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Research Note

Inactivation Kinetics of *Escherichia coli* O157:H7, *Salmonella enterica* Serovar Typhimurium, and *Listeria monocytogenes* in Ready-to-Eat Sliced Ham by Near-Infrared Heating at Different Radiation Intensities

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ABSTRACT

The aim of this study was to investigate the inactivation kinetics of *Salmonella enterica* serovar Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on ready-to-eat sliced ham by near-infrared (NIR) heating as a function of the processing parameter, radiation intensity. Precooked ham slices inoculated with the three pathogens were treated at different NIR intensities (ca. 100, 150, and 200 μW/cm^2/nm). An increase in the applied radiation intensity resulted in a gradual increase of inactivation of all pathogens. The survival curves of the three pathogens exhibited both shoulder and tailing behavior at all light intensities. Among nonlinear models, the Weibull distribution and log-logistic model were used to describe the experimental data, and the statistical results (mean square error and R^2 values) indicated the suitability of the model for prediction. The log-logistic model more accurately described survival curves of the three pathogens than did the Weibull distribution at all radiation intensities. The output of this study and the proposed kinetics model would be beneficial to the deli meat industry for selecting the optimum processing conditions of NIR heating to meet the target pathogen inactivation on ready-to-eat sliced ham.

The consumption of ready-to-eat (RTE) meats, especially precooked sliced ham, has increased because of their convenience *(20)*. However, foodborne illness outbreaks linked to RTE delicatessen meats have increased worldwide. *Salmonella enterica* serovar Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* are the primary foodborne pathogens involved in numerous outbreaks related to delicatessen meats *(3, 8)*. The surface of RTE sliced ham products is the primary site of contamination during postprocessing handling (e.g., conveying, cutting, and slicing). Therefore, an additional decontamination step may be needed as a postlethality intervention to inactivate pathogenic bacteria on sliced ham products before final packaging or after the meats are unwrapped at the delicatessen or other retail outlet. Thermal inactivation is still one of the most effective bacterial control methods for RTE meats *(16)*.

Infrared radiation is a form of electromagnetic energy and is categorized as near infrared (NIR; 0.76 to 2 μm), medium infrared (2 to 4 μm), and far infrared (4 to 1,000 μm). Infrared heating has been gaining wider acceptance because of its higher thermal efficiency and fast heating rate and response time compared with conventional heating *(17)*. Various infrared surface pasteurization technologies have been developed for surface treatment of precooked deli meats *(14–16)*. In our previous study *(14)*, the effectiveness of infrared heat processing for surface pasteurization of RTE sliced ham could have been improved and the long heating time shortened by use of NIR radiation as a heat source.

Several primary mathematical models have been used to describe the kinetics of microbial inactivation by various processing methods in RTE meats *(4, 7, 11)*; however, kinetics models describing the inactivation of major foodborne pathogens on deli meats by NIR heating have not been developed. Predicting the effectiveness of NIR heating against foodborne pathogens based on accurate inactivation kinetics is essential to permit establishment of safe processing conditions and is critical for the effective application of this processing technology in industrial pasteurization.

When survival curves are nonlinear, the *D*-value is usually determined by considering the linear portion of the survival curve. However, this approach is definitely not desirable and would result in over- or underestimation, depending on the shape of the survival curve *(19)*. Significant and systematic deviations from linearity have been frequently observed in many thermal treatments *(12, 18, 19, 21)*. The survival curves of *Salmonella* Typhimurium,

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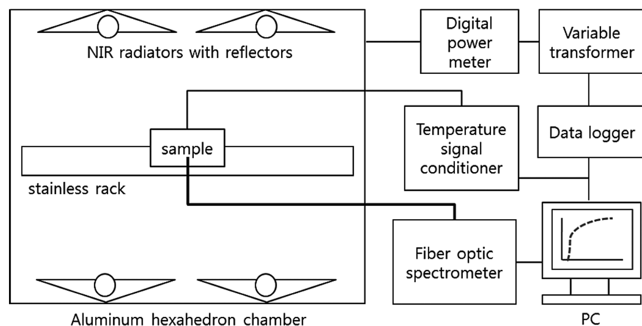

FIGURE 1. *Schematic view of the NIR heating system used in this study.*

E. coli O157:H7, and *L. monocytogenes* on sliced ham during NIR treatment were not log linear and were clearly concave in our previous study *(14)*.

The objective of this study was to find a suitable inactivation kinetics model for *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* on RTE sliced ham by NIR heating as a function of the processing parameter of radiation intensity.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. Strains of *Salmonella* Typhimurium (DT 104), *E. coli* O157:H7 (ATCC 35150), and *L. monocytogenes* (ATCC 19115) were obtained from the School of Food Science bacterial culture collection of Seoul National University (Seoul, Korea). Stock cultures were kept frozen at −80°C in 0.7 ml of tryptic soy broth (TSB; Difco, BD, Sparks, MD) and 0.3 ml of 50% glycerol. Working cultures were streaked onto tryptic soy agar (Difco, BD), incubated at 37°C for 24 h, and stored at 4°C.

Each strain of *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* was cultured in 5 ml of TSB at 37°C for 24 h, centrifuged (4,000 × *g* for 20 min at 4°C), and washed three times with buffered peptone water (BPW; Difco, BD). The final pellets were resuspended in BPW, corresponding to approximately 10^7 to 10^8 CFU/ml. Suspended pellets of each strain of the three pathogenic species were combined to produce mixed culture cocktails. These culture cocktails with a final level of approximately 10^8 CFU/ml were used in this study.

Sample preparation and inoculation. Precooked, vacuum-packaged sliced ham (approximately 90 by 90 by 2 mm) was purchased from a local grocery store (Seoul, Korea). For surface inoculation, 8 ml of the prepared mixed culture cocktail was diluted in 0.8 liter of sterile peptone water. Each ham slice was immersed in the mixed pathogen suspension for 1 min at room temperature (22 ± 2°C), drained on a sterilized rack, and dried in a laminar flow biosafety hood for 20 min with the fan running. Two ham slices (ca. 25 g, inoculum level of 10^6 to 10^7 CFU per sample) were used with each experimental batch.

NIR heating and radiation intensity measurement. A model aluminum chamber (41 by 34 by 29 cm) was used in this study for NIR heating (Fig. 1). Four quartz halogen infrared heating lamps (350 mm; NS-104, NS-TECH Co., Ltd., Gyunggi-do, Korea), each with a maximum power of 500 W for 230 V input, were used as an NIR radiation emitting source. The maximum wavelength (λm) of the infrared heater used in this study was about 1,300 nm, which is in the NIR wave range. The four NIR radiation emitters were arranged horizontally in parallel with the four emitting surfaces facing each other, and four aluminum reflectors were installed behind the emitters to redirect the infrared waves and enhance the efficiency of infrared radiation (Fig. 1). The vertical distance between emitters and sample was 13.5 cm (5.3 in.) at each side. Surface-inoculated ham slices were placed side-by-side in the center of a sterilized stainless rack with the long axis parallel to the NIR lamps. The radiation intensity was selected as the control parameter to provide applicability to other NIR operating conditions. The radiation intensity generated from the NIR heater was measured and recorded by an NIR fiber optic spectrometer (AvaSpec-NIR256-1.7, Avantes, Eerbeek, The Netherlands). At the sample location, the radiation intensity of the four NIR emitters was adjusted to 99.74 μW/cm^2/nm (1.0 kW), 150.03 μW/cm^2/nm (1.4 kW), and 200.36 μW/cm^2/nm (1.8 kW, standard line voltage) using a variable transformer and a data logger (34790A, Agilent Technologies, Palo Alto, CA). The adjusted electric power was simultaneously verified with a digital power meter (WT-230, Yokogawa, Tokyo, Japan).

Temperature measurement. A fiber optic temperature sensor (FOT-L, FISO Technologies Inc., Quebec, Quebec, Canada) connected to a signal conditioner (TMI-4, FISO Technologies) was used to measure real-time temperatures in samples during NIR heating at different radiation intensities. The sensor was placed directly on the surface of the treated ham slices, and the temperature was manually recorded every 5 s. All experiments were replicated three times, and means and standard deviations of sample temperatures for selected radiation intensities were compared to determine the heating rate of samples.

Bacterial enumeration. For enumeration of pathogens, each of two treated ham slices (ca. 25 g) were removed, immediately transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of BPW (detection limit of 10 CFU/g), and homogenized for 2 min with a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml of sample was serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread plated onto each selective medium. Xylose lysine desoxycholate agar (Difco, BD), sorbitol MacConkey agar (Difco, BD), and Oxford agar base with Bacto Oxford antimicrobic supplement (Difco, BD) were used as selective media for the enumeration of *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes,* respectively. When low numbers of surviving cells were anticipated, 1 ml of undiluted stomacher bag contents was equally distributed onto four plates to lower the detection limit. All agar media were incubated at 37°C for 24 to 48 h before colonies were counted. To confirm the identity of the pathogens, random colonies were selected from the enumeration plates and subjected to biochemical and serological tests: the *Salmonella* latex agglutination assay (Oxoid, Ogdensberg, NY), the *E. coli* O157:H7 latex agglutination assay (Remel, Lenexa, KS), and the API *Listeria* test (bioMérieux, Hazelwood, MO).

Modeling of survival curves. All microbial experiments were conducted three times with duplicate samples, and survival curves obtained under nonisothermal conditions were fitted with the Weibull distribution (equation 1) and log-logistic model (equation 2).

The Weibull model *(6)* is given by

$$\log \frac{N}{N_0} = -\left(\frac{1}{2.303}\right)\left(\frac{t}{\alpha}\right)^{\beta} \quad (1)$$

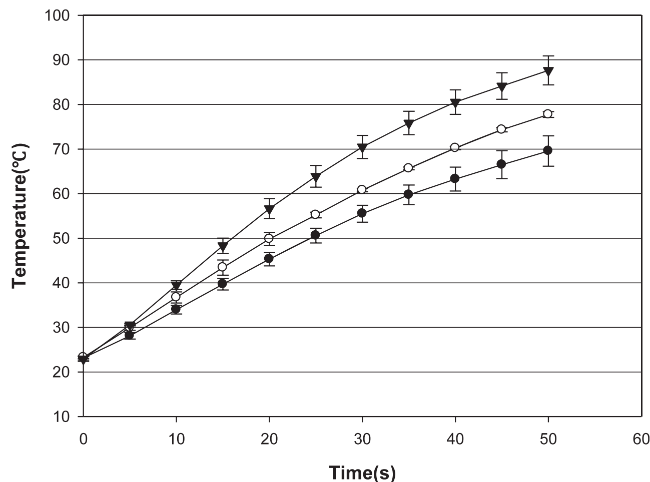

FIGURE 2. *Average temperature-time histories of ham slice surfaces during NIR heating at different radiation intensities. Error bars indicate standard deviations calculated from triplicate results.* ●, *NIR treatment at 100 μW/cm²/nm;* ○, *NIR treatment at 150 μW/cm²/nm;* ▼, *NIR treatment at 200 μW/cm²/nm.*

where α and β are the scale and shape parameters, respectively. The α value represents the time necessary to inactivate the first 0.434 log cycles of the population. The β value accounts for upward concavity of a survival curve ($\beta < 1$), downward concavity of a survival curve ($\beta > 1$), and a linear survival curve ($\beta = 1$).

The log-logistic model *(9)* is described as

$$\log\frac{N}{N_0} = \frac{A}{1+e^{4\sigma(\tau-\log t)/A}} - \frac{A}{1+e^{4\sigma(\tau+6)/A}} \quad (2)$$

where σ is the maximum rate of inactivation (log CFU per milliliter), τ is the log time to the maximum rate of inactivation (log minutes), *t* is the thermal treatment time (seconds), and *A* is the lower asymptote minus the upper asymptote (log CFU per milliliter). Model parameter values and their confidence intervals were obtained by fitting the experimental data to the primary models using GraphPad PRISM (GraphPad Software, Inc., San Diego, CA). The regression coefficient (R^2) and mean square error (MSE) were used to evaluate the goodness of fit of the two models.

RESULTS AND DISCUSSION

Average temperature-time histories of ham slices at different radiation intensities. Average surface temperatures of ham slices during NIR heating at various radiation intensities are shown in Figure 2. The surface temperature rose immediately in response to infrared waves when the ham slice samples were exposed to NIR radiation, and the heating rate of ham slices was dependent on the radiation intensity. The temperature increase at 200 μW/cm²/nm (1.29°C/s) was higher than that at 150 μW/cm²/nm (1.09°C/s) and 100 μW/cm²/nm (0.93°C/s). After 50 s of NIR heating, the surface temperature of ham slices reached ca. 87, 77, and 69°C at 200, 150, and 100 μW/cm²/nm, respectively. For the 150- and 100-μW/cm²/nm treatments, the maximum heating times to reach 87°C from room temperature were ca. 75 and 95 s, respectively.

In our previous NIR heating study *(14),* treatment at 200 μW/cm²/nm (1.8 kW) for 50 s did not significantly change ($P > 0.05$) the quality (color and texture) of ham slices. Significant color and texture changes also were not observed at any other intensity levels used in this study (100 and 150 μW/cm²/nm, data not shown), indicating that NIR treatment at various intensities is acceptable for use in deli meat industry.

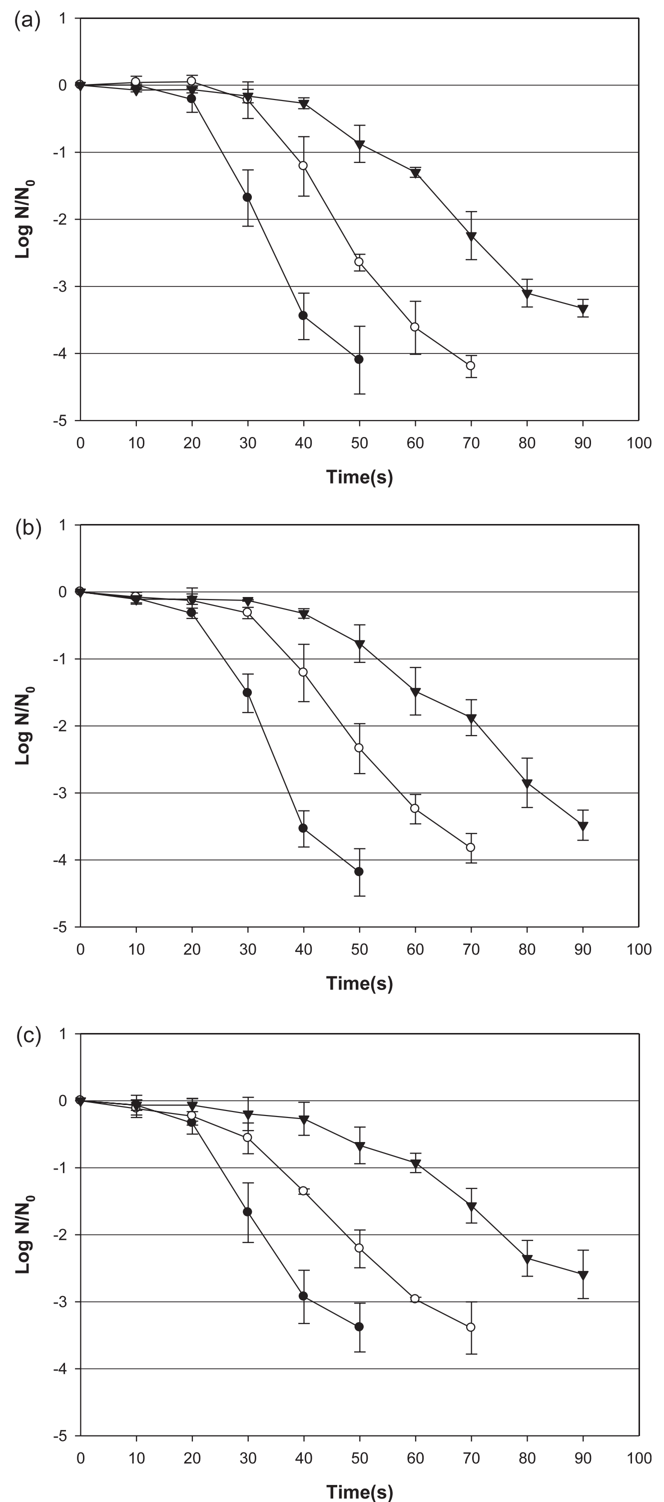

FIGURE 3. *Survival curves for* Salmonella enterica *serovar Typhimurium (a),* Escherichia coli *O157:H7 (b), and* Listeria monocytogenes *(c) on ham slice surfaces treated at different NIR intensities. Error bars indicate standard deviations calculated from triplicate results.* ●, *NIR treatment at 200 μW/cm²/nm;* ○, *NIR treatment at 150 μW/cm²/nm;* ▼, *NIR treatment at 100 μW/cm²/nm.*

TABLE 1. *Kinetic parameters of the Weibull model and log-logistic model for* Salmonella *Typhimurium,* E. coli *O157:H7, and* L. monocytogenes *on ready-to-eat sliced ham treated with near-infrared heating at different radiation intensities*

Pathogen	Radiation intensity (μW/cm²/nm)	Weibull model		Log-logistic model		
		α	β	*A*	σ	τ
Salmonella	100	36.46	2.35	−3.76	−0.09	65.38
Typhimurium	150	23.92	2.19	−4.25	−0.16	46.75
	200	15.19	1.94	−4.16	−0.22	32.06
E. coli O157:H7	100	38.38	2.48	−4.60	−0.08	73.16
	150	23.84	2.08	−4.03	−0.12	47.46
	200	15.28	1.97	−4.33	−0.22	32.88
Listeria	100	41.72	2.41	−3.24	−0.06	69.82
monocytogenes	150	21.12	1.77	−3.69	−0.09	45.67
	200	14.42	1.71	−3.43	−0.17	30.46

Inactivation of pathogenic bacteria by NIR heating at various radiation intensities. The survival curves corresponding to the inactivation of *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* by NIR heating at different radiation intensities on ham slices are shown in Figure 3. An increase in the applied radiation intensity resulted in a faster heating rate and therefore a gradual increase in inactivation of all pathogens. Treatment at 100 μW/cm²/nm for 50 s inactivated populations of *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* by about 0.87, 0.77, and 0.67 log CFU/g, respectively. The 200 μW/cm²/nm treatment for 50 s reduced the three pathogens by an additional 3.22, 3.41, and 2.72 log CFU/g, respectively, more than did the 100 μW/cm²/nm treatment. Especially for *L. monocytogenes,* the 100 μW/cm²/nm treatment required more than 90 s to attain a ca. 3-log reduction. In the first moments of treatment at all intensities, the change in the level of the three pathogens was very small (shoulder of the survival curve) and then the microbial populations declined following a concave downwards curve (when the temperature increased into the >60°C range). The downwardly concaved survival curves exhibited characteristic tailing at 40 to 50 s for the 200 μW/cm²/nm treatment, 60 to 70 s for the 150 μW/cm²/nm treatment, and 80 to 90 s for the 100 μW/cm²/nm treatment.

Suitable model of survival curves. Various models have been proposed to describe non–log-linear survival curves *(2, 5, 12)*. Non–log-linear models assume that bacterial cells in a population do not have identical heat resistances, and a survival curve is the cumulative form of a distribution of lethal agents *(10)*. In general, many non–log-linear models are capable of describing survival curves of a unique shape by using three or more parameters for an adequate description of the data *(22)*. A mathematical model based on the Weibull distribution is often used because of its convenience. The Weibull model uses only two parameters (α and β) to describe the extent of inactivation and degree of curvilinearity, respectively *(19)*.

In the present study, among the non–log-linear inactivation models the Weibull distribution (equation 1) and the log-logistic model (equation 2) were selected to describe the experimental data obtained under nonisothermal conditions, and the fitness of the models was compared. These two models performed better statistically than did other non–log-linear models in our preliminary studies. The model parameter values are presented in Table 1. Table 2 shows the computed MSE and R^2 values to compare the goodness of fit of the Weibull and log-logistic models. The MSE is a measure of the variability remaining in the predictive models, and a lower MSE indicates that the model describes the data adequately *(1)*. The R^2 value (0 <

TABLE 2. *Comparison of goodness of fit of the Weibull model and log-logistic model for the survival curves of* Salmonella *Typhimurium,* E. coli *O157:H7, and* L. monocytogenes *on ready-to-eat sliced ham treated with near-infrared heating at different radiation intensities*[a]

Pathogen	Radiation intensity (μW/cm²/nm)	Weibull model		Log-logistic model	
		MSE	R^2	MSE	R^2
Salmonella	100	0.06	0.96	0.03	0.98
Typhimurium	150	0.16	0.95	0.05	0.98
	200	0.22	0.93	0.08	0.98
E. coli O157:H7	100	0.05	0.97	0.04	0.97
	150	0.10	0.96	0.05	0.98
	200	0.17	0.95	0.04	0.99
Listeria	100	0.05	0.95	0.04	0.96
monocytogenes	150	0.07	0.96	0.04	0.98
	200	0.16	0.93	0.07	0.97

[a] MSE, mean square error; R^2, regression coefficient.

$R^2 < 1$) is often used as an overall measure of predictive models, and a higher R^2 value indicates a better prediction attained by a particular model *(13)*. For *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes,* mean MSE values with the Weibull model were 0.15, 0.11, and 0.1, respectively, and those with the log-logistic model were 0.05, 0.04, and 0.05, respectively. Mean R^2 values of the log-logistic model were 0.98, 0.98, and 0.97, respectively, which were better than the values of 0.95, 0.96, and 0.95, respectively, of the Weibull model. Therefore, for deli meat pasteurization by NIR heating, the log-logistic model more accurately described the survival curves for the three pathogens than did the Weibull distribution at various radiation intensities.

In conclusion, the present work provided the appropriate non–log-linear models describing the inactivation of *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* at different NIR heating intensities (ca. 100, 150, and 200 μW/cm^2/nm). The results of this study can be used to define the optimum operational conditions for NIR heating to achieve adequate microbial population reductions on RTE sliced ham.

ACKNOWLEDGMENTS

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