

Research Article

Predictive Growth Modeling of *Listeria monocytogenes* in Rice Balls and Its Risk Assessment

Seungsoon Yeo and Misook Kim 

Department of Food Science and Nutrition, Dankook University, Cheonan, Republic of Korea

Correspondence should be addressed to Misook Kim; mkim5@dankook.ac.kr

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This study aimed to investigate the growth of *Listeria monocytogenes* in rice balls and to conduct its microbial risk assessment based on the Korean dietary pattern. Each tuna or ham rice ball was mixed with mayonnaise, soy sauce, or gochujang, a Korean traditional fermented red pepper paste, which was artificially contaminated with *L. monocytogenes* and then stored at 7°C–25°C to assess bacterial growth. Growth data were analyzed using three primary models (the Huang, Baranyi, and Gompertz models), and the growth pattern was found to fit well to the Baranyi model based on the following five statistical criteria: root mean square error (0.38–0.56), Akaike's information criterion (−51.55–−26.99), coefficient of determination (0.72–0.97), bias factor (0.97–1.01), and accuracy factor (1.06–1.18). The effects of temperature on bacterial growth rate and lag time were evaluated using the square root model. The minimum growth temperature for *L. monocytogenes* in tuna or ham rice balls was the lowest when they were mixed with mayonnaise (−9.44°C or −15.37°C, respectively). Risk assessment using FDA-iRISK showed that tuna or ham rice balls mixed with gochujang exhibited the highest microbial risk among all the rice balls tested, regardless of the storage temperature. Tuna or ham rice balls mixed with gochujang had the highest disability-adjusted life years per year (0.015) followed by ham rice balls mixed with soy sauce (0.011–0.015) or mayonnaise (0.006–0.015) and then tuna rice balls mixed with soy sauce (0.006–0.008) or mayonnaise (<0.001). In conclusion, our results, determined using predictive growth models, allow the assessment of potential risk ranking associated with the consumption of rice balls contaminated with *L. monocytogenes* based on the number of illnesses experienced per serving and the disease burden.

1. Introduction

Listeria monocytogenes is widely distributed in the natural environment, grows at a wide range of temperatures from 0°C to 45°C, and is resistant to a variety of environmental conditions such as high salinity (10%–12% NaCl) [1], low water activity (minimum 0.83) [2], and low pH (minimum 4.4) [3]. According to the Centers for Disease Control and Prevention, *L. monocytogenes* is accountable for approximately 1,591 cases of listeriosis, 1,455 hospitalizations, and 255 deaths annually in the United States [4]. In general, compared with healthy individuals, the risk of fatal invasive listeriosis is high in vulnerable populations, including pregnant women, newborns, elderly, and immunocompromised patients, resulting in a death rate of 20%–30%

[5, 6]. Reportedly, listeriosis is primarily associated with the consumption of refrigerated ready-to-eat (RTE) foods as these foods are not pasteurized [5, 7]. Thus, controlling the contamination of RTE foods with *L. monocytogenes* is crucial in the food industry.

In Korea, rice balls and kimbab are popular convenience foods and are consumed at a high rate of 54.4% among convenience food consumption as a substitute for meals [8, 9]. Both are made by combining cooked rice with side ingredients such as ham, tuna, meat, cheese, or vegetables. However, these are distinguished in their shape and the number of side ingredients. Korean rice balls are made from rice and fillings such as a few minced ingredients mixed with dressings formed into oval or triangular shapes. Kimbab is a seaweed rice roll containing several ingredients. According to the Institute of Food

Technology [10], convenience foods such as rice balls, kimbab, and sushi that contain cooked rice and various other ingredients are considered potentially hazardous foods and require control of storage time and temperature during their shelf-life. Such foods have been reported to cause many cases of foodborne diseases linked to enteric bacteria in Korea [11]. Foodborne bacteria were detected in 21 out of 77 commercial kimbab as a result of hygiene inspection of convenience foods [12]. There are no reported cases on foodborne disease caused from *L. monocytogenes* in rice balls and kimbab in Korea. However, its outbreak possibility is high due to the rapid growth of HMR (home meal replacement) and CMR (convenient meal replacement) markets.

Several researchers have developed predictive growth models and quantitative microbial risk assessments (QMRAs) for *Staphylococcus aureus* in kimbab [13, 14]. *L. monocytogenes* is another significant pathogen present in refrigerated convenience foods such as kimbab, rice balls, and sushi owing to its ability to grow at low temperatures; however, risk assessment studies on this pathogen have been relatively limited. Rice balls, which contain a single main ingredient (usually tuna, ham, or sauce) other than rice, slightly differ from kimbab, which contains a number of ingredients. In Japan, the contamination risk of RTE foods containing raw minced tuna as a main ingredient with *L. monocytogenes* was 5.7%–12.1% [15]; moreover, a survey revealed that the contamination risk of sausages and ham with *L. monocytogenes* was ranked in top 10 on the necessity of priority management targeting Korean livestock coops [16]. Thus, in this study, the risk of *L. monocytogenes* was assessed in tuna or ham rice balls containing one of three popular sauces (mayonnaise, soy sauce, or gochujang) each as the main ingredient.

Predictive growth models are mathematical expressions that can analyze and predict the growth and survival of microorganisms for assessing food safety [17]. They can be useful in decision-making with respect to critical control points (CCP) in hazard analysis and CCP (HACCP) systems and microbial risk assessment for preventing foodborne illnesses and food spoilage [18]. The growth pattern of *L. monocytogenes* in foods is dependent on nutrients, water activity, pH, the presence of antagonists and synergists, redox potentials in the food matrix, and the environment in which food is stored [19]. The Integrated Pathogen Modeling Program, 2013 (IPMP, 2013) of the United States Department of Agriculture used in this study is one of several predictive software tools that models the growth of microorganisms. QMRA is a scientific method for assessing the risk of foodborne illness after the consumption of foods contaminated with certain pathogenic microorganisms [10, 20]. It includes four steps: hazard identification, exposure assessment, hazard characterization, and risk characterization. Therefore, QMRA can provide food safety standards for foodborne pathogenic microorganisms by estimating the risk of illness due to a certain pathogen in a certain population.

Food and Drug Administration- (FDA-) iRISK, which was developed by the US FDA, is an interactive, web-based, comparative risk assessment tool. It can calculate and determine risk ranks using mathematical logic and Monte Carlo simulations and using data on the number of diseases and then translate the result into public health metrics that

facilitate the comparisons of public health burdens [21]. In this study, we used predictive modeling to investigate the growth characteristics of *L. monocytogenes* in various types of rice balls stored at different temperatures, and the predicted growth parameters obtained from a suitable model were used in risk assessment. QMRA of *L. monocytogenes* in various rice balls was then conducted using FDA-iRISK to compare, evaluate, and rank risk depending on different environmental conditions.

2. Materials and Methods

2.1. Preparation of Rice Balls. Salad mixtures of tuna or ham were first prepared by mixing canned minced tuna (Dongwon F&B Co., Ltd., Changwon, Korea) with mayonnaise (Ottogi, Eumseong, Korea), soy sauce (Sempio, Incheon, Korea), or gochujang (CJ Cheiljedang Co., Ltd., Nonsan, Korea) in the ratios of 3:1, 15:1, or 10:1, respectively, or cubed ham (1 cm pieces; CJ Cheiljedang Co., Ltd., Seoul, Korea) with the same sauces in the ratios (weight: weight) of 5:1, 12:1, or 12:1, respectively. The bacterial cell number of initial rice balls was 2.26 log CFU/g before inoculation of *L. monocytogenes*. An overnight culture of *L. monocytogenes* (KCTC 3710) was inoculated into each salad mixture at approximately 3 log CFU/gram of rice balls. Each salad mixture was wrapped with cooked rice (Chucheong variety, Nonghyup, Anseong, Korea; ratio, 1:4), and each ball was sealed in a sterile plastic bag and stored at 7°C, 15°C, or 25°C. *L. monocytogenes* was enumerated at each sampling time point by incubation on TSA (BD Difco, Sparks, MD) at 37°C for 24 h. All microbial analyses were conducted for each rice ball in four repetitions.

2.2. Primary Model. The growth rate (μ_{\max} and specific growth rate (SGR); log CFU/h) and lag time (LT; h) of *L. monocytogenes* were determined using three primary models (the Huang, Baranyi, and Gompertz models) using IPMP (IPMP, 2013) software.

The Huang model is described by the following equation:

$$Y(t) = Y_0 + Y_{\max} - \ln \left\{ e^{Y_0} + [e^{Y_{\max}} - e^{Y_0}] e^{-\mu_{\max} B(t)} \right\}, \quad (1)$$

$$B(t) = t + \frac{1}{4} \ln \frac{1 + e^{-4(t-\lambda)}}{1 + e^{4\lambda}},$$

where Y_0 , Y_{\max} , and $Y(t)$ are the natural logarithms of the initial bacterial population count, maximum count, and the count at time t , respectively; μ_{\max} is the maximum SGR; and λ is the lag-phase duration.

The Baranyi model is described by the following equation:

$$Y(t) = Y_0 + \mu_{\max} A(t) - \ln \left\{ 1 + \frac{\exp[\mu_{\max} A(t)] - 1}{\exp(Y_{\max} - Y_0)} \right\},$$

$$A(t) = t + \frac{1}{\mu_{\max}} \ln [\exp(-\mu_{\max} t) + \exp(-h_0) - \exp(-\mu_{\max} t - h_0)], \quad (2)$$

where Y_0 , Y_{\max} , $Y(t)$, and μ_{\max} represent the same quantities as those in equation (1) and h_0 is the physiological state of the microorganism under consideration. In the Baranyi model, the lag phase corresponds to h_0/μ_{\max} , and h_0 can be different and unique for each curve. In the Baranyi model, the initial growth curves observed at constant temperature conditions were analyzed to obtain each h_0 value. After analyzing all the growth curves, the h_0 values were averaged to produce a global value. All growth curves were then reanalyzed using the average value of h_0 to determine μ_{\max} at each incubation temperature.

The Gompertz model is described by the following equation:

$$Y(t) = Y_0 + (Y_{\max} - Y_0) \exp \left\{ -\exp \left[\frac{\mu_{\max} e}{Y_{\max} - Y_0} (\lambda - t) + 1 \right] \right\}, \quad (3)$$

where Y_0 , Y_{\max} , $Y(t)$, μ_{\max} , and λ represent the same quantities as those in equation (1).

2.3. Secondary Model. Temperature-dependent variations in the maximum SGR and LT were analyzed using square root and polynomial models, respectively, using Microsoft Excel 2016 (Microsoft Corp, Redmond, Washington). The equations used are shown in equations (4) to (6):

$$\ln(\text{SGR}): a + bT + cT^2, \quad (4)$$

$$\ln(\text{LT}): a + bT + cT^2, \quad (5)$$

$$T_{\min}: \sqrt{\mu_{\max}} = a(T - T_{\min}), \quad (6)$$

where SGR is in log CFU/h and LT is in hours. T is the temperature ($^{\circ}\text{C}$); T_{\min} is the intercept between the slope and the temperature axis; and a , b , and c are regression constants.

2.4. Model Validation. Model acceptability for *L. monocytogenes* in rice balls was evaluated based on the root mean square error (RMSE), coefficient of determination (R^2), Akaike's information criterion (AIC), bias factor, and accuracy factor.

RMSE is the difference between observed and predicted data. For RMSE, values close to zero are desirable as they indicate that the predicted values are very close to the observed values.

R^2 is calculated using the following equation:

$$R^2 = 1 - \frac{\sum e_i^2}{\sum (y_i - \bar{y}')^2}, \quad (7)$$

where e_i is the error of the predictive value, y_i is the predictive data, and \bar{y}' is the average of predictive data.

AIC is calculated using the following equation:

$$AIC_c = -2 \ln(\text{likelihood}) + 2K + \frac{2K(K+1)}{n-K-1}, \quad (8)$$

where n is the number of observations and K is the number of parameters in the model. B_f and A_f were calculated using equations (9) and (10), respectively:

$$B_f = 10 \left\{ \left(\sum \log(\text{pred}/\text{obs}) \right) / n \right\}, \quad (9)$$

$$A_f = 10 \left\{ \sum \log(\text{pred}/\text{obs}) / n \right\}, \quad (10)$$

where obs, pred, and n correspond to the observed value, predicted value, and repetition number of the observed data, respectively. Values near to 1 indicate the suitability of the model, whereas the values <0.7 or >1.5 indicate the unsuitability of the model.

2.5. Risk Assessment for Rice Balls Using FDA-iRISK. Results of risk assessment of all tested rice balls were compared using FDA-iRISK (version 4.0, FDA, US).

3. Results and Discussion

3.1. Growth of *L. monocytogenes* in Tuna or Ham Rice Balls Mixed with Three Sauces. The initial number of *L. monocytogenes* cells in all rice balls was approximately 3.60 ± 0.36 log CFU/g; growth curves of *L. monocytogenes* in six rice balls at different storage temperatures are shown in Figure 1. Bacterial growth in the food matrix is dependent on several factors such as the presence of other microbiota, nutrients, pH, temperature, and water activity [22, 23]. Lower temperatures retarded bacterial growth in all rice balls. Distinctly different growth pattern in LTM (tuna rice balls mixed with mayonnaise; Figure 1(a)) at 7°C from other samples was observed. Mixing mayonnaise negatively affected bacterial growth in both tuna and ham rice balls. The growth rate of *L. monocytogenes* in LTM was slower than that in other samples, and its maximal growth did not exceed 6.5 log CFU/g. Additionally, mixing mayonnaise in ham rice balls resulted in the lowest maximum growth (<7 log CFU/g) of LHM (ham rice balls mixed with mayonnaise). The low growth in mayonnaise may be due to the presence of egg-derived antibacterial substances such as lysozyme and ovotransferrin, although these are present at very low levels [24, 25]. In general, *L. monocytogenes* had a higher growth rate in ham rice balls than in tuna. The maximum population of *L. monocytogenes* at 25°C was 4.5 log CFU/g in LTM, 6.88 log CFU/g in LTS, 7.38 log CFU/g in LTG, 6.84 log CFU/g in LHM, 8.00 log CFU/g in LHS, and 8.18 log CFU/g in LHG. The pH values of all rice balls decreased (from 5.38 to 5.12 in LTM; from 5.40 to 5.09 in LTS; from 5.45 to 5.07 in LTG; from 5.63 to 5.52 in LHM; from 5.55 to 5.47 in LHS; and from 5.67 to 5.59 in LHG) during storage at 7°C for 137 h. pH of tuna rice balls was lower than ham rice balls.

3.2. Primary Modeling of *L. monocytogenes* Growth in Rice Balls. Many researchers agree that no single growth model can produce a consistent goodness of fit for all

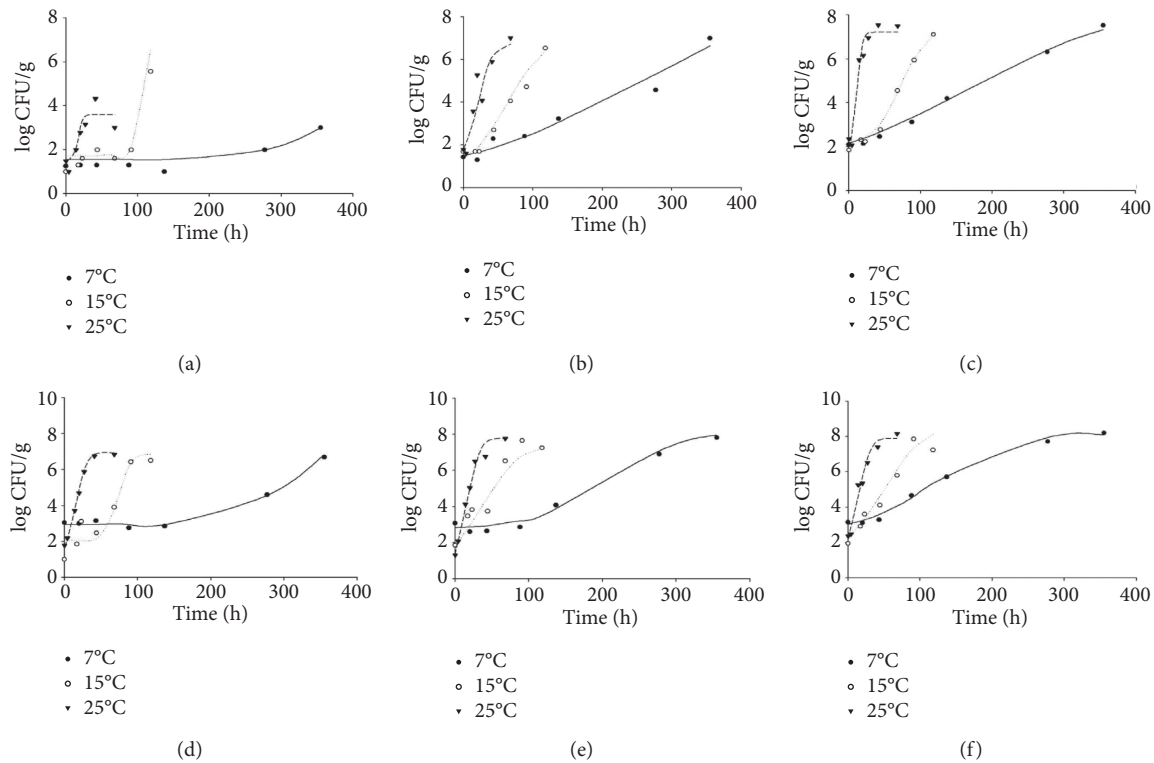


FIGURE 1: Growth of *L. monocytogenes* in rice balls by fitting the Baranyi model: tuna rice balls mixed with mayonnaise (a), soy sauce (b), and gochujang (c) and ham rice balls mixed with mayonnaise (d), soy sauce (e), and gochujang (f). Symbols, experimental data; line, fitted model.

experimental data [26–29]; therefore, most data are assessed using several primary models such as Gompertz, Baranyi, and Huang models (Supplementary Table 1). The microbial growth models such as Gompertz, Baranyi, and Huang models are basically similar. These have been used to calculate microbial growth parameters including lag time, specific growth rate, and maximum population density to estimate the microbial growth in food [29–31]. The results for microbial growth parameters are dependent on which primary model is employed due to the differences of the equations produced from each primary model. The Gompertz model is an empirical equation, the Baranyi model is semitheoretical, and the Huang equation is a theoretical model developed. The results for growth parameters sometimes show that these models were not significantly different [31]. Therefore, they were equally capable of explaining microbial growth in food. In our study, statistical values including R^2 , RMSE, and AIC from all the models were similar for the predictive growth of *L. monocytogenes* in rice balls. In general, the Baranyi model was a better model for predicting *L. monocytogenes* in rice balls than the other models as it exhibited the lowest RMSE and AIC and the highest R^2 . However, the Huang model was suitable for LTS at 15°C and LHG at 7°C, whereas the Gompertz model was acceptable for LTG at 25°C and LHG at 25°C. Even in the same food matrix, the model suitability differed depending on temperature [27]. When compared with the average values for each temperature, the Baranyi model produced the smallest average values for RMSE,

MSE, and AIC and the largest R^2 in most rice balls except in those containing gochujang. LTG and LHG were most accurately modeled using the Gompertz model, but there was no significant difference between the three models. Therefore, we concluded that the Baranyi model was the best primary model for determining the growth of *L. monocytogenes* in rice balls at all temperatures, and so, this model was used to predict the growth pattern of *L. monocytogenes* in rice balls (Figure 1). The experimental observations coincided well with the predicted values, which also predicted the lag and exponential phases.

3.3. Secondary Models for *L. monocytogenes* and Validation. SGR and LT predicted by the Baranyi model were used in a polynomial equation to fit to secondary models. These models were developed to describe the effect of temperature on the SGR and LT, and these equations estimated the SGR and LT of *L. monocytogenes* as a function of temperature (Table 1). Based on SGRs and LTs obtained, mayonnaise was determined as the sauce that most interfered with the growth of *L. monocytogenes* followed by soy sauce and gochujang. As the storage temperature increased, SGR increased (0.02–0.08 at 7°C, 0.06–0.20 at 15°C, and 0.15–0.38 at 25°C). As storage temperature increased, LT decreased (42.55–278.53 h at 7°C, 3.71–93.65 h at 15°C, and 6.96–14.41 h at 25°C) under most growth conditions. These data demonstrate that the growth of the microorganisms is dependent on storage temperature. A short LT was observed for the bacteria in rice balls mixed

TABLE 1: Secondary models developed for the specific growth rate (SGR, log CFU/g/h) and lag time (LT, h) of *L. monocytogenes* in rice balls and statistical validation factors calculated for Baranyi models.

Code	Equation	R^2	T_{\min} (°C)	Storage temperature		
				7°C	15°C	25°C
LTM ¹⁾	SGR = $-0.0109 + 0.0127 \times T + 0.0001 \times T^2$	0.99	-9.43	0.08 ± 0.004	0.20 ± 0.003	0.36 ± 0.02
	LT = $540.2198 - 43.118 \times T + 0.8826 \times T^2$	0.99				
LTS	SGR = $-0.0155 + 0.0039 \times T + 0.0001 \times T^2$	0.97	-3.02	0.02 ± 0.00	0.06 ± 0.01	0.15 ± 0.02
	LT = $87.2021 - 5.5066 \times T + 0.0133 \times T^2$	0.99				
LTG	SGR = $0.0889 - 0.0189 \times T + 0.0012 \times T^2$	0.99	2.67	0.02 ± 0.002	0.08 ± 0.004	0.38 ± 0.01
	LT = $1.7491 + 6.3248 \times T - 0.24796 \times T^2$	0.98				
LHM	SGR = $-0.0721 + 0.0217 \times T - 0.0004 \times T^2$	0.95	-15.40	0.06 ± 0.001	0.16 ± 0.008	0.21 ± 0.03
	LT = $530.4548 - 47.236 \times T + 1.0475 \times T^2$	0.95				
LHS	SGR = $0.0325 - 0.0034 \times T + 0.0004 \times T^2$	0.98	-3.34	0.03 ± 0.001	0.07 ± 0.003	0.19 ± 0.01
	LT = $287.397 - 30.0968 \times T + 0.7449 \times T^2$	0.99				
LHG	SGR = $0.0418 - 0.0049 \times T + 0.0004 \times T^2$	0.99	-2.73	0.03 ± 0.002	0.07 ± 0.006	0.20 ± 0.007
	LT = $87.4372 - 7.5618 \times T + 0.1591 \times T^2$	0.99				

1) LTM, LTS, and LTG are tuna rice balls mixed with mayonnaise, soy sauce, and gochujang contaminated by *L. monocytogenes*; LHM, LHS, and LHG are ham rice balls mixed with mayonnaise, soy sauce, and gochujang contaminated by *L. monocytogenes*.

with gochujang, indicating easy growth. Temperature has been reported to be an important environmental variable affecting microbial growth [32, 33].

R^2 was estimated to evaluate the secondary models; higher R^2 reflected more accurate model predictions. In the Baranyi model, which was the most accurate among the three primary models, R^2 for SGR and LT model equations was >0.95 , indicating good fit. Various developed models have been shown to be capable of predicting the growth of *L. monocytogenes* under the certain conditions [34]. T_{\min} is the minimum temperature that facilitates bacterial growth estimated by the traditional Ratkowsky square model; in this study, we identified slight differences in T_{\min} between tuna and ham rice balls, with ham rice balls exhibiting a lower value than tuna rice balls. Particularly, tuna or ham rice balls mixed with mayonnaise exhibited the lowest T_{\min} (-9.43°C and -15.40°C , respectively) followed by those mixed with soy sauce and gochujang. T_{\min} of *L. monocytogenes* in rice balls is much lower than other reports [34–37]. T_{\min} of *L. monocytogenes* grown in culture media under optimal conditions was reported from 0.5°C to 5°C [38]. T_{\min} for pathogenic bacteria is dependent on the food type owing to differences in nutrients, pH, water activity, salt content, and the presence of antagonists or synergists. The growth of *L. monocytogenes* at low temperatures has also been reported by Devlieghere et al. [36] and Franz et al. [37], who showed that the bacterium can grow at -4.6°C and -3.54°C in green leafy vegetables and cooked ham substrates, respectively. T_{\min} of *L. monocytogenes* is substantially lower than that of other pathogenic bacteria such as *Staphylococcus aureus* in kimbab (2.5°C) [39] and *E. coli* in beef meat (1.5°C to 4.7°C) [40].

3.4. Risk Assessment of Rice Balls Using FDA-iRISK. Table 2 presents the model inputs for risk scenarios of *L. monocytogenes* in rice balls. Due to the absence of the reference literature for rice balls, reports on kimbab were used for determining the initial prevalence of *L. monocytogenes* [41]; among the 30 kimbab samples from

local markets in Korea, 83.3% samples were contaminated with pathogenic bacteria including *L. monocytogenes* (6.7%). Therefore, 0.067 was used as the input data for the initial prevalence. The consumption model included grams per eating occasion and annual eating occasions for kimbab [42, 45]. The initial unit mass was the average serving size of commercial rice balls sold in convenience stores. Data from the Baranyi model were included in FDA-iRISK during the process stage and were designated as “increased” in software. The dose-response model and disease burden were based on a study by Pouillot et al. [43] and Noordhout et al. [44]. The DALY (disability-adjusted life years) is one of the health impact metrics that integrates information on the severity and duration of illness to estimate disease burden [46, 47]. DALY is measured as the sum of years of life lost by premature mortality and years lived with disability. It considers both the probability of experiencing an illness, injury, or even death and the impact of the associated health effect. The WHO estimated that listeriosis resulted in 23,150 illness and 172,823 DALY worldwide in 2010 [48]. Thus, we calculated the DALY per case as 7.46 following DALY divided by illness and listeriosis cases.

Table 3 shows the predictions obtained from FDA-iRISK, including total number of illnesses, mean risk of illness, and total DALY per year for *L. monocytogenes* in tuna or ham rice balls mixed with the three different sauces and stored at different temperatures. In this study, the risk of illness from *L. monocytogenes* in rice balls varied with the main ingredients (i.e., tuna, ham, and the type of sauces) and storage temperature. Their risk rates ranged from 0.000003 to 0.041 based on the mean risk of illness and from 0.00000093 to 0.015 based on the total DALY per year. The highest risk, regardless of the storage temperature, occurred when rice balls were mixed with gochujang. As the storage temperature decreased, the risk of illness from *L. monocytogenes* did not decrease. The risks from LHS and LHM were also highest at 7°C . Despite the low storage temperature at 7°C , the risk of *L. monocytogenes* from rice balls mixed with mayonnaise or soy sauce was higher than that at 25°C .

TABLE 2: Model inputs for food scenarios of *L. monocytogenes* in rice balls in FDA-iRISK 4.0.

Element of risk scenario	Input parameter, iRISK template	<i>L. monocytogenes</i> in rice balls Model input	Reference		
Food Hazard	Rice ball, tuna	Description	—		
	<i>L. monocytogenes</i>	Description	—		
Process model	Initial prevalence	0.067	Cho et al. [41]		
		LTM ¹⁾ : uniform (1.23,1.91/1.50,1.91/1.11,1.62) log CFU ²⁾			
	Initial concentration	LTS: uniform (1.40,1.64/1.26,1.39/1.51,1.99) log CFU			
		LTG: uniform (1.71,2.45/1.94,2.74/2.07,2.14) log CFU			
		LHM: uniform (2.77,3.27/1.95,2.14/1.05,1.92) log CFU		Experiment	
		LHS: uniform (2.58,3.15/1.37,2.05/0.87,1.20) log CFU			
		LHG: uniform (2.58,3.15/2.04,2.79/2.16,2.36) log CFU			
		Initial unit mass	150 g	Experiment	
		Process stage 1: storage at 7, 15, and 25°C, increase by growth	LTM: uniform (3.00,3.01/5.95,6.03/3.31, 4.69) log CFU ³⁾		
			LTS: uniform (7.01,7.02/6.32,8.17/6.29,7.08) log CFU		
LTG: uniform (8.11,9.59/7.09,7.51/7.13,7.32) log CFU			Experiment		
LHM: uniform (6.70,6.98/6.60,7.16/6.81,7.06) log CFU					
LHS: uniform (7.83,8.53/6.81,7.68/7.31,8.26) log CFU					
LHG: uniform (7.83,8.53/7.61,9.98/7.77,7.98) log CFU					
Consumption model	Grams per eating occasion	118.6 g	Shin et al. [42]		
	Eating occasions per year	23.99	Korea Health Statistics: Korea National Health and Nutrition Examination Survey (2016)		
Dose-response model	Healthy people	$7.90E-12$			
	Pregnant women	$2.01E-09$	Pouillot et al. [43]		
	Elderly (more than 65 years old)	$1.49E-10$			
Disease burden	Healthy people	0.015 DALY per case	Noordhout et al. [44]		
	Pregnant women and elderly	12.91 DALY per case			

1) LTM, LTS, and LTG are tuna rice balls mixed with mayonnaise, soy sauce, and gochujang contaminated by *L. monocytogenes*; LHM, LHS, and LHG are ham rice balls mixed with mayonnaise, soy sauce, and gochujang contaminated by *L. monocytogenes*. 2) Input data for the initial concentration input: minimum and maximum concentrations at 7°C/minimum and maximum concentrations at 15°C/minimum and maximum concentrations at 25°C. 3) Process stage input: minimum and maximum concentrations at 7°C/minimum and maximum concentrations at 15°C/minimum and maximum concentrations at 25°C.

Variations in the microbiota population within a rice ball at room temperature may explain the interrupted growth of *L. monocytogenes* under nonsterilized condition compared with that of *L. monocytogenes* alone at a refrigeration temperature [49]. The growth of *L. monocytogenes* in ham rice balls was higher than that in tuna rice balls, even under refrigeration. In general, the use of mayonnaise as a sauce reduced the estimated risk from *L. monocytogenes*. Tuna or ham rice balls mixed with gochujang had the highest value for the mean risk of illness (4.1 cases per hundred servings) regardless of temperature. Ranking of the other products was the same as for total illness, with LTM having the lowest mean risk of illness (3 cases per million servings) stored at 7°C. However, this was still a much higher risk when compared with listeriosis caused by the consumption of

cooked meat (0.000629 cases per million servings) and hot-smoked fish (0.000000989 cases per million servings), as reported by the EFSA [7]. These higher risks may have been due to the large serving size and intake frequency of rice balls in Korea. The total DALY per year, i.e., the annual health burden, of rice balls mixed with gochujang was 0.015 at all storage temperatures, which was the highest identified in this study. LHM exhibited the lowest total DALY (0.006) among ham rice balls. Hong, Kim, and Yoon [50] reported that the DALY of various hams contaminated with *Campylobacter jejuni* ranged from 0.000208 to 0.000346. LTM exhibited the lowest total DALY among all tested rice balls. The susceptibility to listeriosis varies within different population groups; moreover, the risk of illness from consuming rice balls contaminated with *L. monocytogenes* is much

TABLE 3: FDA-iRISK output: risk ranking of six rice balls stored at 7, 15, and 25°C.

Code	Temperature (°C)	Total illnesses ²⁾	Mean risk of illness ³⁾	Total DALY per year
LTM ¹⁾	7	0.0001b	0.000003	0.00000093
	15	0.075	0.003	0.001
	25	0.0005	0.00002	0.004
LTS	7	0.432	0.018	0.006
	15	0.531	0.022	0.008
	25	0.404	0.017	0.006
LTG	7	0.978	0.041	0.015
	15	0.978	0.041	0.015
	25	0.978	0.041	0.015
LHM	7	0.978	0.041	0.015
	15	0.831	0.035	0.012
	25	0.392	0.016	0.006
LHS	7	0.978	0.041	0.015
	15	0.796	0.033	0.012
	25	0.712	0.030	0.011
LHG	7	0.978	0.041	0.015
	15	0.978	0.041	0.015
	25	0.978	0.041	0.015

1) LTM, LTS, and LTG are tuna rice balls mixed with mayonnaise, soy sauce, and gochujang contaminated by *L. monocytogenes*; LHM, LHS, and LHG are ham rice balls mixed with mayonnaise, soy sauce, and gochujang contaminated by *L. monocytogenes*. 2) Total illness is the total number of illnesses generated from the scenario. The total lifespan in the scenario is 70 years (duration of exposure). 3) Mean risk of illness represents the average probability of disease based on one serving.

higher in pregnant (0.20–20.76) and elderly groups (0.02–20.76; Supplementary Table 2) than in the general population.

4. Conclusion

In this study, the growth of *L. monocytogenes* in various rice balls was found to differ depending on the main food ingredients, such as tuna or ham, and sauces, such as mayonnaise, soy sauce, and gochujang. Additionally, the Baranyi model was identified as the optimal model to describe *L. monocytogenes* growth in rice balls and can be useful for conducting future risk assessment. The relative risk of illness from six rice balls was compared using FDA-iRISK based on the dose-response model, consumption, and DALY. Overall, ham rice balls presented a higher risk of listeriosis compared with tuna rice balls. *L. monocytogenes* in rice balls containing mayonnaise had the longest LT, possibly because of less favorable growth conditions such as low pH and the presence of lysozyme and ovotransferrin, which inhibit bacterial growth. Thus, rice balls containing mayonnaise exhibited a lower risk of illness than those containing soy sauce or gochujang. Because the intake of rice balls by Koreans is very high, the risk of listeriosis from rice balls is high. Thus, consistently managing food handling is necessary to control *L. monocytogenes* because it can grow even at low temperatures. These results may be useful in future studies evaluating the growth characteristics of *L. monocytogenes* in rice balls.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Supplementary Materials

Supplementary Table 1: metrics of fit for models of *L. monocytogenes* growth in rice balls, fitted to experimental data ($n = 4$). Supplementary Table 2: risk ranking of six rice balls stored at 7, 15, and 25°C for susceptible population. (Supplementary Materials)

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