

Impact of Chlorine, Temperature and Freezing Shock on the Survival Behavior of *Escherichia coli* O157:H7 on Ready-to-Eat Meats*

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ABSTRACT

Foodborne pathogens continue to pose a potential food safety hazard in ready-to-eat (RTE) meat. Chlorine is commonly used to sanitize processing equipment where *Escherichia coli* O157:H7 (Ec) may survive and contaminate food products. The objective of this study was to characterize the survival behavior of Ec with different stresses on RTE meats. A multi-strain cocktail of Ec was pre-treated with freezing shock for 15 - 20 h and/or chlorine (0, 25, and 50 ppm) for one hour, and then inoculated onto RTE meat surfaces to obtain about 3.0 log CFU/g. Samples were stored at three abuse temperatures (12°C, 18°C, and 24°C) and Ec was enumerated during the storage. The freezing shock impact was studied using the Ec cocktail stored in a freezer overnight followed by chlorine exposure for one hour. The lag phase and growth rate of Ec were estimated using DMFit (Combase, Baranyi's model). Results indicated that Ec growth was suppressed by chlorine treatment. Freezing shock was found to have little impact in terms of lag time and growth rate. The lag phase of Ec after exposure to 0 ppm of chlorine (50.3 h) was shorter than that of Ec treated with 25 ppm (54.6 h) and 50 ppm (164.1 h) at 12°C. However, the lag phase decreased with an increase in temperature, e.g. at 25 ppm, lag times were 54.6, 51.1 and 48.9 h for 12°C, 18°C and 24°C, respectively. Lag times increased with an increase in chlorine concentration. At 24°C, lag times were 15.8, 48.9, and 52.4 h for 0, 25, and 50 ppm, respectively. The growth rate increased with an increase in temperature for 0 and 25 ppm chlorine levels, but decreased at 50 ppm level. Growth rate and lag phase as a function of temperature and chlorine concentration can be described by polynomial models and modified Ratkowsky-type and Zwietering-type models. Results of this study will contribute to risk assessment of RTE meats.

Keywords: Chlorine; Lag Time; Growth Rate; *E. coli* O157:H7; Modeling

1. Introduction

Foodborne pathogen (e.g. *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp.) contamination in foods is one of the safety concerns for the consumers, manufacturers and regulatory agencies. *E. coli* O157:H7 has been isolated from ready-to-eat (RTE) foods and implicated in several outbreaks [1,2]. The Centers for Disease Control and Prevention (CDC) [3] estimated that 48 million people (1 in 6 Americans) get sick, 128,000 are hospitalized, and 3000 die each year from foodborne diseases in the US Outbreaks of food-

borne illness and their economical and public health impacts have been reported by the Foodborne Diseases Active Surveillance Network [4] and Frenzen *et al.* [5]. Due to the high cost of illness and potential threat to public health, both FDA and FSIS have set up rigid standard procedures to monitor the food processes and supply chain to enhance food safety.

With the ubiquity of *E. coli* O157:H7 in the environment, cross-contamination of this microorganism could result from contacts among contaminated processing equipments, workers, and foods which have no further treatment before consumption. In order to reduce the microbial foodborne hazard, surface sanitation using chlorinated water is one of the most common sanitation methods in food processing plants. Chlorine is a very effective agent in water treatment (portable water and

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water used in recreational settings) to eliminate *E. coli* O157:H7 [6]. There are different reports regarding the chlorine resistance of *E. coli* O157:H7 in various foods or media. Sharma *et al.* [7] reported that cells attached to surface or enmeshed in biofilms may have altered sensitivities to cleaners and sanitizers compared to those of planktonic cells. The *E. coli* O157:H7 cells in biofilms were killed more effectively by treatment with an alkaline cleaner than with hypochlorite alone, possibly through a synergistic effect. Virto *et al.* [8] observed that addition of small amount of organic matter to the chlorination medium (150 ppm of trypticase soy broth vs. distilled water) drastically increased the resistance of microorganisms, especially in *E. coli* and *Yersinia enterocolitica*. Their study further indicated that the resistance may depend on changes of permeabilization of the cytoplasmic membrane. Microorganisms adapting to a changing or hostile environment became resistant to the stress has also reported by others. Taormina and Beuchat [9] studied the survival and heat resistance of *L. monocytogenes* after alkali and chlorine exposure. *Listeria* cells survived exposure to processing environments which were not effectively sanitized with alkaline detergents might pose great potentials of cross-contamination. The alkaline-pH-induced cross-protection of *Listeria* against heat may enhance its survival potential in minimally processed foods. Samelis *et al.* [10] reported the survival/growth of *E. coli* O157:H5 in a fresh meat model system with decontamination runoff waste fluid treatment and its resistance to subsequent lactic acid stress (pH 3.5). Wan Norhana *et al.* [11] reported the effects of temperature, chlorine and acids on the survival of *L. monocytogenes* and *Salmonella* on shrimp treated with 100 ppm sodium hypochlorite solution and acetic, hydrochloric and lactic acids. They concluded that pathogens attached to the shrimp surface were more resistant to heat, chlorine and acids when compared to their planktonic counterparts. This conclusion demonstrated that when a model system was used for food safety study, the food model system should be closely monitored to represent the real situations in production or storage. Rajkovic *et al.* [12] investigated the resistance of three pathogens, including *E. coli* O157:H7, after exposure to repetitive cycles of mild bactericidal treatments and concluded that the ability of foodborne pathogens to adapt to mild bactericidal treatments created new challenges in risk assessment and hazard analysis. Aarnisalo *et al.* [13] compared several commercially available disinfectants and chlorinated alkaline cleaners against 10 strains of *L. monocytogenes* at refrigerated temperatures. They concluded that the adaptive response of *L. monocytogenes* to disinfectants or chlorinated alkaline cleaners appeared to be a minor concern if proper concentration was used. However, problems associated with the effectiveness in

disinfection in industrial applications may rise due to the improper use and lack of understanding of specific limitations.

Cold stress is another factor which could affect microbial survival. It is well known that most food pathogens may survive the cold storage which is commonly used in food industry. *E. coli* O157:H7 can multiply very slowly at temperature as low as 44°F (6.7°C) in ground beef [14]. Bollman *et al.* [15] studied the impact of cold shock (10°C, 1.5 h) on survival of *E. coli* O157:H7 in frozen foods. They found that there was no significant difference on *E. coli* O157:H7 survival with and without cold shock in ground beef or pork. In contrast, a cold shock treatment showed a significant enhancement on *E. coli* survival in milk, whole egg or sausage. Grzadzowska and Griffiths [16] concluded that *E. coli* O157:H7 had a greater cold shock effect and cryotolerance than non-O157 strains in their study. The food matrix changed the cold shock response in all their investigated *E. coli* strains. Tasara and Stephan [17] presented an overview of *L. monocytogenes* adaptation to the environmental cold stresses and the molecular adaptive and/or response mechanisms, which provided useful insights regarding how *L. monocytogenes* and other foodborne pathogens may survive a physical or chemical shocking exposure.

The objectives of this study were 1) to investigate the effects of stresses (including freezing shock and chlorine exposure) on the growth of *E. coli* O157:H7 in RTE meat and 2) to model the *E. coli* O157:H7 behavior as affected by the imposed stresses and temperature.

2. Materials and Methods

2.1. *E. coli* O157:H7 Strains and Cocktail Preparation

A cocktail of five *E. coli* O157:H7 strains, which were isolated and obtained from the Food Safety and Inspection Service (FSIS) from pork sandwich (strain OB-90520A), bottom round (strain OB1525C), beef patty (strain OB1423C), ground beef (strain OB1680G), and salami outbreak (strain 380 - 94), was used in this study. A loopful of each strain was transferred from a -80°C stock culture into 10 ml Brian Heart Infusion broth (BHI, Becton, Dickinson and Company, Sparks, MD) and incubated at 37°C for 6 h. A loopful of cell suspension of each strain was then separately transferred to a fresh 10 ml BHI broth and incubated at 37°C for 24 h. Each strain was plated to verify cell counts for equal contribution with adjustment of adding 0.1% peptone water (PW) before the final cocktail preparation. One ml of cell suspension from each strain was combined, and the cocktail was further diluted with sterile 0.1% PW to the targeted level of *E. coli* O157:H7.

2.2. Preparation of Chlorine Solution

Chlorine solutions at 250 ppm and 500 ppm were prepared using a 5.25% sodium hypochlorite solution (Fisher Scientific, Pittsburgh, PA). The 250 ppm chlorine solution was prepared by adding 1 ml of sodium hypochlorite with 99 ml of sterile water in a dark glass bottle and mixed well just before the use. Similarly, the 500 ppm chlorine solution was prepared using the sodium hypochlorite and sterile water volume ratio at 1:49.

2.3. Experimental Design

The experimental conditions used to simulate the survival behavior of *E. coli* O157:H7 exposed to chlorine concentration and abuse temperature were 0 - 50 ppm and 12°C - 24°C, respectively. For model development, the full combinations of chlorine and temperature included 9 sets of combination at chlorine concentrations of 0, 25 and 50 ppm and temperatures of 12°C, 18°C and 24°C (*i.e.* 2 factors \times 3 levels). Each set was repeated twice and with duplicate samples for each set; a total of 18 data sets (9 \times 2) were used in the regression analyses. Freezing shock was performed using the same design with the addition of an overnight storage in a freezer. The developed models were verified by experimental data selected inside and outside the parameter ranges.

2.4. Chlorine Exposure and Freezing Shock

E. coli O157:H7 cocktail was exposed to chlorine solutions of various concentrations for one hour before inoculation onto RTE meat. The cocktail mixture was blended with a chlorine solution and 0.1% PW at a ratio of 1:1:8 (v/v) to attain the targeted microbial counts and chlorine concentrations. For example, a 5 log CFU/ml *E. coli* with 25 ppm chlorine was prepared using a 1 ml of 6 log CFU/ml *E. coli*, 1 ml of 250 ppm chlorine solution and 8 ml of PW. The mixture was vortexed 30 seconds and kept for 1 h to achieve the chlorine exposure time. The pH value of each final mixture was measured by replacing the 1 ml of *E. coli* O157:H7 cocktail with PW, *i.e.* 1 ml chlorine solution and 9 ml of PW, using a Daigger pH meter (Daigger Model 5500 pH Meter, Vernon Hills, IL). Since the *E. coli* O157:H7 survival counts will be reduced during the chlorine shocking treatment, the targeted inoculation counts were attained in several trials. For freezing shock treatment, the prepared cocktail was stored in a freezer overnight before mixing with chlorine. The cold shock was harsher than the treatment at 10°C, 1.5 h by Bollman *et al.* [15] or 10°C, 6 h by Grzadzowska and Griffiths [16]. The ham was purchased at a local manufacturer, sliced, thermal-treated (95°C, 20 min in a sealed pouch) and then cut into 3 cm \times 3 cm size.

One-tenth ml of the prepared cocktail at 3 log CFU/ml level was applied evenly onto one side of the 3 cm \times 3 cm sliced ham surface (weight about 5 g) and was allowed to dry for 10 - 15 min on a tray under a laminar flow hood before vacuum packed (Model CN 200, MULTIVAC, Kansas City, MO).

2.5. Storage and Microbial Analysis

The samples were stored at 12°C, 18°C and 24°C for up to 10 days. Duplicate samples were enumerated for *E. coli* O157:H7 counts during storage using the CT-SMAC selective media. At least two sampling were performed for each treatment. To enumerate *E. coli* O157:H7 count in samples, 0.1 and 0.5 ml of the stomached liquid samples with proper dilutions were evenly spread on the CT-SMAC agar plates and incubated at 37°C for 24 to 48 h.

2.6. Model Consideration and Development

The growth data were analyzed using the DMFit software available on the Combase website (www.combase.cc). Baranyi and Roberts model [18] was applied to fit the growth data to obtain lag times and growth rates of *E. coli* O157:H7 at each chlorine and temperature combination. Two categories of models were considered for modeling the lag time (LAG) and growth rate (GR) to describe the *E. coli* O157:H7 survival after treating with chlorine. One was a typical linear regression or polynomial model based on the experimental design. The other was a secondary model available in the literature which showed the most suitable fit to the current data with necessary modifications. The linear and non-linear regression procedures (SAS, v9.1) were used for data analyses.

3. Results and Discussion

In order to cover a broader range of target parameters, the chlorine upper limit was initially set at 100 ppm. However, it was found difficult to achieve a stable and reliable initial count with 1 h of chlorine exposure. The chlorine was further reduced to 75 ppm and similar results were attained. It implied that chlorine at 75 ppm level and higher imposed a great stress on microbial cells so that a considerable percentage of *E. coli* O157:H7 was eliminated which is similar to the findings for *L. monocytogenes* reported by Sheen *et al.* [19]. At chlorine level, *i.e.* \leq 50 ppm, the initial plate counts on non-selective and selective media showed no significant difference in survival counts ($P > 0.05$) with counts on non-selective media slightly higher (0.2 - 0.3 log CFU/ml) than the selective one. Since meat samples were pre-treated at 95°C for 20 min to eliminate the background microbes, very few

background counts appeared on the non-selective plates. This observation indicated that permanent damage may have occurred to some *E. coli* O157:H7 cells, with one hour chlorine exposure. The *E. coli* O157:H7 count reduction was around 3.0 log CFU/ml at chlorine levels of 25 - 50 ppm. At higher chlorine levels, e.g. ≥ 100 ppm, typically used in sanitization, *E. coli* O157:H7 counts may be reduced by $\geq 10^6$ in seconds or minutes on contact. It should be noted that the chlorine impact on microbial survival depends on many factors, e.g. chlorine concentration, pH, water activity, organic compounds, temperature, contact surface, foods, etc. Peptone (organic compounds) may have enhanced the *E. coli* O157:H7 survival in the current study, which is consistent with the conclusion by Virto *et al.* [8]. However, the results demonstrated *E. coli* O157:H7 behavior and survival, in case of improper cleaning procedures and subsequent RTE meat contamination. The study provides important information for risk assessment.

In this study, the lag phase became longer at low temperatures (*i.e.* 12°C) and increased the potential of other microbes (e.g. mold growth was observed) to outgrow *E. coli* O157:H7 that resulted in making reliable *E. coli* O157:H7 cell counts difficult. The 0 ppm chlorine treatment served as a control for *E. coli* O157:H7 growth on the RTE ham at three different temperatures. An additional step in preparing the stressed *E. coli* O157:H7 sample was performed to further examine *E. coli* O157:H7 growth on RTE meat surface. Following one hour chlorine shock, *E. coli* ($\sim 10^3$ CFU/ml survival) was centrifuged ($3000 \times g$) and washed with 0.1% PW twice and re-suspended, then inoculated on ham surface. No significant difference in either LAG or GR calculations compared with those without wash procedures was observed ($P > 0.05$). Chlorine residuals were tested using Reflectoquant strip and the RQflex plus system (LRE Relais und Elektronik GMBH, Munich, Germany), and the result showed the residual chlorine was less than 1 ppm.

The chlorine exposure or treatment in this study simulated the cross-contamination in a processing facility where equipment was sanitized between two shifts and biofilm is not involved. However, the sanitization was assumed to be incomplete and *E. coli* O157:H7 that survived on equipment surface experienced chlorine stress, and then contaminated the RTE meats and proceeded to the finished product line. The chlorine-stressed *E. coli* O157:H7 level used to inoculate the RTE meat was about 2.7 ± 0.2 log CFU/ml on the non-selective media plate count for each experiment. The cell counts with 50 ppm chlorine treatment were 0.5 log cfu/ml lower ($P < 0.05$, ANOVA, SAS v9.1) than that with 25 ppm exposure. The chlorine exposed *E. coli* O157:H7 was inoculated

onto the ham surface, packed, vacuum sealed, then, incubated at 12°C, 18°C and 24°C. The pH of the inoculum with 0, 25 and 50 ppm chlorine was 7.0 ± 0.2 .

DMFit was applied to evaluate the growth/survival data to obtain lag time and growth rate of each experiment for secondary model development. All growth data fit the Baranyi model well with $R^2 \geq 0.96$. Based on the lag time and growth rate results with freezing shock, it can be concluded that there was no significant impact due to freezing stress ($P > 0.05$). Those data were combined with non-freezing shock data for model development. For modeling purpose, polynomial models are commonly used to describe or predict microbial lag phase and growth rate within ranges of selected parameters with proper experimental design. Cheroutre-Vialette and Lebert [20] reported the *L. monocytogenes* growth behavior with pH shock, osmotic shock (water activity) and their combination using several different types of models including polynomial models. Uljas *et al.* [21] used probabilistic models, which involved logistic regression and response surface models to describe or predict the *E. coli* O157:H7 population in apple cider with seven parameters. Pradhan *et al.* [22] developed a revised risk assessment, which took into account the *L. monocytogenes* contamination and growth of deli meats from production to consumer, with impact of lactate and diacetate on listeriosis cases and death. They further predicted that the human listeriosis cases caused by deli meats could be reduced by 2.5 to 7.8-fold with proper reformulation. Koutsoumanis and Angelidis [23] presented the “probabilistic modeling approach” to evaluate the compliance of RTE foods with new European Union safety criteria for *L. monocytogenes*. Their model system was based on: 1) growth/no growth boundary models, 2) kinetic growth models, 3) product characteristics data, and 4) storage temperature data—a more comprehensive approach for food safety evaluation. Risk assessment typically involves and integrates many different models in which the microbial growth and/or inactivation models are needed to attain the reliable results.

Lag time (*LAG*) and growth rate (*GR*) data from the experimental combinations were suitable for polynomial model development using the general linear regression procedures. Models developed for the *LAG* and *GR* are shown below in Equations (1) and (2), respectively.

$$LAG = 28.7980 + 3.0308CLO - 0.1501(T * CLO) + 0.0241CLO^2 \quad (1)$$

where *LAG* is the lag time in hours, *T* is temperature in °C; *CLO* is chlorine concentration in ppm. The R^2 is 0.91 and the temperature factor in terms of *T* and T^2 were not significant ($P > 0.05$) in the regression analyses.

$$GR^{0.5} = 0.1923 + 0.0084CLO - 0.0005 (T * CLO) + 0.0005T^2 \quad (2)$$

where GR is the growth rate in log cfu/hr and $R^2 = 0.87$. The terms in T and CLO^2 were not significant ($P > 0.05$). GR was expressed in a squared-root which can be easily compared with the results from the modified Ratkowsky-type secondary model [24].

Although the polynomial equations may well describe the relationship among different parameters, the models typically were limited by the ranges of parameters applied in the experimental design. In the current study, temperatures at 12°C - 24°C and chlorine concentrations at 0 - 50 ppm were applied. Furthermore, polynomial models may include quadratic, cubic and quartic equations, in which the quadratic model was most commonly used. It is highly desirable to develop models which can cover a wider range of important factors for applications.

Another secondary model considered and tested for lag time is the hyperbola model, which was proposed by Zwietering *et al.* [25] and applied by Oscar [26] to predict the potential growth behavior of *Salmonella typhimurium* on cooked chicken. Sheen *et al.* [19] also applied the similar principles to model *L. monocytogenes* growth shocked with chlorine.

$$LAG = \lambda = \left(\frac{p}{T - q} \right)^m; \text{ or } \lambda = \prod_{i=1}^n \left(\frac{P_i}{X_i - q_i} \right)^{m_i} \quad (3)$$

where, p is the rate of change of lag time as a function of temperature; q is the temperature at which lag time is infinite; and m is an exponent to be estimated. Other factors (in terms of X_i) may be integrated into the model (using Π operator) and a general multi-factor equation can be generated which also shown in Equation (3). However, this kind of model with applications is little investigated and may be applicable to certain conditions only. For current application, the model is constructed to take into account of both temperature and chlorine factors and shown as:

$$LAG = \lambda = \left[\frac{a}{T - (T_{\min})} \right]^b \cdot \left[\frac{CLO_{\max} + CLO}{CLO_{\max} - CLO} \right]^c \quad (4)$$

Or,

$$LAG = \lambda = \left[\frac{a}{T - (T_{\min})} \right]^b \cdot \left[\frac{CLO_{\max} + CLO}{CLO_{\max} - CLO} \right]^c \quad (5)$$

where, a , b and c are coefficients to be estimated. The temperature (T) and chlorine concentration (CLO) where *E. coli* O157:H7 has infinite lag time were set at 6°C and 100 ppm, respectively. Rutala and Cole [27] reported the lowest concentration of chlorine that reliably inactivated bacteria in hospitals is 100 ppm. The non-linear regres-

sion analyses resulted in Equation (6) where, $a = 799.301$, $b = 0.7516$ and $c = 1.2609$:

$$LAG = \lambda = \left[\frac{799.301}{T - 6} \right]^{0.7516} \cdot \left[\frac{100 + CLO}{100 - CLO} \right]^{1.2609} \quad (6)$$

Statistical analyses showed F-value = 256.36; $Pr > F$ (<0.0001); Sum of squared error/uncorrected total = 1937.8/101294.0. The R^2 is typically not available for non-linear regression analysis. Coefficient “ a ” does not satisfy the definition set for “ p ” in Equation (3), which was not derived based on growth theory but relatively an empirical model. Therefore, “ a ” may only be treated as an unknown to be estimated as well as “ b ” and “ c ”. In Equation (5), if $(100 + CLO)$ was replaced by a coefficient “ k ”, which became similar to temperature factor term, the non-linear regression result was found singular and failed to converge. The T_{\min} set to 4°C or 5°C was also tested, the regression showed similar statistical results.

The secondary model for growth rate consideration was the square-root-type or Ratkowsky-type model [24, 28,29]. McMeekin *et al.* [30] included the water activity term in the Ratkowsky-type model and Adams *et al.* [31] added the pH factor. The general expression (including temperature) is shown below:

$$\sqrt{GR} = \sqrt{\mu_{\max}} = k(T - T_{\min}) \sqrt{X - X_{\min}} \sqrt{Y - Y_{\min}} \sqrt{Z - Z_{\min}} \quad (7)$$

where, k is a coefficient to be determined and X , Y , Z can be water activity or pH or others. T_{\min} is the minimum growth temperature. X_{\min} (or Y_{\min} , Z_{\min}) is the minimum factor X (or Y , Z), e.g. pH, below which growth becomes not possible. A more general model expression was presented by Ross *et al.* [32] to include many other parameters. Equation (7) was analyzed in the current study and acceptable regression results were found not possible. Therefore, a slightly modified form was developed which is:

$$\sqrt{GR} = \sqrt{\mu_{\max}} = k \cdot (T - T_{\min})^g \cdot (CLO_{\max} - CLO)^h \quad (8)$$

where, k , g and h are coefficients to be determined. The non-linear regression results are:

$$\sqrt{GR} = \sqrt{\mu_{\max}} = 0.1182 \cdot (T - 6)^{0.2085} \cdot (100 - CLO)^{0.1455} \quad (9)$$

Statistical analyses showed F-value = 218.45; $Pr > F$ (<0.0001); Sum of squared error/uncorrected total = 0.0553/2.4526.

Since Equation (7) was derived from the general observation of different microbial growth in various conditions, it might not fit all conditions which impact the growth curve. Huang [33] also reported that growth ki-

netics of *Escherichia coli* O157:H7 was better fit by Belehradek-type model (McMeekin *et al.* [34]) than by traditional Ratkowsky model. The author presented the model as $\mu_{max} = k(T - T_{min})^{1.5}$, which can be converted to $(\mu_{max})^{1/2} = k_1(T - T_{min})^{0.75}$. With more parameters involved, the model may become much complicated to achieve acceptable statistical analyses.

Figure 1 indicated that the LAG decreased with increase of temperature and decrease of shocked chlorine concentration. The 0 ppm also showed the growth data at control conditions of *E. coli* O157:H7 on ham surface, which might be further affected by ham composition. Although the LAG can be extended by the chlorine concentration, the higher temperature may offset the chlorine effect, e.g. the LAGs were about the same at 0 ppm chlorine at 12°C and at 50 ppm chlorine at 24°C abuse temperature. It is also interesting to note that the lag times were similar (between 50 and 60 h) at 25ppm chlorine concentration for all three temperatures. **Figure 2** showed that the growth rate slightly increased with increase of chlorine ppm at 12°C and 18°C, but decreased with increase of chlorine at 24°C. Unless the sanitization completely eliminated *E. coli* O157:H7 there exist the risk that microbes might survive and grow when environmental conditions become favorable. **Figures 1** and **2** clearly show that it is difficult to predict the microbial survival behavior under stresses without experimental data support.

Two secondary types of models are presented in this study, *i.e.* the linear regression models and the empirical models based on observations and curve fitting. It is interesting to compare the models in terms of their predicted vs. experimental data. **Figure 3** shows the comparison of linear regression models in which the solid line (slope = 1) indicates the predicted and observed values are equal. When the data points are above or below

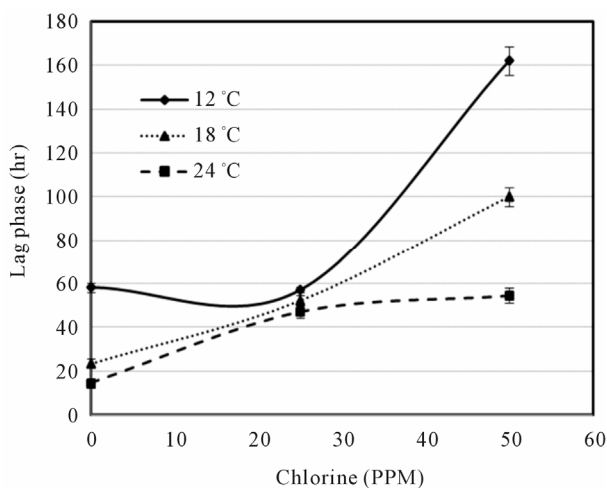


Figure 1. Lag phase vs. chlorine concentration (ppm) at three abuse temperatures.

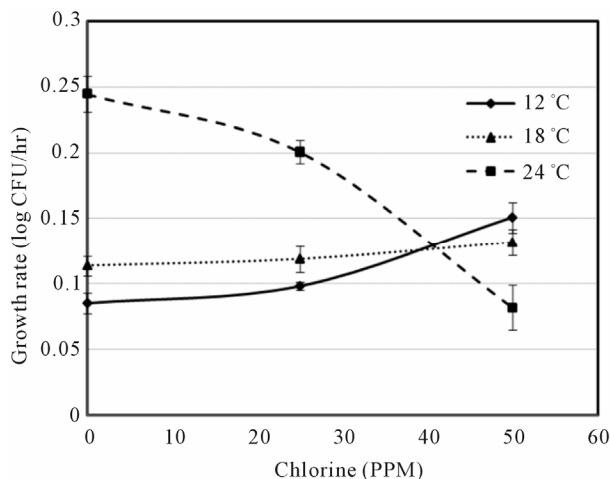


Figure 2. Growth rate vs. chlorine concentration (ppm) at three abuse temperatures.

the solid line, the predicted values are over or under estimated, respectively. Similarly, **Figure 4** demonstrates the results of comparison between the Ratkowsky-type and Zwietering-type models. In **Figure 4**, The LAG data show a narrower 95% confident range than those from **Figure 3**, which indicates that the regression model may predict data closer to the experimental ones. However, the GRs in **Figures 3** and **4** show the reverse trend where the linear regression model may perform better than Ratkowsky-type model.

Model performance in accuracy of prediction was evaluated using a 40 ppm chlorine shock (pH 7.1), then inoculated on ham and stored at 12°C and 18°C. The predicted LAG and GR (for 12°C) from Equations (1), (2), (6) and (9) were 116.542, 0.1298, 115.0272 and 0.0971, respectively. The experimental data were 109.891 (for LAG) and 0.1228 (for GR). For 18°C, predicted values were 80.518, 0.1091, 68.3198 and 0.1296, respectively, and the experimental data were 76.245 (LAG) and 0.1238 (GR). All developed models predicted the LAG and GR within 20% of the experimental values at both 12°C and 18°C. Another experiment was performed to evaluate the developed models outside the chlorine parameter range, *i.e.* chlorine shock at 60 ppm and 18°C abused temperature. The experimental data resulted for LAG and GR were 125.323 h and 0.1254 log cfu/h, respectively. Equations (6) and (9) predicted the values of 134.804 h and 0.1152 log cfu/hr, which were within 10% range of the experiment data. However, Equations (1) and (2) showed the predicted values of 135.298 h (LAG) and 0.1013 log cfu/h (GR). It is generally agreed that the linear regression model with experimental design is only applicable within the designed parameter ranges. In the current case, Equations (1) and (2) were found suitable for applications with chlorine shock at 60 ppm. Since no

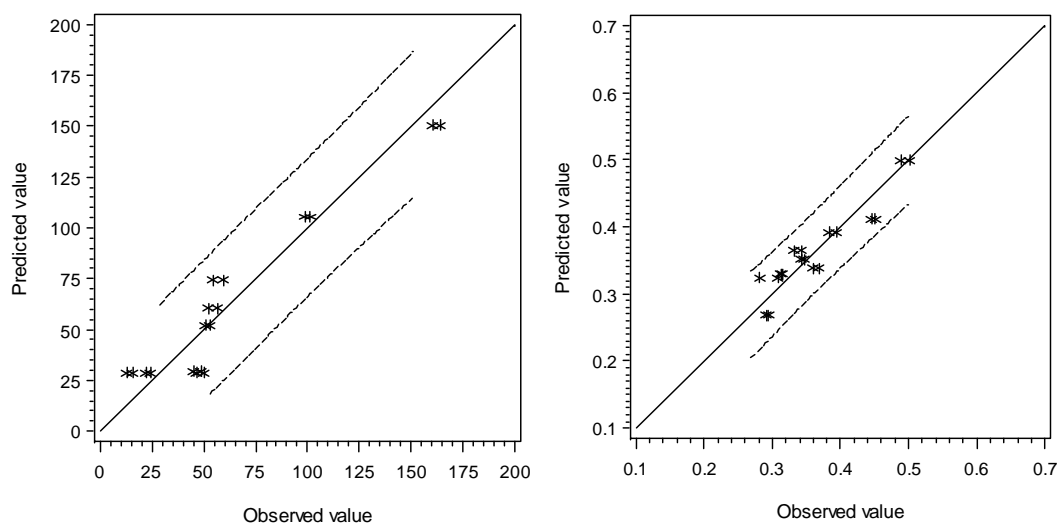


Figure 3. The predicted vs. observed values of LAG (top) and $GR^{0.5}$ (bottom) using Equations (1) and (2), respectively.

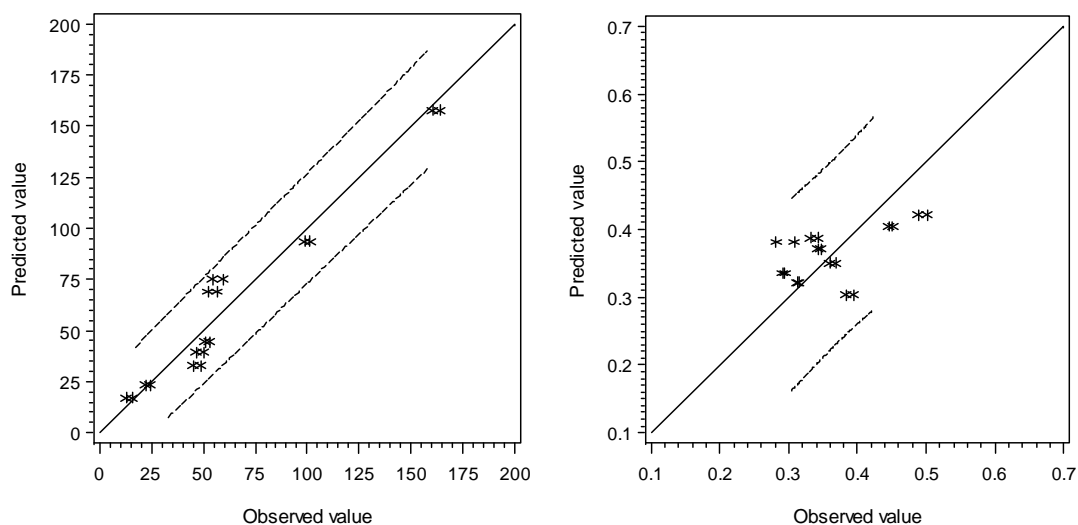


Figure 4. The predicted vs. observed values of LAG (top) and $GR^{0.5}$ (bottom) using Equations (6) and (9), respectively.

reliable data with chlorine shock at 75 ppm were available, Equations (1) and (2) can not be further validated for wider range of chlorine concentration. General speaking, secondary models similar to modified Ratkowsky-type and Zwietering-type might be more suitable for applications with broader parameter ranges.

4. Conclusion

This study provided useful results and information regarding the effects of chlorine concentration, abuse temperatures and freezing shock on the survival and growth behavior of *E. coli* O157:H7 in RTE meat. The freezing shock was found to have little impact on *E. coli* O157:H7 survival behavior in terms of lag time and growth rate. Two types of secondary models, as a function of chlorine

concentration and abuse temperature, were developed and presented, *i.e.* the polynomial regression models, modified Ratkowsky-type and Zwietering-type models. The modified Ratkowsky-type and Zwietering-type models were applicable to a wider range of parameters, although the polynomial regression models were found suitable with chlorine at 60 ppm which is outside the parameter range. Those models may be used for risk assessment, within applicable conditions, to enhance the RTE meat safety.

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Williamson of the Food Safety and Intervention Technologies Research Unit, ERRC/ARS/USDA, Wyndmoor, PA.

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