Korean J. Food Sci. An. Vol. 35, No. 1, pp. 108~113 (2015)
DOI http://dx.doi.org/10.5851/kosfa.2015.35.1.108
ISSN 1225-8563 eISSN 2234-246X
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ARTICLE

Growth Modelling of *Listeria monocytogenes* in Korean Pork *Bulgogi* Stored at Isothermal Conditions

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Abstract

The purpose of this study was to develop predictive models for the growth of *Listeria monocytogenes* in pork *Bulgogi* at various storage temperatures. A two-strain mixture of *L. monocytogenes* (ATCC 15313 and isolated from pork *Bulgogi*) was inoculated on pork *Bulgogi* at 3 Log CFU/g. *L. monocytogenes* strains were enumerated using general plating method on Listeria selective medium. The inoculated samples were stored at 5, 15, and 25°C for primary models. Primary models were developed using the Baranyi model equations, and the maximum specific growth rate was shown to be dependent on storage temperature. A secondary model of growth rate as a function of storage temperature was also developed. As the storage temperature increased, the lag time (LT) values decreased dramatically and the specific growth rate of *L. monocytogenes* increased. The mathematically predicted growth parameters were evaluated based on the modified bias factor (B_f), accuracy factor (A_f), root mean square error (*RMSE*), coefficient of determination (R^2), and relative errors (RE). These values indicated that the developed models were reliably able to predict the growth of *L. monocytogenes* in pork *Bulgogi*. Hence, the predictive models may be used to assess microbiological hygiene in the meat supply chain as a function of storage temperature.

Key words: predictive model, *Listeria monocytogenes*, Baranyi model, pork *Bulgogi*

Introduction

Pork *Bulgogi* is a Korean traditional food made from thin slices of pork, usually picnic ham, shank ham, or marinated ham (Shin *et al*., 2011). When the meat is marinated with spices (containing soy sauce, onion, ginger, garlic, sesame oil, and other seasonings), pathogenic bacteria may contaminate the meat and subsequently be propagated via cut or whole meat that is intended for further processing into meat products. Recently pork *Bulgogi* has been sold as a ready-to-cook (RTC) product in markets. RTC foods, including pork *Bulgogi,* are typically displayed uncovered and hence exposed to contamination by bacterial pathogens. Thus, several food-borne disease outbreaks have been associated with the consumption of contaminated RTC foods (Jo *et al*., 2003). However, the safety of these products during distribution and sale is frequently not monitored (Björkroth, 2005; Nguyen-The and Carlin, 1994). The number of cases of food-borne disease caused by the consumption of contaminated RTC foods is increasing. In 2012, 266 cases of food poisoning were reported in Korea, and 6,058 cases of food poisoning have been reported to date (KFDA, 2013). This has necessitated the development of predictive models to maintain food quality and avoid undesired pathogenic bacteria in food.

Predictive models are mathematical expressions that describe the growth, survival, and inactivation of food-borne microorganisms. Such models have been used extensively to predict the safety of foods under various environmental conditions, including temperature, pH, and composition. Since the growth and survival of microorganisms are greatly affected by the conditions for model development, it is very important to consider the characteristics of the food in the model (Gibson *et al*., 1988; Kang *et al*., 2010).

Listeria monocytogenes is the main pathogen of concern in refrigerated meat products such as RTC meats (Sofos, 2008). This is because such products can be re-

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contaminated during slicing and packaging. The microbiological analysis of *L. monocytogenes* in marinated broiler legs has been reported (Aarnislao *et al*., 2008). In healthy adults, the doses of *L. monocytogenes* required to cause listeriosis have been reported to vary from 10^5 to 10^9 CFU/g (Dalton *et al*., 1997; Miettinen *et al*., 1999). However, in high-risk groups, infectious doses ranging from <10 and 10^4 CFU/g have been reported (Berrang *et al*., 1988; Ericsson *et al*., 1997). In Europe, the acceptable level of *L. monocytogenes* in foods is defined as $<10^2$ CFU/g at the consumption (Anonymous, 2005). The aim of this study was to develop predictive models for *L. monocytogenes*, considering variables of temperature and storage time in RTC pork *Bulgogi*.

Materials and Methods

Bacterial strains

Two strains of *L. monocytogenes* (ATCC 15313 and isolated from pork *Bulgogi*) were used in previous study (Ahn *et al*., 2012). The strains were maintained in Tryptic Soy Broth (TSB, Difco Laboratories, USA) containing 20% glycerol at -80°C. The stock cultures were thawed at room temperature, and 100 μL of culture was then inoculated into 10 mL of TSB and incubated at 35°C for 24 h to reach at concentration of > 8 Log CFU/mL.

Microbiological analysis, pH, and salt concentration of samples

Pork *Bulgogi* samples were purchased from a retail outlet in Seoul. Since *L. monocytogenes* was not detected in these samples, 10 g portions were cut without vegetables, aseptically transferred them into sterile stomacher bag (VWR, USA) and added 90 mL of 0.1% peptone water (Difco Laboratories, USA). Total plate counts were determined on Plate Count Agar (PCA, Difco Laboratories) for 48 h at 35°C, and coliform and *Escherichia coli* were identified on Petrifilms™ Plates (3M™, USA) for 24 h at 37°C.

The pH was measured with a pH meter (inoLab, Germany), and the salt concentration was determined by a saltmeter (Takemura Electric Works Ltd., Japan). Three replicates of each sample were tested.

Inoculation and enumeration

A 100 μL of mixed strains of *L. monocytogenes* was inoculated onto each surface of pork *Bulgogi* and blended. The initial cell counts were adjusted to 3 Log CFU/mL. The inoculated samples were stored at 5, 15, and 25 °C to develop the primary growth models. These samples were then diluted with 90 mL of 0.1% peptone water, plated onto Oxford Agar with Modified Oxford Antimicrobic Supplement (MOX, Difco Laboratories, USA), and incubated at 35°C for 48 h.

Primary modelling

The Baranyi model was used for primary modelling based on the obtained data (Baranyi and Roberts, 1994; Baranyi *et al*., 1995). The growth parameters in the primary model were including time variable (A), bacterial cell density (y), maximum specific growth rate (μ_{max}), and lag time (LT) were determined at each temperature with the Baranyi model using MicroFit version 1.0 software (Advanced and Hygienic Food Manufacturing LINK Programme, UK). The reparameterized model is described by Equations [1], [2], and [3].

$$y(t) = y_0 + \mu_{max}A(t) - \ln\left(1 + \frac{e^{\mu_{max}A(t)-1}}{e^{(y_{max}-y_0)}}\right) \quad (1)$$

$$A(t) = t + \frac{1}{\mu_{max}}\ln\left[\frac{e^{-\mu_{max}t} + q_0}{1 + q_0}\right] \quad (2)$$

$$LT = \frac{\ln\left(1 + \frac{1}{q_0}\right)}{\mu_{max}} \quad (3)$$

Secondary modelling

To describe the effects of temperature (5, 15, and 25°C) on growth of bacteria, lag time (LT), and maximum specific growth rate (SGR), the polynormial model equation was chosen, based on the parameter of primary models. The model is described by Equations [4] and [5]. *a*, *b*, and *c* are constant, and T is temperature.

$$Ln(LT) = a + bT + cT^2 \quad (4)$$

$$Ln(SGR) = a + bT + cT^2 \quad (5)$$

Evaluation of predictive models

To evaluate the model performance of the predicted models, coefficient of determination (R^2), modified bias factor (B_f), accuracy factor (A_f), and root mean square error (*RMSE*) was used in Equations [6], [7], and [8] (Abou-Zeid *et al*., 2009; Ross, 1996).

$$B_f = 10^{\left\{\frac{\Sigma\log\left(\frac{pred}{obs}\right)}{n}\right\}} \quad (6)$$

$$A_f = 10^{\left\{\frac{\Sigma\left|\log\left(\frac{pred}{obs}\right)\right|}{n}\right\}} \quad (7)$$

$$RMSE = \sqrt{\frac{\Sigma(obs-pred)^2}{n}} \quad (8)$$

The *obs*, *pred*, and *n* parameters indicate observed value, predicted value, and repetition number of observed data, respectively. Perfect agreement between predictions and observations leads to bias and accuracy factor equal to 1.0. If A_f value higher than 1, that indicated predicted values are larger than observed values. *RMSE* is effectively the average difference between the model and the data points.

The relative errors (RE) of individual prediction cases was performed as an additional validation (Delignette-Muller *et al*., 1995) :

$$RE\ for\ LT = \frac{(pred-obs)}{pred} \quad (9)$$

$$RE\ for\ SGR = \frac{(obs-pred)}{pred} \quad (10)$$

The acceptable prediction zone of prediction cases were represented as RE values from -0.3 to 0.15.

Statistical analysis

Experiments were repeated twice and the results were analyzed using the Statistical Analysis System (SAS version 9.1, SAS Institute Inc., USA). The data were expressed as mean ± standard deviation (SD).

Results and Discussion

Pork *Bulgogi*'s compositional properties

The compositional properties of pork *Bulgogi* were investigated. The total plate counts and coliform count for pork *Bulgogi* was 5.3 Log CFU/g and 3.5 Log CFU/g, respectively, while *E. coli* was not detected (Table 1). The pH and salt concentration was 5.79 and 1.52%, respectively. A previous report on *Bulgogi* sauce indicated that the pH and salt concentration was 5.41 and 2.01%, respectively, which is similar to our results (Nam *et al*., 2010). *L. monocytogenes* has known as can be survive in pH 4.4-9.4 and 10% NaCl (ICMSF, 1996). The compositional properties of pork *Bulgogi* indicate that it has favorable conditions for the growth of *L. monocytogenes*.

Table 1. Properties of pork *Bulgogi*

Properties	Values (Mean ± SD)
Total plate counts (Log CFU/g)	5.32 ± 0.03
Coliform (Log CFU/g)	3.54 ± 0.47
E. coli (Log CFU/g)	ND[1)]
pH	5.79 ± 0.02
Salt concentrate (%)	1.52 ± 0.12

[1)]Not detected.

Development of predictive models for *L. monocytogenes*

The growth of *L. monocytogenes* inoculated onto pork *Bulgogi* is shown for different storage temperatures (Fig. 1). The initial bacterial count of *L. monocytogenes* was 3.2-3.4 Log CFU/g. Table 2 shows the growth parameters obtained from the Baranyi model. The maximum cell counts (y_{max}) of *L. monocytogenes* at storage temperatures (5, 15, and 25°C) were 3.5-4.0 Log CFU/g within 96 h and not exceeded 4.1 Log CFU/g. After 24 h, *L. monocytogenes* was not detected in pork *Bulgogi* stored at 15 and 25°C. This might be ascribed to be possible changes in the storage conditions. For instance, *L. monocytogenes* was known to be affected by alkaline pH or microbial competition with bacteria such as lactic acid bacteria (Eom *et al*., 2009; Vasseur *et al*., 1999).

The growth curves showed that the storage temperature was decreased, the generation time and LT of microorganisms are increased and the growth is slowed (Fig. 2). The maximum specific growth rate would increase gradually with values of 0.07 Log CFU/g·h, 0.30 Log CFU/g·h, and 0.90 Log CFU/g·h, and that LT would decrease gradually with values of 38.7 h, 8.07 h, and 4.7 h at stor-

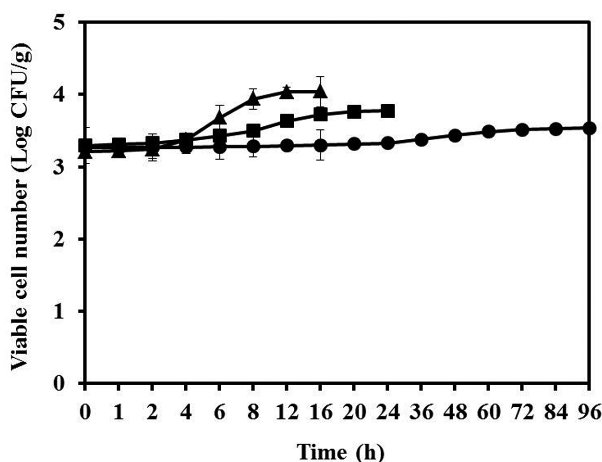

Fig. 1. The growth of *L. monocytogenes* in pork *Bulgogi* at various storage temperature (● : 5°C, ■ : 15°C, ▲ : 25°C).

Table 2. Growth parameters of *L. monocytogenes* in pork *Bulgogi* at various storage temperatures

Temperature (°C)	Growth parameters (Mean±SD)			
	y_0 [1)]	y_{max} [2)]	μ_{max} [3)]	LT [4)]
5	3.25 ± 0.04	3.54 ± 0.09	0.07 ± 0.09	38.7 ± 15.38
15	3.27 ± 0.04	3.78 ± 0.06	0.30 ± 0.15	8.07 ± 1.86
25	3.20 ± 0.04	4.04 ± 0.04	0.90 ± 0.25	4.70 ± 0.52

[1)]Initial cell count (Log CFU/g).
[2)]Maximum cell count (Log CFU/g).
[3)]Maximum specific growth rate (Log CFU/g·h).
[4)]Lag time (h).

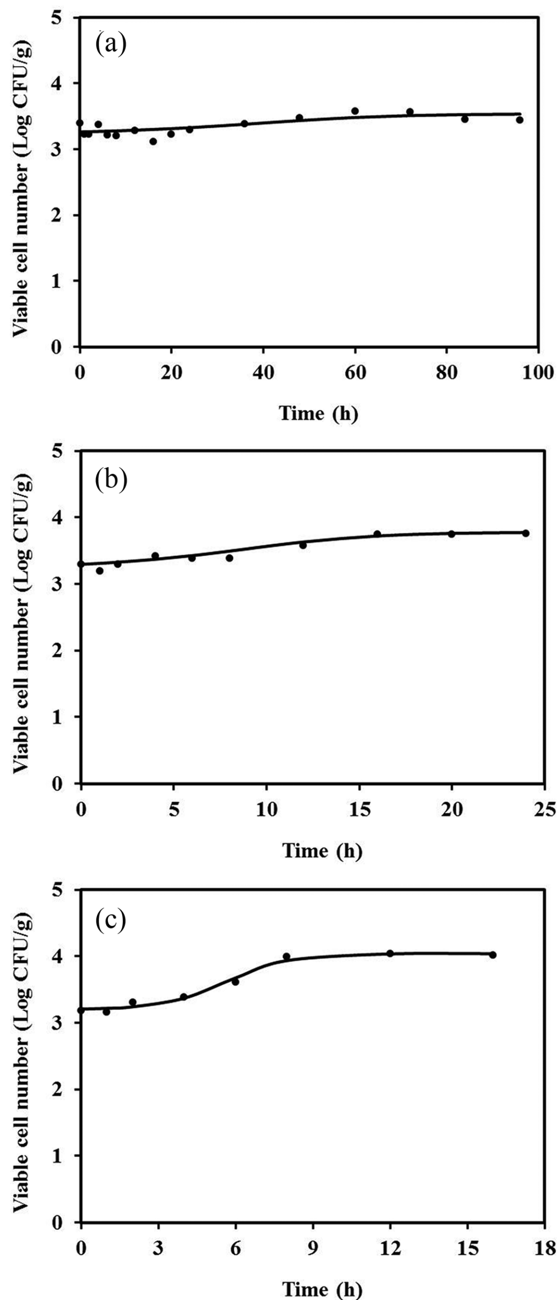

Fig. 2. Primary models at (A) 5°C, (B) 15°C, and (C) 25°C obtained from the predicted model (−) and experimental data (●).

age temperatures of 5, 15, and 25°C, respectively (Table 2). Obtained real results were fit well to predictive line. These results indicate that the growth of microorganisms was influenced by storage temperature. It has been reported that temperature is one of the most important environmental parameters affecting microbial growth and spoilage in meat or meat products (Thomas and Matthews, 2005).

A secondary model was developed to describe how the primary model parameters, including SGR and LT, were affected by temperature (Fig. 3). As storage temperature increased, SGR increased and LT was shortened, according to a secondary model for maximum SGR and LT (Equation (4) and (5)). Other studies have shown that the

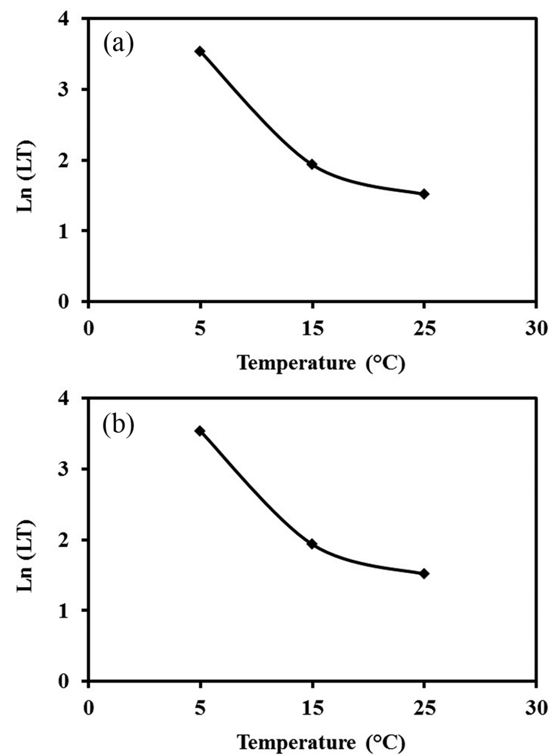

Fig. 3. Secondary models for the effect of storage temperature on growth parameters (a) SGR, specific growth rate and (b) LT, lag time.

Table 3. Evaluation of primary models for *L. monocytogenes* in pork *Bulgogi*

Temperature (°C)	$R^{2,1)}$	$RMSE^{2)}$	$B_f^{3)}$	$A_f^{4)}$
5	0.602	0.08	1.005	1.021
15	0.862	0.06	1.01	1.014
25	0.960	0.05	1.002	1.011

1)Coefficient of determination.
2)Root mean square error.
3)Bias factor.
4)Accuracy factor.

most significant factor for bacterial growth is storage temperature (Gospavic *et al*., 2008; Hong *et al*., 2005).

Evaluation of predictive models

The chemical composition of food changes over time, which can influence the growth of spoilage or pathogenic bacteria. Therefore, a gap between predicted data and observed data can exist. Predicted data are generally based on growth in an aqueous phase, such as broth, and typically indicate faster growth rates than those observed in solid phase, such as, for instance, sausages (Koutsoumanis and Nychas, 2000; Wilson *et al*., 2002). To solve this problem, we have developed models that show a more accurate prediction of bacterial growth in pork *Bulgogi*.

To evaluate the developed predictive models, we used the following indexes used for model performance of predicted and observed data: R^2, *RMSE*, B_f, and A_f. Table 3 shows that the data obtained from the samples stored at 5, 15, and 25°C fit well into the Baranyi model. Although the R^2 values are somewhat low, that is likely because it is not recommended to judge model performance with non-linear regression (Ross, 1996), and the growth of *L. monocytogenes* was insufficient for comparison with previous predictive models. Thus, B_f and A_f are the recommended indices to judge model performance (Baranyi *et al*., 1995). B_f is used to consider whether predictions are in the fail-safe direction or not. Acceptable values for a B_f range from 0.700 to 1.150. By contrast, acceptable values of A_f depend on the number of model variables considered (Oscar, 2005). The B_f values for temperatures of 5, 15, and 25°C were 1.005, 1.01, and 1.002, respectively, and the A_f values were 1.021, 1.014, and 1.011, respectively. The B_f values showed that the predictive bacterial counts exceeded the observed data by approximately. Therefore, the values of the R^2, *RMSE*, B_f, and A_f statistics indicate that the primary model could provide accurate predictions of the growth data and reliably describe the bacterial growth curves in our samples.

The B_f and A_f values were 0.957, 1.045 in the LT model and 1.097, 1.097 in the SGR model (Table 4). B_f and A_f values in both LT and SGR models were close to 1, which indicated a good fit between the observations and the predictions. The *RMSE* value in the LT and SGR model was 0.12 and 0.09, respectively. In addition, RE values for LT and SCR was 0.02 to 0.14 and -0.14 to -0.02. These values was acceptable prediction zone, -0.3 to 0.15 (Oscar, 2005). Therefore, these results demonstrate that the predictions made in this study are reliable. Therefore, the models may be applied to ensure the safety of meat and to establish standards to avoid microbial contamination of meat. In addition, this study could be provide an evaluation index for the safety of products in cases of temperature abuse or process deviations within hazard analysis and critical control points (HACCP) system.

Acknowledgements

This work was supported by the Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2009-0093824).

References

1. Aarnislao, K., Vihavainen, E., Rantala, L., Maijala, R., Suihko, M. L., Hielm, S., Tuominen, P., Ranta, J., and Raaska, L. (2008) Use of results of microbiological analyses for risk-based control of *Listeria monocytogenes* in marinated broiler legs. *Int. J. Food Microbiol.* **121**, 275-284.
2. Abou-Zeid, K. A., Oscar, T. P., Schwarz, J. G., Hashem, F. M., Whiting, R. C., and Yoon, K. S. (2009) Development and validation of a predictive model for *Listeria monocytogenes* scott A as a function of temperature, pH and commercial mixture of potassium lactate and sodium diacetate. *J. Microbiol. Biotechnol.* **19**, 718-726.

Table 4. Evaluation of secondary models for specific growth rate (SGR) and lag time (LT) of *L. monocytogenes* in pork *Bulgogi*

Ln (LT or SGR) = $a + b \times T + c \times T^2$	$RMSE^{1)}$	$B_f^{2)}$	$A_f^{3)}$
Ln (SGR) = $-3.7757 + 0.2041 \times T - 0.0023 \times T^2$	0.09	1.097	1.097
Ln (LT) = $4.7749 - 0.2779 \times T + 0.0059 \times T^2$	0.12	0.957	1.045

1)Root mean square error.
2)Bias factor.
3)Accuracy factor.

3. Ahn, S. H., Lee, Y. J., Lee, J. Y., and Paik, H. D. (2012) Microbiological investigation of ready-to-cook pork bulgogi on Korean markets. *Korean J. Food Sci. An.* **32**, 441-447.
4. Anonymous (2005) Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union, L338, 1-26.
5. Baranyi, J. and Roberts, T. A. (1994) A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* **23**, 277-294.
6. Baranyi, J., Robinson, T. P., Kaloti, A., and Mackey, B. M. (1995) Predicting growth of *Brochothrix thermosphacta* at changing temperature. *Int. J. Food Microbiol.* **27**, 61-75.
7. Berrang, M. E., Frank, J. F., and Brackett, R. E. (1988) Behaviour of *Listeria monocytogenes* in chocolate milk and ice cream mix made from post-expiration date skim milk. *J. Food Prot.* **51**, 823.
8. Björkroth, J. (2005) Microbiological ecology of marinated meat products. *Meat Sci.* **70**, 477-480.
9. Dalton, G. B., Austin, C. C., Sobel, J., Hayes, P. S., Bibb, W. F., Graves, L., Swaminathan, B., Proctor, M. E., and Griffin, P. M. (1997) An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. *New Engl. J. Med.* **336**, 100-105.
10. Delignette-Muller, M. L., Rosso, L., Flandrois, J. P. (1995) Accuracy of microbial growth predictions with square root and polynormial models. *Int. J. Food Microbiol.* **27**, 139-146.
11. Eom, S., Jung, Y., and Yoon, K. (2009) Effect of sanitizer stress response on the growth kinetics of *Listeria monocytogenes* on imitation crabmeat and in broth as a function of temperature. *J. Food Safety* **29**, 564-574.
12. Ericsson, H., Eklöw, A., Danielsson-Tham, M. L., Loncerevic, S., Mentzing, L. O., Persson, I., Unnerstad, H., and Tham, W. (1997) An outbreak of listeriosis suspected to have been caused by rainbow trout. *J. Clin. Microbiol.* **35**, 2904-2907.
13. Gibson, A. M., Bratchell, N., and Roberts, T. A. (1988) Predicting microbial growth: growth responses of *Salmonella* in a laboratory medium as affected by pH sodium chloride, and storage temperature. *Int. J. Food Microbiol.* **6**, 155-178.
14. Gospavic, R., Kreyenschmidt, J., Bruckner, S., Popov, V., and Haque, N. (2008) Mathematical modeling for predicting the growth of *Pseudomonas* spp. in poultry under variable temperature conditions. *Int. J. Food Microbiol.* **127**, 290-297.
15. Hong, C. H., Sim, W. C., Chun, S. J., Kim, Y. S., Oh, D. H., Ha, S. D., Choi, W. S., and Bahk, G. J. (2005) Predictive growth model for native isolated *Listeria monocytogenes* on raw pork as a function of temperature and time. *Korean J. Food Sci. Technol.* **37**, 850-855.
16. ICMSF (International Commission on the Microbiological Specification of Foods) (1996). Choice of sampling plan and criteria for *Listeria monocytogenes*. *Int. J. Food Microbiol.* **22**, 89-96.
17. Jo, C., Han, C. H., Chung, K. H., and Byun, M. W. (2003) Gamma irradiation of ready-to-cook *bulgogi* improves safety and extends shelf-life without compromising organoleptic qualities. *Nutr. Food* **8**, 191-195.
18. Kang, K. A., Kim, Y. W., and Yoon, K. S. (2010) Development of predictive growth models for *Staphylococcus aureus* and *Bacillus cereus* on various food matrices consisting of ready-to-eat (RTE) foods. *Korean J. Food Sci. An.* **30**, 730-738.
19. KFDA (2013) The report of foodborne illness in Korea, Available from http://e-stat.kfda.go.kr/ Accessed April.
20. Koutsoumanis, K. and Nychas, G. J. E. (2000) Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions. *Int. J. Food Microbiol.* **60**, 171-184.
21. Miettinen, M. K., Siitonen, A., Heiskanen, P., Haajanen, H., Brörkroth, K. J., and Korkeala, H. J. (1999) Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. *J. Clin. Microbiol.* **37**, 2358-2360.
22. Nam, J. S., Choi, S. K., and Kim, D. S. (2010) Quality and sensory characteristics of bulgogi sauce with various amount of omija extract juice. *Korean J. Culinary Res.* **16**, 247-259.
23. Nguyen-The, C. and Carlin, F. (1994) The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* **34**, 370-401.
24. Oscar, T. P. (2005) Validation of lag time and growth rate models for *Salmonella* Typhimurium: acceptable prediction zone method. *J. Food Sci.* **70**, M129-M137.
25. Ross, T. (1996) Indices for performance evaluation of predictive models in food microbiology. *J. Appl. Bacteriol.* **81**, 501-508.
26. Sofos, J. N. (2008) Challenges to meat safety in the 21st century. *Meat Sci.* **78**, 3-13.
27. Shin, M. H., Park, J. G., Kim, C. J., and Lee, J. W. (2011) Development ready-to-eat *bulgogi* sauce with no change of sensory properties after high-dose irradiation. *Korean J. Food Sci. An.* **31**, 366-371.
28. Thomas, J. M. and Matthews, K. R. (2005) Food microbiology: an introduction. ASM Press, Washington DC, pp. 247-250.
29. Vasseur, C., Baverel, L., Hébraud, M., and Labadie, J. (1999) Effects of osmotic, alkaline, acid or thermal stresses on the growth and inhibition of *Listeria monocytogenes*. *J. Appl. Microbiol.* **86**, 469-476.
30. Wilson, P. D. G., Brocklehurst, T. F., Arino, S., Thuault, D., Jakobsen, M., Lange, M., Farkas, J., Wimpenny, J. W., and Van Impe, J. F. (2002) Modelling microbial growth in structured foods: towards a unified approach. *Int. J. Food Microbiol.* **73**, 275-289.

(Received 2014.12.10/Revised 2014.12.24/Accepted 2014.12.24)