbiased estimates were obtained for the spline regression, an initial assessment of the linear part of the curve was the line of the second regime (all the data points with time, $t \geq t_0$). In some cases, the assigned linear portion of the curve was not identical to that obtained from the spline regression, rather, one more or less data point was included or deleted to assure that the number of observations deleted was non-decreasing with respect to the fat level, since lag time seem to increase with fat level, or not to delete what would appear to be too many points, as with the case at 58°C for the high-fat turkey matrices. At 62.5°C, the non-linear curves defined by Eq. (4) were used to define linear portion of the survival curves.

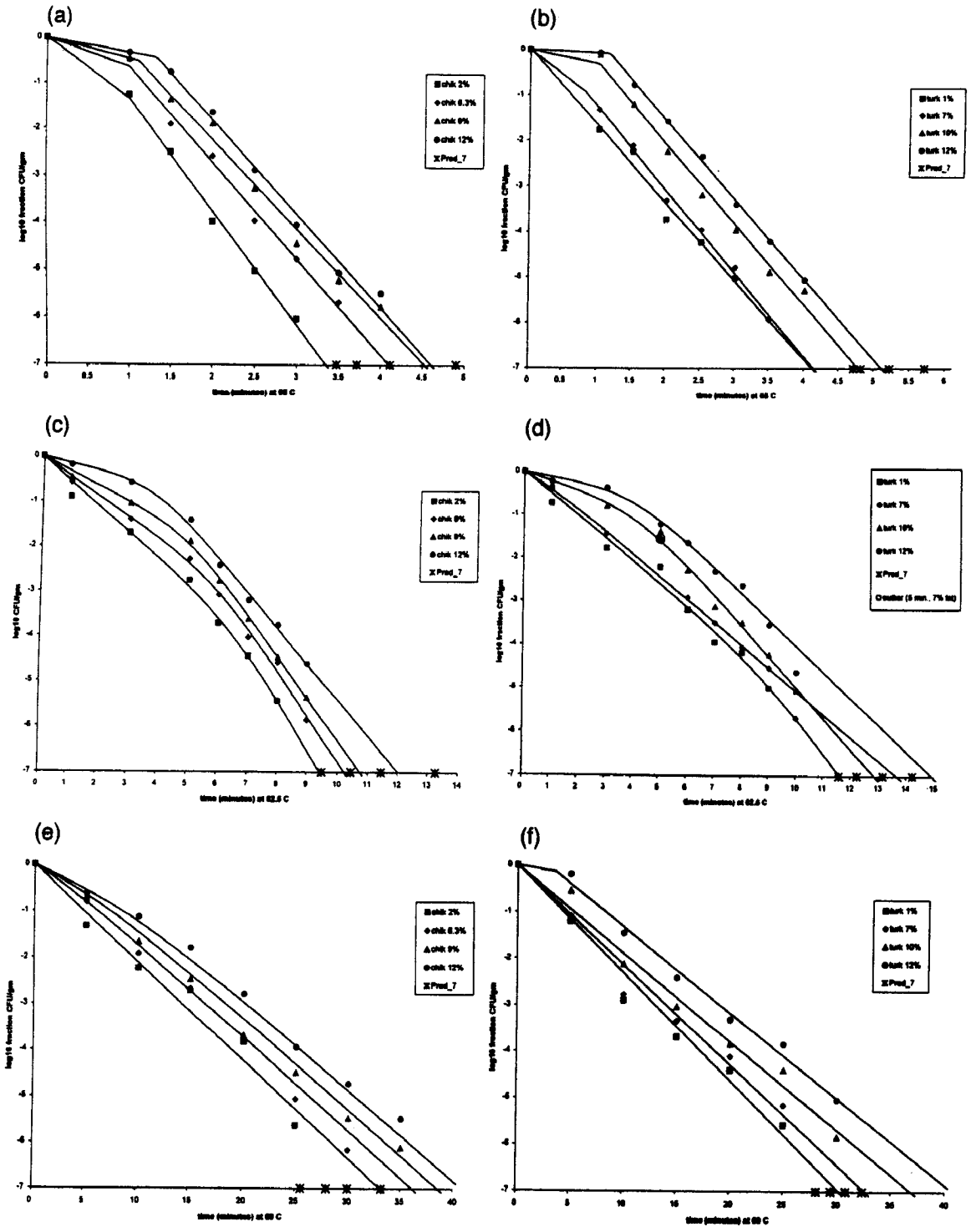
The numbers of observed data points that were excluded from the survival curves from the beginning (time = 0) of the curve are given in Table 2. A value of 0 means that the observed data at time = 0 was included; a value of 1 means that the observed data at time = 0 was excluded; a value of 2 means that the observed data at time = 0 and the subsequent time were excluded, and so forth. The asymptotic D-values and lag times are estimated from the remaining data using simple linear regression, as described above; the number of observations used are given in Table 2. These estimates for each species/fat level matrix are presented in Table 3.

4.1. Secondary model for D-values

Table 4 summarizes the D-values determined from the regressions of "linear" portions of the survival curves. Fig. 2 is a plot of the thermal death curve ($\log_{10}$ asymptotic D-values obtained from the linear regression vs. temperature), together with quadratic

regression lines for the chicken and turkey matrices. The $\log_{10}$ D-values do not appear to lie on a straight line.

To determine the degree of the polynomial in temperature, q, in a secondary model, the $\log_{10}(D)$ values are averaged for a given temperature, T.



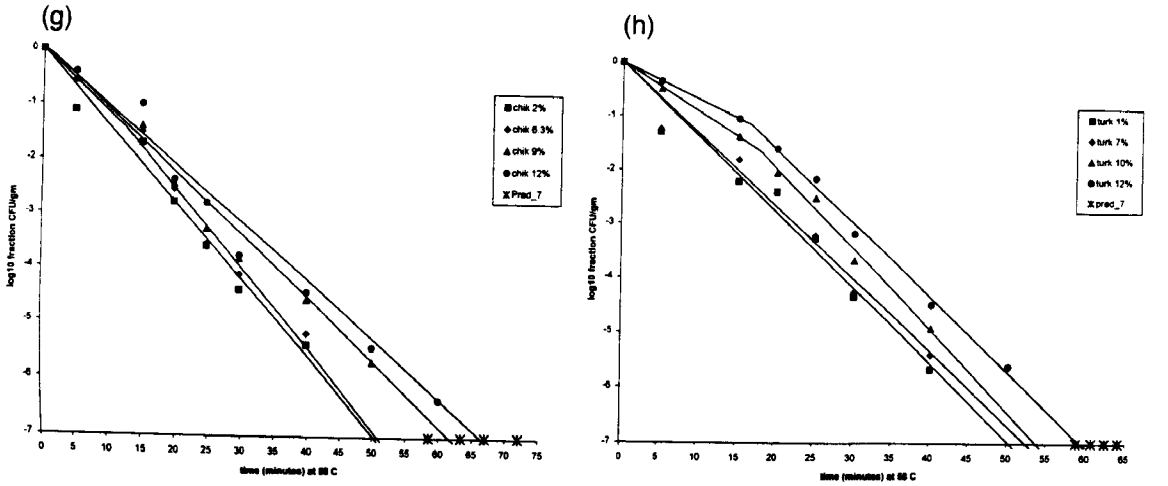


Fig. 1. Observed and fitted non-linear survival curves for given species and temperature. Star points (*) are predicted times needed for a 7 $\log_{10}$ relative reduction, based on secondary models. Each figure has curves for the four fat levels used in the study for each species, as indicated in graphs, e.g. chick 2% represents results for the 2% fat chicken matrix: (a) chicken at 65°C; (b) turkey at 65°C; (c) chicken at 62.5°C; (d) turkey at 62.5°C; (e) chicken at 60°C; (f) turkey at 60°C; (g) chicken at 58°C; (h) turkey at 58°C.

When a regression using a linear term in temperature is performed ($q = 1$), the root mean square error (RMSE, sum of the squared residuals divided by the degrees of freedom associated with the residuals), is 0.0859 $\log_{10}$(min) with 2 *df*. When the quadratic term is included ($q = 2$), then the RMSE increases to 0.1007 $\log_{10}$(min) with 1 *df*. If a squared term of temperature is used alone (with intercept), then the RMSE is 0.0804 $\log_{10}$(min), with 1 *df*. While the latter regression provides a slightly lower RMSE, it is not selected for use here because the usual assumed shape of thermal death curves is linear. In addition, using a quadratic polynomial, while providing in some sense closer predictions to the observed values, results in larger standard errors of the predictions for temperatures above 65°C than those obtained when using a linear term in temperature. Thus, the secondary model will include only linear terms of temperature.

From graphical and statistical examination of the data, the actual model used for predicting asymptotic *D*-values is

$$\log_{10}(D) = a + a\text{Spec}\,\delta_s + (b + b\text{Spec}\,\delta_s)T + cF + c\text{Spec}\,\delta_s F, \quad (9)$$

where δ_s is a dummy variable for the species, defined as -1 for chicken and $+1$ for turkey, F is the adjusted fat level, and a, aSpec, b, bSpec, c, cSpec, are unknown constants to be estimated from the data. The estimates of the values of these six parameters, with standard errors computed from Eq. (8), are given in Table 5. The estimate of the standard deviations and intrablock correlation used in calculating the standard errors from Eq. (8) are: $\sigma = 0.0930$ $\log_{10}$(min), $\sigma_w = 0.0382$ $\log_{10}$(min) and $\rho = 0.831$. Using different powers of fat, such as the square root or square, or adding a term for the fat–temperature interaction did not improve the goodness of fit of the equation. From the moderate significance of the

Table 2
Number of observed data points excluded and, in parentheses, included from observed survival data for a given temperature and poultry matrix, for estimating the *D*-value and lag time in the linear portion of the survival curve

Temperature (°C)	Chicken with fat				Turkey with fat			
	2%	6.3%	9%	12%	1%	7%	10%	12%
58	0 (7)	0 (7)	0 (8)	0 (9)	0 (7)	0 (7)	2 (5)	2 (6)
60	0 (6)	1 (6)	1 (7)	2 (6)	0 (6)	0 (6)	1 (6)	1 (6)
62.5	3 (4)	3 (5)	3 (5)	3 (5)	3 (5)	4[a] (5)	3 (6)	3 (6)
65	1 (5)	2 (5)	2 (6)	2 (6)	0 (6)	1 (6)	1 (7)	1 (7)

[a]Includes outlier data point that was excluded from analysis.

Table 3
Estimates of D-values and lag times (min) for survival curves using linear regression

Temperature (°C)	Species	Fat (%)	Adjusted fat (%) [fat/1.10]	D-value (min)	Lag time (min)
58.0	Chiken	2	1.82	7.38	−0.84
58.0	Chiken	6.3	5.73	7.33	0.87
58.0	Chiken	9	8.18	8.54	−0.25
58.0	Chiken	12	10.91	9.04	0.43
60.0	Chiken	2	1.82	4.83	−0.17
60.0	Chiken	6.3	5.73	4.68	1.55
60.0	Chiken	9	8.18	5.40	1.00
60.0	Chiken	12	10.91	5.50	4.29
62.5	Chiken	2	1.82	1.14	1.82
62.5	Chiken	6.3	5.73	1.16	2.40
62.5	Chiken	9	8.18	1.16	2.79
62.5	Chiken	12	10.91	1.30	2.99
65.0	Chiken	2	1.82	0.41	0.44
65.0	Chiken	6.3	5.73	0.51	0.56
65.0	Chiken	9	8.18	0.53	0.82
65.0	Chiken	12	10.91	0.50	1.09
58.0	Turkey	1	0.91	7.50	−1.24
58.0	Turkey	7	6.36	7.71	−0.85
58.0	Turkey	10	9.09	6.91	5.99
58.0	Turkey	12	10.91	7.41	7.71
60.0	Turkey	1	0.91	4.56	−1.04
60.0	Turkey	7	6.36	4.94	−1.08
60.0	Turkey	10	9.09	5.13	0.59
60.0	Turkey	12	10.91	5.43	2.77
62.5	Turkey	1	0.91	1.53	1.34
62.5	Turkey	7	6.36	1.85	0.56
62.5	Turkey	10	9.09	1.45	2.77
62.5	Turkey	12	10.91	1.78	2.95
65.0	Turkey	1	0.91	0.59	0.00
65.0	Turkey	7	6.36	0.55	0.29
65.0	Turkey	10	9.09	0.57	0.81
65.0	Turkey	12	10.91	0.59	1.03

species–fat interaction, it appears that the effect of fat on D-values is more pronounced for chicken matrices than it is for turkey matrices. The lack of significance of the interactions involving fat and temperature implies that the relationship of the effect of fat on the $\log_{10}$ D-values does not depend significantly upon the temperature; that is, z-values are not dependent upon the fat levels. This lack of statistical significance does not mean, necessarily, that there is actually no temperature–fat interaction effect; for the chicken matrices, the z-values decrease with increasing fat levels for the three fat levels: 6.3%, 9% and 12%. However, for the lowest fat level the estimated

Table 4
D-values (min) determined from linear regression of "linear" portions of survival curves

Fat (%) ranks[a]	Temperature (°C)							
	58		60		62.5		65	
	Chik[b]	Turk[c]	Chik	Turk	Chik	Turk	Chik	Turk
1	7.38	7.50	4.83	4.56	1.14	1.53	0.415	0.589
2	7.33	7.71	4.68	4.94	1.16	1.85	0.514	0.552
3	8.54	6.91	5.40	5.13	1.16	1.45	0.529	0.569
4	9.04	7.41	5.50	5.43	1.30	1.78	0.502	0.592
Mean	8.08	7.38	5.10	5.01	1.19	1.65	0.490	0.575

[a]Increasing order; (Chiken: 1 = 2% fat, 2 = 6.3% fat, 3 = 9% fat and 4 = 12% fat. Turkey: 1 = 1% fat, 2 = 7% fat, 3 = 10% fat and 4 = 12% fat).
[b]Chik: chicken.
[c]Turk: turkey.

z-value was approximately equal to that for the highest fat level, and for the turkey matrices, this relationship did not exist. Thus, the model assumes that z-values are not dependent upon the fat levels.

The estimate of the z-value for chicken ($= -(b - b\text{Spec})^{-1}$) is 5.46°C and for turkey, ($= -(b + b\text{Spec})^{-1}$) it is 6.13°C. From Table 5, bSpec is statistically significant from 0 with corresponding t-value of approximately 2.68 and p-value equal to 0.013 (assuming 24 df). Thus, the two estimated z-values are statistically significantly different. The standard errors of these estimates are both approximately 0.10 min.

The standard errors of the predictions, which were calculated using PROC IML of SAS®-PC, edn. 6.12,

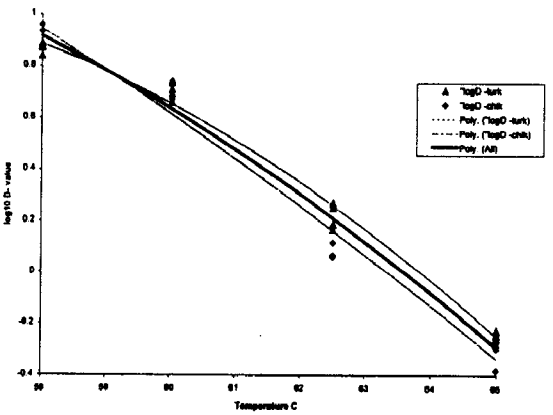


Fig. 2. Plot of $\log_{10}(D\text{-value})$ vs. temperature (°C) and quadratic regression lines for chicken and turkey matrices, separately (dotted lines) and all together (solid line).

Table 5
Estimates of regression coefficients of predicting $\log_{10}(D$-values) with standard errors
The equation is: $\log_{10}(D) = a + a\text{Spec}\,\delta_s + (b + b\text{Spec}\,\delta_s)T + (c + d\text{Spec}\,\delta_s)F$, where T is the temperature (°C), F is the fat level (%) and δ_s is a dummy variable for the species effect (−1 for chicken, +1 for turkey).

Parameter	Estimate	Standard error
a (intercept)	10.962	1.000
aSpec (species effect)	−0.5736	0.1582
b (temperature effect)	−0.1732	0.0163
bSpec (temperature × species effect)	0.01002	0.00257
c (fat effect)	0.00511	0.00191
cSpec (fat × species effect)	−0.00308	0.00191

depend upon the values of the temperature and fat. Within the range of the observed data, the standard errors of the predicted $\log_{10}$ D-values, using Eq. (9), range from 0.047 to 0.075 $\log_{10}$(min). The variance of the predicted values of $\log_{10}(D)$ can be expressed as a quadratic response surface function in temperature and fat.

$$\text{var}(\log_{10}(\text{asym}\,D)) = \sum_{0 \le i+j \le 2} a_{ij} T^i F^j. \qquad (10)$$

The coefficients of the variables in Eq. (10) are, a_{00} (intercept) = 1.0297; a_{10} (coefficient of T) = −0.03348, a_{20} (coefficient of T^2) = 2.728×10^{-4}, a_{01} (coefficient of F) = -9.804×10^{-5}, and a_{02} (coefficient of F^2) = 7.285×10^{-6}. The species effects and the coefficient of the interaction term a_{11} hardly affect the variance estimate, and thus can be ignored. The largest relative difference between the actual and predicted variances is 1.5%, which occurs for a small actual variance and represents a difference, in absolute value, of 0.000031. This difference is also the largest one in absolute value. Thus, Eq. (10), with values of the parameters given above can be used to approximate the variance of the predicted $\log_{10}(D)$.

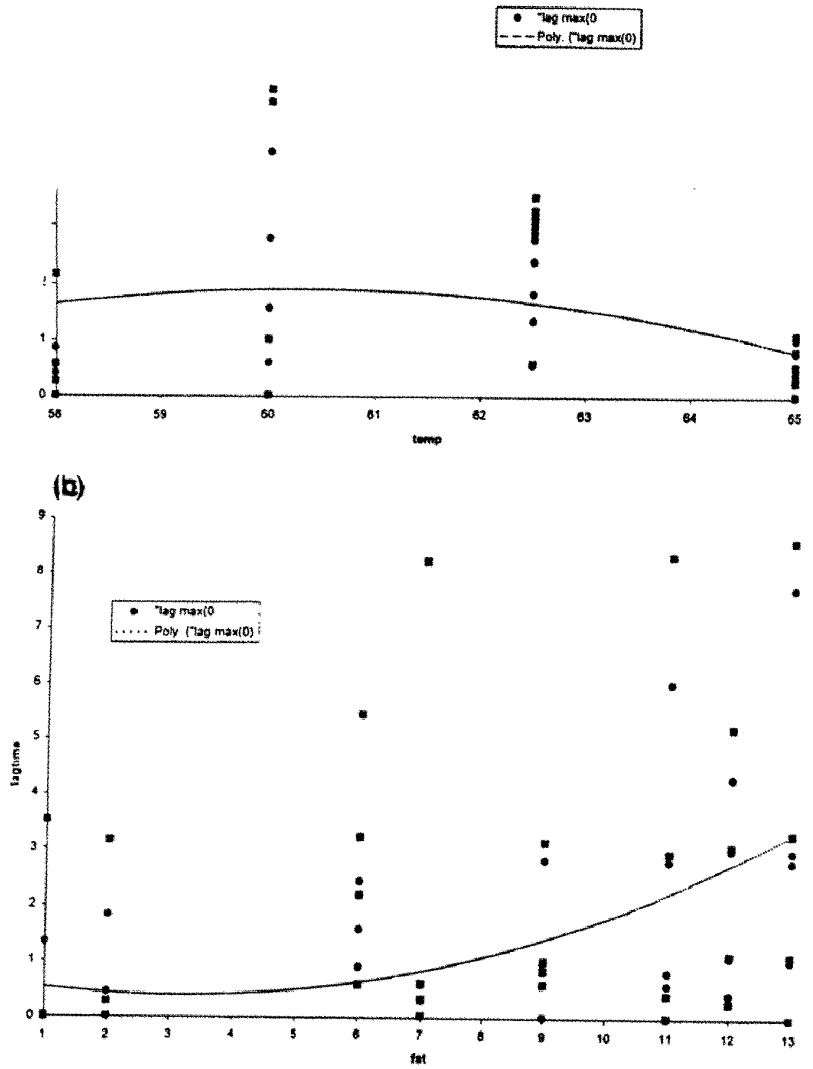
4.2. Secondary model for lag time values

Fig. 3a and b shows the plots of the lag times estimated from the linear regressions together with quadratic polynomial lines. If a lag time is less than 0, it is assigned a value of 0. From these graphs, it can be seen that the lag times increase with fat levels, and decrease with increasing temperatures beyond the maximum value at between approximately 60°C and 62°C.

Regression analysis were performed using lag times, restricted to be greater than or equal to 0. Truncating the lag time estimate creates an only a slight positive bias in the estimate, because most of the estimates are greater than 0, and the ones that are not small, in absolute value, about a minute or less. To help reduce variance, the dependent variable in the regression was the ln(max(0, lag) + 1). The independent variables for consideration are terms of a second-order degree response surface in the fat and temperature plane and a species effect. In the analysis, temperature minus 60°C was used as an independent variable. Analysis of variances were performed using mixed models, where observations for a given temperature and for a given fat level were considered as a random blocks. The residual variance was the largest of the variance components. Because of this, and that the estimate of the lag times are not as important for estimating the times needed to obtain given lethalities, a mixed model, such as the one used for analyzing the D-values, was not used.

The two estimated lag times of 6.0 and 7.7 min for the 10% and 12% turkey matrices and the estimates lag time of 0.42 min for the 12% chicken matrix, all at 58°C, had high influence on the estimates of the model parameters. The R^2 for the full model with all observations is 0.4857, with RMSE equal to 0.4822 ln(min). Deleting the three influential observations increases R^2 to 0.7129 and decreases RMSE to 0.3125 ln(min). Taken together, the three observations do not have a great impact on the predicted lag times; the greatest difference between the predictions with and without these three observations is about 1.5 min for the low-temperature, high-fat matrices. Excluding the three observations, however, would cause an underestimate of the uncertainty of the predicted values, consequently, the parameters of the model are estimated using all observations. The species effect and the linear fat term were not statistically significant, thus the model for estimating the lag time is:

$$\ln(\max(0,\text{lag time}) + 1) = a + b(T-60) + c(T-60)^2 + dF^2 + e(T-60)F. \qquad (11)$$



lag time, assumed ≥ 0, vs. temperature (°C), with quadratic line for chicken and turkey species. (b) Plot lag time, assumed t fat, with quadratic line for chicken and turkey species.

tes of the parameters of Eq. (11) and their rrors are given in Table 6. The standard prediction of the ln(lag time + 1) in the range of the data ranged from 0.12 to 0.24. cting the time needed to obtain a specified the lag times are restricted to be greater equal 0. For fat less than or equal 12% and temperature greater than about 67.5°C, the predicted lag time is 0.

4.3. *Predicted times to obtain a 7 $\log_{10}$ relative reduction of Salmonella*

The time, t_7, at a given temperature during which a 7 $\log_{10}$ relative reduction of *Salmonella* is ob-

Table 6
Estimates of regression coefficients of predicting ln(max(0, lag time) + 1) with standard errors
The equation is: $\ln(\max(0, \text{lag time})+1) = a + b(T-60) + c(T-60)^2 + dF^2 + e(T-60)F$ where T is the temperature (°C) and F is the fat level (%).

Parameter	Estimate	Standard error
a (Intercept)	0.3076	0.1725
b ($T-60$ effect)	0.1954	0.0828
c ($(T-60)^2$ effect)	−0.0334	0.01571
d (Fat^2 effect)	0.00973	0.00214
e ($T-60$, fat interaction)	−0.0144	0.00886

tained is estimated as $t_7 = 7\text{asym}\,D + \text{Lag}$. In terms of the dependent variables, $LD = \log_{10}(\text{asym}\,D)$ and $\text{lnLag} = \ln(\max(0, \text{lag}) + 1)$, the equation for t_7 can be written as:

$$t_7 = 7(10^{LD}) + \max(0, e^{\text{lnLag}} - 1). \quad (12)$$

In Fig. 1a–h, the predicted times, t_7, for the experimental conditions are indicated in the graphs by "*". An approximation of the variance of t_7, can be obtained by using the linear terms of the Taylor series expansion (Rao, 1973) of t_7 as a function of LD and lnLag. The correlation between the two random variables is needed. The approximation would not be applicable at temperatures and fat levels for which there would be more than a negligible probability that the estimated lag times would be zero. However, over all the temperatures, the contribution of the variance of the lag time is small, so that, as an approximation, the stand error of t_7 can be approximated as 7 times the standard error of the estimated asymptotic D-value from the regression (Eq. (10)) and Taylor series linearization.

An upper confidence bound of the estimated times needed to obtain a 7 $\log_{10}$ relative reduction of

Table 7
Estimated times (min) at given temperatures to obtain 7 $\log_{10}$ relative reduction of *Salmonella* spp., for chicken or turkey matrices at selected temperatures and fat levels. Also provided are the 95% upper confidence bounds

(a. chicken)

Temperature (°C)	2% Fat		7% Fat		12% Fat	
	Estimate	Upper bound	Estimate	Upper bound	Estimate	Upper bound
58	58.75	85.98	65.14	94.48	74.56	107.34
60	25.68	33.86	28.95	37.68	35.03	45.07
62	11.59	14.82	13.26	16.73	17.18	21.68
65	3.477	5.154	3.896	5.57	5.390	7.946
67.5	1.067	1.830	1.173	2.005	1.293	3.133
70	0.372	0.730	0.408	0.801	0.449	0.882
71	0.244	0.504	0.268	0.552	0.294	0.608
73	0.105	0.238	0.115	0.261	0.127	0.288
74	0.069	0.163	0.076	0.179	0.083	0.197

(b. turkey)

Temperature (°C)	2% Fat		7% Fat		12% Fat	
	Estimate	Upper bound	Estimate	Upper bound	Estimate	Upper bound
58	59.11	86.44	61.10	88.60	65.56	94.02
60	28.29	37.29	29.73	38.70	33.74	43.28
62	13.88	17.75	14.78	18.68	17.84	22.46
65	4.671	6.844	4.888	7.004	6.153	8.933
67.5	1.664	2.853	1.704	2.913	1.748	3.749
70	0.651	1.277	0.666	1.305	0.682	1.339
71	0.447	0.923	0.457	0.943	0.468	0.967
73	0.211	0.479	0.216	0.489	0.221	0.502
74	0.145	0.344	0.148	0.352	0.152	0.360

Salmonella would provide times that would assure that the 7 $\log_{10}$ relative reduction is obtained. The 95th percentile of *t*-distributions with 2.1–2.5 *df* is approximately 2.6–2.8. The range is not large, thus to simplify the estimation of 95% upper confidence bounds for the predicted time needed to obtain a 7 $\log_{10}$ relative reduction, 2.8 times the standard error of t_7 is added to t_7. In Table 7a,b, estimates of t_7 and the upper 95% confidence bounds are given for selected temperatures and fat levels.

In the published literature, we have not been able to find many articles reporting lethality kinetics for *Salmonella* in poultry. An article (Murphy et al., 1999) reported estimates of *D*-values for a cocktail of *Salmonella* serotypes in ground chicken breast meat at 67.5°C and 70°C of 0.286 and 0.176 min, respectively. Using these estimates of *D*-values, and assuming 0 lag times, the times needed to obtain a 7 $\log_{10}$ relative reduction would be about 2.00 min at 67.5°C and 1.23 min at 70°C. The 2.00-min estimate at 67.5°C is approximately equal to the corresponding 95% upper-bound estimates given in Table 7a for a product fat level equal to 7%, however, the 1.23-min estimate at 70°C is about 50% higher than the corresponding 95% upper-bound estimates ranging from 0.73 to 0.88 min. These comparisons suggest the need for more research, particularly at higher temperatures, and suggest that the upper bound estimates are needed to help assure that the target lethality levels are obtained.

5. Conclusion

Many assumptions used in deriving estimated cooking times, at a given temperature, needed to obtain a specified relative reduction of *Salmonella* need to be explored further. The general shape of the survival curves, covering between 5 and 6 $\log_{10}$ reduction of different *Salmonella* serotypes were assumed to be concave, with an initial shoulder and an asymptotic linear line. This assumption seemed valid for the survival curves observed at 62.5°C and 65°C, however, for the survival curves observed at 58°C and 60°C, this assumption is somewhat less certain. From the observed survival curves, linear portions were selected using fitted non-linear curves.

In deriving *D*-values, the shoulders of the curves were accounted for, and the estimated *D*-values were determined from the selected linear portions of the curves. In the model developed in this paper, the predicted lag times were 0 at temperatures above 67.5°C, when the fat is less than 12%, so that the effect of lag on temperatures above 67.5°C, or even lower temperatures with lower fat products, do not affect the estimated times.

The results of these experiments suggest that the fat levels and the type of species affect the *D*-values and lag times, however, the magnitudes of these effects need more clarification. There was found a statistically significant difference between *z*-values of turkey and chicken, however, no statistically significant effect on *z*-values due to fat level was found. In addition, the results suggest the possibility of non-linear thermal death time curves (plot of $\log_{10}$(*D*-value) vs. temperature), even though, for statistical purposes, a linear curve was assumed. Again, further work is needed to clarify the situation.

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