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Microbial Quality Change Model of Korean Pan-Fried Meat Patties Exposed to Fluctuating Temperature Conditions

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Abstract

Aerobic bacterial growth on Korean pan-fried meat patties as a primary quality deterioration factor was modeled as a function of temperature to estimate microbial spoilage on a real-time basis under dynamic storage conditions. Bacteria counts in the stretch-wrapped foods held at constant temperatures of 0, 5, 10 and 15°C were measured throughout storage. The bootstrapping method was applied to generate many resampled data sets of mean microbial counts, which were then used to estimate the parameters of the microbial growth model of Baranyi & Roberts in the form of differential equations. The temperature functions of the primary model parameters were set up with confidence limits. Incorporating the temperature dependent parameters into the differential equations of bacterial growth could produce predictions closely representing the experimental data under constant and fluctuating temperature conditions.

Key words: bacterial growth modeling, prepared food, bootstrap method, dynamic temperature, confidence limit

INTRODUCTION

Microbial spoilage is usually the most significant quality change affecting the shelf life and acceptability of Korean seasoned side dish products. Microbial spoilage is dependent on intrinsic and extrinsic factors such as water activity, pH, preservatives, temperature, package atmosphere, etc. When a food is manufactured under controlled conditions of processing and packaging, intrinsic factors are usually defined or fixed under limited range, even though there may be chances for them to be modified a little during storage and distribution. Therefore, estimating the shelf life of perishable foods mainly becomes the problem of measuring or estimating the growth of spoilage organisms under the expected distribution conditions. Characterizing their microbial spoilage as a function of environmental variables is required for determining the shelf life and ensuring adequate quality (1,2). Temperature is the most influential environmental factor affecting microbial spoilage and growth throughout the food supply chain. Quantitative characterization of temperature effects on the microbial growth is needed for predicting and controlling the shelf life of foods in distribution channels with diverse temperature conditions (3-5). Because the food storage and distribution methods commonly introduce temperature fluctuations, a quantitative modeling method sufficiently robust to handle dynamic temperature variation is needed for practical application and usefulness. Particularly, a model employing a real-time data transmission using an intelligent package device such as a radio frequency identification (RFID) device would be highly desirable (6): recently Lee et al. (7) proposed a kinetic microbial growth model for a vegetable-based prepared food, which can incorporate instant temperature effects into microbial growth models. Their model needs to be validated for and extended to other prepared foods for comprehensive applicability.

This study therefore aims to develop a microbial spoilage model for pan-fried meat patties, a typical Korean meat-based seasoned side dish. Aerobic bacterial count was used as a primary quality index for the microbial growth modeling from the preliminary storage experiment.

MATERIALS AND METHODS

Pan-fried meat patties

Korean style pan-fried round meat patties, 4 cm (diameter)×1 cm (thickness) were purchased from a shop in Masan, Korea. The seasoned meat patties were prepared by pan-frying the minced mixture of pork and beef (0.95:0.05) which had been coated with liquid-egg after muddling with wheat flour, salt and a minced blend of shiitake mushroom, tofu and onion. The product had a water activity of 0.95, pH of 6.68 and NaCl content of 1.19% (w/w). The products were transferred to the

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laboratory immediately after preparation and used for the storage experiment under static or dynamic temperature conditions

Storage of the meat patties and microbial enumeration

Korean style pan-fried meat patties (150 g) were stored under constant temperature conditions at 0, 5, 10 and 15°C in 18.0 cm × 13.0 cm × 2.0 cm rectangular polystyrene trays. The packages were over-wrapped with 12 μm thick linear low density polyethylene cling film (Clean Wrap, Busan, Korea). Three packages were taken out periodically for microbial enumeration of the food samples.

For the storage experiments under fluctuating temperature conditions, 300 g meat patties were placed in polystyrene trays. In order to record the temperatures of meat patties tray under real-time experimental conditions, an RFID system attached with a thermister sensor was used. The RFID system consists of two MICAz Mote development kits (MOTE-MPR2400) which were designed specifically for an embedded wireless sensor network from Crossbow Technology (San Jose, CA, USA): one set is for real-time measuring, gathering and sending the temperature of trays by wireless signal, and the other set is for receiving the temperature data. During the storage, small amounts of food sample were taken from the package for the microbial enumeration.

For determination of microbial counts on the stored patties, 30 grams of the sample were aseptically transferred to sterile Stomacher bags and diluted with 90 mL sterile 0.05% peptone water. Samples were then homogenized in a stomacher (Stomacher 400 circulator, Seward Limited, UK) for three minutes at 260 strokes/min. Then 1.0 mL aliquots as 10-fold dilutions in 0.05% peptone water were plated on a Petri dish with Plate Count Agar (PCA; Difco Laboratories, Detroit, USA). The number of colony-forming units (cfu) per gram of sample was counted after incubation for 72 hr at 30°C.

Modeling microbial quality change

Differential Equations 1 and 2 proposed by Baranyi and Roberts (8) were adopted to describe the aerobic bacterial growth on the pan-fried meat patties under fluctuating temperature conditions:

$$\frac{d(\log q)}{dt} = \frac{\mu_{max}}{\ln 10} \tag{1}$$

$$\frac{d(\log N)}{dt} = \frac{\mu_{max}}{\ln 10}\left(\frac{1}{1+10^{-\log q}}\right)\left(1-10^{(\log N - \log N_{max})}\right) \tag{2}$$

where q is the normalized concentration of an unknown substance critically needed for cell growth and represents the physiological state of the cell population at time t, μ_{max} is the maximum specific growth rate (1/day), N is the microbial count in cfu/g at time t, and N_{max} is the maximum cell density in cfu/g.

The mean bacterial counts obtained from the bootstrapping procedure were fitted with solution of Equations 1 and 2 with guessed parameter set of q_o, N_o, μ_{max} and N_{max}, where q_o and N_o, are the initial physiological state and density of the microbial cells, respectively (7). One thousand parameter sets were obtained by the repeated procedure of resampling and parameter estimation. Temperature dependence of the parameters was examined and applied to estimating the microbial growth under different temperature conditions.

Microbial growth under constant temperature and dynamic temperature conditions was estimated by solution of differential Equations 1 and 2, whose parameters were substituted with their temperature dependence functions. For dynamically changing temperature conditions, initial q (q_o) in Equation 1 was assumed to be adjusted during the lag time period (t_{lag}) according to Equation 3 (7):

$$\log q_o = \frac{\int_0^{t_{lag}} \log q_o(T)\ dt}{t_{lag}} \tag{3}$$

where all the summation of time variant temperature-dependent contributions to lag time is completed at end of lag time (t_{lag}) which retains constant microbial count.

Variability in temperature dependence of the model parameters was taken into account to give a confidence band of the microbial count according to the method of Park and Lee (9): 95% confidence limit lines were adopted as lower and upper bounds for log q_o and $\sqrt{\mu_{max}}$ vs. temperature, while averages of 2.5% percentile and 97.5% percentile values at all the temperatures were used for log N_o and log N_{max} (see Fig. 2 and Table 1 given later).

RESULTS AND DISCUSSION

Microbial growth model parameters

Fig. 1 shows the total aerobic bacterial counts on meat patties during storage at four different temperatures. Higher temperature resulted in faster growth with significantly reduced lag time. When the microbial counts were supplied for bootstrapped estimation of model parameters, 1000 sets of them were obtained and their average values with 95% bootstrap confidence intervals were given as functions of temperature in Fig. 2. The average parameter sets applied to Equations 1 and 2 could describe the microbial growth well at all four temperatures

Table 1. Regression equations or values describing the 2.5% and 97.5% percentile values of the primary model parameters

Parameter	Equations with coefficients	R^2
log q_o		
2.5% percentile line	log q_o = 0.3152 T − 5.1081	0.899
97.5% percentile line	log q_o = 0.3770 T − 3.8357	0.916
$\mu_{max}^{1/2}$		
2.5% percentile line	$\mu_{max}^{1/2}$ = 0.0508 T + 0.8813	0.864
97.5% percentile line	$\mu_{max}^{1/2}$ = 0.0574 T + 1.0064	0.923
log N_{max}		
2.5% percentile value	log N_{max} = 8.855	
97.5% percentile value	log N_{max} = 9.111	

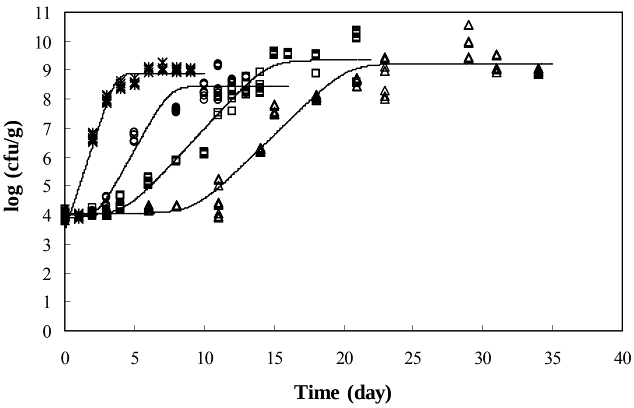

Fig. 1. Total aerobic bacterial counts on pan-fried meat patties stored at 0, 5, 10 and 15°C. The solid lines are estimates derived using Equations 1 and 2 with an averaged parameter set. △: 0°C; □: 5°C; ○: 10°C; ×: 15°C.

as shown in Fig. 1.

The parameters of q_o and μ_{max} were strong functions of temperatures (Fig. 2) as reported for seasoned soybean sprouts (7). Linearized functions based on mean parameters from the bootstrap method can describe their temperature dependence as follows:

$$\log q_o = 0.3100\ T - 4.2894 \quad R^2 = 0.921 \tag{4}$$

$$\sqrt{\mu_{max}} = 0.0545\,T + 0.9402 \quad R^2 = 0.913 \tag{5}$$

where T is temperature in °C.

It is well known that the square root of the maximum specific growth rate increases linearly with temperature, while the increase of q_o with temperature was reported and discussed by Lee et al. (7): initial q_o could be assumed to be readiness to grow which may be adjusted in the microbial lag phase.

Generally values of log N_o and log N_{max} were little affectd by temperature (Fig. 2). As commonly defined, the initial microbial count (N_o) is determined by the contamination during food preparation, and not subsequent storage conditions: average of log N_o from experimental data at four temperatures was 3.864. Even though there was some fluctuation in the estimated maximum cell density of log N_{max} with temperature, its dependence on temperature was not apparent in Fig. 2 (average value: 8.978). Increases in log N_{max} with temperature have been reported in some studies (10,11) but its indifference to temperature is also found and assumed in other works (12,13). Therefore this study assumed that log N_o and log N_{max} of the bacterial growth are constant with some intrinsic variability probably coming from preparation conditions, product characteristics and contaminated microbial flora.

Estimating bacterial growth under constant and dynamic temperature conditions

The parameters of the bacterial growth model can be

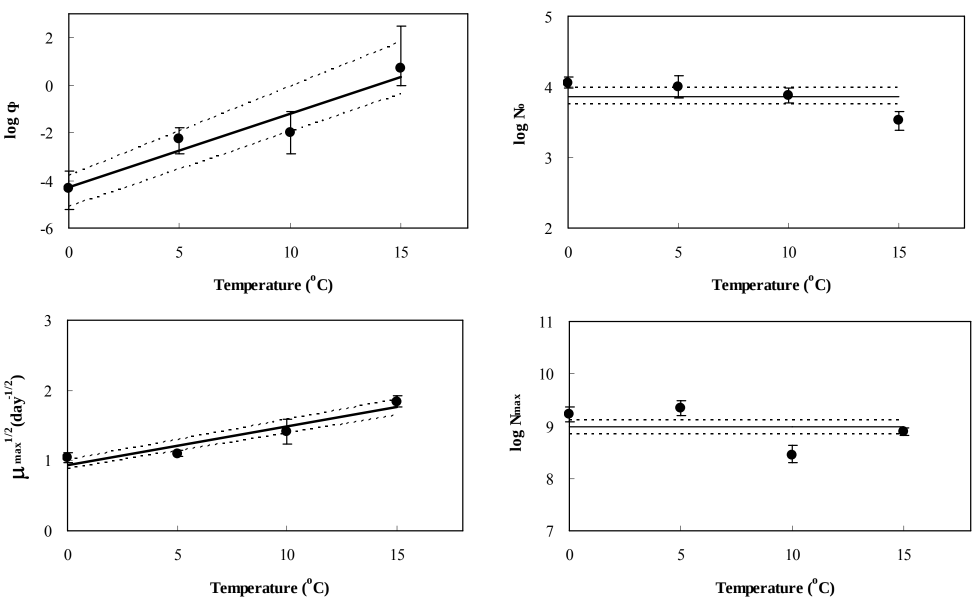

Fig. 2. The primary model parameters vs. temperature. ●: average value at each temperature from the bootstrapping method; Lower and upper dotted lines are linear regression or average of 2.5% and 97.5% percentile values. Solid lines are linear regression or average of mean parameter values at four experimental temperatures.

used for estimating the shelf life based on the criteria of bacterial count. For example, bacterial counts are used for quality criteria of many foods including meat products, dairy products, desserts, meals, cook-chill products and vegetables (14). Substitution of the parameter values into Equations 1 and 2 can estimate the evolution of bacterial count at any temperature. Great value and advantage of differential Equations 1 and 2 are their capability to handle dynamic temperature conditions. If the temperature dependence of primary model parameters is defined mathematically, the microbial growth under fluctuating temperatures can be estimated. Therefore in this study the temperature dependence of log q_o and $\sqrt{\mu_{max}}$ in Equations 4 and 5 was applied to dynamic temperature conditions; log N_o and log N_{max} were assumed to be constant, using average values of those at different experimental temperatures. In order to have the confidence band of the estimated microbial growth, regression lines for 2.5% percentile and 97.5% percentile values in Table 1 (Fig. 2) were supplied in the simulation using Equations 1 and 2 as proposed by Park and Lee (9); the average values of 2.5% and 97.5% percentile values from four experimental temperature conditions were also used as 95% confidence limit values of log N_o (3.747～3.979) and log N_{max} (8.855～9.111), which were assumed to be independent of temperature as discussed above.

Before working with the fluctuating temperature conditions, our model was first tested against constant temperature conditions. Fig. 3 presents the microbial growth band predicted with the overall growth model of aerobic bacteria for the constant temperature conditions where primary model parameters were obtained. The confidence band being a conceptual measure of a region with a 95% chance of encompassing the true estimation (15), and also being understood as confidence intervals of the estimated means (16) described the observed pattern of the microbial growth, covering most of the average experimental data.

Next, the microbial growth model was applied to two different food samples exposed to dynamic temperature conditions: respective initial contamination levels of log N_o from experimental data were supplied to solution of Equations 1 and 2. The predicted bacterial growths under the two fluctuating temperature conditions are compared with experimental counts in Fig. 4. The estimations generally closely describe the behavior of experimental microbial growth, and the confidence band, taking into account parameter variations, comprises most of the bacterial count data throughout the storage time. While there is currently limitation on stochastic prediction of microbial spoilage under dynamic temperature conditions (9), the prediction seems satisfactory to explain the behavior of the bacterial spoilage and estimate shelf life under dynamic temperature conditions.

Even though the prediction with confidence band covers most of the experimental data, the presented

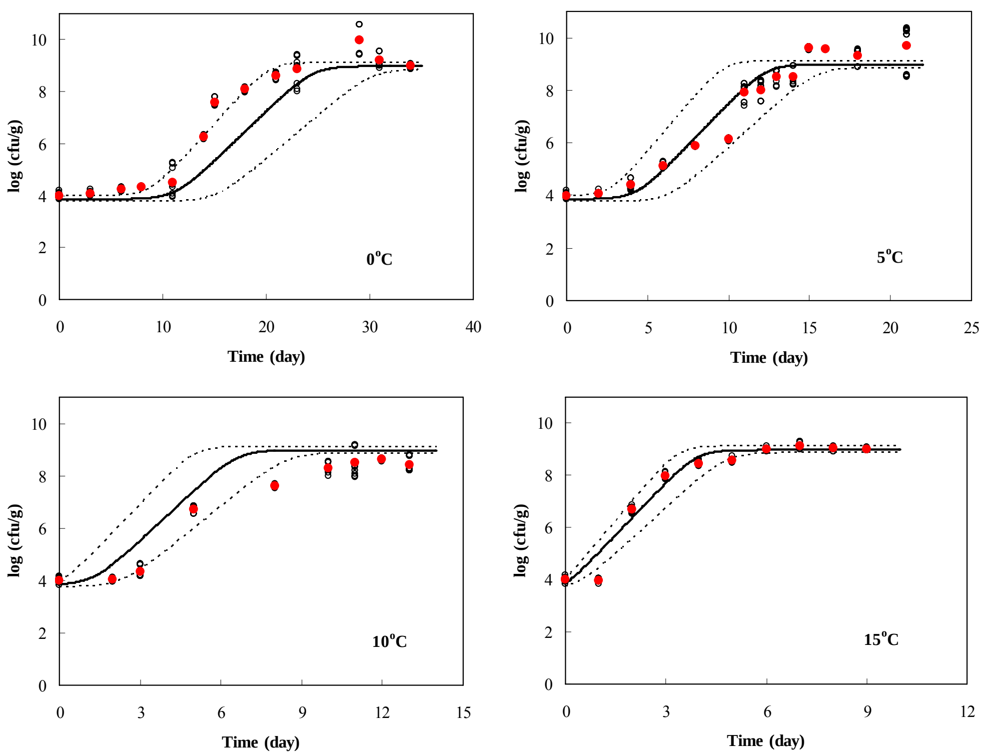

Fig. 3. Bacterial growth estimated with confidence band on pan-fried meat patties at constant temperature conditions. ○: individual plate count data; ●: average of experimental counts. Dotted lines are 95% confidence bands of the estimated growth. Solid lines are the estimation based on average parameters.

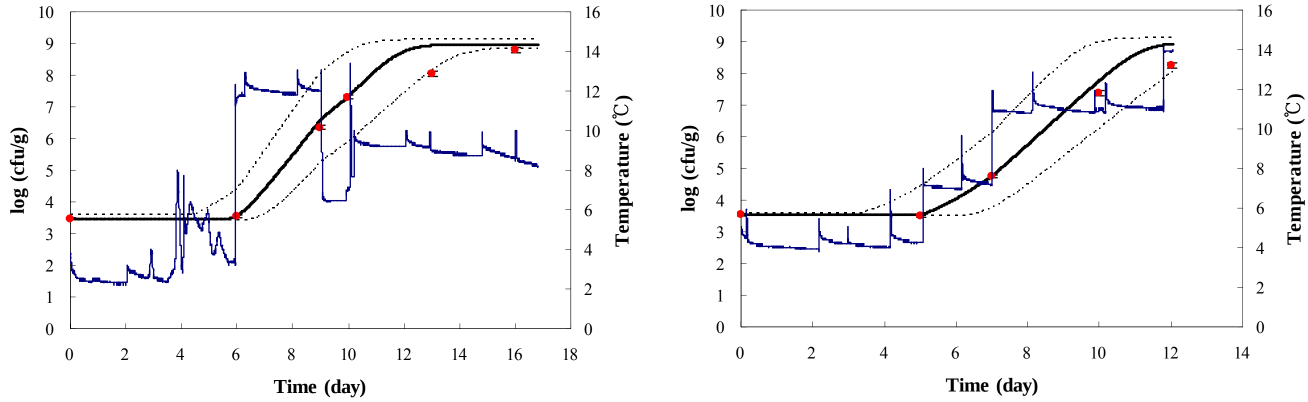

Fig. 4. Two sets of bacterial growth estimation with confidence band on pan-fried meat patties stored under fluctuating temperature conditions. ●: experimental data. The thin line depicts temperature. Dot lines are 95% confidence band of the estimated growth. Thick solid lines are the estimation based on average parameters. Vertical bars depict the standard deviation of the experimental data.

model is better understood to be valid under the constrained conditions of ingredient formulation, food preparation and packaging adopted in this study. Even though more elaborate treatment considering other environmental effects and interactions among microbial species may give more precise estimation of the model parameters and the microbial growth (17-19), the modeling process would be intricate and require much additional time and labor, which would be momentous hurdles and barriers for practical applications. Limitation of the model could be relieved somewhat by consonance between model-building experimental conditions and application domain (1).

CONCLUSION

A kinetic model for aerobic bacterial growth as a primary quality index for pan-fried meat patties, a typical Korean prepared meat side dish was established from storage tests at constant temperatures. Initial physiological state and maximum specific growth rate were formulated as functions of temperature while hypothetical initial contamination level and maximal cell density were independent of temperature. The model could predict the aerobic bacterial count increase throughout storage under static and/or dynamic temperature conditions. The variability of primary model parameters could also be used for providing confidence bands of the predicted microbial growth.

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