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Modeling *Salmonella* Growth in Irradiated Pork for Specific Target Groups and Patients at Isothermal and Dynamic Temperature

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This study developed mathematical models to predict *Salmonella enterica* growth in irradiated pork for specific target groups and immunocompromised patients under constant and dynamic temperature conditions. Irradiated fresh pork slices were prepared by gamma-irradiation at 40 kGy, and inoculated with *Salmonella* followed by storage at 4°C, 15°C, 25°C, and 35°C up to 240 h, depending on storage temperature. *Salmonella* cell counts were enumerated on tryptic soy agar during storage. For calculation of maximum specific growth rate (Log CFU/cm²/h), lag phase duration (h), lower asymptote (Log CFU/cm²), and upper asymptote (Log CFU/cm²), the Baranyi model was fitted to the bacterial cell counts. The model parameters were then further expressed as a function of storage temperature (R^2: 0.8-0.992). In addition, a dynamic model was also developed to predict *Salmonella* growth at changing temperature. Mathematical indices showed that the model had good performance with 1.011 and 1.04 for bias and accuracy factors, respectively, under constant temperature. In addition, graphical comparison showed that *Salmonella* growth simulation under changing temperature was acceptable. The results indicate that the developed models should be useful in predicting *Salmonella* growth in irradiated fresh pork, which could be used for special target groups and immunocompromised patients.

Key words: *Salmonella*, Pork, Irradiation, Predictive microbiology, Dynamic model.

Salmonella spp. colonize the gastrointestinal tract of animals without producing any clinical signs (Bottledoorn *et al.,* 2003; Wong *et al.,* 2007). The pathogen may contaminate pork during slaughter, and they proliferate at storage and distribution (Hansen *et al.,* 2010). In the U.S. retail store, 9.6% pork samples were contaminated with *Salmonella* (Duffy *et al.,* 2000), and the pork in German (1.8%), U.K (3.9%) and Irish retail store (2.6%) were also contaminated with *Salmonella* (Little *et al.,* 2008; Meyer *et al.,* 2010; Prendergast *et al.,* 2009). In slaughterhouses of S. Korea, 10.2% pork carcasses were positive for *Salmonella* (Hwang *et al.,* 2004), and this prevalence of *Salmonella* in S. Korea may increase microbiological risk to consumers.

A preference survey by Kang (2006) showed that patients prefer to have sea foods (45.1%) and meat products (33.3%) in S. Korea, but these foods are hardly allowed to consume to immunocompromised patients because of microbiological food safety. Sterile foods have been suggested for patients as well as military personnel, astronauts and refugees. Thus,

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irradiation has been used in foods for immunocompromised patients and specific target groups in the Philippines, S. Korea, Brazil, Hungary, Netherland, the U.K. and the U.S. (Han *et al.,* 2006; IAEA 2009).

Predictive models have been used to describe growth limits and the kinetic behavior of various foodborne pathogens (Lee *et al.,* 2012; Van Impe *et al.,* 2005). A primary predictive model describes kinetic behavior of bacteria, and a secondary model evaluates the effect of environmental factors on the kinetic parameters (Xanthiakos *et al.,* 2006). To predict *Salmonella* growth in irradiated pork, mathematical modeling would be feasible under constant and changing temperature which is found in storage and distribution (Gumudavelli *et al.,* 2007).

Therefore, the objective of this study was to develop mathematical models to describe *Salmonella* growth in irradiated pork intended for immunocompromised patients and specific target groups under static and dynamic temperature.

MATERIALS AND METHODS

Inoculum preparation

Salmonella enterica subsp. *enterica* ATCC6960 (isolate from pork) was cultured in 10 mL tryptic soy broth (TSB; Difco, Becton Dickinson and Company, Sparks, MD., USA) at 35°C for 24 h. Portions (0.1 mL) of the cultures then were subcultured in 10 mL TSB at 35°C for 24 h. Stationary phase cells were centrifuged (4,629×*g*, 15 min, 4°C) and washed with phosphate buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 L of distilled water). The resulting cell pellets were resuspended in PBS to yield approximately 3 Log CFU/mL.

Pork slice preparation and inoculation

Fresh pork tenderloin was purchased and cut into pieces (0.5×5×2.5 cm) with a flame-sterilized stick knife. Slices were gamma-irradiated at 40 kGy to remove natural flora, using a cobalt-60 irradiator (IR-221; MDS Nordion International Co. Ltd., Ottawa, Ontario, Canada) in Advanced Radiation Technology Institute of Korea Atomic Energy Research Institute (Jeoungeup, Jeonbuk, S. Korea) at 10 kGy/h. Pork slices were then inoculated by spreading a volume of 0.1 mL of the *Salmonella* inoculum. The inoculated samples were kept at 4°C for 15 min to allow cell attachment, and *Salmonella* was inoculated on the second side of the pork slices.

Storage and microbiological analysis

The inoculated pork samples were placed in sterile plastic bags (20×25 cm; Sunkyung Co. Ltd., Seoul, S. Korea) and sealed, and the bags were aerobically stored at 4, 15, 25 and 35°C for 240, 96, 36, and 24 h, respectively. During storage, samples were withdrawn periodically at appropriate sampling time for microbiological analysis; 4°C (0, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h), 15°C (0, 6, 9, 12, 18, 24, 48, 72, and 96 h), 25°C (0, 2, 4, 6, 12, 18, 24, 36, and 48 h), and 35°C (0, 2, 4, 6, 10, 14, 18, and 24 h). A volume of 30 mL saline solution (0.85% NaCl) was added to each sample bag, and it was pummeled (Model 400, Tekmar Co., Los Angeles, CA., USA) for 2 min. After serially diluting each sample with saline solution, 0.1 mL portions were spread-plated on tryptic soy agar (Difco) to enumerate *Salmonella*. All plates were incubated at 35°C for 48 h.

Model development

The study was repeated (replication) twice with two samples for each replication, and microbiological data (CFU/cm^2) were converted to Log CFU/cm^2 before being analyzed. The Baranyi model (Baranyi and Roberts, 1994) was fitted to the microbiological data using a DMFit program (Institute of Food Research, Norwich, UK) for each storage temperature to estimate maximum specific growth rate (μ_{max}; Log CFU/cm^2/h), lag phase duration (LPD; h), lower asymptote (N_0; Log CFU/cm^2), and upper asymptote (N_{max}; Log CFU/cm^2).

To evaluate effects of storage temperature on growth parameters, the parameters were further expressed as a function of storage temperature using the square root function (Ratkowsky *et al.,* 1982) for μ_{max} and 1/LPD, and the second order polynomial function for N_{max}. The square root function is

$$\sqrt{\mu_{max}} = a_{\mu}(T - T_{min}) \quad ...(1)$$

$$\sqrt{\frac{1}{LPD}} = a_{LPD}(T - T_{min}) \quad ...(2)$$

Where a_{μ} and a_{LPD} are the slopes of the fitted lines for the square root of μ_{max} and square

root of 1/LPD, respectively, T is the storage temperature, and T_{min} is the theoretical minimum temperature (°C) for *Salmonella* growth.
The second order polynomial function is

$$\sqrt{N_{max}} = a_1 + a_2 \times T + a_3 \times T^2 \quad ...(3)$$

Where a_i is the coefficient and T is the storage temperature.

In order to predict *Salmonella* growth in irradiated pork under dynamic storage temperature, the mathematical equation described by Baranyi and Robert (1994), and Baranyi et al. (1995) was used. To obtain time-temperature profile for worst-case scenario, *Salmonella*-inoculated fresh pork were stored in arbitrarily set order of following temperature profile; 12°C (24 h), 4°C (30 h), 12°C (12 h), 4°C (30 h), 12°C (48 h), 4°C (66 h), and 25°C (30 h). During dynamic temperature storage, *Salmonella* cell counts in fresh pork were enumerated periodically to evaluate dynamic model performance, and temperature was recorded by an electronic temperature recorder (Giltron® GT340, Gilwoo trading Co., Taipei, Taiwan).

Validation of model

Observed cell counts of *Salmonella* were obtained from an independent experiment, and these data were used to evaluate the model performance in isothermal condition. The observed *Salmonella* cell counts were compared to the predicted *Salmonella* cell counts produced by simulation of the developed models. Bias (*B*) and accuracy (*A*) factors (Ross, 1996) were calculated to evaluate model performance, using equations 4 and 5.

$$\text{B factor} = 10^{\frac{\sum \log\left(\frac{\text{predicted values}}{\text{observed values}}\right)}{n}} \quad ...(4)$$

$$\text{A factor} = 10^{\frac{\sum |\log\left(\frac{\text{predicted values}}{\text{observed values}}\right)|}{n}} \quad ...(5)$$

Where n is the number of observations.

The model performance for changing temperature was evaluated by the graphical comparison.

RESULTS AND DISCUSSION

Salmonella growth was not observed at 4°C (Fig. 1A). The growth curves of *Salmonella* were characterized by a decrease of LPD and increase of μ_{max} as storage temperature increased (Table 1, Fig. 1). Velugoti et al. (2011) predicted *Salmonella* spp. growth in sterile ground pork at 10-45°C. In their study, N_{max} at 15°C was 8.8 Log CFU/g, which was about 2.3 Log unit higher than that of our study, and the pathogen reached stationary phase after about 20 and 9 h of incubation at 25 and 35°C, respectively, which were earlier than those (25°C: 36 h, 35°C: 18 h) found in our study (Table 1). This discrepancy could be caused by different food matrix between ground pork and pork cutting. R^2 values for 15, 25, and 35°C were more than 0.9 from fitting *Salmonella* growth data to the Baranyi model (Table 1). Moreover, all fitted lines also passed through the data points (Fig. 1). This indicates that the primary

Table 1. Coefficients (mean ± standard error) of growth parameters of the primary model obtained by fitting *Salmonella* growth data for each storage temperature

Temperature (°C)	μ_{max} (Log CFU/cm²/h)	LPD (h)	N_0 (Log CFU/cm²)	N_{max} (Log CFU/cm²)	R^2
4	0.001±0.002	240±0.00	2.6±0.2	2.7±0.0	0.133-0.387
15	0.081±0.018	16.34±0.34	2.8±0.0	6.5±0.1	0.985-0.994
25	0.563±0.315	3.87±1.36	2.8±0.2	8.1±0.1	0.934-0.993
35	0.611±0.194	1.28±1.28	2.2±0.4	8.3±0.5	0.980-0.983

μ_{max}: maximum specific growth rate
LPD: lag phase duration
N_0: lower asymptote
N_{max}: upper asymptote

models developed in this study were acceptable to the kinetic behavior of *Salmonella* in irradiated pork. Although *Salmonella* in pork samples at 15°C reached at stationary phase, N_{max} was lower than those at 25°C and 35°C (Table 1, Figs. 1B-1D), because low temperature may inhibit active transport due to decreased affinity for substrate at low temperature (George *et al.,* 1996; McClure *et al.,* 1997; Nedwell and Rutter, 1994).

To improve the goodness of fit of the models, transformation of growth parameters with square root, inverse function, and natural log etc., could be recommended (Lambert and Bidlas, 2007; Yoon *et al.,* 2009). Hence, estimated model parameters (μ_{max}, 1/LPD, and N_{max}) were transformed by square root. The transformed parameters were used to evaluate storage temperature effect on *Salmonella* growth parameters (Fig. 2). As expected square root of μ_{max} and 1/LPD linearly increased as storage temperature increased, and T_{min} was 2.23°C (Figs. 2A and 2B). Other studies (Oscar, 2002; Osiriphun *et al.,* 2004) found that T_{min} were 5.2°C for fresh pork and 3.8°C for chicken, which were higher than that of our study. The lower T_{min} in our study could be caused by no competitions between *Salmonella* and indigenous microflora (Cornu *et al.,* 2011). N_{max} had a sigmoidal increase pattern by temperature (Fig. 2C). Moreover, all observed data points were close to the regression line, and they were placed within 95% confidence interval (Fig. 2). The results show that secondary models may have the acceptable goodness of fit.

Coefficients were calculated with the secondary model under a given temperature (12°C), and the coefficients were used to simulate *Salmonella* growth in accordance with primary model. The predicted *Salmonella* cell counts were compared to the observed cell counts (Fig. 3). The *B* factor was 1.011 and *A* factor was 1.04 for constant storage temperatures. Perfect agreement between predicted *Salmonella* cell counts and observed *Salmonella* cell counts is 1. If a *B* factor is higher than 1, it means that predicted values are larger than the observed values (Tamplin *et al.,* 2005). The result from our study suggests that the

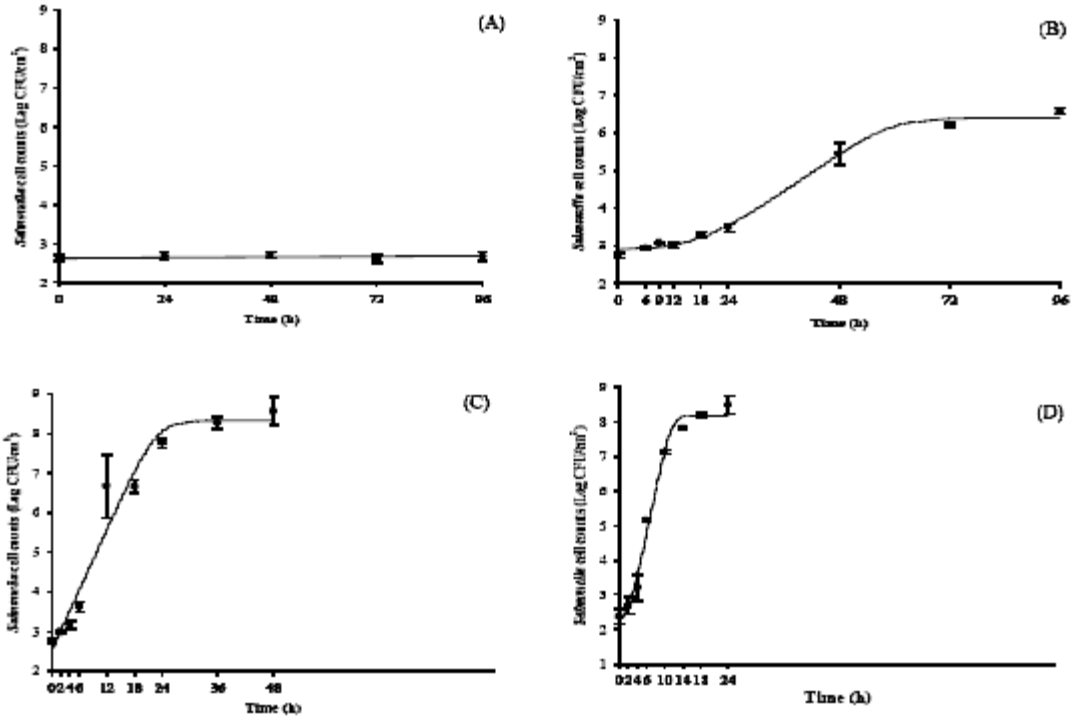

Fig. 1. Bacterial growth (•) of *Salmonella* in irradiated pork, and fitted lines during storage at 4°C (A), 15°C (B), 25°C (C), and 35°C (D)

model predictions exceeded the observations, on average, by approximately 1 %. Thus, models could be considered having good model performance. Tamplin et al. (2005) suggested that models were considered "good model performance" for having a *B* factor of 0.9 to 1.05 and "acceptable" if *B* factors ranged from 0.7 to 0.9 and 1.06 to 1.15.

Salmonella growth under changing storage conditions was also simulated (Fig. 4). The simulation may be useful in predicting the fate of

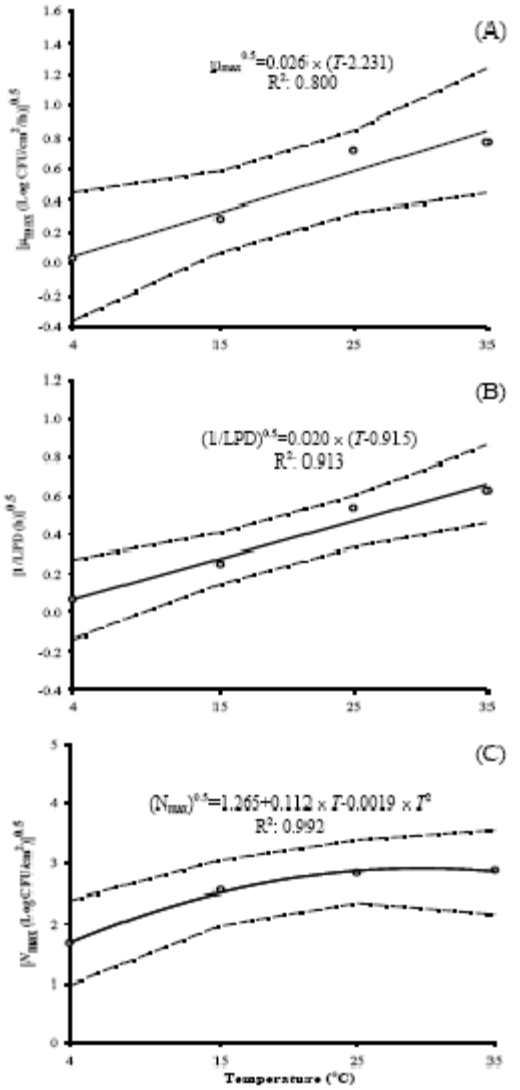

Fig. 2. Effect of storage temperature on maximum specific growth rate (μ_{max}; A), lag phase duration (LPD; B), and upper asymptote (N_{max}; C). Open symbols, observed values; solid line, fitted line; dashed lines, lower and upper 95% confidence intervals

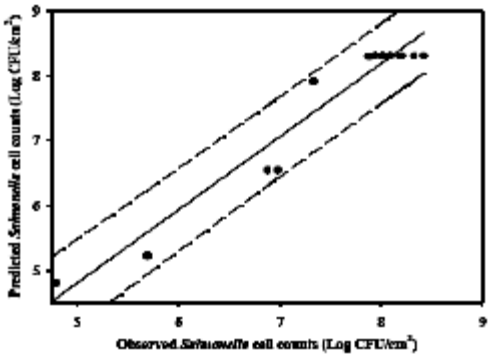

Fig. 3. Comparison of observed *Salmonella* cell counts on irradiated pork with the cell counts simulated by the developed models for constant temperature at 12°C

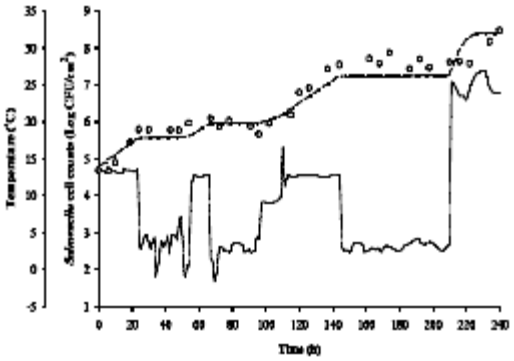

Fig. 4. Simulation of *Salmonella* growth in irradiated pork cutting at the dynamic temperature condition. Open symbols, observed growth; solid line, temperature profile; dashed line, simulated *Salmonella* growth

Salmonella in irradiated pork under changing temperature condition. Fig. 4 shows that most observed data points were around the predicted lines.

In conclusion, the developed models in this study could be used in predicting *Salmonella* cell counts in irradiated pork under constant and dynamic temperature condition, and thus, the prediction may improve the food safety of irradiated fresh pork.

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