Models of the behavior of *Escherichia coli* O157:H7 in raw sterile ground beef stored at 5 to 46 °C

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Received 24 September 2004; accepted 6 October 2004

**Abstract**

*Escherichia coli* O157:H7 can contaminate raw ground beef and cause serious human foodborne illness. Previous reports describe the behavior of *E. coli* O157:H7 in ground beef under different storage conditions; however, models are lacking for the pathogen’s behavior in raw ground beef stored over a broad range of temperature. Using sterile irradiated raw ground beef, the behavioral kinetics of 10 individual *E. coli* O157:H7 strains and/or a 5- or 10-strain cocktail were measured at storage temperatures from 5 °C to 46 °C. Growth occurred from 6 to 45 °C. Although lag phase duration (LPD) decreased from 10.5 to 45 °C, no lag phase was observed at 6, 8, or 10 °C. The specific growth rate (SGR) increased from 6 to 42 °C then declined up to 45 °C. In contrast to these profiles, the maximum population density (MPD) declined with increasing temperature, from approximately 9.7 to 8.2 log cfu/g. Bias (Bf) and accuracy (Af) factors for an *E. coli* O157:H7 broth-based aerobic growth model (10 to 42 °C) applied to the observations in ground beef were 1.05, 2.70, 1.00 and 1.29, 2.87, 1.03, for SGR, LPD and MPD, respectively. New secondary models increased the accuracy of predictions (5 to 45 °C), with Bf and Af for SGR, LPD, and MPD of 1.00, 1.06, and 1.00 and 1.14, 1.33, and 1.02, respectively. These new models offer improved tools for designing and implementing food safety systems and assessing the impact of *E. coli* O157:H7 disease.

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**Keywords:** *E. coli* O157:H7; Ground beef; Models

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**1. Introduction**

The US Centers for Disease Control & Prevention report that *Escherichia coli* O157:H7 has
an incidence rate of 2.9 reported infections per 100,000 population (Anonymous, 2001c). There are an estimated 60,000 illnesses in the US each year due to this pathogen, with 1800 associated mortalities (Mead et al., 1999). The impact of this disease has also resulted in regulations that prohibit the presence of *E. coli* O157:H7 in raw ground beef (Anonymous, 1996). Although the beef processing industry and government inspection agencies have implemented interventions to reduce the incidence of *E. coli* O157:H7 in ground beef, microbiological results of ground beef products show that 0.8% are contaminated with *E. coli* O157:H7 (Anonymous, 2001b). Consequently, modeling tools are needed to estimate the fate of *E. coli* O157:H7 that may contaminate ground beef towards the design of improved intervention methods.

The USDA/ARS Pathogen Modeling Program (PMP; www.arserrc.gov/mfs/pathogen.htm) is utilized by food industries to estimate the behavior of certain bacterial pathogens as a function of intrinsic and extrinsic environmental factors. In PMP version 6.1, the models for *E. coli* O157:H7 are derived from bacteriological broth-based data, resulting in uncertainty about the accuracy of these model predictions when they are extrapolated to food matrices, such as ground beef. Such potential variation in lag phase duration (LPD) and growth rate among broth- and food-based models has been reported by Ross (1999) through examination of an extensive number of data sets from independent laboratories. These observations reinforce the need to validate models in specific food matrices so that model utility is recognized for process operations and risk assessment.

The present study measures the growth of *E. coli* O157:H7 in sterile raw ground beef over a temperature range that includes both bacterial growth and inactivation. The primary model parameters of specific growth rate (SGR), (LPD), and maximum population density (MPD) are compared to predictions of a PMP model for the aerobic growth of *E. coli* O157:H7 in brain heart infusion (BHI) broth (Buchanan et al., 1993). In addition, new secondary models are proposed that improve the estimation of *E. coli*O157:H7 behavior in sterile raw ground beef.

### 2. Materials and methods

#### 2.1. Bacterial strains and working cultures

A total of 10 *E. coli* O157:H7 strains were used in the experiments, either as individual strains or in a 5-strain or 10-strain cocktail. Nine of the strains (OB1340, OB90520A, OB141412, OB1525C, OB1423C, OB1514C1, OB1680G, OB1533A, and DB1538) have been previously described (Tamplin, 2002) and originated from beef associated with human *E. coli* O157:H7 illness. A tenth strain (GFP 80EC; courtesy of P. Fratamico, USDA/ARS) produced a plasmid-encoded green fluorescent protein; this strain was derived from strain SEA13B88 that was associated with human *E. coli* O157:H7 illness linked to contaminated apple juice (Anonymous, 2002). A cocktail was prepared with strains OB90520A, OB1525C, OB1423C, OB1680G, and GFP 80EC and was used in experiments performed from 8 to 44 °C. For experiments near the growth–no growth temperature boundary (≤8 °C and ≥44 °C), growth was studied separately for the 10 strains and with a cocktail of the 10 strains. A complete description of the methods for storing and culturing the bacterial strains has been reported (Tamplin, 2002). In brief, the strains were grown individually in brain heart infusion broth (BHI; Difco Laboratories Detroit, MI) to stationary phase at 37 °C, the broth diluted in peptone water (PW), and then dilutions were added to ground beef for a final concentration of approximately 3 to 4 log colony-forming units (cfu)/g. In experiments examining the effect of the previous growth phase in BHI broth on a subsequent lag phase in ground beef, the five-strain *E. coli* O157:H7 cocktail was prepared in BHI at 37 °C as described above, added to 10 °C ground beef at different phases of growth in BHI broth, and then levels of *E. coli* O157:H7 were measured over 12 days of incubation at 10 °C.

#### 2.2. Ground beef preparation

Ground beef (~5% to 7% fat, double-ground) was produced from beef trimmings using a home kitchen grinder or purchased as ground beef from a local retail outlet. The fat content of the ground beef was measured by the modified Babcock procedure (Kosikowski, 1982). Previous studies showed low variation
in strain growth parameters among different batches of ground beef with the same fat level at the same incubation temperature (Tamplin, 2002). Ninety-gram portions of ground beef were aseptically weighed into Stomacher bags (Koch Industries, Kansas City, MO), frozen at −30 °C, sterilized by a commercial irradiation treatment company (IBA, Brentwood, NY) or at the USDA/ARS Eastern Regional Research Center (Wyndmoor, PA) with a Cs$^{137}$ source at a dose of 42 kGy, and then maintained at −20 °C until experimentation.

2.3. Growth studies

The methods used to inoculate, store, sample, and isolate *E. coli* O157:H7 from experimental irradiated ground beef have been described (Tamplin, 2002). In brief, 90-g portions of irradiated ground beef were inoculated with 10 ml of PW containing an *E. coli* O157:H7 cocktail or individual strains. The inoculum was mixed in the ground beef, and then 90-g samples were stored at selected temperatures (5, 6, 8, 10.5, 11, 12.5, 15, 17, 18, 20, 25, 30, 37, 40, 42, 45, and 46 °C). Data for 10 °C storage were derived from previously published studies that used the same methods (Tamplin, 2002). The incubation temperature was measured over the duration of the experiment using a Dickson model FT121 or D100 temperature data recorder (Dickson, Addison, IL) with a temperature probe inserted into a separate sample bag of ground beef. The temperature recorder was calibrated against a National Institute of Standards and Technology certified thermometer (ERTCO Precision Ever Ready Thermometer West Patterson, NJ). At selected time intervals, 3-g portions of ground beef were transferred to a Stomacher bag and diluted in PW. The samples were stomached, and whole and/or diluted preparations were made in PW and spiral-plated onto tryptic soy agar (TSA; Difco) using an Autoplate 400 (Spiral Biotech, Boston, MA). Agar plates were incubated at 37 °C for 16 to 18 h, and then colonies were enumerated. In studies of the lower temperature growth boundary, 10 ml of BHI broth was also inoculated with the same inoculum and at the same level, stored, and sampled in the same manner as the ground beef to control for experimental variation. Each temperature study consisted of two or three sample replicates of ground beef. At each time interval, each replicate sample was plated on two TSA plates. The cfu data were transformed to log values and recorded in an Excel spreadsheet (Microsoft, Redmond, WA). Means ($\bar{x}$) and standard deviations (std) were calculated for growth parameters and curves goodness-of-fit.

2.4. Primary and secondary models

The model described by Baranyi and Roberts (1994) was used to fit curves to the experimental data and to estimate values for the primary growth parameters (LPD [h], growth rate [ln h$^{-1}$] and MPD [log cfu/g]) using DMFit curve-fitting software (courtesy of J. Baranyi, Institute of Food Research, Norwich, UK). The growth rate derived from the DMFit curve-fitting software was converted to specific growth rate (SGR) by multiplying the growth rate by the natural logarithm of 10 and expressing the units in ln h$^{-1}$. TableCurve 2D version 5.0 (SPSS Chicago, IL) was used to select certain candidate models for the distributions of SGR, LPD, and MPD as a function of temperature. The SGR was also modeled with the extended Ratkowsky model (Ratkowsky et al., 1983).

2.5. Measurement of model performance

Model performance for LPD and MPD was measured by the method of Ross (1996) who describes the calculation of a bias factor ($B_f$) for generation time (GT) as

$$ B_f = 10^{\left(\frac{\sum \log (GT_{predicted}/GT_{observed})}{n}\right)} $$

where $n$ is the number of discrete measurements. In Eq. (1), a value of 1.0 is a perfect average agreement between the model and the observations. The value can also be less than or greater than 1. Values for LPD and MPD were substituted for GT to measure the performance of predictions for these parameters.

Ross also described the measurement of a model accuracy factor ($A_f$) as

$$ A_f = 10^{\left(\frac{\sum \log (GT_{predicted}/GT_{observed})}{n}\right)} $$

In Eq. (2), $A_f$ is the absolute value of the logarithm of the ratio, and the value is always greater than or equal to 1. Perfect agreement between model and
observations is a value of 1.0. As in Eq. (1), LPD and MPD were substituted for GT to measure model performance.

Model performance for SGR was calculated by the method of Baranyi et al. (1999) where

\[
B_t = \exp \left( \frac{\sum_{k=1}^{m} \left( \ln f(x^{(k)}) - \ln \mu^{(k)} \right) }{m} \right)
\]

(3)

\[
A_t = \exp \left( \frac{\sum_{k=1}^{m} \left( \ln f(x^{(k)}) - \ln \mu^{(k)} \right)^2 }{m} \right)
\]

(4)

In Eqs. (3) and (4), \(m\) is the set of observations, and \(\mu\) is the maximum specific growth rate. Perfect agreement between predict and observation is a value of 1.

3. Results and discussion

3.1. Behavior of E. coli O157:H7 in ground beef at 5 to 46°C

A cocktail of 10 E. coli O157:H7 strains was added to sterile raw ground beef at approximately \(10^3\) to \(10^4\) log cfu/g to facilitate enumeration by direct agar plate counts. This inoculum level likely represents a higher level of contamination than that which normally occurs in contaminated retail ground beef (Anonymous, 2001b). Previous studies by our laboratory have shown that lag phase duration is longer, and growth rate is lower at lower inoculum levels of \(10^1\) log cfu/g compared to levels of \(10^2\) log cfu/g and higher. The cocktail of 10 E. coli O157:H7 strains did not grow at 5°C in sterile ground beef over 23 days of incubation. Instead, the population showed an inactivation rate of \(-0.013\) ln h\(^{-1}\) (Table 1). At the next higher incubation temperature, 6°C, growth was measured separately for the 10 strains over 25 days. Overall, the growth profiles were nonsigmoidal in shape, indicating that this temperature was near the strain growth/no growth boundary.

More specifically, nine strains grew over the 25 days of incubation, with an average SGR of 0.003 ln h\(^{-1}\) and MPD of 4.71 log cfu/g. One strain, OB1340, was inactivated (SGR = -0.002 ln h\(^{-1}\)).

Other reports describe the behavior of E. coli O157:H7 in ground beef at lower storage temperatures. Barkocy-Gallagher et al. (2002) report that the levels of five E. coli O157:H7 strains increased in nonsterile ground beef between 0.9 and 1.5 log cfu at 7°C over a 14-day period. In BHI broth studies, Palumbo et al. (1995) examined the minimum and maximum growth temperatures for 16 E. coli O157:H7 strains using a temperature gradient incubator and shaking flask cultures. In the gradient incubator study, the minimum growth temperatures for the 16 strains were 7.3 (one strain), 9.2 (seven strains), 9.4 (five strains), 11.3 (one strain), and 11.6°C (two strains). In the shaken flasks experiments, 4 of the 16 strains grew at 8 but not 5°C, and the remaining strains grew at 10 but not 8°C. Therefore, our observations of growth in ground beef

<table>
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<tr>
<th>Temperature</th>
<th>SGR(^a)</th>
<th>LPD</th>
<th>MPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>-0.013</td>
<td>np</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0.003</td>
<td>np</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0.022</td>
<td>np</td>
<td>0</td>
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<td>0</td>
</tr>
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<td>0.14</td>
<td>9.38</td>
</tr>
<tr>
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<td>0.19</td>
<td>11.6</td>
</tr>
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<td>0.29</td>
<td>10.6</td>
</tr>
<tr>
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</tr>
<tr>
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<td>0.99</td>
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</tr>
<tr>
<td>30</td>
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<td>1.39</td>
<td>1.19</td>
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<td>8.13</td>
</tr>
<tr>
<td>46</td>
<td>np</td>
<td>np</td>
<td>np</td>
</tr>
</tbody>
</table>

\(\text{SGR}—\text{specific growth rate (ln h}^{-1}\text{), LPD—lag phase duration (h), MPD—maximum population density (log cfu).}\)

\(\text{np—no prediction.}\)

\(\text{ng—no growth.}\)

\(\text{log cfu at 25 days without a demonstrable stationary phase.}\)
at 6 °C extend previous reports of the minimum growth temperature for E. coli O157:H7. It is noteworthy that many studies describing bacterial growth temperatures do not describe the calibration of temperature-measuring devices to substantiate the accuracy of temperature measurements, particularly near the growth/no growth boundary.

At 6, 8, and 10 °C, a lag phase was not observed, as previously reported by Tamplin (2002) for E. coli O157:H7 in sterile and nonsterile raw ground beef stored at 10 °C. Fig. 1 shows a representative growth profile at 8 °C. The absence of a lag phase was also observed with more intensive sampling over shorter time intervals (not shown). The lack of a lag phase may indicate that the transition of E. coli O157:H7 from a 37 °C BHI stationary phase culture to raw refrigerated ground beef may require little physiological adjustment for exponential growth (Baranyi and Roberts, 1994). Since it was possible that the previous phase of growth in BHI broth may have influenced the lack of a lag phase in 10 °C ground beef, in separate experiments, a five-strain cocktail of E. coli O157:H7 was transferred from 37 °C BHI broth at 1 (lag phase), 2 and 3 (exponential phase), and at 22 h (late stationary phase) to 10 °C ground beef. A lag phase was not observed in ground beef for any of these transfer times (not shown), indicating that the previous phase of growth in BHI at 37 °C had less of an effect on the lack of a lag phase than the influence of the ground beef matrix. Further studies should also examine the effect of other previous environmental factors (e.g., temperature) to determine their influence on the E. coli O157:H7 lag phase in ground beef.

Similar to the present studies, the aerobic growth model for E. coli O157:H7 used for comparative purposes was developed in BHI broth and used late stationary phase cells grown in BHI at 37 °C. Unlike our observations for ground beef, the model for E. coli O157:H7 in BHI does not predict growth below 9 or above 42 °C. Therefore, the differences observed between the broth model growth parameters and those observed in ground beef could have resulted from the different test matrices. We hypothesize that matrix-related differences in E. coli O157:H7 behavior might be accentuated near the lower growth/no growth temperature boundary, and, therefore, we also tested the fate of E. coli O157:H7 in BHI broth at 5, 6, and 8 °C. To decrease experimental variation for test matrix comparisons, ground beef and BHI broth were inoculated simultaneously with the same preparation of E. coli O157:H7, stored in the same incubator, and sampled at the same time intervals. Similar to observations in ground beef, the 10-strain cocktail was inactivated (SGR=−0.036 ln h⁻¹) in BHI broth at 5 °C (not shown). However, different behaviors were observed between the two test matrices at 6 °C, whereby all 10 strains were inactivated (SGR,

Fig. 1. The experimental growth profile of E. coli O157:H7 at 8 (A), 15 (B), 25 (C), and 40 °C (D) in sterile raw ground beef, with primary curve-fitting.
\[ \dot{x} = -0.014 \ln h^{-1} \] in BHI broth compared to growth although at a low rate (SGR, \( \dot{x} = 0.003 \ln h^{-1} \)) in ground beef. In the same manner, at 8 \(^\circ\)C, all 10 individual E. coli O157:H7 strains were inactivated (SGR, \( \dot{x} = -0.054 \ln h^{-1} \)) in BHI broth, whereas all strains grew (SGR, \( \dot{x} = 0.022 \ln h^{-1} \)) in ground beef. These contrasting observations reinforce the suggestion that bacteriological broth is not always appropriate for predicting microbial behavior in food matrices, particularly near the growth/no growth boundary.

At a temperature range of 10.5 to 45 \(^\circ\)C, the growth of E. coli O157:H7 displayed a typical sigmoid-shaped bacterial growth curve (Fig. 1). The SGR increased from 6 to 42 \(^\circ\)C then declined from 42 to 45 \(^\circ\)C, the latter likely reflecting a reduction in the physiological condition of the bacteria as they approached the growth/no growth boundary. In contrast, the broth model predicted a stationary SGR from 37 to 42 \(^\circ\)C. The maximum SGR observed in ground beef was between 40 and 42 \(^\circ\)C, a range encompassing the optimal growth temperature of 40.2 \(^\circ\)C in Muller–Hinton broth reported by Gonthier et al. (2001).

An LPD was not observed at 8 or 10 \(^\circ\)C, and then a sharp increase occurred between 10 and 10.5 \(^\circ\)C (Fig. 1). Thereafter, the LPD decreased from 10.5 to 45 \(^\circ\)C. In contrast to the distribution of SGR and LPD, the MPD displayed a relatively moderate reduction from 8 to 45 \(^\circ\)C (Fig. 1).

At 45 \(^\circ\)C, there was relatively low variation among the growth parameters for the 10 strains tested: SGR, \( \dot{x} = 2.07 \ln h^{-1} \), std=0.05; LPD, \( \dot{x} \geq 0.83 \) h, std=0.34; MPD, \( \dot{x} = 8.13 \log cfu/g \), std=0.20; \( R^2 = 0.99 \), std=0.00 (Table 1). Unlike at 45 \(^\circ\)C, the higher variation (\( R^2 = 0.39 \)) among the 10 strains at the lower temperature growth boundary of 6 \(^\circ\)C may be due to greater strain variation in conformations of enzymes and structural compounds, whereas, at the upper-temperature growth boundary, strong thermal effects may result in more uniform and irreversible degradation of cell structures. At temperature \( \geq 46 \) \(^\circ\)C, the 10-strain cocktail of E. coli O157:H7 did not grow in ground beef. Similarly, Palumbo et al. (1995) reported that all 16 E. coli O157:H7 strains grew at 45 \(^\circ\)C in BHI broth, although growth data for higher temperatures were not reported. Salter et al. (2002) showed that some Shiga-toxin-producing E. coli strains can grow at 46 and 47 \(^\circ\)C in broth.

### 3.2. Broth-based model performance for raw ground beef

The method of Ross (1996) was used to measure \( B_f \) and \( A_f \) for LPD and MPD. Model \( B_f \) and \( A_f \) was determined for SGR using the method of Baranyi et al. (1999).

Over a temperature range of 10 to 42 \(^\circ\)C, the temperature limits in common between the aerobic broth model for E. coli O157:H7 and the present study, the broth model predicted an SGR that was 0.79 to 1.92 times greater than the rate observed in sterile ground beef (Table 2). The overprediction could have resulted from Gompertz-derived growth rate estimates used in the broth model, which have been shown to be higher than those derived from the Baranyi model (Baranyi et al., 1993; Ross, 1999; Whiting and Cygnarowicz-Provost, 1992). Reducing the broth model-predicted SGR by 13% showed that the broth model SGR was 0.69 to 1.40 times greater than the rate observed in ground beef over a temperature range (10 to 42 \(^\circ\)C) common to both the broth model and experimental conditions. The predicted LPD by the broth model was 0.74 to >56.0 times greater than the observed LPD (Table 2). In general, the greatest differences between the predicted and observed LPD were at temperatures less than 15 \(^\circ\)C. This was

<table>
<thead>
<tr>
<th>Temperature(^a)</th>
<th>SGR</th>
<th>LPD</th>
<th>MPD</th>
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<tbody>
<tr>
<td>10</td>
<td>1.11</td>
<td>&gt;56.0(^b)</td>
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<td>0.94</td>
<td>0.92</td>
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</tr>
<tr>
<td>42</td>
<td>0.79</td>
<td>0.74</td>
<td>1.08</td>
</tr>
</tbody>
</table>

\(^a\) 10 to 42 \(^\circ\)C was a temperature range common to the broth model predictions and present experiments in ground beef.

\(^b\) No lag phase was detected in ground beef at 10 \(^\circ\)C; therefore, the LPD value was assumed to be 1.0 for the calculation of \( B_f \).
especially true for 10 °C, where the broth model predicted an LPD of 56 h, yet no lag phase was observed in ground beef. Furthermore, the broth model did not predict growth at temperatures less than 9 °C, whereas growth was observed in ground beef at 6 °C. The broth model-predicted MPD was 1.0 to 1.1 times greater than that observed for ground beef.

Bias ($B_f$) and accuracy ($A_f$) factors for the E. coli O157:H7 broth-based aerobic growth model (10 to 42 °C) applied to the observations in ground beef were 1.05, 2.70, 1.00 and 2.87, 1.03, 1.00, for SGR, LPD and MPD, respectively. When the Gompertz-based PMP prediction for SGR was reduced by 13%, the $B_f$ and $A_f$ values were 0.94 and 1.34, respectively.

Considering Ross’ (1999) description of “good” model performance for GT being a $B_f$ of 0.9 to 1.05 and an “acceptable” $B_f$ being 0.7 to 0.9 and 1.06 to 1.15, the extrapolation of the broth model to ground beef did not meet either of these criteria for LPD. In addition, Ross (1999) reported that a single-variable model with a good $B_f$ would have an $A_f$ of 1.15, which was not observed for SGR (i.e., 1.34) or LPD (i.e., 2.87).

3.3. New secondary models

The lognormal (Eq. (5)) and extended Ratkowsky (Eq. (6)) models were evaluated for SGR as a function of temperature.

$$y = a + b \exp \left[ -\ln(2) \frac{\ln \left( \frac{x - c}{d} \right)}{\ln(e)} + \frac{e}{d} \right]^2$$

For SGR, $a = -0.366$, $b = 3.003$, $c = 0.366$, $d = 15.653$, and $e = 0.296$. For LPD, $a = -1.033$, $b = 14.957$, $c = 10.641$, $d = 6.376$, $e = 11.253$. $R^2$ for SGR and LPD=0.98 and 0.85, respectively.

$$\sqrt{k} = b(T - T_{\min})(1 - \exp(c(T - T_{\max})))$$

where $b = 0.0280$, $c = 0.7524$, $T_{\min} = 3.7942$, $T_{\max} = 47.1646$, $R^2 = 0.99$.

The extended Ratkowsky model (Ratkowsky et al., 1983) was subsequently chosen to model SGR due to better goodness-of-fit ($R^2 = 0.99$ vs. 0.98; Fig. 2) and a more mechanistic basis. LPD, as a function of temperature, showed a lognormal distribution. Therefore, a lognormal model was evaluated (Eq. (5); Fig. 2). A cubic model was used to fit the distribution of MPD (Eq. (7); Fig. 2).

$$y = a + bx^3$$

where $a = 9.411431$, and $b = -1.2337693^{-5}$. $R^2 = 0.68$.

Model performance measured over an experimental temperature range of 5 to 45 °C showed $B_f$ and $A_f$ for SGR, LPD and MPD of 1.00, 1.06, 1.00, and 1.14, 1.33, 1.02 respectively. For SGR, the $B_f$ and $A_f$ were 1.00 and 1.14, respectively. For comparative purposes, the $B_f$ and $A_f$ of the models for SGR, LPD, and
MPD over the growth temperature range common to the broth model and the ground beef studies (i.e., 10 to 42 °C) were 1.00, 0.99, and 1.00 and 1.17, 1.35, and 1.02, respectively (Fig. 3).

Therefore, the overall performance of the new models from 5 to 45 °C met the definition of “good” or “acceptable” models as defined by Ross (1999). However, as a “good” model, the $A_f$ would be expected to be <1.15 since there was only one model variable (i.e., temperature); this condition was not met for LPD (i.e., $A_f=1.33$) from 5 to 45 °C. Although model performance can be somewhat improved, the ability to make predictions at temperatures of less than 9 °C or greater than 42 °C, compared to the current PMP model, is a marked improvement.

3.4. Relationship to risk assessment

The findings of these experiments have a number of significant implications for risk assessment of foods contaminated with *E. coli* O157:H7. The specific findings of (a) growth at 6 °C for 9 out of 10 strains and (b) the absence of an appreciable lag period at 6, 8, and 10 °C suggest that more occurrences of growth at refrigeration temperatures should be expected than are typically assumed in risk assessment models. For fresh meat, such as ground beef that is stored under refrigeration temperatures by the producer, the retailer, and the consumer, the range of temperatures around the minimum growth temperatures may be very important. A survey by Audits International (1999) demonstrates the importance of understanding the behavior of pathogens in this temperature range. According to this survey of storage conditions of fresh meat at retail, 17% of the measured cases had temperatures between 42 (5.6 °C) and 45 °F (7.2 °C), and a further 8% were held at temperatures between 45 (7.2 °C) and 50 °F (10 °C). Very similar percentages for these temperature ranges (17% and 6%, respectively) were found in home-refrigerated...
storage. Another key finding of this survey was the average time-out-of-refrigeration (i.e., between retail and home storage) of 65 min (range: 11 to 380 min), with 47% of the product being in the temperature range of 42 (5.5°C) to 50°F (10°C) as measured before being placed in the home refrigerator. Given the current findings of a lack of lag period in the temperature range of 8 to 10°C, this would suggest that a significant portion of the product could experience growth during transportation to the home.

As an example, a draft risk assessment for *E. coli* O157:H7 in ground beef (Anonymous, 2001a) assumes that there is no growth below 46°F (7.7°C). The assessment also applies an equation for LPD that predicts an LPD of approximately 80 h at 8°C. Similarly, a more recent risk assessment of *E. coli* O157:H7 in steak tartare (Nauta et al., 2001) assumes an LPD of 123 h at 7°C, with the corresponding statement that “storage time should exceed 5 days before the onset of growth.” A third and earlier risk assessment (Cassin et al., 1998) assumes that there is no growth below 7.7°C.

Recent studies of the maximum population density add further complexity to the situation whereby the maximum population observed in 10°C raw ground beef was not observed to exceed 6.4 log cfu/g (Tamplin, 2002). This is in sharp contrast to the assumptions employed in risk assessments of up to 10 log cfu/g. Such an overprediction of the maximum population size, particularly in product which is later pooled, might produce significant overestimates of risk.

These apparent mismatches between experimental findings and risk assessment assumptions underline the challenge of predicting the performance of food safety systems, particularly with a shifting and sometimes conflicting evidence base. It further implies that experimental resources might be best applied to points in the system that represent a combination of both frequently encountered and important environmental conditions such as those representing the conditions of refrigeration of fresh meat.

This study is part of an overall research program to improve the performance of models for bacterial pathogens in food. The new models proposed in this research address the growth of *E. coli* O157:H7 in a sterile food and consequently predict the upper limits, or worst-case scenario, for growth in ground beef.

New models which consider the effects of competitive flora (Nissen et al., 2001; Tamplin, 2002; Walls and Scott, 1996) will provide more conservative estimates of *E. coli* O157:H7 growth and may be useful for estimating the lower limits of risk. However, it is prudent for food industries to consider models for sterile foods since the quality and quantity of the native competitive flora is not typically known.

The PMP software package currently contains bacteriological broth-based models for aerobic and anaerobic growth, as well as inactivation, of *E. coli* O157:H7. The present ground beef models will be incorporated into the PMP, and the data sets from these studies will be incorporated into a relational database, ComBase (www.combase.cc) (Baranyi and Tamplin, 2004). These resources will provide ground beef operations and risk managers with better strategies to reduce the impact of *E. coli* O157:H7 disease on public health.

**Acknowledgments**

The author wishes to acknowledge József Baranyi of the Institute of Food Research, Norwich, UK for helpful discussions.

**References**


