pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 36, No. 6, pp. 504~509 (2021)
https://doi.org/10.13103/JFHS.2021.36.6.504

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Prevalence and Kinetic Behavior of *Escherichia coli* in Smoked Duck at Changing Temperature

Eunyoung Park[1], Yujin Kim[1], Yewon Lee[1], Yeongeun Seo[1], Joohyun Kang[1], Hyemin Oh[1,2], Joo-Sung Kim[3], Yohan Yoon[1,2]*

[1]*Department of Food and Nutrition, Sookmyung Women's University, Seoul, Korea*
[2]*Risk Analysis Research Center, Sookmyung Women's University, Seoul, Korea*
[3]*Research Group of Consumer Safety, Research Division of Strategic Food Technology, Korea Food Research Institute, Wanju, Korea*

(Received October 26, 2021/Revised December 26, 2021/Accepted December 27, 2021)

ABSTRACT - The objective of this study was to develop dynamic model to describe the kinetic behavior of *E. coli* in sliced smoked duck. *E. coli* was detected in 2 sliced smoked duck samples (16.7%) at 1.23 log CFU/g. The maximum specific growth rate (μ_{max}) of *E. coli* ranged from 0.05 to 0.36 log CFU/g/h, and lag phase duration (*LPD*) ranged from 4.39 to 1.07 h, depending on the storage at 10-30°C, and h_0 value ranged from 0.24 to 0.51. The developed model was validated with observed values obtained at 13°C and 25°C. The model performance was appropriate with 0.130 of root mean squared error (*RMSE*), and the dynamic model also described properly kinetic behavior of *E. coli* in sliced smoked duck samples. These results indicate that *E. coli* can contaminate sliced smoked ducks and the models developed with the *E. coli* isolates are useful in describing the kinetic behavior of *E. coli* in sliced smoked duck.

Key words: *Escherichia coli*, Smoked duck, Dynamic model

Duck meat has been usually consumed in roasting and soup in Korea and the consumption of smoked ducks has been increased because of convenient preparation (Lee et al.[1]). However, *Escherichia coli* have been isolated from poultry product. Kim et al.[2] also suggested that smoked duck was not microbiologically safe enough. Because smoked ducks are usually consumed without additional heating, there is high possibility for foodborne pathogen contamination.

E. coli is facultative anaerobic, Gram-negative bacilli that belongs to the Enterobacteriaceae family (Adriane et al.[3]). They are indicator bacteria for hygiene (Samie[4]), and the pathogenic *E. coli* are also major cause of foodborne illness. *E. coli* may contaminate duck carcasses during evisceration or by workers hands in slaughter houses, and the *E. coli* are cross contaminated to smoked duck during processing environments. The pathogen may gradually proliferate during transfer and storage. Thus, the kinetic behavior of *E. coli* in sliced smoked duck needs to be investigated.

*Correspondence to: Yohan Yoon, Department of Food and Nutrition, Sookmyung Women's University, Seoul 04310, Korea
Tel: +82-2-2077-7585, Fax: +82-2-710-9479
E-mail: yyoon@sookmyung.ac.kr

To describe kinetic behavior of foodborne pathogens, the kinetic parameters for the foodborne pathogens should be calculated, and predictive models can be used to do so (Ha et al.[5]). Most predictive models are developed using a constant temperature, but variable such as temperature and humidity change during food storage and transfer. Thus, a dynamic model should be used to describe the kinetic behavior of bacteria under changing conditions (Ha et al.[5]).

Therefore, the objective of this study was to develop a dynamic model to describe the kinetic behavior of *E. coli* in sliced smoked duck at changing temperatures.

Materials and Methods

Detection of *E. coli* in sliced smoked duck

To evaluate *E. coli* prevalence and contamination levels, 12 sliced smoked duck samples were purchased from grocery stores in Republic of Korea. Twenty five-gram portions of sliced smoked duck were aseptically cut by a flame-sterilized knife and placed into sterile filter bags (3M, St. Paul, MN, USA), and 225-mL aliquots of 0.1% buffered peptone water (BPW; Becton Dickinson and Company, BD, Sparks, MD, USA) were added to the filter bags. The samples were then pummeled using a pummeler (BagMixer; Interscience, St. Nom, France) for 60 s. One milliliter aliquots of

the homogenates were dispensed into the Petrifilm™ *E. coli*/Coliform Count Plates (3M), and the plates were then incubated at 37°C for 24 h. Blue colonies, having gas bubbles were manually counted. The blue colonies were identified with 16S rRNA sequencing after amplification with primers [27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3')]. The PCR reaction was performed with 20 ng genomic DNA as the template in a 30 μL reaction mixture by using EF-Taq (Solgent, Daejeon, Korea). Activation of Taq polymerase was performed at 95°C for 2 min, followed by 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min and a final stage of 72°C for 10 min. The amplified products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA, USA). The mixture was incubated at 95°C for 5 min and placed on ice for 5 min, and then analyzed using ABI Prism 3730XL DNA analyzer (Applied Biosystems) for sequencing.

Preparation of inoculum

Four *E. coli* strains NCCP14038, NCCP14039, NCCP15661, and NCCP11142 were cultured in 10-mL tryptic soy broth (TSB; BD, Sparks, MD, USA) at 37°C for 24 h. One milliliter of the cultures was transferred into 10-mL fresh TSB and subcultured at 37°C for 24 h. The subcultured strains were mixed in a centrifuge tube, and then centrifuged at 1,912×*g* and 4°C for 15 min. The supernatants were discarded, and the cell pellets were washed twice with phosphate-buffered saline (PBS: pH 7.4; 0.2 g KH_2PO_4, 1.5 g $Na_2HPO_4 \cdot 7H_2O$, 8.0 g NaCl, and 0.2 g KCl in 1-L distilled water). The cell pellets were then resuspended in PBS and diluted with PBS to obtain 5 log CFU/mL of concentration.

Development of predictive models

Sliced smoked duck samples were cut into 25 g portions. The sliced smoked duck samples were dipped into 1.5-L *E. coli* inoculum for 3 min, drained for 10 min, and then placed in sterile filter bags. The sample bags were stored at 10°C, 20°C, 25°C, and 30°C up to 96 h, 48 h, 48 h, and 48 h, respectively. During storage, samples were analyzed regularly for enumeration. Fifty milliliter of 0.1% BPW was added into the sample bag and homogenized for 1 min, using a pummeler (BagMixer; Interscience, St. Nom, France). The homogenates were serially diluted with 9 mL BPW, and 0.1 mL aliquotes of the diluents were spread-plated on tryptic soy agar (TSA; Becton, Dickinson and Company) for total bacteria and Petrifilm™ *E. coli*/Coliform Count Plate for *E. coli.* The plates were incubated at 35°C for 24 h, and the colonies were then manually counted. The primary model was developed by fitting the *E. coli* cell count data to the Baranyi model (Baranyi and Roberts[6]), using DMFit curve fitting software 3.5 (Institute of Food Research, Norwich, UK), and kinetic parameters such as lag phase duration (*LPD*; h) and maximum growth rate (μ_{max}; log CFU/g/h) were calculated. The Baranyi model was as follows;

$$N_t = N_0 + \mu_{max} \times A_t - \ln\left[1 + \frac{\exp(\mu_{max} \times A_t) - 1}{\exp(N_{max} - N_0)}\right] \quad (1)$$

Where N_t is the bacterial cell count at time t, and N_0 and N_{max} are the initial and final bacterial cell counts in a growth curve, respectively. A_t is the adjustment function, which denotes the physiological status of bacterial cells (Baranyi and Roberts. 1994)[6]. The A_t is as follows;

$$A_t = \frac{q(t)}{1 + q(t)} \quad (2)$$

Where $q(t)$, a new variable, representing the physiological state of the cell is introduced[6]. The *LPD* and μ_{max} values were further-analyzed, using a polynomial model as a function of temperature to develop a secondary model as follows;

$$LPD \text{ or } \mu_{max} = a_0 + a_1 T + a_2 T^2 \quad (3)$$

Where a_i are the coefficient values and T is the storage temperature (°C). Also, h_0 values were calculated for describing the initial physiological status of bacterial cells (Grijspeerdt and Vanrolleghem)[7].

Validation of predictive model performance

To evaluate the model performance, observed *E. coli* cell counts were collected from the additional experiment. Given that *E. coli* growth data on smoked ducks was not available in the published literature, additional experiments at 15 and 23°C were conducted. During storage, the *E. coli* cell counts were enumerated with the methods described above. These data were then compared to the predicted *E. coli* cell counts calculated using the developed predictive models. The differences between the observed and predicted data were then quantified by calculating the root mean square error (*RMSE*), bias factor, and accuracy factor shown in Eq. 4, Eq. 5, and Eq. 6, respectively

$$\text{RMSE} = \sqrt{1/n \times \Sigma(\text{observed data} - \text{predicted data})^2} \quad (4)$$

$$\text{Bias factor} = 10^{\left(\Sigma \text{Log}\left(\frac{\text{predicted}}{\text{observed}}\right)/n\right)} \quad (5)$$

$$\text{Accuracy factor} = 10^{\left(\Sigma \left|\text{Log}\left(\frac{\text{predicted}}{\text{observed}}\right)\right|/n\right)} \quad (6)$$

Where n represents the number of data points.

Development of dynamic model

To describe *E. coli* growth in sliced smoked duck samples at changing temperatures, a dynamic model was developed with the equation suggested by Baranyi and Roberts (1994)[6] in accordance with the developed primary and secondary models. To evaluate the performance of the dynamic model, *E. coli*-inoculated sliced smoked duck samples were stored at changing temperature in the range of 14°C-25°C, and *E. coli* cell counts were enumerated with the method described above. These cell counts were then compared with the predicted cell counts, calculated with the dynamic model.

Statistical analysis

LPD, μ_{max}, and h_0 data were analyzed with a general linear model, using procedure of SAS® (Version 9.4, SAS Institute, Inc., Cary, NC, USA). The mean comparisons among storage temperature were performed, using a pairwise *t*-test at $\alpha = 0.05$.

Results and Discussion

As a result of the prevalence investigation in sliced smoked duck samples, *E. coli* was detected in 2 of 12 samples (16.7%) at 1.2 log CFU/g. *E. coli* has been detected in raw poultry, and even in cooked poultry products in previous studies. Eyi and Arslan[8] reported that *E. coli* was detected in 49 (87.5%) of the poultry meat samples. Coliforms were detected in duck meat (ready-to-eat product) at 1.30 log CFU/g (Kang[9]). Also, Adeyanju and Ishola[10] reported *E. coli* (43.4%) were detected in poultry meat. Thus, *E. coli* can be considered as an important risk in sliced smoked duck samples and the fate of *E. coli* in sliced smoked ducks should be investigated.

The fitted lines with the Baranyi model passed through *E. coli* cell counted data points, (Fig. 1.) and R^2 value were 0.890-0.986 (Table 1). It indicates that the primary model was developed properly. During storage, μ_{max} of *E. coli*

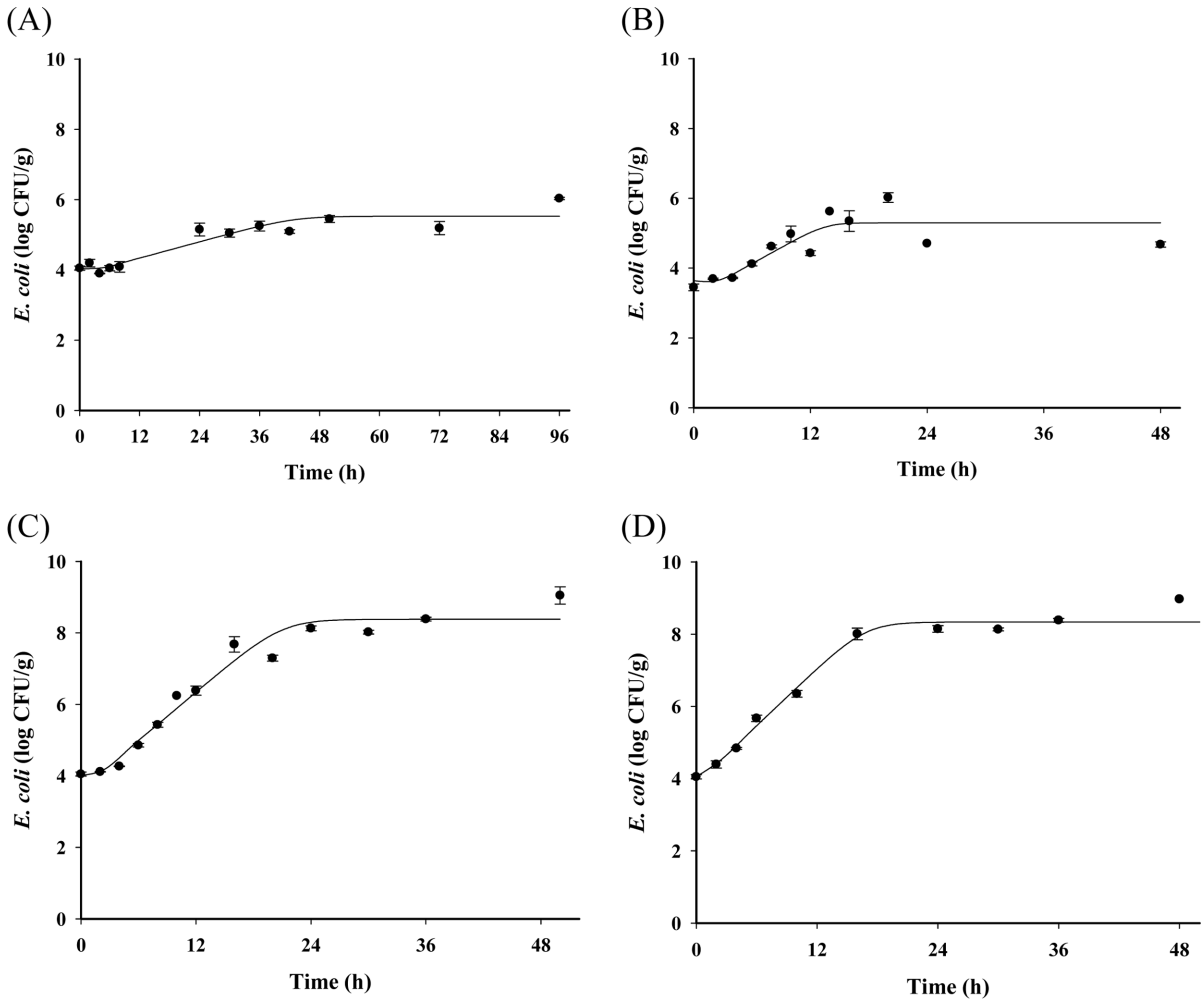

Fig. 1. Bacterial populations of *Escherichia coli* in sliced smoked duck samples during storage at 10°C (A), 20°C (B), 25°C (C), and 30°C (D) for 96, 48, 48, and 48 h respectively. • : observed value, - : fitted line.

Table 1. The parameters calculated by Baranyi model for the *Escherichia coli* in sliced smoked duck

Storage temperature (°C)	(h)	μ_{max} [2] (log CFU/g/h)	h_0 [3]	R^2
10	4.39±0.14 [A]	0.05±0.01 [D]	0.24±0.07 [B]	0.890
20	2.80±0.10 [B]	0.18±0.02 [C]	0.51±0.07 [A]	0.953
25	1.69±0.31 [C]	0.30±0.02 [B]	0.50±0.07 [A]	0.938
30	1.07±0.28 [D]	0.36±0.02 [A]	0.38±0.09 [A]	0.986

[1] Lag phase duration.
[2] Maximum specific growth rate.
[3] Parameter specifying the initial physiological state of cells.
[A-D] Means within the same column with different superscript letters are significantly different ($P < 0.05$).
Data shown are mean±standard deviation.

increased (P<0.05) from 0.05 to 0.36 log CFU/g has storage temperature increased (Table 1). Even at 10°C, which is legal maximum temperature for refrigeration (MFDS[11]), gradual increases in *E. coli* cell counts were observed at 0.05 log CFU/g/h of μ_{max} after 4.39 h of *LPD* (Table 1). The *LPD* of *E. coli* decreased from 4.39 to 1.07 h as storage temperature increased (Table 1). The h_0 can be thought of as a suitability indicator of the microorganism population to the actual environment (Barayi and Roberts[12]); this measure was higher at temperatures above 20°C (0.38-0.51) (P<0.05) (Table 1). This indicates that cells grown at temperatures over 20°C can adapt to the actual environment more quickly, and thus, storing contaminated sliced smoked duck samples above 20°C may increase the risk. To evaluate the effect of storage temperature on the kinetic parameters such as μ_{max} and *LPD*, secondary models were developed (Fig. 2). The fitted lines produced by the secondary models were close to the experimentally observed kinetic data with high R^2 values (0.967-0.972), indicating that the secondary models were appropriate for describing the effect of storage temperature on kinetic parameters. To validate the developed model, predicted μ_{max} and *LPD* values were calculated with the secondary model at specific temperatures (15°C and 23°C). These predicted kinetic parameters were then used to predict *E. coli* cell counts at given storage times in accordance with the primary model. The predicted *E. coli* cell counts were then compared with the observed *E. coli* cell counts, which were obtained from the additional experiments at 15°C and 23°C. The *RMSE* was calculated to be 0.13; where a value close to zero indicates that the predicted data are the same as the observed data (Chai and Draxler[13]). Also, bias and accuracy factors were 1.003 and 0.999, respectively; 1 of bias and accuracy factors indicate a perfect match between predicted and observed values. These results of parameters (*RMSE*, bias, and accuracy factors) indicate that the developed models were appropriate for describing the kinetic behavior of *E. coli* in sliced smoked duck samples.

Using the model, *E. coli* cell counts were predicted at changing temperatures (14°C-25°C), simulating storage, transfer and preparation, and the simulated values were similar to the observed cell counts (Fig. 3.). This result indicates that this developed dynamic model could predict

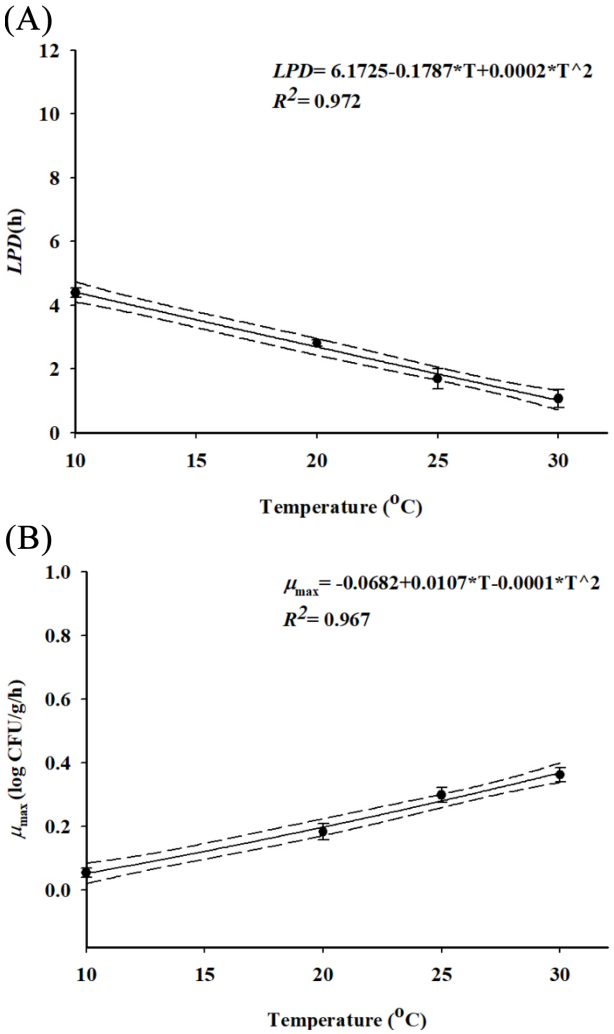

Fig. 2. Secondary models for lag phase duration (*LPD*) (A) and maximum specific growth rate (μ_{max}) (B) of *Escherichia coli* in sliced smoked duck samples as a function of temperature.

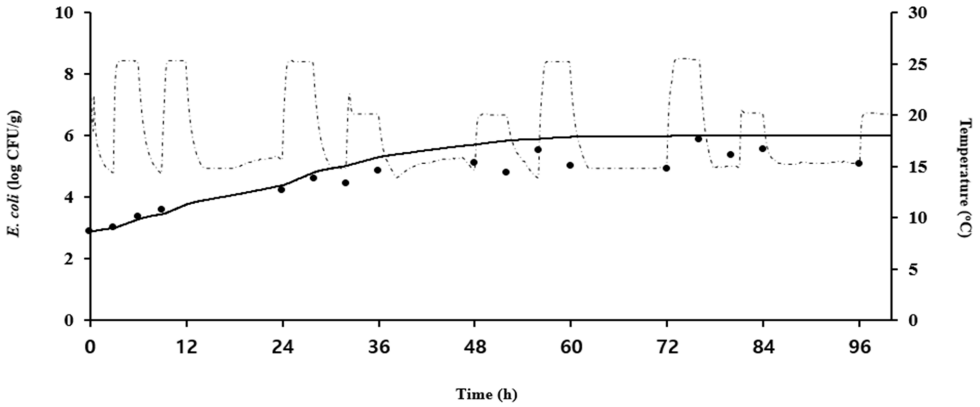

Fig. 3. Change in *Escherichia coli* counts in sliced smoked duck samples under changing temperature. • : observed value, - : fitted line.

the growth of *E. coli* in sliced smoked duck at simulating conditions for transfer and storage.

In conclusion, *E. coli* was detected in 16.7% sliced smoked duck samples with an average contamination level of 1.2 log CFU/g. Kinetic models were developed by applying various temperatures, simulating real transfer and storage environments. The developed model indicates that *E. coli* can multiply at changing temperatures and grow rapidly in smoked duck above 20°C, the preparation temperature, with very high h_0. *E. coli* may cause foodborne illness if smoked duck slices are consumed without additional heating. Therefore, the development of dynamic model to predict *E. coli* growth should be useful for the consumer who eat sliced smoked duck without additional heating consequence.

Acknowledgement

This research was supported by Main Research Program E0142101-02 of the Korea Food Research Institute (KFRI) funded by the Ministry of Science, ICT & Future Planning.

국문요약

본 연구에서는 훈제오리 슬라이스에서 *Escherichia coli* 유통 중 생장 예측을 위한 dynamic model을 개발하였다. *E. coli*는 2개의 훈제 오리 시료(16.7%) 에서 1.23 log CFU/g검출되었다. 10-30°C 보관에 따라 *E. coli*의 μ_{max}는 0.05-0.36 log CFU/g/h, LPD는 4.39-1.07h, h_0 값은 0.24-0.51을 나타내었다. 개발된 모델의 검증은 15°C, 23°C에서 수행하였다. 모델 검증 결과 *RMSE*값이 0.130으로 개발된 모델이 다른 온도에 적용하기에 적합하다고 판단하였다. 이러한 결과는 *E. coli*로 개발된 모델은 훈제오리 슬라이스에서 *E. coli*의 변화하는 온도에 따른 생장을 예측하는 데 유용하다.

Conflict of interests

The authors declare no potential conflict of interest.

ORCID

Eunyoung Park https://orcid.org/0000-0001-8331-1848
Yujin Kim https://orcid.org/0000-0002-0903-9871
Yewon Lee https://orcid.org/0000-0001-8715-1140
Yeongeun Seo https://orcid.org/0000-0001-8715-1140
Joohyun Kang https://orcid.org/0000-0002-7341-8526
Hyemin Oh https://orcid.org/0000-0002-8073-7242
Joo-Sung Kim https://orcid.org/0000-0002-8173-9805
Yohan Yoon https://orcid.org/0000-0002-4561-6218

References

1. Lee, H.L., Koo, B.J., Choi, S.I., Sung, S.H., Park, J.H., Lee, C.W., Jo, C.U., Jung, S., Quality property of the smoked breast meat produced with fresh and frozen-thawed duck meat. *CNU Journal of Agricultural Science*, **42**, 131-139 (2015).
2. Kim, H.B., Lee, J.Y., Jang, Y.H., Chang, B.J., Kim, A.R., Choe, H.N., Prevalence and antimicrobial resistance of Salmonella spp. and Escherichia coli isolated from ducks in Korea. *Korean J. Vet. Res.*, **56**, 91-95 (2016).
3. Adriane, L.V., Cardoso, R., Pádua, R., Siqueira, V., High prevalence rate of extended-spectrum beta-lactamases (ESBL) among Enterobacteriaceae in a small Brazilian public hospital. *Braz. J. Pharm. Sci*., **47**, 701-707 (2011).
4. Samie, A., 2017. *Escherichia coli*: Recent advances on physiology, pathogenesis and biotechnological applications. INTECH, London. United Kingdom, pp. 125-132.
5. Ha, J.M., Kim, W.I., Ryu, J.K., Lee, J.Y., Kim, S.J., Lee, H.Y., Lee, S.M., Yoon, Y.H., A dynamic model for toxin-producing bacteria on tomatoes. *J. Bioanal. Biostat*., **1**, 1-4 (2015).

6. Baranyi, J., Roberts, T.A., A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.*, **23**, 277-294 (1994).
7. Grijspeerdt, K., Vanrolleghem, P., Estimating the parameters of the Baranyi model for bacterial growth. *Food Microbiol*., **16**, 593-605 (1999).
8. Eyi, A., Arslan, S., Prevalence of Escherichia coli in retail-poultry meat, ground beef and beef. *Med. Weter.*, **68**, 237-240 (2012).
9. Kang, M.S., The Growth of Salmonella enteritidis, Staphylococcus aureus and Escherichia coli on ready-to-eat meats under various temperature conditions. Master's thesis, University of Myongji, Seoul, Korea (2008).
10. Adeyanju, G.T., Ishola, O., Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springerplus*, **3**, 1-9 (2014).
11. Ministry of Food and Drug Safety, (2021, March 26). Food code. Retrieved from https://www.foodsafetykorea.go.kr/foodcode/index.jsp
12. Baranyi, J., Roberts, T.A., Mathematics of predictive food-microbiology. *Int. J. Food Microbiol.*, **26**, 199-218 (1995).
13. Chai, T., Draxler. R., Root mean square error (RMSE) or mean absolute error (MAE)?–Arguments against avoiding RMSE in the literature. Geosci. *Model Dev.*, **7**, 1247-1250 (2014).