Korean J. Food Sci. Ani. Resour.
Vol. 31, No. 6, pp. 899~906(2011)
DOI http://dx.do.org/10.5851/kosfa.2011.31.6.899

ARTICLE

The Changes of Natural Microflora in Liver Sausage with Kimchi Powder during Storages

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Abstract

The objectives of this study were to apply the Baranyi model to predict the growth of natural microflora in liver sausage with added kimchi powder. Kimchi powder was added to the meat products at 0, 1, 2, and 3% levels. To determine and quantify the natural microflora in the meat products, total plate counts and counts of anaerobic bacteria and lactic acid bacteria were examined throughout the 28 d of storage. The obtained data were applied to the Baranyi growth model. The indices used for comparing predicted and observed data were B_f, A_f, root mean square error (RMSE), and R^2. Twelve predictive models were characterized by a high R^2 and small RMSE. The Baranyi model was useful in predicting natural microflora levels in these meat products with added kimchi powder during storage.

Key words: liver sausage, kimchi powder, Baranyi model, microflora

Introduction

With the recent growth of the Korean agro-food industry, Korean consumers have shown increasing concerns over agro-food safety and have put more emphasis on the diversity, quality, and health effects of agro-food. The increase in the volume of international agro-food trade complicates agro-food safety issues. A recent survey demonstrated that Korean consumers tend to choose safer agro-foods over cheaper ones, indicating that they are particularly concerned about agro-food safety (Choi and Kim, 2006).

Recently, health-conscious consumers have been demanding functional and healthy meat products. Functional meat and meat products are produced by feeding functional material to livestock or by adding functional materials to meat products, respectively (Jimenez Colmenero, 2000; Kim *et al*., 2011, Yang *et al*., 2002; Youssef and Barbut, 2011). Available functional meat products are diverse and include products such as jerky, sausage, liver sausage, etc., due to the different sources (various types of meat and poultry; organ meats, including liver; and other sources, such as back fat; etc.), spices and other functional additives, processing procedures (comminution, curing, smoking, drying, and packaging), and processing equipment used in their production (Cheo, 2009). The large diversity in processing parameters can cause variations in the effectiveness of the processing procedures used to inactivate bacterial populations that may be present in the raw materials. Moreover, adding functional additives such as kimchi powder to meat products may influence the microbial populations present in the product, and thus, the microbial safety of meat products should be evaluated. However, lactic acid bacteria (LAB) are known to be major lactic acid producers in food products and have been characterized as the main spoiling flora of many cooked meat products (Borch *et al*., 1996; Samelis *et al*., 2000a; Vasilopoulos *et al*., 2007; Matamoros *et al*., 2010). When stored anaerobically and under refrigeration, e.g. through vacuum packaging or modified atmosphere packaging, LAB will dominate the spoilage process (Vermeiren *et al*., 2004). The metabolic activity of LAB results in spoilage appearing as sour, off-flavors, off-odors, milky exudates, slime production, swelling of the package through gas production and discoloration such as greening (Samelis *et al*., 2000b).

Traditionally, the microbiological safety of foods has been established via challenge tests. These tests simulate the effect of environmental conditions on food, in terms

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of growth and proliferation of spoilage and pathogenic microorganisms (Roberts, 1995). Challenge tests can provide data useful in determining the safety and shelf-life of food under set conditions. However, challenge tests have been criticized as an expensive, labor intensive, time-consuming, and non-cumulative research tool (McDonald and Sun, 1999). More recently, challenge tests have been considered as only giving modest assurance of product safety in the food chain (Notermans and Veld, 1994; Baranyi and Roberts, 1995; Roberts, 1997). The inadequacy of challenge tests and the desire for a safe and wholesome food supply has led to the development of a relatively new discipline called predictive microbiology (McMeekin *et al*., 1993).

In this context, the objectives of this study were to predict the growth of natural microflora in liver sausage to which kimchi powder had been added at 0, 1, 2, and 3%. The obtained data were applied to the Baranyi model for bacterial growth, and predicted and observed data were compared for the B_f, A_f, root mean square error (RMSE), and R^2.

Materials and Methods

Preparation of kimchi powder

Commercially produced kimchi was purchased from a local market and fermented at 4°C for 30 d. Fermented kimchi was minced with a cutter (Cutter C4 VV, Sirman, Italy) and vacuum-packaged in polyethylene bags. The vacuum-packaged kimchi was immediately frozen at -20 ±1°C until used. In order to prepare kimchi in powder form, it was heat-treated with a hot air dryer (Enex-Co-600, Enex, Korea) at 60±1°C. Samples were dehydrated until a constant weight was reached (<15% final moisture) and were then ground to <0.5 mm (35 mesh) in size. The powder was stored in a deep freezer (-70°C) until further use.

Preparation of liver sausage

Fresh pork meats were purchased from a pilot plant at Konkuk University and pork back fat, pork liver, and pork skin were also collected from a slaughter house, in Seoul, Korea. The manufacturing process was divided into 2 steps, involving production of cured and emulsified portions of pork meats including fresh pork meat, pork fat, pork skin and pork liver. The cured portion, emulsified portion, and pork liver were then mixed and chopped using a 3 mm-plate. Two percent isolated soy protein, 0.44% nitrite pickle salt (NPS), and kimchi powder were added to this mixture, which was then blended for 15 min before being stuffed into fibrous casings (approximate diameter of 72 mm), using a motorized sausage stuffer (IS-8, Sirman, Italy). The kimchi powder was added at levels of 0, 1, 2, and 3%, based on the control formula weight. After stuffing, the liver sausages were dried in a smoker (ES-1, ETL Testing Laboratories Inc., USA) at 55°C for 30 min, smoked at 65°C for 60 min, and cooked at 75°C for 2 h. The cooked liver sausages were then cooled in cold water for 3 min and stored at 4°C until testing.

Microbial analysis

To quantify the natural microflora in a meat product, the total plate counts (TPC), anaerobic bacteria, and LAB were enumerated throughout the storage (28 d) at 5°C. Preparation and microbial analyses were based on standard methods described in the FDA Bacteriological Analytical Manual (BAM; FDA, 2010). For each sampling, 10 g of meat product was aseptically transferred into a sterile stomacher bag, and 90 mL of sterile 0.1% peptone water was added. The sample was then homogenized in a stomacher (Masticator-Paddle-Blender, IUL Instruments, Spain) for 2 min at normal speed and aliquots were plated out directly or as 10-fold dilutions in 0.1% peptone water. After serially diluting each sample in sterile peptone water, 0.1 mL aliquots of each sample were separately plated onto each of 2 duplicate agar plates. Total plate count (TPC) for aerobic bacteria was evaluated by incubating inoculated Plate Count Agar (PCA; Difco, USA) at 35±2°C for 24±2 h, and anaerobic bacteria were determined by spread-plating on PCA using a BBL anaerobic jar (Difco) at 35±2°C for 24±2 h. LAB counts were determined using lactobacilli MRS agar (Difco) at 35±2°C for 24±2 h. After incubation, plates with 30-300 colonies were chosen for counting. All analyses were performed 3 times, with 2 samples for each replication, and counts were expressed as colony-forming units per gram (CFU/g).

Application of Baranyi model in liver sausage

The growth data were analyzed using Baranyi and Roberts's equation (Baranyi and Roberts, 1994). The growth model was expressed mathematically in the following equations (1) and (2). The logarithm of the cell numbers, log N, is given as follows:

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

Where, A is defined as

$$A(t) = t + \frac{1}{v}\ln\left(\frac{e^{-vt}+q_0}{1+q_0}\right) \quad (2)$$

Where q_0 is the normalized concentration of an unknown substance critically needed for cell growth and represents the initial physiological state of the cell population, μ_{max} is the maximum specific growth rate (log CFU/g/d), y is the bacterial count in log (CFU/g) units at time t, and y_0 is the initial bacteria count in log (CFU/g) units at time 0, y_{max} is the maximum cell density in log (CFU/g) units. The parameter q_0 is related to lag time (t_{lag}), which is calculated from the value of q_0 and μ_{max} as in equation (3). For convenience, if the environment is constant, a lag parameter can also be derived in the Baranyi model. Consider the formula (2) given for $A(t)$. $A(t)$ approximates the function t-λ more and more as t increases, where

$$\lambda = \frac{\ln\left(1+\frac{1}{q_0}\right)}{v} \quad (3)$$

λ is gradual delay in time. Baranyi *et al.* (1993) proved mathematically that if the asymptote of A(t) is a function of the form t–t_0, where t_0 is a constant, then the classical definition of the lag time (Pirt, 1975) is very close to t_0. Therefore, it is reasonable to define t_{lag} by the equation (3).

The average parameters of y_0, μ_{max}, y_{max}, and lag time (t_{lag}) in this study were determined using the MicroFit® version 1.0 (Institute of Food Research, UK), which fitted the integrated form (Baranyi and Roberts, 1994, 1995) to the data set of average plate counts during storage.

To assess the liver sausage microflora, the significant difference of the averages of the obtained parameters, was statistically examined by least squares analysis using PROC GLM of SAS version 9.1.

Evaluation of experimental data

The variability of the data was evaluated, with means and standard deviations calculated with Microsoft Excel and the MicroFit® program, under given conditions and at given storage times in accordance with the Baranyi and Roberts (Baranyi and Roberts, 1994, 1995). The indices used for comparisons of predicted and observed data were the determination coefficient (R^2), RMSE, and the modified bias factors and accuracy factors (B_f, A_f, respectively); see equation (4) and (5) (Skandamis and Nychas, 2000).

$$B_f = \exp\left[\frac{\sum(\ln y_{predicted} - \ln y_{observed})^2}{n}\right] \quad (4)$$

$$A_f = \exp\left\{\sqrt{\left[\frac{\sum(\ln y_{predicted} - \ln y_{observed})^2}{n}\right]}\right\} \quad (5)$$

where y is the response variable and n is the number of observations. Perfect agreement between predictions and observations leads to bias and accuracy equal to 1.0. An A_f value higher than 1 indicates that predicted values are larger than observed values (Yoon *et al.*, 2006).

The RMSE is the average difference between the model and the data points. It provides a more intuitive measure of how well the model fits the data. It has the same units as the data (typically log CFU/g) (equation (6)).

$$RMSE = \frac{RSS}{df}\sqrt{\frac{\sum(\mu_{observed} - y_{predicted})^2}{df}} \quad (6)$$

Where RSS is the residual sum of squares and df is the degree of freedom. F-values were also calculated and compared with F-table values.

Results and Discussion

Analysis of parameters on the Baranyi model

To determine the number of the natural microflora in the liver sausage, the TPC, and the counts of anaerobic bacteria, and LAB in the sausage, to which different amounts of kimchi powder (0, 1, 2, and 3%) had been added, were enumerated during the 28 d in which the sausage was stored at 5°C. On the basis of the observed data, microbial growth models of TPC, anaerobic bacteria, and LAB were estimated by the function of Baranyi and Roberts.

Table 1 shows the estimated parameters, viz. the mean± SD of y_0, y_{max}, μ_{max}, and t_{lag}, obtained from predictive models, at different kimchi powder levels. Because the kimchi powder could contribute to the initial bacterial load, the initial bacterial counts (y_0) for the TPC, anaerobic bacteria, and LAB differed after drying (day 0), depending on the level of kimchi powder.

Based on all 4 statistics, we can conclude that the Baranyi model can predict microbial growth very well and is reliable for predicting the effects of adding kimchi powder to liver sausage on TPC, and anaerobic bacteria and LAB counts. The number of natural bacteria (TPC, anaerobic bacteria, and LAB) in the control sausage gradually increased during 28 d of storage, and the LAB of the control sausage exceeded 6.0 log (CFU/g). All the kimchi

Table 1. Parameters (mean±SD) of growth of the total plate counts, anaerobic, lactic acid bacterial counts estimated on the basis of the Baranyi model in liver sausage

Bacterial type	Treatments[1]	Parameters			
		y_0	y_{max}	μ_{max}	t_{lag}
		Log CFU/g		Log CFU/g/day	Day
Total plate counts	C	2.23±0.05[d]	4.62±0.03[d]	0.65±0.03[b]	13.79±0.39[a]
	T1	2.41±0.05[b]	4.89±0.03[c]	0.53±0.03[c]	13.14±0.39[c]
	T2	2.34±0.05[c]	14.8±0.03[a]	0.34±0.03[d]	10.22±0.39[d]
	T3	2.63±0.05[a]	5.08±0.03[b]	0.70±0.03[a]	13.39±0.39[b]
Anaerobic bacterial counts	C	1.97±0.05[d]	3.37±0.03[d]	0.39±0.03[d]	10.99±0.39[d]
	T1	2.26±0.05[b]	5.25±0.03[a]	0.84±0.03[c]	12.16±0.39[c]
	T2	2.26±0.05[b]	4.42±0.03[b]	1.06±0.03[b]	13.01±0.39[b]
	T3	2.52±0.05[a]	4.30±0.03[c]	2.60±0.03[a]	13.75±0.39[a]
Lactic acid bacterial counts	C	2.01±0.05[d]	5.39±0.03[c]	1.57±0.03[b]	12.62±0.39[c]
	T1	2.28±0.05[c]	6.58±0.03[a]	1.26±0.03[d]	12.04±0.39[d]
	T2	2.50±0.05[b]	6.57±0.03[b]	2.05±0.03[a]	13.21±0.39[b]
	T3	2.52±0.05[a]	5.12±0.03[d]	1.48±0.03[c]	13.49±0.39[a]

All data is given as mean±SD.

[1]Treatments comprised dried kimchi powder. Control, meat batter without kimchi powder; T1, meat batter with 1% kimchi powder; T2, meat batter with 2% kimchi powder; T3, meat batter with 3% kimchi powder.

[a-d]Means within a column with unlike superscript letters are significantly different ($p<0.05$).

treated sausages (T1, T2, and T3) showed similar tendencies to the control (C) in terms of TPC, anaerobic bacteria, and LAB. In the range of 1, 2, and 3% kimchi powder, treatments did not show any particularly hazardous results, when these y_{max}, μ_{max}, t_{lag} were compared to the control values.

Application of Baranyi model

The predictive models for aerobic bacteria in liver sausage are shown in Fig. 1. The TPC of control and all treated sausages increased between day 0 and 28 d of storage, reaching a maximal population (y_{max}) between 4.62 and 14.8 log CFU/g (Table 1). Adding kimchi powder to 1% (T1) and 2% (T2) resulted in lower μ_{max} values than in the control (Table 1), and T2 showed the slowest bacterial growth rate at 0.34 log CFU/g/d. However, 3% kimchi powder (T3), at 0.70 log CFU/g/d, showed a faster bacterial growth rate than did the control. Control samples showed the longest t_{lag}, 13.79±0.39 d, while all treated samples showed shorter t_{lag} than control (Table 1).

Fig. 2 shows predictive models for anaerobic bacteria in liver sausage during the 28 d of storage. The parameters for anaerobic bacteria showed similar tendencies to the aerobic bacteria for initial (y_0) and maximal bacterial

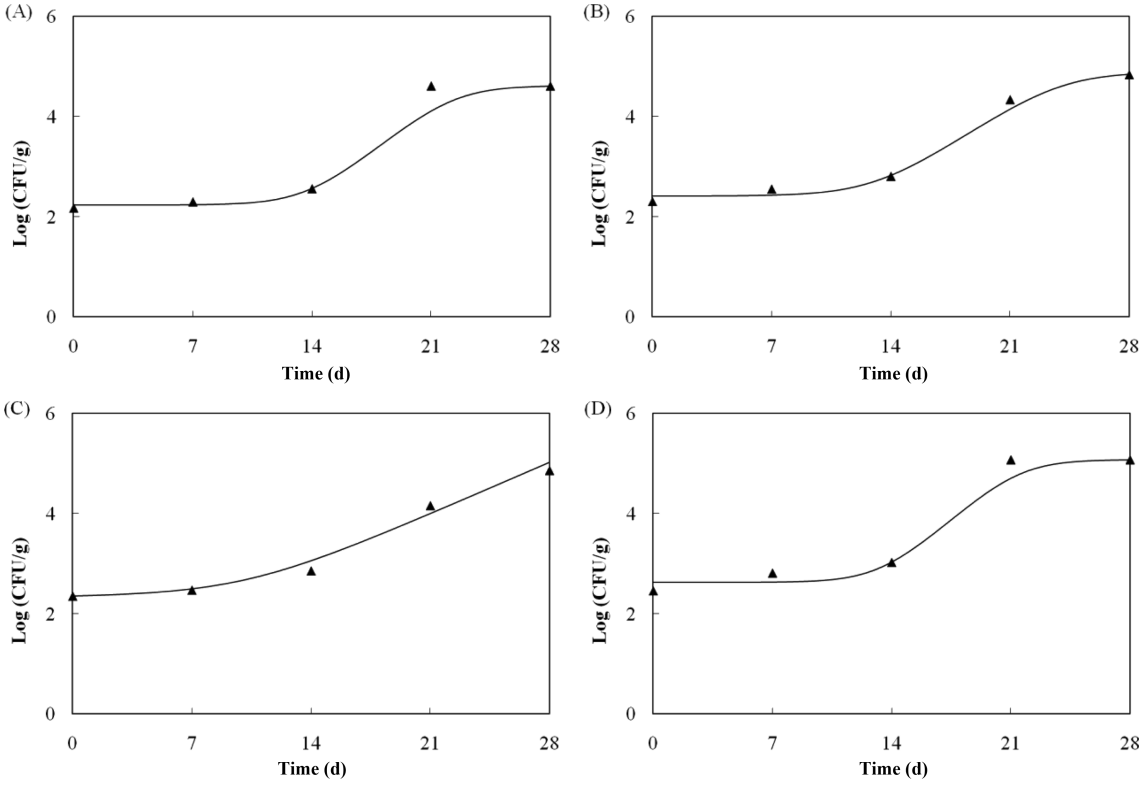

Fig. 1. Changes of aerobic bacteria in liver sausage containing kimchi powder [(A) Control, (B) T1, (C) T2, and (D) T3], estimated on the basis of the Baranyi model (—, predicted data; ▲, experimented data).

counts (y_{max}) (Table 1). All parameters in the μ_{max} value were significantly different between the 4 groups in the anaerobic bacteria model ($p<0.05$). The t_{lag} of T3 was the highest at 13.75 d (Table 1).

The predictive models for LAB in liver sausage are shown in Fig. 3. LAB growth curves showed that control, T1, T2, and T3 have tendencies similar to each other, independent of the level of kimchi powder, and distinct from the curves for TPC and anaerobic bacteria (Fig. 3). There was little difference in the initial counts (y_0) and the maximal counts (y_{max}) between control and all kimchi-treated sausages. T2 showed the highest growth rate (2.05 Log CFU/g/d), and T1 showed the longest t_{lag} (13.49 d).

The Baranyi model assumed that during lag phase, the specific growth rate depended on the need of each cell to synthesize intracellular substances, for instance, RNA or other cytoplasmic components such as ribosomes (Baranyi and Roberts, 1994). Under constant environmental growth conditions, the Baranyi model considers that the lag time, t_{lag}, is inversely proportional to the maximum specific growth rate, μ_{max}. In models of TPC and anaerobic bacteria, for control and treated sausages, the t_{lag} value was inversely proportional to the μ_{max} value (Table 1). Difficulties in estimating the length of the lag phase were not caused by the lack of proper models but by lack of detailed knowledge of the physiological phase of bacterial growth. A population may include cells at the stages of active growth or lag phase, or they may be damaged, busy repairing damage, or at a stage of dying from severe damage (McMeekin and Ross, 2002).

Evaluation of experimented data

To evaluate the models, the B_f, A_f, RMSE, and R^2 indices were used for comparisons of predicted and observed data (Table 2). The predictive parameters of TPC, anaerobic bacteria, and LAB were characterized by a high determination coefficient R^2; high R^2 values result from a small number of degree of freedom.

The R^2 values for the model of TCP in liver sausage were 1.007 (control), 0.988 (T1), 0.908 (T2), and 0.978 (T3) (Table 1). The higher the R^2 values ($0<R^2<1$), the better the prediction by the model (Duffy *et al.*, 1994; Ross 1999). The bias factor (B_f) and accuracy factor (A_f) values in the control deviated slightly from 1.0, which would have indicated perfect agreement (Table 1). The B_f of 0.999 in the control sausage indicated that the predictive total plate counts exceeded the observed counts by

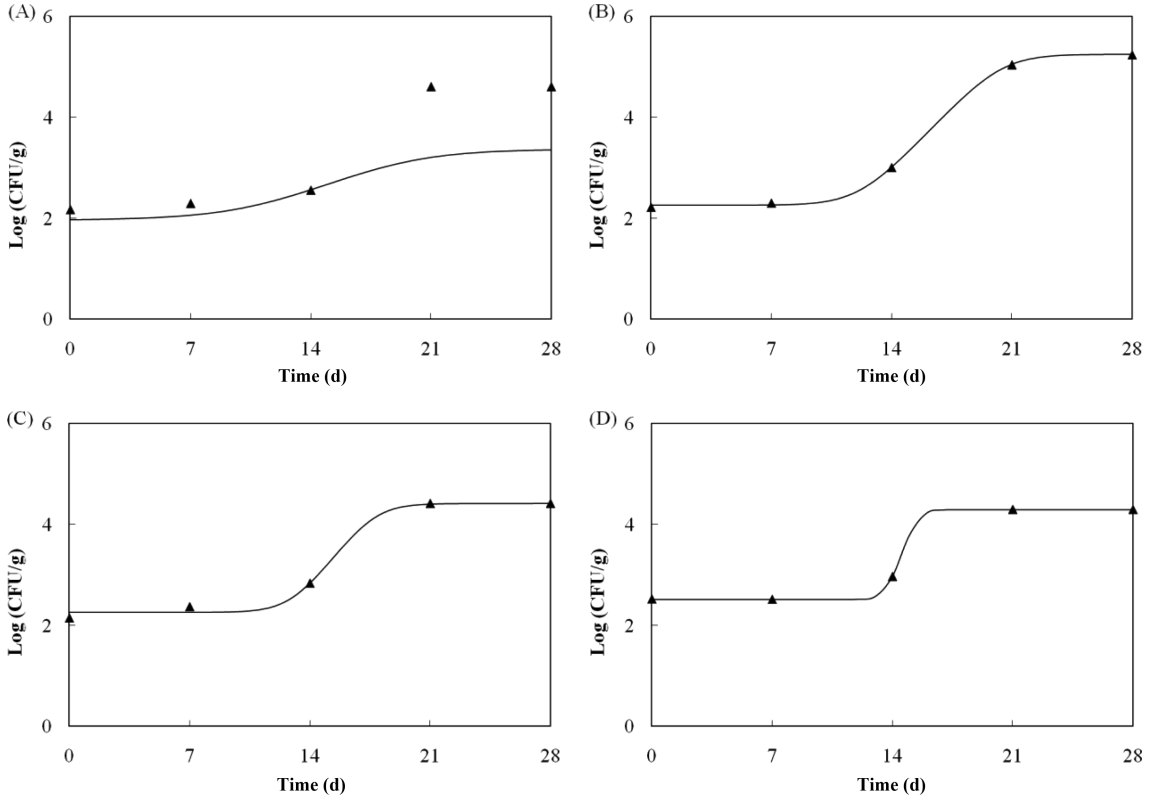

Fig. 2. Changes of anaerobic bacteria in liver sausage containing kimchi powder [(A) Control, (B) T1, (C) T2, and (D) T3], estimated on the basis of the Baranyi model (—, predicted data; ▲, experimented data).

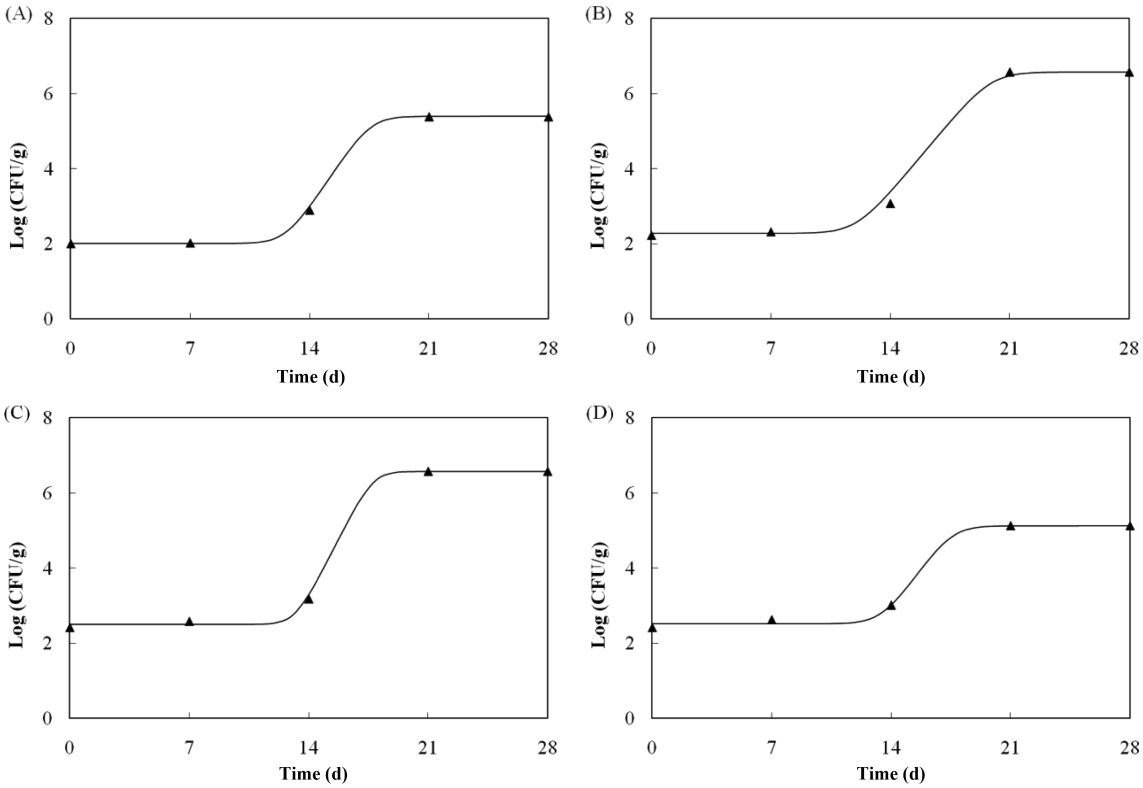

Fig. 3. Changes of LAB in liver sausage containing kimchi powder [(A) Control, (B) T1, (C) T2, and (D) T3], estimated on the basis of the Baranyi model (—, predicted data; ▲, experimented data).

Table 2. Comparison of statistical indices obtained from predictive models in liver sausage

Bacterial type	Treatment[a]	Statistical indices[b]			
		R^2	B_f	A_f	RSME
Total plate counts	C	1.007	0.9987	1.0012	0.03
	T1	0.988	1.0007	1.0045	0.03
	T2	0.908	1.0184	1.0066	0.34
	T3	0.978	1.0010	1.0102	0.11
Anaerobic bacterial counts	C	0.940	1.0055	1.0008	0.03
	T1	0.991	1.0015	1.0005	0.02
	T2	0.983	1.0012	1.0042	0.07
	T3	1.000	0.9997	1.0000	0.03
Lactic acid bacterial counts	C	0.977	1.0084	1.0017	0.01
	T1	0.927	1.0250	1.0165	0.03
	T2	0.966	1.0088	1.0041	0.05
	T3	0.990	1.008	1.0036	0.07

[a]Treatments comprised dried kimchi powder. Control, meat batter without kimchi powder; T1, meat batter with 1% kimchi powder; T2, meat batter with 2% kimchi powder; T3, meat batter with 3% kimchi powder.

[b]B_f, bias factors; A_f, accuracy factors; RMSE, root mean square error; R^2, determination of coefficient

approximately 0.15%. A B_f lower than 1 (B_f <1) indicates a "fail safe" model (Paluumbo *et al.*, 1991), while a B_f higher than 1 (B_f >1) indicates a "fail dangerous" model (Nolan *et al*., 1992). Ross *et al.* (2000) also noted that for models describing pathogen growth rate, B_f in the range of 0.7-0.9 or 1.06-1.15 is considered acceptable, and B_f <0.7 or B_f >1.5 is considered unacceptable. Thus, the results for TPC, anaerobic bacteria and LAB models indicated that good predictions can be made by Baranyi models.

A mathematical modeling process usually begins with first-order models, which are mathematical formulae describing microbial growth or survival curves, expressed by a total plate count, toxin production, and level of substrates or level of metabolites (Kajak and Kolozyn-Krajewska, 2006). It can also be used to estimate the effects of various combinations of variables in food environments (Whiting, 1995). The modeling of specific pathogens in meat and meat products under various temperature ranges or different marination methods has been reported many times in the context of growth inactivation (Heo *et al.*, 2009; Ross, 1999). However, the effectiveness of adding kimchi powder on microbial content of meat products has not been studied much to date.

In this study, only the primary model was applied to liver sausage with added kimchi powder. However, for more detailed assurance of microbiological safety and to

estimate a more stable shelf-life, further estimations of the effects of various combinations of variables, such as storage temperature, salt level, or additives, should be taken into account in secondary modeling. Furthermore, data should be also applied to other predictive modeling equations, such as the modified logistic model and the Gompertz model, to find models more reliable and suitable for food products.

Acknowledgments

This work was funded by the Ministry of Agriculture and Forestry (106115-02-1-SB010), and the Rural Development Administration (PJ007399) in Korea. This study was also supported by the Priority Research Centers Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2009-0093824).

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(Received 2011.12.8/Accepted 2011.12.12)